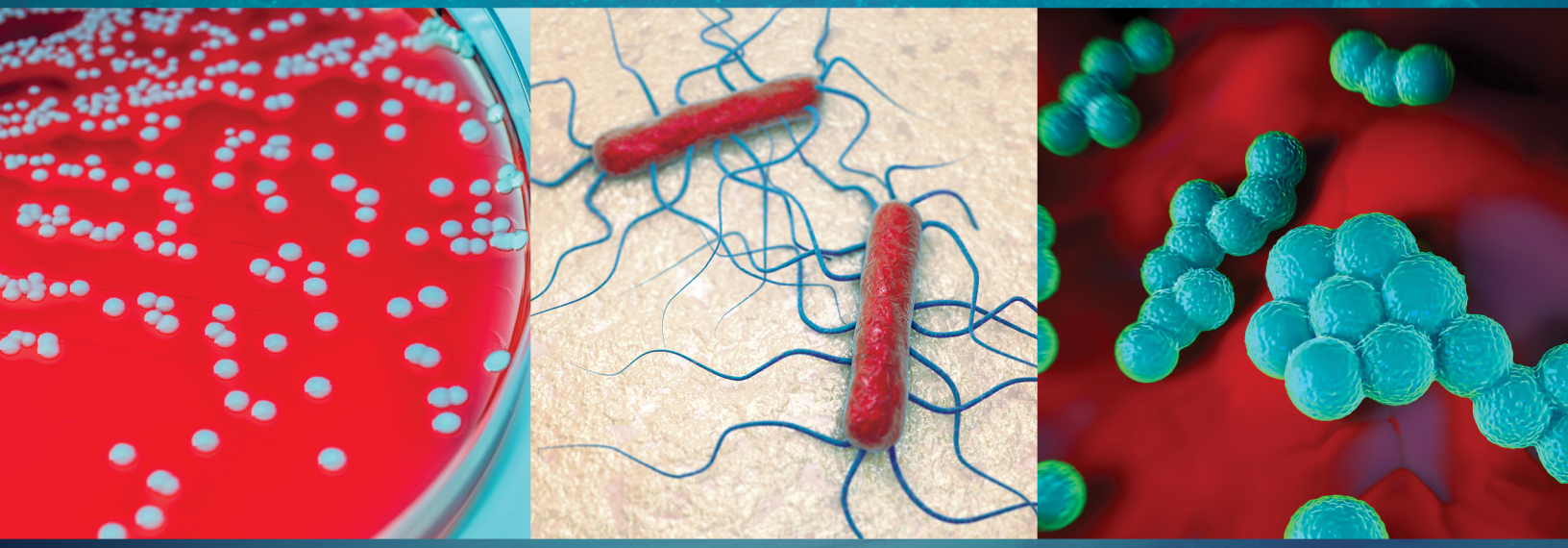
Antimicrobial Resistance

in Bacteria from Livestock and Companion Animals



EDITORS
Stefan Schwarz, Lina Maria Cavaco, and Jianzhong Shen
with Frank Møller Aarestrup

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Washington, DC

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Contents

Contributors *xi*
Foreword *xxiii*
Preface *xxv*
About the Authors *xxix*

1. History and Current Use of Antimicrobial Drugs in Veterinary Medicine 1
   John F. Prescott
2. Antimicrobial Susceptibility Testing of Bacteria of Veterinary Origin 17
   Jeffrey L. Watts, Michael T. Sweeney, and Brian V. Lubbers
3. Molecular Methods for the Detection of Antimicrobial Resistance 33
   Muna F. Anjum, Ea Zankari, and Henrik Hasman
4. Mechanisms of Bacterial Resistance to Antimicrobial Agents 51
   Engeline van Duijkeren, Anne-Kathrin Schink, Marilyn C. Roberts, Yang Wang, and Stefan Schwarz
5. Resistance to Metals Used in Agricultural Production 83
   Christopher Rensing, Arshnee Moodley, Lina M. Cavaco, and Sylvia Franke McDevitt
6. Resistance of Bacteria to Biocides 109
   Jean-Yves Maillard

7. Antimicrobial Resistance among Staphylococci of Animal Origin 127
STEFAN SCHWARZ, ANDREA T. FEßLER, IGOR LONCARIC, CONGMING WU, KRISTINA KADLEC, YANG WANG, AND JIANZHONG SHEN

8. Antimicrobial Resistance in *Streptococcus* spp. 159
MARISA HAENNI, AGNESE LUPO, AND JEAN-YVES MADEC

9. Antimicrobial Resistance in *Enterococcus* spp. of Animal Origin 185
CARMEN TORRES, CARLA ANDREA ALONSO, LAURA RUIZ-RIPA, RICARDO LEÓN-SAMPEDRO, ROSA DEL CAMPO, AND TERESA M. COQUE

10. Antimicrobial Resistance in *Rhodococcus equi* 229
STEEVE GIGUÈRE, LONDA J. BERGHAUS, AND JENNIFER M. WILLINGHAM-LANE

11. Antimicrobial Resistance in *Listeria* Species 237
LAURA LUQUE-SASTRE, CRISTINA ARROYO, EDWARD M. FOX, BARRY J. MCMAHON, LI BAI, FENGQIN LI, AND SÉAMUS FANNING

12. Antimicrobial Resistance in Nontyphoidal *Salmonella* 261
PATRICK F. MCDERMOTT, SHAOHUA ZHAO, AND HEATHER TATE

13. Antimicrobial Resistance in *Escherichia coli* 289
LAURENT POIREL, JEAN-YVES MADEC, AGNESE LUPO, ANNE-KATHRIN SCHINK, NICOLAS KIEFFER, PATRICE NORDMANN, AND STEFAN SCHWARZ

14. Antimicrobial Resistance in *Campylobacter* spp. 317
ZHANGQI SHEN, YANG WANG, QIJING ZHANG, AND JIANZHONG SHEN

15. Antimicrobial Resistance in *Pasteurellaceae* of Veterinary Origin 331
GEOVANA B. MICHAEL, JANINE T. BOSSÉ, AND STEFAN SCHWARZ

16. Antimicrobial Resistance in *Bordetella bronchiseptica* 365
KRISTINA KADLEC AND STEFAN SCHWARZ

17. Antimicrobial Resistance in *Acinetobacter* spp. and *Pseudomonas* spp. 377
AGNESE LUPO, MARISA HAENNI, AND JEAN-YVES MADEC

18. Antimicrobial Resistance in *Corynebacterium* spp., *Arcanobacterium* spp., and *Trueperella pyogenes* 395
ANDREA T. FEßLER AND STEFAN SCHWARZ

19. Antimicrobial Resistance in *Stenotrophomonas* spp. 409
YANG WANG, TAO HE, ZHANGGI SHEN, AND CONGMING WU

20. Antimicrobial Resistance in *Mycoplasma* spp. 425
Anne V. Gautier-Bouchardon

21. Antimicrobial Resistance in *Clostridium* and *Brachyspira* spp. and other Anaerobes 447
Marie Archambault and Joseph Rubin

22. Antimicrobial Resistance in *Leptospira*, *Brucella*, and Other Rarely Investigated Veterinary and Zoonotic Pathogens 471
Darren J. Trott, Sam Abraham, and Ben Adler

23. Antimicrobial Resistance in *Chlamydiales*, *Rickettsia*, *Coxiella*, and Other Intracellular Pathogens 485
Daisy Vanrompay, Thi Loan Anh Nguyen, Sally J. Cutler, and Patrick Butaye

24. Antimicrobial Drug Resistance in Fish Pathogens 501
Ron A. Miller and Heather Harbottle

25. Antimicrobial Resistance: a One-Health perspective 521
Scott A. McEwen and Peter J. Colignon

26. Licensing and Approval of Antimicrobial Agents for Use in Animals 549
Constança Pomba, Boudewijn Catry, Jordi Torren Edo, and Helen Jukes

27. Monitoring Antimicrobial Drug Usage in Animals: Methods and Applications 569
Nicole Werner, Scott A. McEwen, and Lothar Kreienbrock

28. Present and Future Surveillance of Antimicrobial Resistance in Animals: Principles and Practices 595
Shabbir Simjee, Patrick F. McDermott, Darren J. Trott, and Chuanchuen Rungtip

29. Source Attribution and Risk Assessment of Antimicrobial Resistance 619
Sara M. Pires, Ana Sofia Duarte, and Tine Hald

30. Optimization of Antimicrobial Treatment to Minimize Resistance Selection 637
Luca Guardabassi, Mike Apley, John Elmerdahl Olsen, Pierre-Louis Toutain, and Scott Weese

31. Antimicrobial Stewardship in Veterinary Medicine 675
David H. Lloyd and Stephen W. Page

Index 699

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Foreword

I am honored to be asked to write a preface for the collection of articles that comprise *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals*. The authors are all leaders in the field and have provided comprehensive summaries of their knowledge in all aspects of the use of antibiotics and development of resistance in animal health.

It is regrettable that it has taken 50 years to recognize that this worldwide plague of misuse and resistance development cannot be reversed. Given recent publications, the extent of antibiotic use in commercial fish farming in Southeast Asia reminds us that there are still many hurdles to be overcome if we want to control the misuse of antibiotics in humans.

Finding new antimicrobials has always been difficult, and it is to be hoped that modern molecular biology and its constant technical improvements will save the day and provide humans with reliable agents for disease treatment without the inevitability of drug resistance. Improving knowledge of the science of low-molecular-weight microbial compounds will perhaps, in time, teach us how to use antibiotics for human use. Antimicrobial discovery must continue apace such that a source of reliable agents will always be available, whether for human or animal health.

This compendium of interesting and focused articles is valuable, and it promises to provide expert advice on all aspects of antimicrobial resistance and ways of controlling it. Remember that in the 1950s antibiotics were miracles, but they have become menaces! It is up to us to focus on intelligent use of small molecules as cures and to finally eliminate infectious diseases in humans.

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Preface

Antimicrobial agents are indispensable for the treatment of bacterial infections not only in humans, but also in animals. In veterinary medicine, no new classes of antimicrobial agents are expected to be licensed in the coming years. In fact, virtually all antimicrobial agents that have been approved during the past 2 decades for veterinary applications are derivatives of already approved antimicrobial agents. Given this situation, it is most important to use the currently available antimicrobial agents as wisely as possible to preserve their efficacy for the future. To this end, it is important to better educate all those people who prescribe antimicrobial agents in human and veterinary medicine about antimicrobial resistance, resistant bacteria, management practices, and measures to prevent infections as well as prudent use of antimicrobial agents. On one hand, it is important to refresh continuously the knowledge on the aforementioned topics, and on the other hand, it is necessary to raise awareness on antimicrobial resistance of bacteria from livestock and companion animals so that measures to counteract resistance development will be implemented.

This book, *Antimicrobial Resistance of Bacteria from Livestock and Companion Animals*, is intended to become a valuable reference for persons who treat, research, or monitor resistance in bacteria of animal origin. The first book of this kind—*Antimicrobial Resistance in Bacteria of Animal Origin*, edited by Frank M. Aarestrup—was published in 2006 by ASM Press. More than a decade later, we felt that it was time to compile a new book for ASM Press that would include the wealth of data on antimicrobial resistance in bacteria from animals that has been published in the meantime.

The new book is composed of 31 individual chapters, some of which have been updated and expanded from the 2006 book by including in part new authors and coauthors. Other chapters have been included for the first time and represent a valuable addition to this book.

The first six chapters introduce the reader to general aspects of antimicrobial resistance. Chapters 1 to 3 describe the history and current use of antimicrobial drugs in veterinary medicine; the particularities of antimicrobial susceptibility testing of bacteria from animals and veterinary-specific breakpoints to evaluate the results; and new and molecular methods for the detection of antimicrobial resistance (including MALDI-TOF mass spectrometry, next-generation sequencing, and DNA microarrays), respectively. Chapter 4 provides a comprehensive overview of the multiple mechanisms by which bacteria become resistant to antimicrobial agents, while chapters 5 and 6 give important information on resistance to metals used in livestock production and bacterial resistance to biocides. Both metals and biocides represent important substances that can coselect for antimicrobial resistance in bacteria.

The next 18 chapters provide the latest information on antimicrobial resistance in specific groups of bacteria that represent important obligate or facultative pathogens in livestock and companion animals. In comparison to the book published in 2006, separate chapters describing antimicrobial resistance in staphylococci (inclu ding the livestock-associated methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius*, which have gained much public health attention during the past decade) and streptococci have been included. Moreover, new chapters on antimicrobial resistance in *Listeria* spp., *Rhodococcus equi*, *Bordetella bronchiseptica*, *Stenotrophomonas* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Trueperella pyogenes*, *Arcanobacterium* spp., and *Corynebacterium* spp. were added. In addition, chapters were included that deal with antimicrobial resistance in *Chlamydia*, *Chlamydophila*, *Rickettsia*, *Coxiella*, and other intracellular pathogens, as well as *Leptospira*, *Brucella*, and other rarely investigated veterinary and zoonotic pathogens. The remaining chapters on antimicrobial resistance in *Enterococcus*, nontyphoidal salmonellae, *Escherichia coli*, *Campylobacter* spp., *Pasteurellaceae*, and fish pathogens as well as *Clostridium* spp., *Brachyspira* spp., and other anaerobes were completely revised and expanded.

The final seven chapters provide insight into antimicrobial resistance with a One Health perspective, licensing and approval of antimicrobial agents for use in animals, and methods and applications for monitoring of antimicrobial drug usage in animals and provide an overview of principles and practices of monitoring antimicrobial resistance in bacteria from animal origin. In addition, single chapters describe risk assessment and source attribution models, the optimization of treatment of animals to avoid resistance selection, and last but not least, antimicrobial stewardship in veterinary medicine.

All in all, this book provides a comprehensive and up-to-date overview of the various aspects associated with antimicrobial resistance. The information provided in this book will be useful to all people interested in this topic, including veterinary practitioners, students, researchers, industry, and decision-makers. To compile all this information, we recruited the very best experts worldwide in antimicrobial resistance of livestock and companion animals to write the corresponding chapters. We are deeply indebted to the 84 authors and coauthors from institutions in 16 countries, including Australia, Austria, Belgium, Canada, China, Denmark, France, Germany, Ireland, Portugal, St. Kitts and Nevis, Spain, Switzerland, Thailand, the United Kingdom, and the United States, who rendered

this book a truly international collaboration product. This book would not have been possible without their invaluable input and participation. We also thank our many colleagues who reviewed the various chapters. We also thank ASM Press, not only for the opportunity to compile this book, but also for being patient with us during this process.

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His research group is involved in the analysis of the molecular genetics of antimicrobial resistance, antimicrobial susceptibility testing methods, and monitoring of antimicrobial resistance. He has published more than 400 papers in international peer-reviewed journals and has received several research awards. Prof. Schwarz acts as editor of or serves on the editorial board of six international journals. A cat lover, he spends his free time taking care of abandoned cats at a small animal shelter in Berlin.

Lina M. Cavaco, PhD, is Portuguese but was born in Germany in 1976 and began her education there. When she moved to Portugal in 1984 and spent part of her childhood in a small town in the south of Portugal, she became attracted to the country life and the veterinary profession. Dr. Cavaco received a degree in Veterinary Science from the Veterinary Faculty of the University of Lisbon (formerly the Technical University) in 2000 and began research into the diagnosis of bacterial diseases in domestic and zoo species at the bacteriology section of the University of Lisbon, where she obtained an MSc degree in Veterinary Public Health.

At the end of 2005, Dr. Cavaco moved to Denmark to start studies on the mechanisms and selection of antimicrobial resistance at Copenhagen University and obtained her PhD degree in September 2008. From October 2008 to May

2017, she worked at the Technical University of Denmark, focusing her research on antimicrobial resistance mechanisms and selection of resistance to antimicrobials and metals in Enterobacteriaceae and MRSA while also teaching undergraduates and performing proficiency testing and training. This peaked her interest in online education, and she developed and launched a massive open online course (MOOC) on antimicrobial resistance methods and participated in the preparation of another MOOC on whole-genome sequencing, both of which are accessible to a global audience.

Dr. Cavaco has been at the Statens Serum Institute and Copenhagen University since June 2017, where her primary research is focused on new antimicrobial candidate compounds and studies assessing efficacy for treatment of infections with multiresistant bacteria.

Prof. Jianzhong Shen is a member of the Chinese Academy of Engineering and Dean of the College of Veterinary Medicine at China Agriculture University (CAU). He received his bachelors, masters, and doctoral degrees from CAU and has been working at CAU for 30 years, since 1988. He has actively served as a committee member for multiple national organizations and scientific communities, including the Veterinary Drug Evaluation Commission of the Ministry of Agriculture of China, the Veterinary Drug Residue Commission of China, the Feed Additive Evaluation Commission of the Ministry of Agriculture of China, and the Chinese Association of Animal Science and Veterinary Medicine.

Dr. Shen's research focuses on the development of detection methods for veterinary drug residues as well as on the emergence, transmission, and molecular mechanisms of antibiotic resistance in bacteria of animal origin. In the past decade, he has published more than 200 peer-reviewed papers in international journals and received more than 40 patents. He has also received several major Chinese national awards, including the National Technology Invention Award (2015), the National Agriculture Science and Technology Award (2013), the National Agricultural Scientific Research Talent award (2012), and the National Excellent Scientific and Technological Workers award (2010).

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History and Current Use of Antimicrobial Drugs in Veterinary Medicine

1

John F. Prescott[1]

INTRODUCTION

The introduction of antimicrobial drugs into agriculture and veterinary medicine shortly after the Second World War caused a revolution in the treatment of many diseases of animals. In the "wonder drug era" of the late 1940s and early 1950s, the effective treatment of many infections that were previously considered incurable astonished veterinarians, such that some even feared for their livelihoods. Not all use of antimicrobial drugs in food animals is yet under veterinary prescription globally, despite repeated recommendations by the World Health Organization and other responsible organizations, so that the term "veterinary medicine" is used here rather generically to suggest use in animals rather than just use by veterinarians.

A broad overview of key features of the history of antimicrobial drug use in animals is given in Table 1, which traces developments from the preantibiotic era to the present day, where there are arguable fears that we are moving into the "postantibiotic" era but which may better be described as the antimicrobial stewardship era. Much of this overview will focus on antimicrobial use in food animals, the subject of an earlier review that partly focused on the public health aspects of the use of antimicrobials in food animals (1), but will include important aspects of use in companion animals.

The table illustrates many important features in the history of the use of antimicrobials in animals: (i) The development of resistance to antimicrobial drugs followed soon after their introduction. (ii) Resistance was usually dealt with by the development of new classes of antimicrobials by the isolation from nature of novel antibiotics within a particular class or by development of synthetic analogs of an existing class. (iii) The antimicrobial drugs used in animals were the same as those in human medicine, although a number of them that were rejected by human medicine because of toxicity problems (e.g., bacitracin) became growth-promoting feed antimicrobials in food animals. At least one of these drugs (colistin) is now being reclaimed for systemic use in humans. (iv) Antimicrobials were used in agriculture in feed as growth promoters, or for subtherapeutic purposes, almost as soon as they were discovered. (v) The majority of antimicrobial drugs used in animals (and shared with human medicine) belong to a small number of major classes, and only one major new class of antimicrobial drugs (fluoroquinolones; pleuromutilins are an exception, but have very restricted use) has been introduced for food animal use in the past 30 years. (vi) Significant antimicrobial contamination in carcasses or selected tissues was detected in the 1970s and 1980s, leading either to the banning of potentially toxic (e.g., carcinogenic or idiosyncratic effects) drugs or to rigorous, ongoing programs of detection in carcasses after slaughter. This was the major focus of regulations of use in food animals in those decades, and there is still confusion in some quarters about the difference between residues and resistance. (vii) The public health impacts resulting from the development of resistance, and especially because of transmissible resistance, have been a major battleground between agriculture and medicine for nearly 50 years. (viii) The resistance crisis in human medicine has led to unprecedented concern at the highest political levels globally about the threat of resistance to humanity, to an unprecedented focus on stewardship, and to major ongoing reduction and ongoing changes in agricultural use of antibiotics, at least in the developed world. (ix)

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Table 1 Historical time line of important events and trends in the use of antimicrobial drugs in animals, with emphasis on food animals

Timeline	Feature of period	Antimicrobial drug development	Important events
1925–1935	Antiseptic era	Discovery of sulfonamides	Discovery of penicillin, first beta-lactam, by Alexander Fleming
1936–1940	Antiseptic, sulfonamide period	Penicillin efficacy shown in humans	Sulfonamides introduced into food animal use
1941–1945	Dawn of "wonder drug" era	Streptomycin, first aminoglycoside, discovered	Second World War is impetus for antimicrobial drug discovery for treatment of war wounds
1946–1950	"Wonder drug" era	Discovery bacitracin, chloramphenicol, neomycin, polymyxin, streptogramins, tetracycline antibiotics, all natural products of microorganisms, usually fungi	Penicillin, streptomycin released from military use for civilian population and animal use; widespread use in animals by 1950, largely empirical; far more wonder than science
1951–1955	"Wonder drug" era	Discovery of erythromycin, first macrolide; introduction of neomycin, aminoglycoside, for topical or intestinal infections in animals Introductions of nitrofurans into clinical use, especially for intestinal infections	Tetracyclines, chloramphenicol used therapeutically in animals; widespread, largely empirical, use Intramammary use of antibiotics for mastitis treatment widespread Discovery of and extensive use of antibiotics for growth promotion in food animals, pioneered in the USA
1956–1960	"Wonder drug" era	Discovery of vancomycin, first glycopeptide Tylosin, novel macrolide Virginiamycin, streptogramin, used as growth promoters	More science and less wonder, but drug dosage still largely empirical Early studies of drug excretion in Denmark
1961–1965	Emerging resistance period	Methicillin and other penicillinase-resistant penicillins Introduction of spiramycin, a macrolide, into animal use Gentamicin, antipseudomonal aminoglycoside	Discovery of transmissible, plasmid- or "R" factor-based, multiple drug resistance in *Enterobacteriaceae* Studies of drug residues and withdrawal periods in animals
1966–1970	New drug analog period	Cephalothin, first-generation cephalosporin Ampicillin, first broad-spectrum penicillin, used in food and companion animals, example of a successful synthetic alteration of side chains of basic beta-lactam ring to expand activity Amikacin, for gentamicin-resistant infections Flavomycin introduced as growth promoter	New drug analogs successfully address resistance problem Transmissible, multiple-drug resistant, serious *Salmonella* infections, transmission from calves to human in UK Because of transmissible resistance, Swann Report in UK removes drugs important in human medicine as feed antibiotics, allows their veterinary prescription-only use therapeutically in food animals
1971–1975	New drug analog period	Carbenicillin, antipseudomonal penicillin Other first-generation cephalosporins introduced Trimethoprim-sulfonamide combination	New drug analogs successfully address resistance problem FDA report (1972) suggests stopping feed use of subtherapeutic penicillin, tetracyclines; not implemented
1976–1980	Tissue drug residue problems in food animals; early pharmacokinetic period	Cexotin, first extended-spectrum (second-generation) cephalosporin Moxalactam, unusual beta-lactam Introduction in Europe of avoparcin, glycopeptide, for growth promotion in food animals	Chloramphenicol use in food animals banned in USA and Denmark because of potential human toxicity through residues, followed by other countries Transmissible, multiple-drug resistant, *Salmonella typhimurium* phage type 204

(Continued)

Table 1 *(Continued)*

Timeline	Feature of period	Antimicrobial drug development	Important events
			spreads from calves to humans *Journal of Veterinary Pharmacology and Therapeutics* started, to improve drug use in animals Focus on pharmacokinetics and drug metabolism in food animals by J.D. Baggot (Ireland, USA), L.E. Davis (USA), P. Nielsen and F. Rasmussen (Denmark)
1981–1985	Pharmacokinetic, drug dosage prediction period	Cefotaxime, antipseudomonal cephalosporin, and other third-generation cephalosporins Broad-beta-lactamase inhibitors combined with aminopenicillins, e.g., sulbactam-ampicillin used in food animals Imipenem-cilastatin, unusual broad-spectrum beta-lactam	Antimicrobial drug dosage prediction based on pioneering pharmacokinetic approach in food animals developed by Ziv in Israel, Hjerpe in USA, and others Changes in sulfamethazine use in swine to address residue issue Ban on nitrofuran and nitroimidazole drugs for food animals in USA because of mutagenicity
1986–1990	Increasing resistance problems in humans: MRSA emerges	Quinolones, fluoroquinolones introduced into human medicine Ceftiofur, a second- to third-generation cephalosporin, introduced for food animals	Development of Food Animal Residue Avoidance Database (FARAD) in USA Moratorium on sulfamethazine use in dairy cows in USA Moratorium on most use of aminoglycosides in food animals in USA because of kidney residues
1991–1995	Increasing resistance problems in humans: VREs emerge	Azithromycin and other improved macrolides introduced into human medicine Tilmicosin (macrolide), tiamulin (pleuromutilin), florfenicol (nontoxic introduced for food animal selected use Fluoroquinolones introduced for selected use in food animals in Europe	First fluoroquinolone, enrofloxacin, introduced into food animal (poultry) use in USA, with severe restrictions; includes resistance monitoring through National Antimicrobial Resistance Monitoring System that integrates food animal and medical data National Council on Clinical Laboratory Standards (NCCLS) in USA establishes veterinary subcommittee to develop susceptibility testing methods and interpretations Animal Medicines Use Clarification Act in USA allows veterinary prescription extra-label use of certain approved drugs
1996–2000	Resistance crisis in medicine; now includes penicillin-resistant *Streptococcus pneumoniae*	Oral, third-generation cephalosporins in human medicine may partially drive resistance crisis Effective antivirals introduced into human medicine Fluoroquinolone introduced for treatment of acute pneumonia in cattle in USA	VRE emergence linked to avoparcin use in food animals in Europe; ban of avoparcin and four other growth promoters in Europe WHO (1998) recommends withdrawal of growth-promoting antimicrobials if significant for human medicine Global emergence of multidrug-resistant *S. typhimurium* DT104 Japanese Veterinary Antimicrobial Resistance Monitoring Program started NCCLS guidelines for veterinary bacterial susceptibility testing published Center for Veterinary Medicine of FDA proposes "an approach for establishing thresholds in association with the use of antimicrobial drugs in food-producing animals" (Framework document)

(Continued)

Table 1 *(Continued)*

Timeline	Feature of period	Antimicrobial drug development	Important events
			FDA Center for Veterinary Medicine proposes withdrawal of fluoroquinolones in poultry because of resistance in *Campylobacter*
2001–2005	Resistance crisis in medicine continues, expands	No new antimicrobial drugs introduced for food animals	WHO Global Strategy for Containment of Antimicrobial Resistance calls for prescription-only use of antimicrobials in food animals, national usage and resistance monitoring, phasing out of growth promoters if drugs important for humans Withdrawal of fluoroquinolones for use in poultry in USA because of emerging resistance in *C. jejuni* Spread of multidrug resistance including cephalosporinase (CMY2) genes among certain *Salmonella* serovars. Development of prudent use guidelines by practitioner specialty groups at national levels
2005–2010	Resistance crisis in medicine continues; MRSA and MRSP emerge in animals, spread partly by people	No new antimicrobial drugs introduced for food animals Voluntary ban on use of ceftiofur in pigs in Denmark, and on use of ceftiofur in chickens in Canada	Resistance crisis in medicine focuses intense effort on improved infection and antimicrobial drug use control by physicians; some benefits observed Veterinary "prudent" or "judicious" use approaches increasingly replaced by emerging concept of stewardship Global spread of food animal-associated MRSA, driven by zinc oxide use in food animals Voluntary ban on ceftiofur use in Danish pigs followed by marked reduction in extended-spectrum cephalosporinase *E. coli* excretion MRSA emerges in Dutch animal workers; Holland proposes 50% reduction in antibiotic use in food animals within 5 years MRSP emerges in dogs, clonal spread in Europe and North America
2011–2016	Resistance crisis in medicine reaches highest political levels United Nations affirms global collective action	Avilamycin introduced as first-in-class, animal-use-only, orthosomycin antibiotic to prevent mortality due to necrotic enteritis of broilers under veterinary prescription; first "new" antimicrobial drug for food animals in 10 years	Transatlantic Task Force on Antimicrobial Resistance to cooperate between USA and European Union on resistance Innumerable calls for global solutions to antibiotic resistance WHO Options for Action promotes development of national action plans incorporating human and animal health sectors Human medicine antibiotic resistance threats identified by microorganism level in USA, most unrelated to animal use Consumer demands for "antibiotic-free" animal production; McDonald's Corporation adopts antimicrobial stewardship requirements for sourcing meat G7 agrees to address the resistance crisis O'Neill Report in UK's final recommendations includes reducing unnecessary use in agricul-

(Continued)

Table 1 *(Continued)*

Timeline	Feature of period	Antimicrobial drug development	Important events
			ture as one of 10 planks to fight resistance Plasmid-mediated colistin resistance gene *mcr1* related to agricultural use in animals and humans identified in China, shown to be globally widespread United Nations High-Level Meeting on the antimicrobial resistance threat unprecedented meeting
2017–	Stewardship era	Intense activity to find alternatives to antibiotics or "animal only" antibiotics for food animals	Political will to address the resistance crisis continues in place Anticipated enhancement of surveillance, stewardship, and innovation as global response to antimicrobial resistance crisis Innovation in numerous fields relating to use of antibiotics anticipated Increasing adoption of a "One Health" approach to resistance may remove some of the conflict between agriculture and human medicine The story continues

The science and practices supporting optimal antimicrobial drug use in animals and in humans has developed relatively slowly and is not complete.

DISCOVERY OF ANTIBIOTICS AND EARLY USAGE

Antimicrobial drugs were introduced for animal (and human) use with a minimum of controlled experimental studies, so that from the start of their use there were frequent calls to move from the wonder to the science. As in human medicine, much of the early dosage used was empirical and based on inadequately controlled small-scale trials (2, 3), so that there was a "confusing hodge-podge of widely divergent optimum dose-ranges for the many livestock diseases allegedly amenable to the activity of penicillin" (4). In the United States such empiricism led to a licensed dosage of penicillin G in cattle that was clearly inadequate. It took four or five decades before the licensed drug dosage was more scientifically determined, based on quantitative understanding of the interaction of drug with the target microorganism (dosage, pharmacokinetic and pharmacodynamic parameters, *in vitro* susceptibility) as well as clinical data (Table 1). Clinical evaluation is still an important component used in the licensing of antimicrobial drugs, in part because the predictive science is imperfect.

In retrospect, for drugs whose use "has advanced the practice of medicine farther than any other single factor of any of the previous centuries" (5), the time taken to establish the science of the clinical use of antimicrobial drugs seems astonishing. As human medicine's poor cousin, veterinary medicine lagged in the development of the science of optimal antimicrobial drug use, but the lag was only relative since the same delay was clearly visible in human medicine. In general, the science and practice of antimicrobial usage in animals has largely paralleled that in human medicine, in the same way that most antimicrobial drugs used were the same or were in the same drug class. There have been, however, a number of features unique to animal agriculture, as discussed below.

In most countries, approval by the appropriate regulatory authority must be obtained before an antimicrobial drug can be legally sold, and this depends on extensive testing to ensure safety and efficacy, as well as, in the case of food animals, studies of safety for people consuming their products. Registration requirements for veterinary medical products have been largely harmonized internationally under the International Cooperation on Harmonization of Technical Requirements of Veterinary Medicinal products (VICH) of the World Organization for Animal Health, membership of which includes the European Union and the United States. A harmonized VICH guideline, GL 27, defines the data

requirements for risk of transfer of resistant bacteria or resistance determinants from foods of animal origin to humans. These data are assessed in terms of exposure of food-borne pathogens and commensal bacteria, and the "qualitative probability" that human exposure to resistant bacteria results in adverse human consequences. As part of this assessment, many countries are attempting to stratify the stringency of regulatory requirements by how important a drug is to public health, also discussed below. This is a highly contentious issue, since most antimicrobial drugs can, under various criteria, be claimed as "critically important." A more useful and a more rational approach, which could be adopted in both human and veterinary medicine, is categorization into "lines" based in part on culture and susceptibility results (Table 2) (6). This approach has the advantage of simplicity (for example, labels on bottles could indicate the category) and would enhance the use of laboratory diagnosis. Research is needed into whether such a categorization scheme would be accepted in the animal (and human) health world, including the barriers to acceptance and what it would take to implement such a system so that it would be widely accepted.

The regulation of antimicrobial drug use in animals is a complex process that has jurisdictional differences. Regulation is more stringent for use of these drugs in food animals, although "off- or extra-label" use (use of the product in any manner not specified on the label) is often approved under specific circumstances and constraints. Use of antimicrobial drugs in companion animals is subject to less stringent regulation, and there is likely more off-label use in companion animals (as there is in human medicine).

Although the situation is changing, there has been historically no formal interest by regulatory authorities in postapproval use (or periodic relicencing) of antimicrobial drugs in animals. Some of the label claims for some antimicrobial drugs list approval for use in bacteria that have had their names changed several times since approval or for diseases that have subsequently been shown to be caused by other agents, so that reading the labels can be like reading a outdated veterinary microbiology textbook from the 1960s. The postapproval monitoring of resistance in *Campylobacter jejuni* following the introduction of fluoroquinolones for use in broiler chickens in the United States, and the subsequent withdrawal of fluoroquinolones from use in poultry in the United States is, however, a well-known example of postapproval monitoring of the approved use of a drug.

PRACTICES IN ANTIMICROBIAL DRUG USAGE UNIQUE TO ANIMAL HUSBANDRY

The greatest differences in usage of antimicrobial drugs between animal husbandry and human medicine were, and in many countries still are, in the use in agriculture of antimicrobials for growth promotion and for long-term disease prophylaxis, although the situation is changing relatively rapidly. This has occurred particularly in countries where livestock, notably chickens and pigs, are reared intensively.

Table 2 Suggested categorization of antimicrobial drugs for veterinary use (6)

Class	Definition	Examples
First-line (primary)	Initial treatment of known or suspected bacterial infection in absence of culture and susceptibility results These drugs may commonly be used in human medicine but are usually considered less important for treating serious human (and animal) infections or raise less concern about development of resistance	Penicillin, most cephalosporins, trimethoprim-sulfonamides, tetracyclines
Second-line (secondary)	Used when culture and susceptibility testing, plus patient or infection factors, indicate that no first-line drugs are reasonable choices Drugs in this class may be more important for treatment of serious human (and animal) infections, or there may be particular concern about development of infection	Fluoroquinolones, third and later generation cephalosporins
Third-line (tertiary)	Used in serious, life-threatening infections, with the support of culture and susceptibility results, when no first-line or second-line drugs are indicated Not for use in food animals	Carbapenems
Restricted, voluntarily prohibited	Used only in life-threatening infections when culture and susceptibility testing indicate no other options Not for use in food animals Additional requirements may be indicated, or use may be voluntarily prohibited	Vancomycin

The growth-promotional benefits of adding low concentrations of many antibacterial drugs to feed was recognized almost as soon as antibiotics were introduced. The enhancement of growth rates and improved efficiency of use of feed were noted when pigs and poultry were fed the fungal waste derived from antibiotic production, originally intended as a source of vitamins and protein, but mostly as an efficient way to use the waste. The effect was originally attributed to the presence of vitamin B_{12} ("animal protein factor") in the mycelial mass, but with time it was recognized to be a direct effect of residual antibiotic. Interestingly, how antimicrobial drugs improve growth rate and efficiency of utilization is still unknown, although it is thought to be through an inhibitory or metabolic effect of some kind on the Gram-positive intestinal microflora. Curiously, until about the mid-1950s, low prolonged oral dosage of tetracycline was even used to improve the growth of underweight human infants and children, but this practice was dropped because of both resistance and discoloration of teeth. In animals, the growth-promotional and disease-prophylactic benefits appear to have remained constant over the years (7), supporting the idea that these effects result from metabolic rather than antibacterial activities. As the use of antibiotics as growth promoters in intensively reared livestock becomes illegal in much of the world, alternative approaches to manipulation of the microbiome may replace their growth-promoting effects.

Not only have antimicrobial drugs been used for growth promotion, but some drugs were and in some countries still are administered in feed for prolonged periods at somewhat higher concentrations, the "subtherapeutic levels" (defined in the United States as less than 200 g per ton of feed), which are lower concentrations than those approved for therapeutic purposes. The historical origin of subtherapeutic usage and indeed the meaning of this term are obscure, but it seems to have both beneficial growth-promotional and disease-prophylactic effects, particularly against pathogens that do not readily develop or acquire resistance. Drugs such as tetracyclines are administered "subtherapeutically" for many defined, licensed purposes at a range of concentrations varying with the drug, the food-animal species, and the purpose. Such usage, which can often be prolonged and thus inconsistent with important general principles of antimicrobial drug dosage (6), has been particularly widespread in the swine industry in countries in which the drugs are still allowed for this purpose (8). The practice is coming under increased scrutiny and will likely also be banned and replaced by short-term antimicrobial prophylaxis or short-term treatment targeted to specific pathogens. As noted earlier, a number of antimicrobial drugs (such as the streptogramins) that were too toxic for parenteral use in humans were relegated to growth-promotional and disease-prophylactic use in food animals.

Another practice that has historically been far more common in food animal use than in human medicine has been short-term mass medication with therapeutic concentrations of drugs immediately before an outbreak of disease can be anticipated, or immediately at the onset of disease in a population (9). This type of prophylaxis has been commonly practiced in beef feedlot and swine medicine and is most akin to the prophylactic use of antimicrobial drugs to prevent *Haemophilus* or *Neisseria* meningitis in humans. Prophylactic use of intramammary antimicrobial drugs to prevent development of new infections and to treat existing infections has become a standard practice in dairy cows in the two months before calving and re-entering the milking herd ("dry cow treatment"), with no apparent adverse effect on resistance development, perhaps in part because the very high concentrations of drugs achieved in the udder result in rapid killing of the target bacteria. Blanket use of dry cow treatment is also coming under scrutiny and is being replaced by use only when udders are known to be infected and likely to carry an infection over to the next lactation.

USE OF ANTIMICROBIAL DRUGS IN ANIMALS

Food Animals

Data on the quantities and types of antimicrobial used in food animals are increasingly available in highly developed countries, with countries such as Denmark and Sweden leading the way. A global assessment of trends in antimicrobial use in food animals recognized the relative lack of reliable quantitative data globally but forecasted a marked increase as livestock production practices intensified in middle- and low-income countries (10). In Europe, the Danish Integrated Antimicrobial Monitoring and Research Program (DANMAP), started in 1995, is a ground-breaking and very high-quality program that monitors both resistance in selected food-animal indicator organisms and pathogens and usage of antimicrobials in human and animal medicine. DANMAP can accurately record national antimicrobial use in animals to the kilogram level. In Sweden, the Swedish Veterinary Antibiotic Resistance Monitoring organization has had a similar program since 2000 and has integrated this with Swedish Antibiotic Utiliza-

tion and Resistance in Human Medicine since 2011. In the United States, the National Antimicrobial Resistance Monitoring System has monitored resistance in food-animal indicator organisms and select human and animal pathogens nationally, but has not monitored use, since 1996. In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance has a similar program but has struggled for national jurisdictional reasons to obtain accurate food animal use data. In Europe, the European Medicines Agency collects data on antimicrobial use in animals from member countries (European Surveillance on Veterinary Antimicrobial Consumption).

These data have great value in "benchmarking" comparisons between antimicrobial use in food animals between different countries, which can vary widely, but there is uncertainty about the best way to compare antimicrobial use between different species (e.g., milligram/population corrected unit, animal daily dose) and about the validity of some of the comparisons based on sales data, differences in dosages, and differences in animal demographics including weight estimates (11, 12). This is discussed further in chapter 28. The importance of benchmarking cannot be underestimated as a driver for reducing antimicrobial use in food animals at the farm and the veterinarian level, as illustrated by the use of the "yellow" card system in Denmark and the experience of the value of benchmarking in Holland in its 50% reduction of use in food animals between 2007 and 2012 (13, 14). Reduction in use in food animals is associated with reduction of resistance in indicator bacteria (15), and a correlation between antimicrobial use in animals and resistance in indicator bacteria is well established (16). A robust approach to benchmarking has been developed (17).

The documentation of antimicrobial drug use in food animals nationally and internationally is a rapidly growing, fast moving, and evolving field that can only be briefly touched on here but is reviewed in detail in chapter 28. As promoted by the World Health Organization and others, surveillance and documentation of use, and of the impacts of reduction in use in animals, is an essential element in addressing the resistance crisis and improving how antimicrobials are used in animals.

There are now numerous studies of the antimicrobial-prescribing habits of veterinarians and factors influencing those habits (18, 19) which can be expected to continue as veterinary medicine embraces antimicrobial stewardship.

Companion Animals

The use of antimicrobials in companion animals essentially mirrors their use in human medicine, a discussion of which is far beyond the scope of this chapter. Only in recent years have antimicrobial use practices in companion animals come under scrutiny, both as sources of important emerging resistance issues (such as methicillin-resistant *Staphylococcus aureus* [MRSA] and *Staphylococcus pseudintermedius* [MRSP]) (20) and as potential sources for multidrug-resistant pathogens for humans. The rapid emergence and clonal spread of methicillin-resistant *S. pseudintermedius* has been a "wake-up call" for companion animal practice (20–22). Untreatable multidrug-resistant hospital-associated infections are now being encountered in companion animals. Studies of the use of antimicrobials in primary care companion animal veterinary practice have been characterized by their small sample size and labor-intensive nature. However, studies are now being reported that involve analysis of mega-data on usage obtained from shared practice software to obtain that involving large numbers of animals (e.g., one million dogs) (23). However, documentation of usage alone is not particularly useful since it may not be appropriate to the infections being treated. For example, in Canada, one study found that there was overuse of cefovecin and of fluoroquinolones for the treatment of cat and dog diseases for which antibiotics were either not indicated or for which first-line antimicrobials were appropriate (24). The potential value of companion animal usage data obtained electronically is that, as has been shown for food animals, it can be used for benchmarking purposes as part of a broader approach to improved antimicrobial stewardship.

PUBLIC HEALTH ASPECTS OF ANTIMICROBIAL DRUG USE IN ANIMALS

Food Animals

The effect of antimicrobial drug use in food animals on the development of resistance in bacteria that can cause disease in humans has been the subject of prolonged, acrimonious, and ongoing debate. The major and most accessible reviews of this issue are summarized in Table 3, which shows that the intensity of the criticism of agricultural usage of antimicrobial drugs intensified from the mid-1990s and has now reached a crescendo, paralleling the antimicrobial resistance crisis in human medicine (Table 1).

The first major review of the effect of antimicrobial drug use on resistance in human and animal pathogens was carried out in the United Kingdom under the chairmanship of M.M. Swann. The impetus for the review was a combination of recognition of the increasing im-

Table 3 Historical time line of major reports and their conclusions or recommendations relating to the public health aspects of antimicrobial drug use in food animals

Date	Report/country	Major conclusions or recommendations
1962	Netherthorpe Committee *UK, Joint Committee, Agricultural and Medical Research Councils*	Recognized economic benefit of antimicrobials as growth promoters, saw no reason to discontinue However, continue to examine situation and substitute penicillin and tetracycline use if alternative nontherapeutic growth promoters become available
1969	Committee on Antibiotic Uses in Animal Husbandry and Veterinary Medicine; The Swann Report *UK, Report to Parliament*	Restriction of use of antimicrobials into prescription-only therapeutic use and nonprescription feed additives Growth-promotional and subtherapeutic use of drugs important in human medicine were banned
1972	The Use of Antibiotics in Animal Feeds *USA, FDA Task Force*	Use of antimicrobial drugs in food animals may promote resistance in *Salmonella*; manufacturers to show this is not a problem Sufficient evidence to stop use of penicillin and chlortetracycline as growth promoters
1979	Drugs in Livestock Feed *USA, Office of Technology Assessment*	Stop use of penicillin and tetracyclines as growth promoters, even though this would have short-term economic cost
1980	The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animals *USA, National Research Council*	Could not conclude from data available that there was a direct relationship between subtherapeutic drug use in animal feed and human health Insufficient data from UK that implementation of Swann report had reduce postulated hazards to human health
1981	Antibiotics in Animal Feeds *USA, Council for Agricultural Sciences and Technology*	Irrational to ban subtherapeutic dosage without also banning therapeutic use Cost of a ban on feed antimicrobials: about $3.5 billion
1989	Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feed *USA, Institute of Medicine*	Unable to find substantial direct evidence of definite human health hazard in the use of subtherapeutic concentrations of penicillins and tetracyclines in animal feeds
1995	Impacts of Antibiotic-Resistant Bacteria *USA, Office of Technology Assessment*	Need to collect more data to resolve the issue of the effect of feed antimicrobials in animals on human health A further report will not resolve the issue
1997	Antimicrobial Feed Additives *Sweden, Ministry of Agriculture, Commission on Antimicrobial Feed Additives*	As part of negotiations leading to European Union membership, Sweden, which had banned use of antimicrobial growth promoters in 1985, re-reviewed benefits of antibacterial feed additives and again concluded that benefits did not outweigh the risks
	The Medical Impact of the Use of Antimicrobials in Food Animals *WHO*	Stop using antimicrobials for growth promotion or subtherapeutic purposes in animals if used in human therapeutics or if they select for cross-resistance to antimicrobials used in human medicine
1998	A Review of Antimicrobial Resistance in the Food Chain *UK, Ministry of Agriculture, Fisheries, and Food*	Resistance in animal pathogens and commensal bacteria is selected for by antimicrobial drug use, can reach people through food chain, may cause disease or colonize people, can transfer resistance to human pathogens, *Campylobacter* and *Salmonella*, and certain antimicrobials are especially problematic
1999	The Use of Drugs in Food Animals: Benefits and Risks *USA, Committee on Drug Use in Food Animals: Panel on Animal Health, Food Safety and Public Health, National Research Council and Institute of Medicine*	Use of drugs in food animals does not appear to constitute an immediate public health concern; additional data may alter conclusion, but data are lacking Recommended integrated national databases to support rational, visible, science-driven decision-making and policy development for regulatory approval and use of antimicrobials in food animals Estimated cost of ban on nontherapeutic use in animals: between $5 and 10 per person per year in USA
2000	The Use of Antibiotics in Food-Producing Animals: Antibiotic-Resistant Bacteria in Animals and Humans	Stop using growth promoters if same drugs important in human medicine

(Continued)

Table 3 *(Continued)*

Date	Report/country	Major conclusions or recommendations
	Australia: Joint Expert Advisory Committee on Antibiotic Resistance	All antimicrobials for animals prescription only Predetermine "resistance thresholds" for animal antimicrobials that trigger investigation or mitigation Develop a comprehensive and integrated resistance surveillance system Monitor antimicrobial usage Find alternatives to antimicrobials for food animals
2001	Risk Assessment on the Human Health Impact of Fluoroquinolone Resistant *Campylobacter* Associated with the Consumption of Chicken *USA, Center for Veterinary Medicine*	Risk assessment by highly detailed mathematical model with numerous explicit assumptions suggested that in 1998 mean estimate of 8,678 U.S. citizens had fluoroquinolone-resistant *Campylobacter* illnesses acquired from chicken and received fluoroquinolones for treatment
2002	The Need to Improve Antimicrobial Use in Agriculture: Ecological and Human Health Consequences *USA, Alliance for the Prudent Use of Antibiotics. Clinical Infectious Diseases 34; Supplement 3*	Elimination of nontherapeutic use of antimicrobials in food animals will lower burden of antimicrobial resistance in the environment, with benefits to human and animal health
	Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health *Canada, Health Canada Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health*	Make all antimicrobials for disease control prescription only Develop extra-label policy Control an importation of drugs "loop-hole" Stringently reassess growth-promotional use of drugs Develop national surveillance of resistance and use
	Food Safety and Pig Production in Denmark. Controls on antibiotics, veterinary medicines and *Salmonella* Verner Wheelock Associates Limited; Danish Bacon and Meat Council	Control of antimicrobial-resistant bacteria by banning antimicrobial growth promoters, and *Salmonella* control programs, has made Denmark a model and given the Danish pig industry competitive economic advantage
2003	Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment *WHO*	Clear evidence of adverse human health consequences due to resistant organisms resulting from nonhuman usage Surveillance of usage and resistance important to identify problems and choose interventions Magnitude of impact accompanied by considerable uncertainty
	Impacts of Antimicrobial Growth Promotion Termination in Denmark *WHO*	Review of the "Danish experiment" of terminating use of growth promoters on efficiency of food animal production, animal health, food safety, and consumer prices concluded that there have been no serious negative effects Very beneficial in reducing total quantity of antimicrobials used and reducing antimicrobial resistance in important food animal reservoirs
2004	Second Joint FAO/OIE/WHO Expert Panel on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Management Options *WHO*	Establish national surveillance programs on use and resistance; follow WHO/OIE guidelines on responsible use; implement strategies to prevent transmission through food etc.
	Antibiotic Resistance: Federal Agencies Need to better Focus Efforts to Reduce Risk to Humans from Antibiotic Use in Animals *U.S. General Accounting Office*	Expedite risk assessments in animals of antibiotics critically important to humans; develop plan to assess and mitigate risk
2008	Antimicrobial Resistance from Food Animals. *World Health Organization*	Continues to press for multiple approaches to improve antimicrobial use in food animals, prescription only, integrated use and resistance surveillance, identify barriers to implementation of international guidelines, etc.
	Putting Meat on the Table: Industrial Farm Animal Production in America	Phase out nontherapeutic antimicrobial use in food animals, restrict use to veterinary oversight and prescription, require

(Continued)

Table 3 *(Continued)*

Date	Report/country	Major conclusions or recommendations
	USA, Pew Commission on Industrial Farm Animal Production	reporting of annual sales, review previously approved antibiotics for animals etc. General approach very critical of modern intensive agriculture
2009	The American Veterinary Medical Association Response to the Final Report of the Pew Commission on Industrial Farm Animal Production *American Veterinary Medical Association (AVMA)*	Described Pew Report as having some value but "dangerous and uninformed" and "shocking" The Pew Report and the AVMA response highlights the highly conflictive nature of the debate in the USA
2010–2014	No major reports during this period but numerous scientific papers investigating development and spread of livestock-associated MRSA, spread of extended-spectrum β-lactamase-producing *E. coli* or *Salmonella* in food animals treated with ceftiofur, and possible or definite spread to humans Many examples during this period of small groups of engaged academics reviewing the issue in different forums and making recommendations	
2015	Antimicrobials in Agriculture and the Environment: Reducing Unnecessary Use and Waste *UK, Review on Antimicrobial Resistance: O'Neill Report*	Scale of antimicrobial use in global agriculture is massive A review of the evidence supports a link between antimicrobial use in animals and resistance in human pathogens Proposed global targets for antimicrobial use in animals, restrictions on use of certain antimicrobials, and improved surveillance
2016	Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. *UK, Review on Antimicrobial Resistance: O'Neill Report*	Influential economist's view of the present and future scale and costs, call for urgent global action Highlights agricultural area as one of 10 major recommendations

portance of (i) the phenomenon of "infectious," transferable, drug resistance associated in part with the pioneering work of the distinguished British veterinary microbiologist H. Williams Smith, (ii) the emergence and dissemination in calves in Britain of multidrug-resistant *Salmonella enterica* serotype Typhimurium and its spread to humans, and (iii) experiences around this time of a difficult-to-control epidemic of chloramphenicol-resistant *S. enterica* serovar Typhi in Central America. Chloramphenicol was then the drug of last resort for typhoid fever in humans (25). The 1969 Swann Report to the British government gave a careful analysis of how different usage of antimicrobial drugs in animals might lead to selection of resistant bacteria and resistance plasmids and how such resistant bacteria, or their transmissible resistance traits, could lead to difficult to treat infections in humans. The major recommendations of the committee were as follows: (i) "Feed" antimicrobial drugs could only be used for growth promotion without prescription if they had little or no implication as therapeutic agents in humans, would not impair the value of prescribed drugs, and produced an economic benefit. Since penicillin and tetracyclines did not meet these criteria, they were withdrawn from growth-promotional use and could only be used therapeutically by veterinary prescription. (ii) The "therapeutic" antimicrobials (those other than growth-promotional antimicrobials) tylosin, sulfonamides, and nitrofurans should no longer be used without veterinary prescription. The spirit of the Swann report was to restrict the use of therapeutically effective antibiotics to only therapeutic use on a veterinary prescription basis. Withdrawal of penicillin and tetracyclines for growth-promotional and subtherapeutic purposes was, however, soon followed by their substitution by bacitracin, flavomycin, nitrovin, and virginiamycin for similar purposes.

It was perhaps unfortunate that little effort was made in Britain following the Swann report to improve the scientific base of understanding of the effect of antimicrobial drug use in animals on human health, or to document the effect of implementation of the report. Nevertheless, the sustained work of A.H. Linton (26) and that of his colleagues led to important conceptual understanding of the routes of movement of resistant bacteria between animals and humans, and the factors which enhanced the movement, although the scale of the movement still has considerable uncertainty (Fig. 1).

In the United States, the response to the issues raised in the Swann report was largely unenthusiastic and critical (Table 3). Resistance to Swann's recommendations was based on the estimates of the considerable economic contribution that growth-promoting and subtherapeutic (feed) antimicrobial drugs made to agriculture in comparison to what was criticized as the inadequate evidence, the dubious and slender risk, and the "special case pleading" on which the recommendations of the

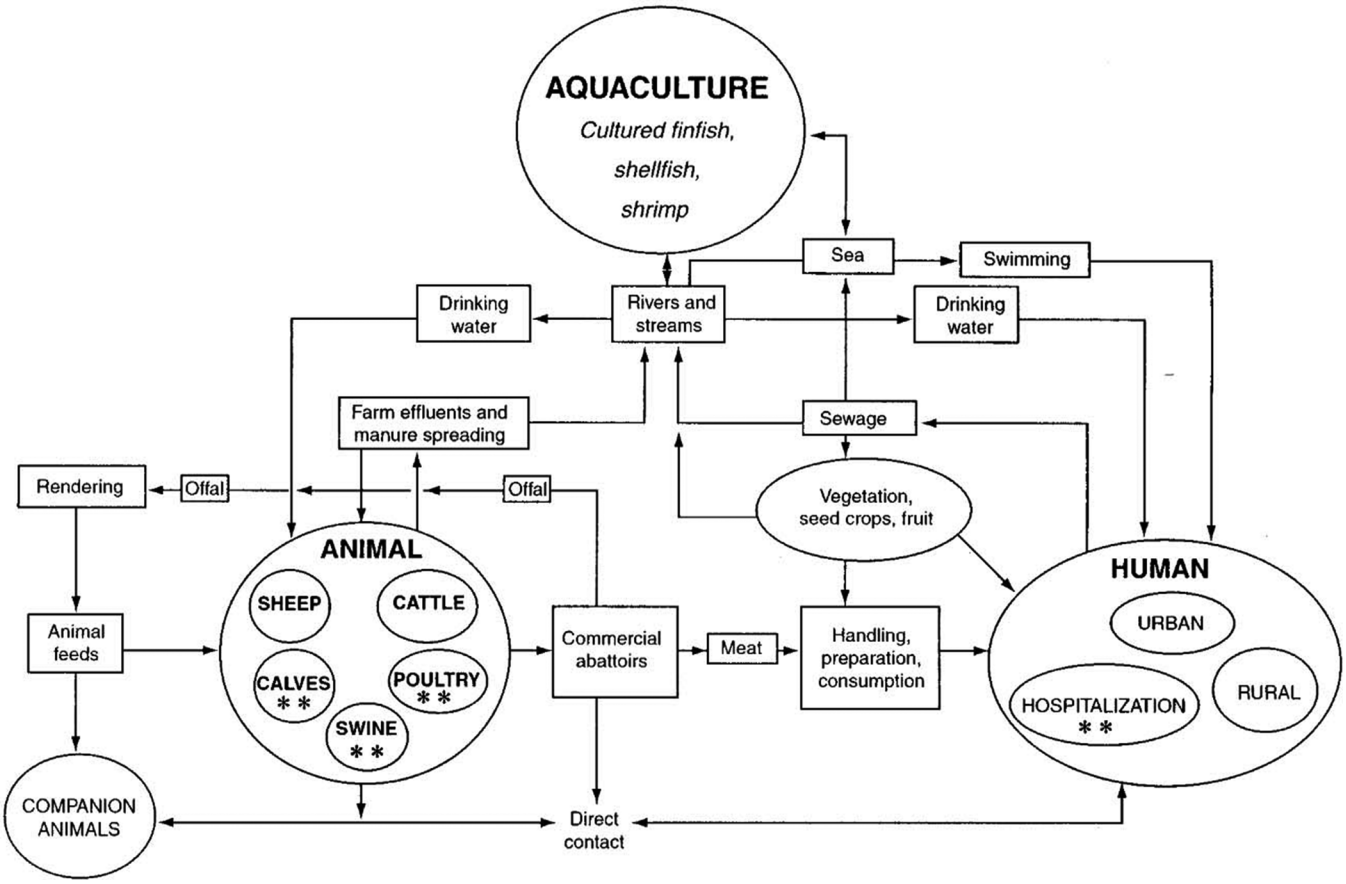


Figure 1 Routes of exchange of *Escherichia coli* between animals and humans. Note the areas where antimicrobial drug selection for resistance is most likely. The size of the circles or boxes does not indicate the extent of the scale of the movement. After Linton (26), modified by R. Irwin; reproduced with permission.

Swann report were regarded as based. The strong lobby of antimicrobial drug manufacturers and the absence in the United States of a national health system (i.e., the patient pays for illness, whereas in Europe it is the nation that bears the cost) may have helped to fuel the criticism. The data were regarded as inadequate to make clear judgements, but the scale of the problem was also thought to be minor. For example, the 1989 Institute of Medicine study (Table 3) suggested that use of subtherapeutic or growth-promoting drugs might contribute to perhaps 26 human deaths a year from antimicrobial-resistant *Salmonella*. For perspective, these numbers would have compared to about 40,000 automobile accident and 10,000 gunshot fatalities in the United States in the same year.

Despite the inconclusive nature of many of the reports in the United States in the period between 1972 and 1995, the issue refused to die. There were periodic highly publicized reports throughout this period of serious human illness caused by *Salmonella* carrying resistance genes thought to be acquired from subtherapeutic, or even therapeutic, use of antimicrobials in animals. One of several examples was that of Spika and others (27) of chloramphenicol-resistant *S. enterica* serotype Newport traced from hamburger meat to dairy farms. Such reports led to apparently carefully orchestrated media and even major science journal frenzies about the discovery of the "smoking gun," with consequent fervent denials by the animal antimicrobial drug industry. Given the existing well-established understanding of the epidemiology of the movement of resistant intestinal bacteria (Fig. 1), these periodic frenzies seemed at the time both astonishing and somehow hysterical. The periodic surges in public interest, however, produced no political will in the United States to re-examine the problem.

The reason for the extensive re-examination of the issue from the mid-1990s was related to several factors. The most important of these was the antimicrobial

resistance crisis in medicine, in which for the first time resistant bacteria moved "out of the hospital and into the community." The very serious nature of the crisis led to a re-examination within the human medicine community of all aspects of antimicrobial use and even to the apparent rediscovery of the importance of basic infection control procedures such as hand-washing. The antimicrobial resistance crisis in medicine again focused the medical establishment on agricultural usage of antimicrobials, in some cases almost to the extent of using it as the scapegoat for the crisis in medicine.

Improvements in understanding of the microbiology of infectious diseases acquired from animals were less important, but also critical, forces in the re-examination of antimicrobial usage in agriculture. For example, at the time of the Swann report, *C. jejuni* was not recognized as a human pathogen, although it subsequently became identified as the most common cause of bacterial gastroenteritis in humans. The emergence of fluoroquinolone resistance in *C. jejuni* of poultry origin in the United States because of the use of fluoroquinolones to treat *Escherichia coli* infections in chickens (28) subsequently led to the ban of all use of this class of drug in chickens in the United States (Table 3). A subsequent risk analysis in the United States suggested that 8,678 citizens treated for this illness with fluoroquinolones had fluoroquinolone-resistant *C. jejuni* illness acquired from chickens (Table 3), a huge number compared to the "26 possible deaths because of resistant *Salmonella*" identified in the 1989 Institute of Medicine report.

Similarly, at the time of the Swann report, vancomycin-resistant enterococci (VREs) were also unknown, although subsequently, enterococcal infections emerged as major nosocomial, largely hospital-acquired, infections in humans, with vancomycin as the "drug of last resort" in such infections. Acquisition of transmissible vancomycin-resistance genes by these hospital-associated bacteria made them essentially untreatable, again raising the specter of the postantibiotic era (Table 1). Work by Aarestrup and his colleagues in Denmark was important in identifying avoparcin, a glycopeptide antimicrobial related to vancomycin, as selecting for the massive presence of VREs in the intestine of poultry and swine fed this drug as a growth promoter (29). For the first time, there was convincing large-scale evidence that eliminating the use of antimicrobial drugs in food animals could dramatically reverse the rise of resistant bacteria in these animals (30). Convincing molecular genetic typing evidence showed that VREs from animals colonized humans (31) and, most dramatically, the marked decline in human intestinal colonization by VREs in Europe following the withdrawal of avoparcin as a growth promoter (32) suggested that the scale of the movement of resistant intestinal bacteria from animals to humans, which had always been a matter of great uncertainty, was far larger than generally suspected previously. Molecular genetic typing and whole-genome sequencing of resistance genes and gene regions were unavailable at the time of the Swann Report but were subsequently used extensively to characterize the relatedness (and therefore sometimes the source) of both bacteria and their resistance genes obtained from animals and humans.

More recent application of whole-genome sequencing to antimicrobial-resistant extrapathogenic *E. coli* from human urinary tract infections and comparison to isolates from healthy chickens clearly indicates that some resistant human isolates derive from chickens (33). Recognition of the likelihood of such a previously unsuspected "insidious epidemic" suggests that the scale and importance of the movement of resistant bacteria and their genes into the human population may be far greater than suspected. One group suggested that cephalosporin use in poultry was responsible for about 1,500 human deaths annually in Europe (34). A voluntary ban on ceftiofur use in Danish swine production was shown to effectively reduce extended-spectrum cephalosporinase-producing *E. coli* in slaughter pigs (35). It seems likely that similar bans, voluntary or involuntary, may be adopted in different jurisdictions in agriculture. The World Health Organization ranking of antimicrobials according to their importance in human medicine (36) remains an important issue for animal use, since the World Health Organization classification of "critically important" includes drugs such as penicillin, and essentially all antibiotics are classified as important, highly important, or critically important. Further discussion, which will likely focus on "highest-priority critically-important antimicrobials" (36), is clearly required as one of the steps in addressing the global resistance crisis.

It is highly ironic that the recent emergence and global dissemination of MRSA in livestock, particularly of clonal complex 398 associated with swine, and the emergence and dissemination of livestock-associated MRSA infections in humans (37, 38) has been linked to the use of zinc oxide in the feed of intensively reared livestock (39). Following the European ban on antimicrobial growth promoters, zinc oxide was introduced as an alternative to to prevent enteric infections in young animals.

Companion Animals

There has been no systematic study of the effect of antimicrobial drug use in companion animals (meaning,

particularly, dogs and cats) and transfer of resistant bacteria or their genes to humans. As a generalization, resistance to antimicrobials is growing among bacteria that cause infection in pets, such as *S. aureus*, *S. pseudintermedius*, and *E. coli* (40), and such bacteria can be transmitted between pets, owners, and veterinary staff both directly and indirectly. Practicing veterinarians are far more likely than controls to be nasally colonized by *S. aureus* (41). Companion animals have been documented to act as reservoirs of some of the high-risk multidrug-resistant clones of *Enterobacteriaceae* (42–44), some of which are likely to be acquired from their human owners. Infection with such resistant bacteria may be amplified by antimicrobial use in veterinary clinics or hospitals and subsequently spread back to animal owners in the dance of infection (Fig. 1).

THE EMERGING CONCEPT AND PRACTICE OF ANTIMICROBIAL STEWARDSHIP IN VETERINARY MEDICINE

As the science of antimicrobial resistance moves onto the political stage, the past 2 years have seen dramatic changes in the global response to the antimicrobial resistance crisis, culminating most recently in the September 2016 United Nations High-Level Meeting and the commitment of members to address the issue in a multifaceted way. This follows earlier similar commitment by members of the G7 and numerous important analyses of how to address the crisis (45, 46) (Table 1). In the United States, there has been game-changing commitment (Guidance 213, Guidance for Industry 233) to remove antibiotics from use as growth promoters and to bring all antibiotics used in feed or water of food animals under veterinary oversight. Canada has followed suit. Another major change has been adoption of a "One Health" approach to resistance (reviewed in chapter 26), an approach that involves multidisciplinary and multi jurisdictional approaches to very complex problems involving people, animals, and the environment (47). An evolving concept and practice, a One Health approach may reduce some of the conflict between the use of antimicrobials in human and veterinary medicine by focusing efforts and energy on resolving resistance issues in a collaborative rather than blaming manner.

Antimicrobial resistance is a multifaceted problem with all the complexities of climate change, to which it is highly analogous. It has multiple causes, with no single actor or factor that can be blamed, has the well-established ability to be self-sustaining, and has the potential to be catastrophic. No single intervention will address the problem, but the combination of multiple interventions and approaches has the potential to have a cumulative impact that will help in its control. A stewardship approach which integrates so much of what we now know about effective antimicrobial use (6), and about infection generally, is the best approach for first-line veterinary practitioners to address the resistance crisis. "Antimicrobial stewardship," reviewed in chapter 32, is the term increasingly used in medicine to describe the multifaceted approaches required to sustain the efficacy of antibiotics and minimize the emergence of resistance. The concept and practice of antimicrobial stewardship continues to evolve in human and veterinary medicine, but it is an approach that takes an active, dynamic process of continuous improvement encapsulated in the idea of good stewardship practice (GSP) (6, 48). Only a GSP mind-set will ensure the long-term sustainability of antimicrobial drugs. Antimicrobial stewardship and GSP involve coordinated approaches and interventions designed to promote, improve, monitor, and evaluate the judicious use of antimicrobials to preserve their future effectiveness and promote and protect human and animal health. This involves a "5R" approach of responsibility, reduction, refinement, replacement, and review (49). Critically, a GSP approach to stewardship also could be evaluated quantitatively as a standard of veterinary practice.

The question for the future is how to preserve existing and develop new drugs in the face of bacterial pathogens, some of which appear to have become particularly adept at developing or acquiring resistance over the past 60 years. Using some of the tools available as we enter the "golden age of microbiology" to improve the way we diagnose infections and develop new, likely targeted, antimicrobials is promising (50). However, as noted by one writer in respect to resistant bacterial infections in companion animal practice (40), resolving the issue of multidrug-resistant endemic bacterial infections will not be through development of new antibiotics if current hygiene practices remain and if we don't undertake good stewardship practices to preserve our existing drugs.

With respect to the changing relationship to antimicrobial drugs in human and veterinary medicine that the resistance crisis has produced, there's a sense that humanity is perhaps in the intermission after the first act of a three-act play, and we're still trying to determine if the play is a comedy or a tragedy. It currently feels like both. There's a huge amount to be done.

Citation. Prescott JF. 2017. History and current use of antimicrobial drugs in veterinary medicine. Microbiol Spectrum 5(6):ARBA-0002-2017.

References

1. **Prescott JF.** 2006. History of antimicrobial usage in agriculture: an overview, p 19–27. *In* Aarestrup FM (ed), *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
2. **Little RB, Bryan CS, Petersen WE, Plastridge WN, Schalm OW.** 1946. INTRAMAMMARY therapy of bovine mastitis. *J Am Vet Med Assoc* **108:**127–135.
3. **Roberts SJ.** 1953. Antibiotic therapy in large animals, p 39–48. *Conference Proceedings, Am Vet Med Assoc*.
4. **Collins JH.** 1948. The present status of penicillin in veterinary medicine. *J Am Vet Med Assoc* **113:**330–333.
5. **Hussar AE, Holley HW.** 1954. *Antibiotics and Antibiotic Therapy*. MacMillan, New York, NY.
6. **Weese JS, Page S, Prescott JF.** Antimicrobial stewardship in animals, p 117–132. *In* Giguère S, Prescott JF, Dowling PM (ed), *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley Blackwell, Ames, IA.
7. **Shryock T, Page S.** 2013. Performance uses of antimicrobial agents and non-antimicorbial alternatives, p 379–394. *In* Giguère S, Prescott JF, Dowling PM (ed), *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley Blackwell, Ames, IA.
8. **Burch DGS.** 2013. Antimicrobial drug use in swine, p 553–568. *In* Giguère S, Prescott JF, Dowling PM (ed), *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley Blackwell, Ames, IA.
9. **Apley MD, Coetzee JF.** 2013. Antimicrobial drug use in cattle, p 495–518. *In* Giguère S, Prescott JF, Dowling PM (ed), *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley Blackwell, Ames, IA.
10. **Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R.** 2015. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci USA* **112:**5649–5654.
11. **Grave K, Torren-Edo J, Muller A, Greko C, Moulin G, Mackay D, Fuchs K, Laurier L, Iliev D, Pokludova L, Genakritis M, Jacobsen E, Kurvits K, Kivilahti-Mantyla K, Wallmann J, Kovacs J, Lenharthsson JM, Beechinor JG, Perrella A, Mičule G, Zymantaite U, Meijering A, Prokopiak D, Ponte MH, Svetlin A, Hederova J, Madero CM, Girma K, Eckford S, ESVAC Group.** 2014. Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. *J Antimicrob Chemother* **69:**2284–2291.
12. **Dupont N, Fertner M, Sonne Kristensen C, Toft N, Stege H.** 2016. Reporting the national antimicrobial consumption in Danish pigs: influence of assigned daily dosage values and population measurement. *Acta Vet Scan* **58:**27.
13. **Speksnijder DC, Mevius DJ, Bruschke CJM, Wagenaar JA.** 2015. Reduction of veterinary antimicrobial use in the Netherlands. The Dutch success model. *Zoonoses Public Health* **62**(Suppl 1)**:**79–87.
14. **Speksnijder DC, Jaarsma DAC, Verheij TJM, Wagenaar JA.** 2015. Attitudes and perceptions of Dutch veterinarians on their role in the reduction of antimicrobial use in farm animals. *Prev Vet Med* **121:**365–373.
15. **Dorado-Garcia A, Mevius D, Jacobs JJ, Van Geijlswijk M, Mouton JW, Wagenaar J, Heederik DJ.** 2016. Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. *J Antimicrob Chemother* **71:** 3607–3619.
16. **Chantziaras I, Boyen F, Callens B, Dewulf J.** 2014. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. *J Antimicrob Chemother* **69:**827–834.
17. **Bos MEH, Mevius DJ, Wagenaar JA, van Geijlswijk IM, Mouton JW, Heederik DJJ, Netherlands Veterinary Medicines Authority (SDa).** 2015. Antimicrobial prescription patterns of veterinarians: introduction of a benchmarking approach. *J Antimicrob Chemother* **70:**2423–2425.
18. **De Briyne N, Atkinson J, Pokludová L, Borriello SP, Price S.** 2013. Factors influencing antibiotic prescribing habits and use of sensitivity testing amongst veterinarians in Europe. *Vet Rec* **173:**475.
19. **De Briyne N, Atkinson J, Pokludová L, Borriello SP.** 2014. Antibiotics used most commonly to treat animals in Europe. *Vet Rec* **175:**325.
20. **Lloyd DH.** 2012. Multi-resistant *Staphylococcus pseudintermedius*: a wake-up call in our approach to bacterial infection. *J Small Anim Pract* **53:**145–146.
21. **Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L.** 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J Antimicrob Chemother* **65:**1145–1154.
22. **McCarthy AJ, Harrison EM, Stanczak-Mrozek K, Leggett B, Waller A, Holmes MA, Lloyd DH, Lindsay JA, Loeffler A.** 2015. Genomic insights into the rapid emergence and evolution of MDR in *Staphylococcus pseudintermedius*. *J Antimicrob Chemother* **70:**997–1007.
23. **Buckland EL, O'Neill D, Summers J, Mateus A, Church D, Redmond L, Brodbelt D.** 2016. Characterisation of antimicrobial usage in cats and dogs attending UK primary care companion animal veterinary practices. *Vet Rec* **179:**489.
24. **Murphy CP, Reid-Smith RJ, Boerlin P, Weese JS, Prescott JF, Janecko N, McEwen SA.** 2012. Out-patient antimicrobial drug use in dogs and cats for new disease events from community companion animal practices in Ontario. *Can Vet J* **53:**291–298.
25. **Randall CJ.** 1969. The Swann Committee. *Vet Rec* **85:** 616–621.
26. **Linton AH.** 1977. Antibiotic resistance: the present situation reviewed. *Vet Rec* **100:**354–360.
27. **Spika JS, Waterman SH, Hoo GW, St Louis ME, Pacer RE, James SM, Bissett ML, Mayer LW, Chiu JY, Hall B, Greene K, Potter ME, Cohen ML, Blake P.** 1987. Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms. A major persisting source of human salmonellosis in California. *N Engl J Med* **316:**565–570.

28. **Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA, Osterholm MT, Investigation Team.** 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. *N Engl J Med* **340:**1525–1532.
29. **Bager F, Madsen M, Christensen J, Aarestrup FM.** 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* **31:**95–112.
30. **Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F.** 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* **45:**2054–2059.
31. **Jensen LB, Hammerum AM, Poulsen RL, Westh H.** 1999. Vancomycin-resistant *Enterococcus faecium* strains with highly similar pulsed-field gel electrophoresis patterns containing similar Tn*1546*-like elements isolated from a hospitalized patient and pigs in Denmark. *Antimicrob Agents Chemother* **43:**724–725.
32. **Klare I, Badstübner D, Konstabel C, Böhme G, Claus H, Witte W.** 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microb Drug Resist* **5:**45–52.
33. **Nordstrom L, Liu CM, Price LB.** 2013. Foodborne urinary tract infections: a new paradigm for antimicrobial-resistant foodborne illness. *Front Microbiol* **4:**29.
34. **Collignon P, Aarestrup FM, Irwin R, McEwen S.** 2013. Human deaths and third-generation cephalosporin use in poultry, Europe. *Emerg Infect Dis* **19:**1339–1340.
35. **Agersø Y, Aarestrup FM.** 2013. Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing *Escherichia coli* in slaughter pigs. *J Antimicrob Chemother* **68:**569–572.
36. **Collignon PC, Conly JM, Andremont A, McEwen SA, Aidara-Kane A, Agerso Y, Andremont A, Collignon P, Conly J, Dang Ninh T, Donado-Godoy P, Fedorka-Cray P, Fernandez H, Galas M, Irwin R, Karp B, Matar G, McDermott P, McEwen S, Mitema E, Reid-Smith R, Scott HM, Singh R, DeWaal CS, Stelling J, Toleman M, Watanabe H, Woo GJ, World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR).** 2016. World Health Organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies to control antimicrobial resistance from food animals. *Clin Infect Dis* **63:**1087–1093.
37. **Smith TC, Pearson N.** 2011. The emergence of *Staphylococcus aureus* ST398. *Vector Borne Zoonotic Dis* **11:** 327–339.
38. **Cuny C, Wieler LH, Witte W.** 2015. Livestock-associated MRSA: the impact on humans. *Antibiotics (Basel)* **4:** 521–543.
39. **Cavaco LM, Hasman H, Aarestrup FM, Wagenaar JA, Graveland H, Veldman K, Mevius D, Fetsch A, Tenhagen B-A, Concepcion Porrero M, Dominguez L, Granier SA, Jouy E, Butaye P, Kaszanyitzky E, Dán A, Zmudzki J, Battisti A, Franco A, Schwarz S, Gutierrez M, Weese JS, Cui S, Pomba C, Members of MRSA-CG.** 2011. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* **150:**344–348.
40. **Lloyd DH.** 2007. Reservoirs of antimicrobial resistance in pet animals. *Clin Infect Dis* **45**(Suppl 2)**:**S148–S152.
41. **Jordan D, Simon J, Fury S, Moss S, Giffard P, Maiwald M, Southwell P, Barton MD, Axon JE, Morris SG, Trott DJ.** 2011. Carriage of methicillin-resistant *Staphylococcus aureus* by veterinarians in Australia. *Aust Vet J* **89:** 152–159.
42. **Ewers C, Bethe A, Stamm I, Grobbel M, Kopp PA, Guerra B, Stubbe M, Doi Y, Zong Z, Kola A, Schaufler K, Semmler T, Fruth A, Wieler LH, Guenther S.** 2014. CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? *J Antimicrob Chemother* **69:**1224–1230.
43. **Ewers C, Stamm I, Pfeifer Y, Wieler LH, Kopp PA, Schønning K, Prenger-Berninghoff E, Scheufen S, Stolle I, Günther S, Bethe A.** 2014. Clonal spread of highly successful ST15-CTX-M-15 *Klebsiella pneumoniae* in companion animals and horses. *J Antimicrob Chemother* **69:** 2676–2680.
44. **Abraham S, Wong HS, Turnidge J, Johnson JR, Trott DJ.** 2014. Carbapenemase-producing bacteria in companion animals: a public health concern on the horizon. *J Antimicrob Chemother* **69:**1155–1157.
45. **O'Neill J.** 2016. *Tackling Drug Resistant Infections Globally: Final Report and Recommendations*. The Review on Antimicrobial Resistance. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf44.
46. **Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O.** 2013. Antibiotic resistance-the need for global solutions. *Lancet Infect Dis* **13:**1057–1098.
47. **Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S, Laxminarayan R, Lubroth J, Magnusson U, Thi Ngoc P, Van Boeckel TP, Woolhouse MEJ.** 2016. Antibiotic resistance is the quintessential One Health issue. *Trans R Soc Trop Med Hyg* **110:**377–380.
48. **Guardabassi L, Prescott JF.** 2015. Antimicrobial stewardship in small animal veterinary practice: from theory to practice. *Vet Clin North Am Small Anim Pract* **45:**361–376, vii.
49. **Page S, Prescott J, Weese S.** 2014. The 5Rs approach to antimicrobial stewardship. *Vet Rec* **175:**207–208.
50. **Prescott JF.** 2014. The resistance *tsunami*, antimicrobial stewardship, and the golden age of microbiology. *Vet Microbiol* **171:**273–278.

Antimicrobial Resistance in Bacteria from Livestock and Companion Animals
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Antimicrobial Susceptibility Testing of Bacteria of Veterinary Origin

2

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INTRODUCTION

The emergence of multidrug-resistant bacteria in both human and veterinary medicine over the past decade has limited the therapeutic choices available to clinicians (1–4). This has, in turn, led to increased pressure on the veterinary community to reduce the overall use of antimicrobial agents, with an emphasis on those deemed critically important for use in human health (4–6). Thus, selection of the most appropriate agent for treatment is essential in preserving the future utility of the currently available agents in both human and veterinary medicine while also ensuring animal welfare. Antimicrobial susceptibility tests (ASTs) can provide essential information to guide the veterinarian in selecting the most appropriate agent and are considered an essential component of responsible use and antimicrobial stewardship programs (2, 7).

The foundational characteristics of ASTs are as follows: (i) a standardized, reproducible test methodology; (ii) quality control (QC) guidelines to ensure day-to-day and interlaboratory assay performance; and (iii) an established relationship between the test outcome and the clinical outcome following treatment (8–12). While standardized test methods have been available in human medicine since the 1960s, the development of rigorous AST methods for use in veterinary medicine was not undertaken until the early 1990s (9, 13). This is not surprising given the variety of host species (companions, food animals, and exotics) and the differences in the application of test results. AST results for a pathogen from a canine, for example, will be used to select treatment for the individual animal, while the same information for a swine isolate may be used to devise therapy for the entire herd. This diversity in host animals and the application of test results has made the development of ASTs that provide accurate, reproducible, clinically relevant results for veterinary pathogens daunting, but substantial progress has been made over the past 2 decades.

Historical Perspective

The use of ASTs in veterinary medicine closely paralleled the development of ASTs in human medicine (as reviewed in reference 7). Because the AST methods for human pathogens such as *Staphylococcus aureus* or *Escherichia coli* could be readily applied to the same organisms isolated from animal diseases with equivalent values, the use of these methods for testing veterinary isolates was a logical extension of the human AST methodologies. However, the use of these methods for the more fastidious veterinary pathogens, as well as the use of human interpretive criteria, was problematic. For example, four different media were recommended by various authors for susceptibility testing of *Histophilus somni* ("*Haemophilus somnus*"), and five different media were recommended for testing of *Actinobacillus pleuropneumoniae* (as reviewed in reference 7, 13). This not only made comparison of AST results from different laboratories difficult, but no single medium would reliably support the growth of all strains. Moreover, the only interpretive criteria available for categorizing isolates as susceptible or resistant were those developed for use with human pathogens, and veterinary laboratories routinely used these breakpoints for similar animal pathogens regardless of the host species. By the early 1980s, it had become apparent that the use of human interpretive criteria did not reliably predict clinical outcomes when applied to veterinary pathogens and antimicrobial agents. This led some authors to question the use of AST methods

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in veterinary medicine, while other investigators attempted to develop veterinary-specific interpretive criteria (as reviewed in references 7, 14). It was apparent by the late 1980s that there was a need for the development of veterinary-specific AST methods and interpretive criteria.

In 1993, the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) formed the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (V-AST) with the task of developing veterinary-specific AST standards (9). This group decided that rather than attempt to address all uses of antimicrobial agents in veterinary medicine (such as growth promotion, prophylaxis, and extra-label usage), it would limit its efforts to therapeutic uses for systemic diseases. This allowed the V-AST to use experience gained from human pathogens for the development of veterinary-specific methods and interpretive criteria. The group also decided it would limit the development of interpretive criteria to the approved indication and pathogens for specific agents. At the time, this eliminated the problems associated with establishing interpretive criteria for extra-label antimicrobial uses and allowed the development of host/pathogen/antimicrobial-specific interpretive criteria (9, 13); however, this has become increasingly difficult recently as the V-AST has attempted to create international standards and guidelines because approved indications vary considerably by jurisdiction.

By 1999, the V-AST had published the first standard for the performance of ASTs with veterinary pathogens, M31 (now VET01), as well as the first guideline on the data requirements for interpretive criteria development, M37 (now VET02) (9, 10, 13). The first version of VET01 included a standardized test method for *H. somni* and *A. pleuropneumoniae* that utilized a common test medium, veterinary fastidious medium, as well as QC organisms for use when testing these organisms (9, 13). The document also included the first publication of veterinary-specific interpretive criteria for several veterinary-use-only antimicrobial agents such as ceftiofur and tilmicosin. The current version adds a standardized method for testing of *Campylobacter* spp. as well as veterinary-specific interpretive criteria for antimicrobial agents in each of the major antimicrobial classes (9). In addition to the sponsor-supported interpretive criteria, the V-AST formed a working group in 1998 to address the development of interpretive criteria for older, generic agents. Since its inception, the Generic Working Group has developed host-pathogen-specific interpretive criteria for several generic compounds such as tetracycline and penicillin. The FDA-approved antimicrobial agents in the United States with veterinary-specific interpretive criteria are listed in the CLSI VET01 document. VET01 is the accepted worldwide standard for susceptibility testing of veterinary pathogens and is used by laboratories accredited by the American Association of Veterinary Laboratory Diagnosticians (9). Additionally, the V-AST has developed standards for the antimicrobial susceptibility testing of pathogens that cause diseases of fish and other aquatic animals and has developed guidelines for laboratories to use when testing infrequently encountered or unusual organisms (15, 16).

At present, there are only a few groups other than the CLSI that are developing standards for antimicrobial susceptibility testing of veterinary pathogens. In 1996, the European Society for Clinical Microbiology and Infectious Diseases established the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to harmonize antimicrobial breakpoints across Europe (17, 18). Since its inception, EUCAST has addressed susceptibility testing of human pathogens, and clinical breakpoints have been harmonized for a large number of agents (19, 20). In 2014, EUCAST expanded into the antimicrobial susceptibility testing of veterinary pathogens with the formation of the VETCAST group (http://www.eucast.org/ast_of_veterinary_pathogens/). The VETCAST group has defined its responsibility for developing veterinary-specific testing and clinical breakpoints for new and generic agents exclusively in Europe. At present, VETCAST is developing its standards and operating processes for antimicrobial susceptibility testing of veterinary pathogens. Additionally, veterinary-specific methods and interpretive criteria have been developed for the calibrated dichotomous sensitivity method widely used in Australia (21).

AST METHODS

Antimicrobial susceptibility testing is performed when the antimicrobial susceptibility of a bacterial pathogen cannot be determined solely from its identification or if bacterial resistance is suspected due to poor clinical response (9). Bacterial pathogens from infected animals are normally cultured from clinical specimens (such as skin or lung tissue) using selective or nonselective growth media. However, only potential pathogens, not contaminants or normal bacterial flora, are selected for antimicrobial susceptibility testing, and the identification of any isolated bacterial pathogen may be determined using biochemical and/or automated methods. It is highly recommended that isolation and identification

procedures be performed prior to *in vitro* antimicrobial susceptibility testing.

Veterinary diagnostic laboratories can select from several methodologies for antimicrobial susceptibility testing of veterinary pathogens, with most laboratories using the agar disk diffusion (ADD) method or the broth microdilution MIC method or both (10). To ensure the generation of accurate, reproducible results, laboratories should adhere to a standard, well-defined method that includes the appropriate QC information. The purpose of an AST method is not to mimic *in vivo* conditions but to provide reproducible results that can be correlated to clinical outcome. In the United States, the majority of veterinary laboratories use either a CLSI reference method or a commercial method that adheres to this standard (9, 12). The method a laboratory selects depends on a variety of factors, including the cost per test, the availability of appropriate antimicrobial agents (since veterinary-specific agents are not available in all test systems), and the volume of samples to be handled by the laboratory. In general, smaller laboratories with relatively low test volumes tend to prefer the ADD method, and larger laboratories prefer the more semiautomated MIC methods.

ADD Method

The ADD method remains a flexible, low-cost means of conducting antimicrobial susceptibility testing and is widely used in many veterinary laboratories. In general, appropriate agar plates are inoculated with a standardized concentration of a pure bacterial culture, to which antimicrobial-impregnated paper disks are then added to the test plate. The outcome of the assay is a "zone of inhibition," and although results are read to the nearest millimeter, the final results are reported categorically as susceptible, intermediate, or resistant to the antimicrobial agent of interest. If ADD test results are to be reliable, the methodology must be standardized and controlled as originally described (22), from which interpretive criteria (susceptible, intermediate, resistant) have been developed. Care must be taken when reading zones with hazy endpoints or the presence of small colonies within the larger defined zone of inhibition. Advantages of the ADD test include its lower material cost per test compared to other susceptibility test methods, flexibility in antimicrobial selection, and ease of setting up the assay. Disadvantages may include inherent test and user variabilities such as inoculum size used, measuring of zones, labor requirements, and stability of disks. Guidelines for proper media preparation and storage, as well as proper storage of antimicrobial susceptibility disks, are available for veterinary laboratories (9). The ADD zone diameters inversely correlate (larger zone is correlated to a lower MIC) with MIC values from a standard dilution test (usually from a broth microdilution method), and the CLSI provides the most up-to-date zone diameter (and MIC) interpretive criteria for clinical breakpoints (10).

Numerous veterinary-specific antimicrobial agents are commercially available in antimicrobial-impregnated disks used with the ADD method (9). However, certain veterinary-specific agents or infrequently tested disks may only be available from the pharmaceutical manufacturer. Additionally, smaller veterinary laboratories may have difficulties in standardizing the inoculum used in this method, but commercial systems are available for this purpose.

MIC Methods

A variety of MIC methods ranging from the agar dilution method to a commercially available gradient strip method, as well as semiautomated broth microdilution methods, are available for veterinary laboratories (9, 10). Both broth and agar dilution techniques can be used to quantitatively determine the *in vitro* activity of an antimicrobial agent against a bacterial culture of interest. In general, a standardized inoculum of a pure culture of the isolate of interest is added to a series of tubes, agar plates, or wells that contain a broth or agar medium with increasing concentrations of an antimicrobial agent based on a log2 scale (for example, 0.5 g/ml, 1.0 g/ml, 2.0 g/ml, etc.). Commercial microdilution systems are available that may contain dried or frozen antimicrobial agents in a dilution series, and appropriate QC tests should be performed for any method that is used. The MIC method provides a value that is expressed in micrograms per milliliter and allows categorization of the organism as susceptible, intermediate, or resistant based on available clinical breakpoints.

The techniques described are primarily for testing commonly isolated pathogens that grow well after overnight incubation when using Mueller-Hinton broth. However, Mueller-Hinton broth is not adequate to support the growth of many fastidious pathogens encountered in veterinary medicine. Media that are supplemented with additional growth factors such as yeast or lysed blood may adequately support growth of certain fastidious organisms. When an appropriate medium has been identified for use, QC procedures and interpretive criteria need to be established for testing that organism. Standardized methods for testing the more-fastidious organisms such as anaerobes and *Campylobacter* spp. have been developed for the MIC methodology (9, 10).

The MIC method is preferred for use in surveillance or epidemiological programs because it allows the calculation of quantitative summary statistics. Of the various MIC formats, the broth microdilution method is the most widely used. However, the primary disadvantage of the available systems is inflexibility in antimicrobial selection and dilution test ranges unless the laboratory is willing to bear the cost of custom panels. The number of dilutions tested in the MIC method may vary from laboratory to laboratory depending on the final use of the results. Laboratories involved in surveillance programs usually prefer to test a smaller number of antimicrobial agents over an extended number of drug dilutions, while many diagnostic laboratories choose to use a "clinical breakpoint" panel. These breakpoint panels allow the laboratory to test a larger number of compounds with more limited dilution ranges spanning the interpretive criteria or clinical breakpoint values for each antimicrobial agent. Care should be taken in selecting a breakpoint panel that has in-range values for at least one QC strain to allow for validation of test results.

Antimicrobial Combination (Checkerboard) Test Methods

Laboratories may want to understand the activity of antibiotic combinations on pathogens in specific situations, especially where a multidrug-resistant phenotype is exhibited by the pathogen. Although not considered a standardized technique, the checkerboard assay is an *in vitro* method that may be used to evaluate synergy, indifference, or antagonism between two antimicrobial agents when they are combined. Antimicrobial combinations are considered to be synergistic if the effect of the antimicrobial combination is greater than the effect of either agent alone or greater than the sum of the effects of the individual agents. Indifference is defined as a drug combination that has an effect equal to the effect of either agent alone, and antagonism is defined as a combination that has an effect less than the effect of either agent alone (23). In the checkerboard assay, one antimicrobial agent is added vertically at 2-fold dilutions to a 96-well plate while another agent is added horizontally to produce a "checkerboard" of various combined drug concentrations (Fig. 1). The outcome of the checkerboard assay is the fractional inhibitory concentration (FIC). FIC values are determined by identifying the MIC values for each drug alone, as well as the lowest MIC of the drugs in combination (Fig. 1). FICs can be determined by the following formula:

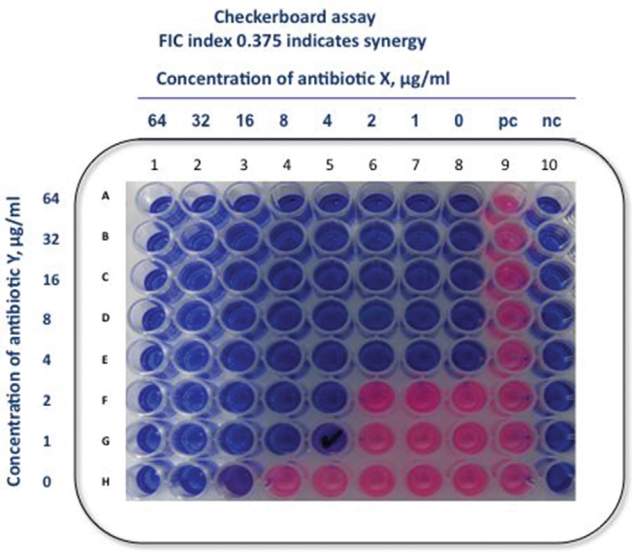


Figure 1 Example of a checkerboard plate setup that shows a synergistic reaction between two combined antibiotics (http://www.ibg.kit.edu/nmr/548.php).

$$\begin{aligned}&(\text{MIC of drug X in combination}/\\&\text{MIC of drug X alone})\\+\ &(\text{MIC of drug Y in combination}/\\&\text{MIC of drug Y alone})\end{aligned}$$

An FIC value that is ≤ 0.5 is considered to be synergistic, while values of >4.0 would be considered antagonistic (23).

Although checkerboard assays are laborious and time-consuming, this method is valuable in understanding the potential effect of two drugs when administered closely together to help avoid interference from either drug (24). Additionally, this method is helpful when determining if synergistic activity of combination therapy would be expected in the therapy of drug-resistant pathogens. Synergism and antagonism may be drug-pathogen specific, so test results should be considered case by case, and it should be noted that most tests will result in indifference instead of synergism or antagonism for the majority of drug-pathogen combinations. Any evidence of synergy or antagonism based on the checkerboard method should be confirmed by time-kill studies with the combined agents, followed by animal and clinical studies to establish whether combination therapy is a truly effective option for treatment.

QC and Test Standardization

Regardless of the susceptibility test method that is selected, the routine administration of QC testing is an

essential component of AST. The routine testing of QC strains allows the veterinary laboratory to verify that personnel, incubation conditions, test media, and antimicrobial agents are performing at an acceptable, standardized level. Numerous QC strains have been defined by various standards organizations for use in their systems (17, 25, 26). Any QC ranges should be developed using a multilaboratory study that examines both inter- and intralaboratory reproducibility such as those defined by the CLSI (9, 10) or by using an external quality assurance system (25). The overall performance of the susceptibility test system should be monitored using those ranges listed by testing the appropriate control strains each day (or week) the test is performed. The interval at which QC strains are tested varies among laboratories. While inclusion of QC strains on each day of testing is preferred, this may be too costly and time-consuming for smaller veterinary laboratories, and these laboratories may choose to run these QC strains on a weekly basis or less often. A procedure for conversion from daily to weekly QC testing is described in document VET01 (9).

REPORTING OF AST RESULTS

The overall goal of AST is to assist the clinician with antimicrobial selection; therefore, a primary responsibility of the veterinary laboratory is to select the most appropriate agents for routine testing and reporting. Reporting AST results for veterinary pathogens is more complicated than for human pathogens due to the differences in approved antimicrobial agents and approved interpretive criteria for the various animal host species. For this reason, most veterinary laboratories have predefined panels of compounds specifically used for testing pathogens from specific hosts or specific disease indications. This may include a specific panel for testing bovine/porcine pathogens, a bovine mastitis panel, an equine panel, or a companion animal (canine/feline) panel. In addition to species-specific drug panels, there are separate Gram-negative and Gram-positive panels for both diagnostic and surveillance purposes.

The veterinary laboratory should not report specific antibacterial agents in certain host animals, and the reporting of agents that do not have specific approvals in a host species (termed "extra-label use" in the United States) should only be done with extreme care after consultation with a veterinarian. Extra-label use is defined as an antimicrobial agent used in any manner not specifically listed on the drug packaging and may include use in a different animal species; for treatment of a different disease; at a different dose, route, or frequency of administration; or with a different withdrawal time (27). For example, federal regulations in the United States prohibit the extra-label use of chloramphenicol, fluoroquinolones and glycopeptides in food-producing animals, and AST results for these agents should not be reported when a clear extra-label use situation exists (28). However, the individual laboratory must make decisions to report antimicrobial results for certain antimicrobials, such as aminoglycosides in cattle. This antimicrobial is not strictly prohibited from use in cattle and may be used at the discretion of a practicing veterinarian; however, this use requires a significantly extended withdrawal time, and bovine practitioners in the United States have therefore adopted a voluntary "no use" policy (29). Similar types of regulations exist for regions outside of the United States, and in some countries the restrictions on extra-label use are even more stringent. Antimicrobial products in the European Union with formularies and drug classes that are deemed critically important for human health may therefore be ranked accordingly for first-line, second-line, or third-line use in animals and should be reported accordingly by diagnostic laboratories in those jurisdictions.

Preference in reporting should be given to those agents with veterinary-specific interpretive criteria for the host-pathogen combination being tested, because these are the most clinically relevant values (Table 1). The use of clinical breakpoints developed for a specific disease in one host species for categorizing isolates from another host species as susceptible or resistant may not accurately predict clinical outcome and should be minimized whenever possible. If agents are to be reported using interpretive criteria developed for other animal species or humans, then this should be indicated to the veterinarian. For example, if a *Pasteurella multocida* isolate from a case of bovine respiratory disease is tested against tetracyclines, it is inappropriate to report this isolate as susceptible based on swine breakpoints for this drug-pathogen combination; rather, it should be reported based on the interpretive criteria for the bovine breakpoint. In this example, a *P. multocida* isolate with a MIC of 2 g/ml would be considered resistant for swine respiratory disease but susceptible for bovine respiratory disease. While each laboratory bears the responsibility for developing specific reporting cascades for the various host animals and pathogens to be tested, most veterinary microbiology laboratories use the information provided in Table 1 of CLSI document VET01 as a guideline for selecting antimicrobial agents that could be considered for routine testing (9). It is preferred to test and report those antimicrobial agents that have veterinary-specific interpretive criteria (called

Table 1 Summary of antimicrobial agents with veterinary-specific interpretive criteria[a]

Antimicrobial agent	Host species with approved interpretive criteria
Amikacin	Dogs, horses
Amoxicillin-clavulanate	Dogs, cats
Ampicillin	Dogs, horses, swine
Cephalothin	Dogs
Cefazolin	Dogs, horses
Cefpodoxime	Dogs
Ceftiofur	Horses, swine cattle, bovine mastitis
Chloramphenicol	Dogs
Clindamycin	Dogs
Danofloxacin	Cattle
Difloxacin	Dogs
Doxycycline	Dogs
Enrofloxacin	Dogs, cats, cattle, chickens, turkeys
Florfenicol	Swine, cattle
Gamithromycin	Cattle
Gentamicin	Dogs, horses
Marbofloxacin	Dogs, cats
Orbifloxacin	Dogs, cats
Penicillin	Dogs, horses, swine, cattle
Penicillin-novobiocin	Bovine mastitis
Pirlimycin	Bovine mastitis
Pradofloxacin	Dogs, cats
Spectinomycin	Cattle
Tetracycline	Dogs, swine, cattle
Tilmicosin	Swine, cattle
Tiamulin	Swine
Tildipirosin	Swine, cattle
Tulathromycin	Swine, cattle

[a]From reference 10.

group A agents) over those antimicrobial agents that use human interpretive criteria (group B) since the relationship from human to veterinary application has not been demonstrated. Antimicrobial agents that have neither veterinary-specific nor human-specific CLSI-approved interpretive criteria (group C) should be selectively reported due to differences in dosage and pharmacokinetics, and those agents listed in group D should be selectively tested and selectively reported. Reporting in this manner allows the clinician to evaluate the clinical relevance (or lack thereof) of the test results.

Cumulative AST Data

The preferred method for reporting cumulative MIC results is to report all data in a frequency distribution table or graph, because this allows the user to understand the activity of a specific agent against a given population of target organisms. Only a single bacterial species should be used to understand the activity of a particular antibiotic, and the laboratory should not combine distributions from different bacterial species. When large numbers of antibacterial agents and pathogens need to be reported in a succinct manner, summary statistics are often the most practical method for reporting MIC data (4). The most common summary statistics would include total number of isolates, MIC_{50} (the MIC at which 50% of the isolates are inhibited), MIC_{90} (the MIC at which 90% of the isolates are inhibited), and the MIC test range. These are based on populations of bacterial isolates and are not the concentration of antimicrobial that inhibits 50% or 90% of the growth from a single culture. A minimum of 10 isolates, but ideally at least 50 isolates, should be used and tested to calculate MIC_{50}, MIC_{90}, and MIC range values from the population of isolates used. Only on-scale dilution values should be reported, and the MIC_{50} and MIC_{90} values may be indicated when MIC distribution data are presented (http://aac.asm.org/site/misc/ifora.xhtml). If data for a single interpretive category are to be summarized, the percent susceptible is preferred because it avoids the issue of handling the intermediate and resistant categories. Additional analyses can be performed, but direct comparison of MIC data across antimicrobial classes should not be done due to potential differences in the relative *in vitro* activity of these two classes on a per-weight basis and differences in the dosing and pharmacokinetics of the two antimicrobials. For example, if ceftiofur has an MIC_{90} of $\leq$0.06 g/ml for *Mannheimia haemolytica*, compared to an MIC_{90} of 16.0 g/ml for tulathromycin, the efficacy of these two compounds may still be comparable (because both values are below the susceptible breakpoints for each drug) when used to treat bovine respiratory disease. When summary comparisons between antimicrobials are made, it is more appropriate to use the percent-susceptible data.

Class Testing

Veterinary diagnostic laboratories may report AST results for a particular antimicrobial agent within an antibiotic class to predict the susceptibility of other agents within the same class, and this is often referred to as "class testing." The CLSI has recommended the use of certain antimicrobial agents to predict antimicrobial susceptibility to multiple agents within the same class as a means of increasing testing efficiencies in diagnostic laboratories (9). For example, ampicillin, tetracycline, and clindamycin are routinely tested as the representatives for their respective antimicrobial classes. In contrast, there are significant differences in the

activity of other antimicrobial agents within a class in which class testing cannot be performed. Such is the case when using the veterinary macrolide tilmicosin as the class representative for tulathromycin and other veterinary macrolides. In this example, no information is available that supports the use of tilmicosin as the class representative for other veterinary macrolides since veterinary macrolides have different substituted ring structures, as well as different *in vitro* and *in vivo* properties with regard to MIC activity, dosage regimen, and pharmacokinetics. Additionally, there is evidence that the presence of different macrolide resistance genes has a differential effect on the MICs of drugs in this class (30). Therefore, any susceptibility results for tilmicosin to predict the activity and potential treatment outcome for tulathromycin, gamithromycin, or tildipirosin is strongly discouraged due to the unique attributes of each antibiotic.

ESTABLISHMENT OF VETERINARY-SPECIFIC INTERPRETIVE CRITERIA

The methodology for establishing veterinary interpretive criteria can be found in the most current version of CLSI document VET02 (10). In establishing veterinary-specific interpretive criteria, the CLSI V-AST committee evaluates a tripartite database: MIC distribution data, pharmacokinetic/pharmacodynamic (PK/PD) data, and clinical outcome data (10).

MIC Distribution Data

In general, bacteria that possess a genetic resistance element will have increased MICs relative to bacteria without that same resistance mechanism. By plotting a MIC distribution (histogram) for a single bacterial species-antimicrobial combination, the bacterial population can be divided into two groups: (i) bacteria with low MIC values for that antimicrobial that likely do not possess a resistance mechanism, also known as the wild-type population, and (ii) bacteria with high MIC values indicating the presence of an antimicrobial resistance mechanism, the non-wild-type population. The terms "low MIC values" and "high MIC values" are not absolute values but are relative and based on the specific bacteria-antimicrobial combination. For example, a low MIC for a population of *M. haemolytica* isolates to danofloxacin would be ≤0.25 g/ml, while a low MIC for the same population of isolates to tulathromycin would be ≤16 g/ml.

For some antimicrobial-pathogen combinations, a bimodal MIC distribution may not exist, and determination of a distinct non-wild-type population is not possible. This is most often found in cases where specific resistance mechanisms are rare in that particular bacterial species. In these cases, the CLSI and V-AST may only delineate a "susceptible" category. If a laboratory isolate test result is "nonsusceptible," the identity of the organism and antimicrobial susceptibility should be reconfirmed. For example, ampicillin has only a "susceptible" category for β-hemolytic streptococci, as does danofloxacin for *M. haemolytica* and *P. multocida*, although in the latter case, the "intermediate" and "resistant" categories were recently added based on new data demonstrating a resistant subpopulation (31).

It is also worth noting that in some instances, rather than a clear distinction between wild-type and non-wild-type populations, a particular resistance mechanism will cause small incremental increases in the MIC, leading to a less distinct division of the population.

Because the MIC distribution data (and the subsequent interpretive criteria on which it is built) are based on a specific pathogen-antimicrobial combination, attempts to extrapolate interpretive criteria to a different pathogen or antimicrobial should not be done for reasons further discussed below.

PK/PD Data

Evaluating only the *in vitro* microbiological response to an antimicrobial ignores the role of the animal host, so interpretive criteria must also consider some of the animal host factors that impact disease outcome. Considering that the total effect of an antimicrobial in treating an infection is a reflection of how the antimicrobial is absorbed by the animal following administration, distributed to the site of infection, metabolized (this can increase or decrease the antimicrobial's activity) by the animal, and finally eliminated from the animal, it seems intuitive that pharmacokinetics would be part of establishing veterinary interpretive criteria. The effect of the antimicrobial on the bacterial pathogen is the pharmacodynamic response of interest (Table 2). Together, the host animal contribution to the change in drug exposure and the bacterial response over time becomes the PK/PD relationship and is generally categorized as either the time that antimicrobial concentrations in the plasma are greater than the MIC of the infecting pathogen ($fT > MIC$), the ratio of the total antimicrobial exposure (expressed as the area under the drug plasma concentration curve) to the MIC of the infecting pathogen ($fAUC/MIC$), or the ratio of peak drug plasma concentrations to the MIC of the infecting pathogen ($fCmax/MIC$). The PK/PD index associated with veterinary antimicrobial classes can be found in Table 3 (32–40).

Table 2 Definitions of pharmacodynamic parameters

Time above MIC (*f*T > MIC)
Antimicrobial activity is based on the time that the active (non-protein bound) concentration of the drug is above the MIC of the infecting bacterium.
Area under the curve to MIC ratio (*f*AUC/MIC)
Activity for antimicrobials associated with this PK/PD index is based on the total exposure, as measured by the area under the drug plasma concentration curve (pharmacokinetic profile) of active (non-protein bound) antimicrobial in relation to the MIC of the infecting bacterium.
Peak plasma concentration to MIC ratio (Cmax/MIC)
Some antimicrobials have activity based on the ratio of the peak plasma concentration of active (non-protein bound) antimicrobial to the MIC of the infecting bacterium.

During the breakpoint development process, a statistical process known as Monte Carlo simulation is used to capture the variation in host animal pharmacokinetics (41). In doing so, an interpretive category is based not on the average plasma concentrations of an antimicrobial for that animal species, but rather on the concentrations that will likely be achieved by 90% of animals in the population treated with that particular antimicrobial dosing regimen.

Clinical Outcome Data

The third and final piece of data used to establish veterinary interpretive criteria comes from clinical studies in which animals affected with the particular disease and infecting pathogen are treated with the specific antimicrobial and dosing regimen. Clinical outcomes are compared to the MIC distribution of isolates from that study. Generally, clinical response will be good at low MIC values (again, low is relative to the antimicrobial-pathogen combination) and will decrease as MIC values increase. Clinical outcome data in support of interpretive criteria are usually based on a small number of animals (and isolates); therefore, these data generally become supportive in nature to the more robust microbiological distribution and PK/PD data. As with microbiological distribution and PK/PD data, clinical outcome data similarly constrain the ability to extrapolate interpretive criteria to other situations, for example, other disease conditions, caused by other pathogens, in different host species (classes), treated with different dosing regimens.

Table 3 Mechanism of action and associated pharmacokinetic/pharmacodynamics (PK/PD) indices of antimicrobial agents used in veterinary medicine

Antimicrobial class	Representative antimicrobials	Mechanism of action	PK/PD index associated with activity	Reference for PK/PD
Macrolides/azalides/ lincosamides/ketolides	Erythromycin, clarithromycin Azithromycin Clindamycin Telithromycin	Protein synthesis inhibitor	AUC/MIC	37
Beta-lactams	Penicillins, cephalosporins, carbapenems	Cell wall synthesis inhibitor	Time > MIC	38
Glycopeptides	Vancomycin	Cell wall synthesis inhibitor	Time > MIC, Cmax/MIC	39
Fluoroquinolones	Enrofloxacin, danofloxacin, marbofloxacin, pradofloxacin	DNA replication/transcription inhibitor	AUC/MIC Cmax/MIC	40 36
Aminoglycosides	Gentamicin, amikacin, neomycin, spectinomycin	Protein synthesis inhibitor	Cmax/MIC	41
Tetracyclines	Oxytetracycline, chlortetracycline, doxycycline, minocycline	Protein synthesis inhibitor	Time > MIC	42
Phenicols	Florfenicol, chloramphenicol	Protein synthesis inhibitor		
Trimethoprim		Folic acid synthesis inhibitor		
Sulfonamides	Sulfadimethoxine, sulfamethazine	Folic acid synthesis inhibitor		
Oxazolidinones	Linezolid	Protein synthesis inhibitor	AUC/MIC	43
	Novobiocin	Exact mechanism unknown; proposed mechanisms include: Cell wall synthesis inhibitor Nucleic acid synthesis inhibitor Protein synthesis inhibitor		

Re-Evaluation of Clinical Breakpoints

Over time, breakpoints may need to be revised due to the emergence of new bacterial resistance mechanisms and subsequent changes in clinical response rates. In human medicine, case reports/case series documenting clinical failures and description of the resistance mechanism may initiate revision of a clinical breakpoint; however, there is no formal timeline in place for re-evaluation of veterinary breakpoints (42–44). In veterinary medicine, breakpoint revision could be considered by the CLSI V-AST upon presentation of microbiological, PK/PD, and clinical cutoff data; however, there seems to be a significant communication gap between clinicians, diagnostic laboratories, and the V-AST subcommittee that would signal the need for breakpoint revision. Closing this gap should be considered a priority if breakpoints are to maintain their clinical relevance.

ROLE OF AST FOR CLINICAL OUTCOME

The role of AST in managing patients was best summarized by Doern et al. (45) with the statement "Susceptibility testing is not done in a vacuum." Microbiology laboratories often go to great lengths to ensure standardization and repeatability of the methods they use. While this is an extremely important aspect of AST, laboratories need to recognize that the utility of AST for the clinician is predicated on two important factors: the test must be timely enough to be clinically useful, and the test must have predictive value. Provided that these criteria are met, AST becomes a powerful tool in the judicious selection of antimicrobial therapy.

Testing Timing

Broth dilution and disk diffusion susceptibility testing of most veterinary pathogens requires a minimum of 36 hours from specimen submission to AST result: approximately 18 hours of incubation for the initial culture and 18 hours to perform the actual AST. Specimen transport, mixed cultures, and contaminant growth and slow-growing organisms can add significantly to the testing turnaround time. Direct susceptibility testing of clinical specimens has been reported to reduce turnaround time and in studies evaluating its use for urine and blood culture was nearly equivalent to the standardized method; however, because this is a nonstandard method, results should be reported as preliminary and confirmed with the standard method (46). The primary disadvantage to direct testing of clinical specimens is that the purity of the culture and bacterial inoculum cannot be controlled. Testing bacterial inocula above 5×10^5 CFU/ml has been reported to lead to an increase in MIC values (47, 48), which could lead to a false-resistant test result and lead the clinician to rule out the use of an appropriate antimicrobial, perhaps encouraging the use of a more advanced or more toxic antimicrobial, while testing a lower bacterial inoculum could lead to a false-susceptible result and the use of an antimicrobial that would be clinically inappropriate for that infection. Alternative AST methods with the potential to reduce the turnaround time associated with testing are currently being investigated. A brief discussion of these methods can be found at the end of this chapter under "Future Directions and Trends."

Correlation of AST Results with Clinical Outcome

While cost and turnaround time certainly influence the decision to pursue diagnostic testing, the primary benefit, or limitation, of culture and susceptibility testing rests on the predictive ability of the test. The "90-60 rule" has been used to summarize the predictive value of AST: infections due to susceptible isolates will respond approximately 90% of the time, while infections due to resistant isolates will respond approximately 60% of the time (49). The actual evidence for and against the predictive value of AST is quite sparse and often contradictory. The predictive value of AST for predicting therapeutic outcomes for mastitis in dairy cows was reviewed by Constable and Morin (50), who "do not currently recommend the use of susceptibility testing to guide treatment decisions for individual cows," despite reporting several studies that did, indeed, demonstrate statistically significant associations between AST and therapeutic outcomes. Conversely, a review by Barlow (51), which evaluated many of the same references as the review by Constable and Morin, reported that many of these studies had limited statistical power due to high rates of spontaneous bacteriological cure and low proportions of resistant isolates. The Barlow review highlighted the need for "unbiased randomized clinical trials with sufficient sample size" to evaluate the correlation between AST and clinical outcomes for mastitis therapy.

In a publication by McClary et al. (52), the authors also did not demonstrate a correlation between AST and bovine respiratory disease treatment response. However, as with the mastitis studies, this study also reported a low proportion of resistant isolates (6/745 for *M. haemolytica*; 16/231 for *P. multocida*). Additionally, in this study, the authors used bacterial isolates obtained by deep nasopharyngeal swab to measure the pretreatment MIC values. This may have impacted the correlation of AST result to clinical outcome, because it

has been demonstrated in several studies that bacterial pathogens from the upper respiratory tract may be different from those cultured from the lower respiratory tract of bovine respiratory disease-affected calves (53–55).

Reports from the human medical experience are also not definitive. Studies have been reported that demonstrate clear statistical associations between AST result and clinical outcome (56–58). Some reports have highlighted situations where the categorical interpretation (susceptible, intermediate, resistant) does not accurately predict clinical outcome, but an association between increasing MIC values and worsening of patient outcomes occurs; many of these papers call for revision of the established breakpoints (42, 59–61). Other studies fail to demonstrate any relationship at all between the *in vitro* result and the patient outcome (62, 63).

It is important for readers to understand that all veterinary-specific breakpoints have been evaluated for correlation to clinical outcome through the breakpoint development process. Through an expert consensus process, over 90 antimicrobial, pathogen, and host species combinations have been approved to provide guidance to clinical decision-making.

While even staunch proponents of AST will readily admit that an *in vitro* test cannot fully account for all the variables within an individual animal that will ultimately impact clinical outcome, the clinician needs to know, "Is there value in the test?". Our contention is that AST has value in the antimicrobial selection process. Therefore, what are the factors/considerations that potentially increase or decrease the relationship between the *in vitro* result and the clinical outcome?

Factors Impacting the Relationship Between *In Vitro* Result and Clinical Outcomes

Laboratory factors

Assuming that a standardized susceptibility test method is being utilized, one of the most important factors that can potentially affect the *in vitro-in vivo* relationship is selecting the right isolate, that is, the isolate responsible for clinical disease, for susceptibility testing. Both the clinician collecting the specimen and the laboratory microbiologist can have a profound impact on selecting the right isolate. The veterinary clinician has the responsibility to submit samples that limit normal flora/contaminant growth and are representative of the disease process in that individual or group of animals. For example, selecting the right isolate for AST when an *E. coli* isolate is recovered from a cystocentesis sample from a canine patient showing clinical signs of a urinary tract infection that then grows in pure culture in the laboratory would be a relatively straightforward process. The selection of the right isolate becomes less clear if the *E. coli* is recovered from a mixed culture and the sample was collected by free catch in a patient showing signs of polyuria. In the latter case, the *E. coli* could be a primary urinary tract infection pathogen, or it could be a contaminant that results in antimicrobial treatment when none was indicated and misdiagnosis of the underlying disease condition.

In the laboratory, the microbiologist will generally select a single CFU from the original culture to perform AST. The degree to which this impacts the *in vitro-in vivo* relationship is generally dependent on the degree to which there are mixed-strain infections (64).

Extrapolating breakpoints

Clinical interpretive categories are designed to predict the contribution of the antimicrobial to therapeutic success (or failure) (65). This correlation is imperfect, at best, at the individual patient level but does allow the clinician to evaluate the relative odds for success in a population of patients (66, 67). Because a clinical interpretive criterion is designed for a specific bacterial species-antimicrobial-host species-disease process-dosing regimen combination, extrapolation of any of those factors would potentially affect the predictive value of the test result. While in most situations this would seem intuitive, i.e., a clinical interpretive category for *S. aureus* may or may not apply to *M. haemolytica*, oftentimes, the clinician may not be aware of which host species-disease process-dosing regimen was used to determine the "S"/"I"/"R" on the report. Clinical microbiology laboratories should be encouraged to make this more transparent on reports.

Host factors

An *in vitro* AST will not account for all of the factors that ultimately lead to clinical outcome in a patient. The test is not intended to mimic the disease process in an animal, but rather to provide a result that can provide the clinician with an expectation of the biology of the specific pathogen and what the drug can (or cannot) contribute to clinical outcome. In cases where the immune function contributes significantly to disease outcome, the predictive value of AST will be reduced.

ROLE OF SURVEILLANCE AND ANTIMICROBIAL STEWARDSHIP

Surveillance is defined as the continuous collection of data to understand the prevalence of antimicrobial-resistant bacteria, while monitoring is defined as the

routine measurement and analysis of antimicrobial susceptibility testing information to detect trends (60, 68, 69). Data generated from surveillance and monitoring studies can be used to detect the emergence of antimicrobial-resistant phenotypes, to understand any trends for reduced susceptibility to a certain antimicrobial agent, to determine mechanisms of resistance in bacteria, and to provide information for prudent use recommendations. Surveillance and monitoring programs are one component in providing an overall framework or plan of action for antimicrobial stewardship and the judicious use of antibacterial agents.

Numerous government and industry-sponsored veterinary surveillance programs already exist, and particular thought should given to designing and implementing a veterinary surveillance program. Components related to sampling strategies such as deciding on active or passive surveillance, which animal species to sample, which bacterial organisms from what body sites should be selected, and the species of bacteria to be collected (animal bacterial target pathogens, zoonotic, and/or commensal bacteria), as well as the maximum number of isolates per geographical location or disease outbreak, are just a few key considerations to help address the defined objective of any long-term surveillance study. Additionally, selecting the most appropriate antimicrobial agents for testing, the type of AST method used, the standardized testing method and appropriate QC, and reporting and analysis of data should be well thought out prior to program initiation (70).

The proper design and methodology of any surveillance program helps to address the biggest challenge for cross-comparison of surveillance studies, which is harmonization. Therefore, comparing surveillance data across programs may be difficult since each surveillance study may not be measuring the same parameter. This is especially true for surveillance programs that use epidemiological cutoff values (ECVs) rather than clinical breakpoints or breakpoints from different host species to determine bacterial resistance (65). CLSI document VET05 offers guidance on areas in which harmonization can be achieved in veterinary antimicrobial surveillance programs (65).

Judicious antimicrobial use is the attempt to both maximize therapeutic efficacy and minimize the selection of resistant microorganisms (71), and antimicrobial stewardship is defined as a coordinated program that promotes the appropriate use of antimicrobials, improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms (http://www.apic.org/Professional-Practice/Practice-Resources/Antimicrobial-Stewardship). As concern about antimicrobial resistance in human and veterinary pathogens has increased, so has the number of surveillance and monitoring activities in government, academic, and industry settings which have led to improved judicious use and stewardship of antimicrobial agents. The most recent example in the United States centers on The President's Council of Advisors on Science and Technology (PCAST), which released a report in 2014 recommending steps the federal government can take to combat antibiotic resistance. The PCAST report includes action steps for human health care, animal agriculture, drug development, and surveillance, with the goal of preventing infections, improving surveillance, encouraging appropriate use of antibiotics in people and animals, and developing new antimicrobial drugs (https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_carb_report_sept2014.pdf).

EPIDEMIOLOGIC CUT-OFF VALUES

Veterinary diagnosticians and practitioners will be most familiar with using standardized AST methods and clinical breakpoints to guide the treatment of individual veterinary patients. However, another important use of AST is in the area of antimicrobial resistance surveillance. While the testing methodology is exactly the same for both clinical diagnostics and antimicrobial resistance surveillance, the overall goal and intended use of test results are slightly different. Where the goal of clinical diagnostics is to predict therapeutic outcome in a patient (or group of patients in production settings) and the test result is an interpretive category, the goal of surveillance is to detect new and emerging antimicrobial resistance mechanisms within a population of bacteria as soon as possible, and the test result is an epidemiological cutoff value, also known as an ECV or ECOFF. As such, clinical breakpoints and ECVs should not be used interchangeably (72). ECVs are determined through evaluation of the distribution of MICs, very similar to the process of evaluating MIC distributions to establish a clinical interpretive criteria (73, 74). However, ECVs only separate the microbial populations into wild-type and non-wild-type categories based on the presence or absence of resistance mechanisms. Thus, ECVs do not consider PK/PD data or clinical outcome data and are often set lower than the "susceptible" clinical interpretive criteria, again, to facilitate detection of emerging antimicrobial resistance within a population of bacterial isolates as early as possible. A comparison of ECVs and clinical breakpoints is provided in Fig. 2.

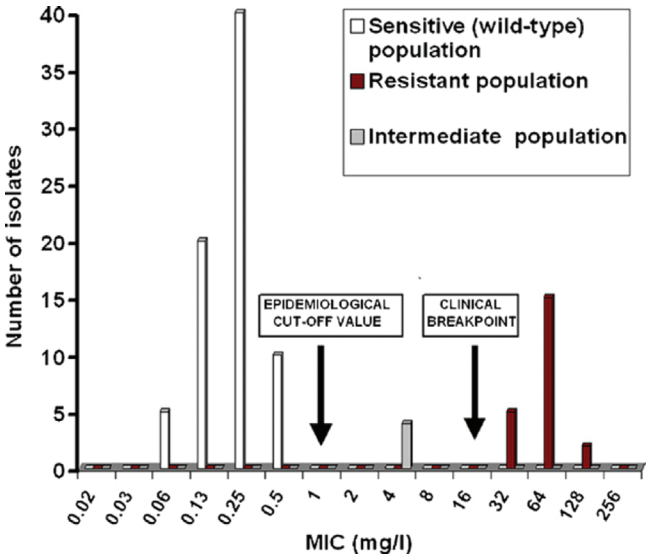


Figure 2 Illustrated differences between an epidemiological cutoff value and a clinical breakpoint (67).

FUTURE DIRECTIONS AND TRENDS

Much progress has been made in the past decade in the development of veterinary-specific testing methods and interpretive criteria. Test methods for additional veterinary pathogens including mycoplasma species commonly encountered in veterinary medicine, such as *Mycobacterium bovis*, need to be developed. A test method for susceptibility testing of *Haemophilus parasuis* has been published recently (73). The development of veterinary-specific interpretive criteria for older, generic agents has increased greatly over the past 10 years, but much work still remains in which additional clinical breakpoints for these older drugs are often used in veterinary surveillance and monitoring programs. At present, standardized methods for susceptibility testing of *Campylobacter* spp. and *Arcobacter butzleri* have been developed, but no interpretive criteria for these organisms or other enteric pathogens are available (75). The standardization of test methods for *Campylobacter* spp. has been particularly beneficial to monitoring programs such as the National Antimicrobial Resistance Monitoring System. Additionally, the relevance of AST results to antimicrobial agents used in control (prophylaxis) or growth promotion has not been established. The continued development of veterinary-specific interpretive criteria will play an increasingly important role in the prudent use of antimicrobial agents, and the pharmaceutical industry must be encouraged to participate in this process. While much of the interpretive criteria to date have been developed by CLSI for agents approved in the United States, this work needs to be expanded to include agents approved in Europe and elsewhere. As other organizations such as EUCAST and the World Organisation for Animal Health begin to address veterinary AST methods and interpretive criteria, the need for collaboration and harmonization among the various standards organizations will become critical.

Alternative test methodologies to traditional phenotypic AST have been evaluated with the primary goal of increasing the speed at which test results are delivered to the clinician, and these have been discussed in detail elsewhere (76). Those methodologies that are currently used in a limited capacity or hold the most potential for future adoption are PCR, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), and next-generation sequencing. The utility and limitations of these three methods are briefly discussed here. Currently, PCR testing is being used primarily as an adjunct method to phenotypic susceptibility testing. In this manner, PCR is used to confirm the presence of a specific resistance gene, such as the *mecA* gene that confers resistance to beta-lactamase stable penicillins in *S. aureus*. As a primary testing method, PCR can be performed more rapidly than MIC or disk diffusion testing because it does not require overnight incubation. Use of PCR can decrease the turnaround time for clinicians compared to traditional AST methods (77). However, the primary limitations of PCR are that the "target" gene must be known—a challenging proposition for new, emerging, or rare resistance mechanisms—and the presence of a gene may not accurately predict activity of the genetic element.

MALDI-TOF mass spectrometry has also been recently evaluated as a method to improve the turnaround time associated with bacterial identification. There are three primary methods for determining antimicrobial susceptibility using MALDI-TOF. When bacterial isolates are incubated in the presence of the antimicrobial, MALDI-TOF can be used to measure antimicrobial breakdown products from bacterial enzyme hydrolysis (78). MALDI-TOF has also been used in combination with a PCR amplification step to detect resistance elements and to measure the protein spectral changes that occur with antimicrobial exposure, although the latter use has primarily focused on fungi (79, 80). While the use of MALDI-TOF as an AST method requires further comparison to current reference methods, the technology holds promise since many laboratories currently have the instrumentation because they are using this equipment for bacterial identification.

Yet another technology that is being evaluated for antimicrobial susceptibility testing is whole-genome sequencing (WGS). Its role is more limited at this time,

but it has the potential to be transformative for the veterinary diagnostic laboratory, especially for susceptibility testing of bacterial species that are difficult to culture. One challenge for WGS will be its cost compared to phenotypic testing, which is relatively inexpensive. However, the cost of WGS seems to decrease yearly as the technology advances and becomes easier to perform. Additionally, speed, accuracy, and resolution are improving, thereby potentially allowing this technology to compete with conventional techniques. A specific undertaking for WGS will be its ability to equal the sensitivity and robustness of phenotypic susceptibility testing due to the currently incomplete database information that links genotype to phenotype (81). If WGS can provide accurate results that are faster than the 18 to 24 hours required for standard disk or broth susceptibility testing, it could supplement phenotypic testing, which would still be necessary to detect any novel mechanisms of resistance, but could be used to quickly identify resistance for certain antibiotics where known drug-resistance genes are present before results from the currently used phenotypic AST methods become available (82). Other possible applications for WGS may include detecting multidrug-resistant veterinary and zoonotic pathogens as well as serving as a useful tool in the monitoring and spread of bacterial pathogens in epidemiological studies (76, 77).

Though not related to technologies, but rather to the need for improved responsible use of anti-infectives, another component of antimicrobial stewardship that will receive more attention in the future is the monitoring of actual antimicrobial usage. Because the need for harmonization in surveillance and monitoring programs continues to be challenging, there will also be a need for harmonization when recording and reporting on antimicrobial usage to more accurately understand the overall sales and consumption of antimicrobial agents in veterinary medicine and the associations between antimicrobial consumption, use, and resistance at the individual patient/herd level.

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References

1. **Michael GB, Freitag C, Wendlandt S, Eidam C, Feßler AT, Lopes GV, Kadlec K, Schwarz S.** 2015. Emerging issues in antimicrobial resistance of bacteria from food-producing animals. *Future Microbiol* **10:**427–443.
2. **Guardabassi L, Prescott JF.** 2015. Antimicrobial stewardship in small animal veterinary practice: from theory to practice. *Vet Clin North Am Small Anim Pract* **45:**361–376, vii.
3. **DeDonder KD, Harhay DM, Apley MD, Lubbers BV, Clawson ML, Schuller G, Harhay GP, White BJ, Larson RL, Capik SF, Riviere JE, Kalbfleisch T, Tessman RK.** 2016. Observations on macrolide resistance and susceptibility testing performance in field isolates collected from clinical bovine respiratory disease cases. *Vet Microbiol* **192:**186–193.
4. **Levy SB.** 2001. Antibiotic resistance: consequences of inaction. *Clin Infect Dis* **33**(Suppl 3)**:**S124–S129.
5. **Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightingale C, Preston R, Waddell J.** 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother* **53:**28–52.
6. **Seyfarth AM, Wegener HC, Frimodt-Møller N.** 1997. Antimicrobial resistance in *Salmonella enterica* subsp. *enterica* serovar typhimurium from humans and production animals. *J Antimicrob Chemother* **40:**67–75.
7. **Watts JL, Yancey RJ Jr.** 1994. Identification of veterinary pathogens by use of commercial identification systems and new trends in antimicrobial susceptibility testing of veterinary pathogens. *Clin Microbiol Rev* **7:**346–356.
8. **Ferraro MJ.** 2001. Should we reevaluate antibiotic breakpoints? *Clin Infect Dis* **33**(Suppl 3)**:**S227–S229.
9. **Clinical and Laboratory Standards Institute (CLSI).** 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard—4th ed. CLSI document VET01-A4. CLSI, Wayne, PA.
10. **Clinical and Laboratory Standards Institute (CLSI).** 2013. Development of *in vitro* susceptibility testing criteria and QC parameters for veterinary antimicrobial agents; approved guideline—3rd ed. CLSI document VET02-A3. CLSI, Wayne, PA.
11. **Clinical and Laboratory Standards Institute (CLSI).** 2015. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals—3rd ed. CLSI supplement VET01S. CLSI, Wayne, PA.
12. **Turnidge JD, Ferraro MJ, Jorgensen JH.** 2003. Susceptibility test methods: general considerations, p 1102–1107. *In* Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (ed), *Manual of Clinical Microbiology*, 8th ed. ASM Press, Washington, DC.
13. **National Committee on Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard—2nd ed. NCCLS document M31-A2, NCCLS, Wayne, PA.
14. **Woolcock JB, Mutimer MD.** 1983. Antibiotic susceptibility testing: caeci caecos ducentes? *Vet Rec* **113:** 125–128.
15. **Clinical and Laboratory Standards Institute (CLSI).** 2013. Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals—2nd ed. CLSI document VET04-A2. CLSI, Wayne, PA.
16. **Clinical and Laboratory Standards Institute (CLSI).** 2017. Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated

from Animals - 1st ed. CLSI Supplement VET06. CLSI, Wayne PA.

17. **European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Dieases (ESCMID).** 2000. EUCAST definitive document E.DEF 3.1, June 2000: determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *Clin Microbiol Infect* **6:**509–515.
18. **European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).** 2000. EUCAST definitive document E.DEF 2.1, August 2000: determination of antimicrobial susceptibility test breakpoints. *Clin Microbiol Infect* **6:**570–572.
19. **Kahlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, Rodloff A, Steinbakk M, Urbaskova P, Vatopoulos A.** 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. *J Antimicrob Chemother* **52:**145–148.
20. **Kahlmeter G, Brown D.** 2004. Harmonisation of European breakpoints: can it be achieved. *Clin Microbiol Newsl* **26:**187–192.
21. **Bell SM, Gatus BJ, Pham JN, Rafferty DL.** 2002. Antibiotic susceptibility testing by the CDS method. The Antibiotic Reference Laboratory, Department of Microbiology, The Prince of Wales Hospital, New South Wales, Australia.
22. **Clinical and Laboratory Standards Institute (CLSI).** 2014. Analysis and presentation of cumulative antimicrobial susceptibility test data; approved guideline—4th ed. CLSI document M39-A4. CLSI, Wayne, PA.
23. **Sweeney MT, Zurenko GE.** 2003. *In vitro* activities of linezolid combined with other antimicrobial agents against staphylococci, enterococci, pneumococci, and selected Gram-negative organisms. *Antimicrob Agents Chemother* **47:**1902–1906.
24. **Sweeney MT, Brumbaugh GW, Watts JL.** 2008. *In vitro* activities of tulathromycin and ceftiofur combined with other antimicrobial agents using bovine *Pasteurella multocida* and *Mannheimia haemolytica* isolates. *Vet Therap* **9:**212–222.
25. **Aarestrup F.** 2004. *Antibiotic Resistance in Bacteria of Animal Origin II: External Quality Assurance Systems*. Danish Inst. Food Vet. Res., Copenhagen, Denmark.
26. **Andrews JM, BSAC Working Party on Susceptibility Testing.** 2001. BSAC standardized disk susceptibility test method. *J Antimicrob Chemother* **48**(suppl 1):S43–S57.
27. **AVMF.** Animal Medicinal Drug Use Clarification Act (AMDUCA). https://www.avma.org/KB/Resources/Reference/Pages/AMDUCA.aspx.
28. **U.S. GPO.** 2018. Electronic Code of Federal Regulations. http://www.ecfr.gov/cgi-bin/text-idx?SID=054808d261de27898e02fb175b7c9ff9&node=21:6.0.1.1.16&rgn=div5#21:6.0.1.1.16.2.1.2.
29. **AVMA.** AVMA policies. https://www.avma.org/KB/Policies/Pages/default.aspx.
30. **Michael GB, Eidam C, Kadlec K, Meyer K, Sweeney MT, Murray RW, Watts JL, Schwarz S.** 2012. Increased MICs of gamithromycin and tildipirosin in the presence of the genes erm(42) and msr(E)-mph(E) for bovine *Pasteurella multocida* and *Mannheimia haemolytica*. *J Antimicrob Chemother* **67:**1555–1557.
31. **Sweeney MT, Papich MG, Watts JL.** 2017. New interpretive criteria for danofloxacin antibacterial susceptibility testing against Mannheimia haemolytica and Pasteurella multocida associated with bovine respiratory disease. *J. Vet. Diagn. Invest.* **29:**224–227.
32. **Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ.** 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* **37:**1073–1081.
33. **Drusano GL, Johnson DE, Rosen M, Standiford HC.** 1993. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas* sepsis. *Antimicrob Agents Chemother* **37:**483–490.
34. **Ambrose PG, Bhavnani SM, Rubino CM, Louie A, Gumbo T, Forrest A, Drusano GL.** 2007. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clin Infect Dis* **44:**79–86.
35. **Turnidge JD.** 1998. The pharmacodynamics of beta-lactams. *Clin Infect Dis* **27:**10–22.
36. **Knudsen JD, Fuursted K, Espersen F, Frimodt-Møller N.** 1997. Activities of vancomycin and teicoplanin against penicillin-resistant pneumococci *in vitro* and *in vivo* and correlation to pharmacokinetic parameters in the mouse peritonitis model. *Antimicrob Agents Chemother* **41:**1910–1915.
37. **Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ.** 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* **37:**1073–1081.
38. **Blaser J, Stone BB, Groner MC, Zinner SH.** 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrob Agents Chemother* **31:**1054–1060.
39. **van Ogtrop ML, Andes D, Stamstad TJ, Conklin B, Weiss WJ, Craig WA, Vesga O.** 2000. *In vivo* pharmacodynamic activities of two glycylcyclines (GAR-936 and WAY 152,288) against various Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother* **44:**943–949.
40. **Andes D, van Ogtrop ML, Peng J, Craig WA.** 2002. *In vivo* pharmacodynamics of a new oxazolidinone (linezolid). *Antimicrob Agents Chemother* **46:**3484–3489.
41. **Rey JF, Laffont CM, Croubels S, De Backer P, Zemirline C, Bousquet E, Guyonnet J, Ferran AA, Bousquet-Melou A, Toutain PL.** 2014. Use of Monte Carlo simulation to determine pharmacodynamic cutoffs of amoxicillin to establish a breakpoint for antimicrobial susceptibility testing in pigs. *Am J Vet Res* **75:**124–131.
42. **Shukla BS, Shelburne S, Reyes K, Kamboj M, Lewis JD, Rincon SL, Reyes J, Carvajal LP, Panesso D, Sifri CD, Zervos MJ, Pamer EG, Tran TT, Adachi J, Munita JM, Hasbun R, Arias CA.** 2015. Influence of minimum inhib-

itory concentration in clinical outcomes of *Enterococcus faecium* bacteremia treated with daptomycin: is it time to change the breakpoint? *Clin Infect Dis*. **62:**1514–1520.

43. **Turnidge J, Paterson DL.** 2007. Setting and revising antibacterial susceptibility breakpoints. *Clin Microbiol Rev* **20:**391–408.
44. **Turnidge JD, Subcommittee on Antimicrobial Susceptibility Testing of the Clinical and Laboratory Standards Institute.** 2011. Cefazolin and *Enterobacteriaceae*: rationale for revised susceptibility testing breakpoints. *Clin Infect Dis* **52:**917–924.
45. **Doern GV, Brecher SM.** 2011. The clinical predictive value (or lack thereof) of the results of *in vitro* antimicrobial susceptibility tests. *J Clin Microbiol* **49**(Supplement)**:** S11–S14.
46. **Breteler KBK, Rentenaar RJ, Verkaart G, Sturm PDJ.** 2011. Performance and clinical significance of direct antimicrobial susceptibility testing on urine from hospitalized patients. *Scand J Infect Dis* **43:**771–776.
47. **Egervärn M, Lindmark H, Roos S, Huys G, Lindgren S.** 2007. Effects of inoculum size and incubation time on broth microdilution susceptibility testing of lactic acid bacteria. *Antimicrob Agents Chemother* **51:**394–396.
48. **Udekwu KI, Parrish N, Ankomah P, Baquero F, Levin BR.** 2009. Functional relationship between bacterial cell density and the efficacy of antibiotics. *J Antimicrob Chemother* **63:**745–757.
49. **Rex JH, Pfaller MA.** 2002. Has antifungal susceptibility testing come of age? *Clin Infect Dis* **35:**982–989.
50. **Constable PD, Morin DE.** 2003. Treatment of clinical mastitis. Using antimicrobial susceptibility profiles for treatment decisions. *Vet Clin North Am Food Anim Pract* **19:**139–155.
51. **Barlow J.** 2011. Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. *J Mammary Gland Biol Neoplasia* **16:**383–407.
52. **McClary DG, Loneragan GH, Shryock TR, Carter BL, Guthrie CA, Corbin MJ, Mechor GD.** 2011. Relationship of *in vitro* minimum inhibitory concentrations of tilmicosin against *Mannheimia haemolytica* and *Pasteurella multocida* and *in vivo* tilmicosin treatment outcome among calves with signs of bovine respiratory disease. *J Am Vet Med Assoc* **239:**129–135.
53. **Allen JW, Viel L, Bateman KG, Rosendal S, Shewen PE, Physick-Sheard P.** 1991. The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. *Can J Vet Res* **55:**341–346.
54. **DeRosa DC, Mechor GD, Staats JJ, Chengappa MM, Shryock TR.** 2000. Comparison of *Pasteurella* spp. simultaneously isolated from nasal and transtracheal swabs from cattle with clinical signs of bovine respiratory disease. *J Clin Microbiol* **38:**327–332.
55. **Godinho KS, Sarasola P, Renoult E, Tilt N, Keane S, Windsor GD, Rowan TG, Sunderland SJ.** 2007. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. *Vet Rec* **160:**22–25.
56. **Nguyen MH, Yu VL, Morris AJ, McDermott L, Wagener MW, Harrell L, Snydman DR.** 2000. Antimicrobial resistance and clinical outcome of *Bacteroides* bacteremia: findings of a multicenter prospective observational trial. *Clin Infect Dis* **30:**870–876.
57. **Bastos ML, Hussain H, Weyer K, Garcia-Garcia L, Leimane V, Leung CC, Narita M, Penã JM, Ponce-de-Leon A, Seung KJ, Shean K, Sifuentes-Osornio J, Van der Walt M, Van der Werf TS, Yew WW, Menzies D, Collaborative Group for Meta-analysis of Individual Patient Data in MDR-TB.** 2014. Treatment outcomes of patients with multidrug-resistant and extensively drug-resistant tuberculosis according to drug susceptibility testing to first- and second-line drugs: an individual patient data meta-analysis. *Clin Infect Dis* **59:**1364–1374.
58. **López-Góngora S, Puig I, Calvet X, Villoria A, Baylina M, Muñoz N, Sanchez-Delgado J, Suarez D, García-Hernando V, Gisbert JP.** 2015. Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for *Helicobacter pylori* infection. *J Antimicrob Chemother* **70:**2447–2455.
59. **Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM.** 2004. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* **42:**2398–2402.
60. **Mavros MN, Tansarli GS, Vardakas KZ, Rafailidis PI, Karageorgopoulos DE, Falagas ME.** 2012. Impact of vancomycin minimum inhibitory concentration on clinical outcomes of patients with vancomycin-susceptible *Staphylococcus aureus* infections: a meta-analysis and meta-regression. *Int J Antimicrob Agents* **40:**496–509.
61. **Silley P, Simjee S, Schwarz S.** 2012. Surveillance and monitoring of antimicrobial resistance and antibiotic consumption in humans and animals. *Rev Sci Tech* **31:** 105–120.
62. **Venugopal AA, Riederer K, Patel SM, Szpunar S, Jahamy H, Valenti S, Shemes SP, Khatib R, Johnson LB.** 2012. Lack of association of outcomes with treatment duration and microbiologic susceptibility data in *Clostridium difficile* infections in a non-NAP1/BI/027 setting. *Scand J Infect Dis* **44:**243–249.
63. **Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL.** 2003. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* **123:**1495–1502.
64. **Zetola NM, Modongo C, Moonan PK, Ncube R, Matlhagela K, Sepako E, Collman RG, Bisson GP.** 2014. Clinical outcomes among persons with pulmonary tuberculosis caused by *Mycobacterium tuberculosis* isolates with phenotypic heterogeneity in results of drug-susceptibility tests. *J Infect Dis* **209:**1754–1763.
65. **Silley P.** 2012. Susceptibility testing methods, resistance and breakpoints: what do these terms really mean? *Rev Sci Tech* **31:**33–41.
66. **Giguère S, Lee E, Williams E, Cohen ND, Chaffin MK, Halbert N, Martens RJ, Franklin RP, Clark CC, Slovis NM.** 2010. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin

in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R equi*. *J Am Vet Med Assoc* **237:**74–81.

67. **Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y.** 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis* **36:**53–59.
68. **Hunter PA, Reeves DS.** 2002. The current status of surveillance of resistance to antimicrobial agents: report on a meeting. *J Antimicrob Chemother* **49:**17–23.
69. **Franklin A, Acar J, Anthony F, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, Vose D, van Vuuren M, White DG, Wegener HC, Costarrica ML, Office International des Epizooties Ad hoc Group.** 2001. Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. *Rev Sci Tech* **20:**859–870.
70. **Clinical and Laboratory Standards Institute (CLSI).** 2011. Generation, presentation, and application of antimicrobial susceptibility test data for bacteria of animal origin; a report. CLSI document VET05-R. CLSI, Wayne, PA.
71. **U.S. Food and Drug Administration.** Judicious use of antimicrobials. http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/.
72. **Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, Gaastra W.** 2010. Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother* **65:**601–604.
73. **Prüller S, Turni C, Blackall PJ, Beyerbach M, Klein G, Kreienbrock L, Strutzberg-Minder K, Kaspar H, Meemken D, Kehrenberg C.** 2016. Towards a standardized method for broth microdilution susceptibility testing of *Haemophilus parasuis*. *J Clin Microbiol* **55:**264–273.
74. **Miller RA, Reimschuessel R.** 2006. Epidemiologic cutoff values for antimicrobial agents against *Aeromonas salmonicida* isolates determined by frequency distributions of minimal inhibitory concentration and diameter of zone of inhibition data. *Am J Vet Res* **67:**1837–1843.
75. **Riesenberg A, Frömke C, Stingl K, Feßler AT, Gölz G, Glocker E-O, Kreienbrock L, Klarmann D, Werckenthin C, Schwarz S.** 2017. Antimicrobial susceptibility testing of *Arcobacter butzleri*: development and application of a new protocol for broth microdilution. *J Antimicrob Chemother* **72:**2769–2774.
76. **van Belkum A, Dunne WM Jr.** 2013. Next-generation antimicrobial susceptibility testing. *J Clin Microbiol* **51:**2018–2024.
77. **Waldeisen JR, Wang T, Debkishore M, Lee LP.** 2011. A real-time PCR antibiogram for drug-resistant sepsis. *PLoS One* **6:**e28528. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0028528
78. **Hooff GP, van Kampen JJA, Meesters RJW, van Belkum A, Goessens WHF, Luider TM.** 2012. Characterization of β-lactamase enzyme activity in bacterial lysates using MALDI-mass spectrometry. *J Proteome Res* **11:**79–84.
79. **Marinach C, Alanio A, Palous M, Kwasek S, Fekkar A, Brossas J-Y, Brun S, Snounou G, Hennequin C, Sanglard D, Datry A, Golmard J-L, Mazier D.** 2009. MALDI-TOF MS-based drug susceptibility testing of pathogens: the example of *Candida albicans* and fluconazole. *Proteomics* **9:**4627–4631.
80. **De Carolis E, Vella A, Florio AR, Posteraro P, Perlin DS, Sanguinetti M, Posteraro B.** 2012. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for caspofungin susceptibility testing of *Candida* and *Aspergillus* species. *J Clin Microbiol* **50:**2479–2483.
81. **Köser CU, Ellington MJ, Cartwright EJP, Gillespie SH, Brown NM, Farrington M, Holden MTG, Dougan G, Bentley SD, Parkhill J, Peacock SJ.** 2012. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. *PLoS Pathog* **8:**e1002824.
82. **Punina NV, Makridakis NM, Remnev MA, Topunov AF.** 2015. Whole-genome sequencing targets drug-resistant bacterial infections. *Hum Genomics* **9:**19.

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Molecular Methods for Detection of Antimicrobial Resistance

3

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INTRODUCTION

Molecular characterization of the genetic mechanism(s) underlying a given phenotypic result, obtained by traditional antimicrobial sensitivity testing, is now an integral part of many clinical investigations in relation to bacterial infections, whether in humans or animals. In some cases, when phenotypic results are too time-consuming, nonconclusive, or unavailable, molecular analysis can be used to investigate the presence of a given gene or point mutation and thereby give direct support to ensure that an optimal treatment or control strategy is undertaken in a timely manner. In addition, molecular characterization is frequently used as an indirect method to aid in epidemiological investigations following an outbreak, when phenotypic data is not sufficiently detailed to control possible outbreaks involving resistant bacteria. Finally, molecular characterization of antimicrobial resistance (AMR) determinants is also used for local, national, or even global surveillance of AMR. Currently, the European Food Safety Authority (EFSA) and the European Center for Disease Control (ECDC) are involved in monitoring and coordinating surveillance of AMR in important zoonotic bacteria from food animals and humans, and systems such as the European Antimicrobial Resistance Surveillance System (EARSS) have helped generate data regarding the prevalence of AMR in many European countries. However, most of the data are based on phenotypic characterization of isolates, although genotypic detection of AMR genes is also being increasingly performed by member states.

Discussing phenotypic detection of AMR in bacteria is not meaningful unless the purpose of the analysis is defined. In relation to treatment of clinical infections, parameters such as sites of infection, clinical manifestation, and toxic concentration of the antimicrobial agent used need to be considered to define a relevant clinical breakpoint, which distinguishes between treatable (sensitive), potentially treatable (intermediate resistant), and nontreatable (resistant) infections. Alternatively, the epidemiological cutoff, or ECOFF, can be used to differentiate between the wild type, fully susceptible populations of bacteria, and bacteria with reduced susceptibility due to an acquired resistance mechanism.

Acquired resistance to antimicrobial agents is correlated with a multitude of molecular mechanisms depending on the organism and the antimicrobial agent involved. These mechanisms include very different genetic events such as constitutive or inducible expression of (acquired) resistance genes, upregulated expression of resistance genes because of mutations in the promoter/regulator region, and insertion of strong active promoters as part of, e.g., insertion elements upstream of the resistance gene, mutations in housekeeping genes acting as targets for antimicrobial agents, and loss-of-function mutations in regulatory elements or specific porins (1). In addition, bacteria can be intrinsically resistant to certain types or even whole classes of antimicrobial agents when these are given in therapeutic concentrations. For example, enterococci are inherently resistant to cephalosporins, partly due to low binding affinity of the penicillin binding protein 5, which is involved in cell wall synthesis in this organism (2). Common causes of intrinsic resistance are lack of (or low affinity to) the target for the antimicrobial agent, an inability of the drug to access the target, expression of chromosomally located resistance genes encoding enzymes, and the presence of multidrug efflux pumps (3).

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Because of the large diversity of possible mechanisms involved in reduced antimicrobial susceptibility, it is not a trivial task to transform all of these mechanisms or genes into sequence-based detection algorithms if genotypic methods are eventually to substitute for phenotypic methods, especially because new genes or allelic variants of current resistance mechanisms are continuously being discovered. In the case of intrinsic resistance, *a priori* knowledge of a given organism is required to predict if it is intrinsically resistant to certain antimicrobials, which makes translation of sequence-based data into predicted susceptibility even more complicated. For example, anaerobic bacteria are intrinsically resistant to aminoglycosides, while *Helicobacter pylori* is intrinsically resistant to metronidazole and *Pseudomonas aeruginosa* shows natural resistance to sulfonamides and trimethoprims (4–6). Conversely, some genetic resistance mechanisms are inherently difficult to detect by classical *in vitro* phenotypic methods and can therefore remain undetected, even though they can lead to treatment failure *in vivo*, thereby increasing the importance of detecting these mechanisms by molecular methods. This is evident for Gram-negative pathogens carrying the $bla_{\text{OXA-48}}$ gene, often conferring only a minor reduction in their susceptibility to carbapenems (7), and some clonal lineages of methicillin-resistant *Staphylococcus aureus* (MRSA), where heterologous expression of the *mecA* gene can lead to inconclusive interpretation of the organism's susceptibility to beta-lactams (8).

Despite the varied challenges posed by genotypic detection of mechanisms leading to reduced susceptibility to different antimicrobial agents, molecular methods are being used extensively by both research and reference laboratories. Some of the methods employed, such as PCR and hybridization techniques, have been used for decades, while new methods such as whole-genome sequencing (WGS) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) are just emerging. The most commonly used modern molecular methods in relation to detection of determinants involved in conferring reduced susceptibility to antimicrobial agents are discussed in this article. The main advantages and disadvantages of using the different genotypic methods for detecting AMR will be described individually below; a more elaborate discussion of these methods has been published recently (9).

PCR

PCR is a technique that was developed in the 1980s by Kary Mullis (10) and has revolutionized molecular biology, enabling rapid and exponential amplification of target DNA sequences using a forward and reverse PCR primer and an enzyme known as DNA polymerase in the presence of deoxyribonucleotides. Conventional PCR comprises three steps: (i) denaturing of the double-stranded DNA at 95°C, (ii) annealing of the PCR primers at 50 to 60°C, and (iii) extension of the DNA at 72°C. PCR is used routinely in microbiology laboratories for detecting any genes that may be present within bacteria, as long as a DNA sequence is available for the whole or partial gene which can be used to design the PCR primers. The PCR-amplified gene product can be visualized by running agarose gels and staining DNA with ethidium bromide or other fluorescent DNA-chelating dyes. The whole process, including amplification and visualization, can take between 4 and 5 h.

Since the development of PCR, there have been several advances, which include real-time PCR (RT-PCR) and isothermal amplifications, e.g., loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA). The main difference between conventional PCR and RT-PCR is that in the latter, amplification of the target DNA sequence is monitored in real time as it occurs, rather than at the end, due to the presence of fluorescent dyes in the reaction, and thus is also known as quantitative PCR (qPCR). Therefore, in RT-PCR, agarose gel electrophoresis is not required; this can save considerable time and is safer, because the use of ethidium bromide, which is a carcinogen, is not required. RT-PCR can use either (i) nonspecific dyes that intercalate with any double-stranded DNA or (ii) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence (11). Isothermal PCR techniques such as LAMP and RPA, in contrast, differ from conventional or RT-PCR in that the whole process is performed at a constant temperature and does not require ramping up and down of temperatures. For LAMP, the temperature is around 65°C, while it is around 40°C for RPA; up to six different primers are used for LAMP, whereas RPA uses two primers (12, 13). The presence of intercalating dyes in the reaction allows fluorescent detection of target DNA amplification in real time using a real time PCR machine, which can be considerably faster than PCR or RT-PCR. However, LAMP and RPA PCR amplification can also occur using a simple water bath or heating element and be measured by photometry for turbidity, which makes both methods amenable for point-of-care applications and use in low-resource settings (14).

Multiplex PCR, in which several target DNA fragments are amplified simultaneously, can be performed using either conventional or RT-PCR. The application of PCR for monitoring multiple AMR genes in bacteria has become easier with the use of multiplexing, and this technique is widely used today, where appropriate, to replace PCR and RT-PCR applications to amplify single genes. In a multiplex PCR assay, several resistance genes can be detected simultaneously with different primers included in the assay mix. The products must be of different sizes and can be visualized either by gel electrophoresis, if from conventional PCR, or by addition of different dyes for RT-PCR. Multiplex PCRs are often designed to detect different genes, all relating to the same resistance phenotype such as detection of the most prevalent beta-lactamases present in Gram-negative bacteria which are involved in resistance to cephalosporins (15) or carbapenems (16, 17). Therefore, by screening for these genes simultaneously, considerable time and effort can be saved in detecting the possible mechanism(s) responsible for the resistance phenotype.

The robustness of any PCR strategy relies on multiple factors such as optimal primer design, GC content of the target template, and presence of inhibitory molecules in the sample. The primers require close to 100% identity to the target DNA and are thus sensitive to the presence of single nucleotide polymorphisms within the binding sequence, especially at the 3′ end of the primer, where elongation initiates. It is therefore important to select primers in binding areas with low or no nucleotide variation. To ensure the robustness of new PCR assays, a thorough validation process should always be performed to evaluate the specificity (number of false-positive results) and sensitivity (number of false-negative results) of the assay. Designing primers is relatively easy, but to ensure that these primers have both a high specificity and high sensitivity is not a trivial task, because this requires access to a diverse sequencing dataset representing the variation expected to be present in the test material. If multiplexing is also required, software programs can be employed to help in defining relevant sets of primer pairs (18). Finally, both a positive and a negative control are required every time a PCR assay is performed, to ensure that the sensitivity and specificity are maintained each time the assay is performed.

Conventional PCR assays are well suited to detect the presence or absence of (resistance) genes but are less suited for detection of point mutations within target genes, unless subsequent Sanger sequencing is performed to detect these mutations. RT-PCR can detect single point mutations in a given gene if sequence-specific DNA probes targeting the mutation area are used. However, an advantage that conventional PCR has over RT-PCR is that the latter can only be used to detect the presence of short fragments of DNA, optimally up to 150 bp, whereas conventional PCR can easily detect much larger fragments.

AMR Gene Detection

The use of PCR to detect the presence of AMR genes in a bacterial isolate or even in samples from different environments is commonplace. Due to the ease of designing conventional PCR primers, there are a plethora of PCRs that have been used to detect the presence of different AMR genes from bacteria, in both aerobes and anaerobes. A highly cited paper in this area is by Schwartz et al. (19), who discuss PCR used to monitor for the presence of *vanA* (encoding vancomycin resistance), *mecA* (encoding methicillin resistance), and *ampC* (encoding ampicillin resistance) in wastewater systems and water biofilms. The authors found the *vanA* gene in both wastewater and drinking water biofilms, with the *vanA* gene being found in the latter in the absence of enterococci. While *mecA* was only detected in hospital wastewater, *ampC* was the most widespread and was present in wastewater, surface water, and drinking water biofilms. Another paper is one by Lévesque et al. (20), which describes PCR mapping used to determine AMR genes and the order in which they were present in integron cassettes. In 1995, when this paper was published, integrons were a new type of mobile element, and this work was quite novel. A comprehensive, although not exhaustive, list of primers to detect AMR genes is available at the website of the European Reference Laboratory for Antimicrobial Resistance (EURL AMR) in bacteria from animals and food (http://www.eurl-ar.eu/data/images/faq/primerliste%20til%20web_07.11.2013.pdf).

Conventional PCR has also been used to detect resistance genes in bacteria during infection to antibiotics used in first-line therapy such as those associated with extended-spectrum beta-lactamase (ESBL) resistance (21–23). These PCR assays are often performed in addition to antimicrobial susceptibility testing of the isolates because PCR results can be obtained more quickly, enabling control measures or accurate treatment. More recently, both PCR and RT-PCR have been used widely across Europe and for rapid large-scale epidemiological surveillance of archived bacterial isolates to look for the presence of the plasmid-mediated *mcr-1* and *mcr-2* genes harboring plasmid-mediated resistance to antibiotics of last resort such as colistin, which has attracted

much interest since its detection and reporting in 2015 (24–31). As demonstrated by the colistin outbreak, the use of RT-PCRs as a rapid, easy, and cheap method for targeting AMR gene detection during outbreak response remains unrivalled by any other technology (28).

Examples of the application of multiplex PCR for detecting AMR genes in clinical samples include studies by Strommenger et al. (32) and Chung et al. (33), in which the resistance present in multidrug-resistant *S. aureus* was determined by conventional PCR or in which a multiplex RT-PCR was employed to detect the presence of *mecA* and species-specific genes. Another example is a multiplex PCR which is used routinely for surveillance in reference laboratories to detect some of the most prevalent ESBL genes: *bla*TEM, *bla*SHV, *bla*CTX-M, and *bla*OXA (34). Using this multiplex PCR, Randall et al. (35) determined the prevalence of ESBL-producing *Escherichia coli* strains present in pigs at slaughter in the United Kingdom in 2013 to be 23.4%. They also showed that *bla*CTX-M was the most common ESBL type present in these isolates; Sanger sequencing of the PCR product showed *CTX-M-1* to be the most prevalent variant to be carried by these isolates, although some *CTX-M-15* were also detected.

LAMP assays for the detection of resistance include several assays which have been developed for detecting genes encoding resistance to antibiotics for first-line therapeutics or antibiotics of last resort. Examples include LAMP assays developed for the detection of ESBLs, AmpC genes, and carbapenemases in bacteria purified from both humans and animals (12, 36). Due to the rapidity of LAMP assays and the ease of conducting them at a constant temperature, several LAMP assays have been developed (*bla*VIM, *bla*NDM, *bla*KPC, *OXA-48* family, *CTX-M-1* family, and *CTX-M-9* family) to detect carbapenemase and ESBL-producing *Enterobacteriaceae* using the eazyplex SuperBug CRE system (Amplex Biosystems GmbH, Giessen, Germany) (36). As with PCR, LAMP assays can be used to look for the presence of ESBL genes in a bacterial community rather than in single isolates. For example, Kirchner et al. (37) have used LAMP assays to determine the presence of *bla*CTX-M genes in lysates made from the mix of bacteria present in overnight enrichment broths prepared from neck flap and cecal samples from poultry carcasses collected at an abattoir. The results showed that just less than a third of the ceca from poultry carcasses were contaminated with bacteria harboring the *bla*CTX-M gene, but this figure was much higher in skin flaps (>60%), probably due to cross-contamination.

DNA MICROARRAY TECHNOLOGY

DNA microarrays are genomic tools which have been used successfully in the past decade to assess bacterial genomic diversity by detecting the presence or absence of genes in a test organism in comparison to a reference strain or genes. The DNA microarray technology was initially based on glass slides that were spotted with thousands of specific DNA probes based on genes present in one reference strain for which a whole-genome sequence was available. As more isolates of a particular species or genus were whole-genome sequenced, the numbers of probes present on the microarray slide increased substantially to represent accessory genes not present in the reference strain that were part of the "pan-genome." Comparative genomic hybridizations were performed whereby test and reference isolate DNA were fluorescently labeled and hybridized to a microarray slide (38). The presence or absence of genes in the test isolate, in comparison to a reference, was determined by analyzing the hybridization results. This method enabled comparison of the genomic diversity in a relatively large number of test isolates (tens to hundreds) for which WGS was not available. Examples include *E. coli* and *Salmonella*, for which a large number of studies were performed looking at AMRs (39–41).

However, the use of glass slides and fluorescent dyes made the process expensive and time-consuming. Furthermore, there were several advancements in the technology. Although a number of different technologies were available and are reviewed in more detail elsewhere, especially in the context of AMR (42), in this section we will concentrate on that available from Alere Technologies. The Alere microarrays had several advantages that made them suitable for use in routine diagnostic laboratories, which may receive hundreds of samples. Advantages included adaptation of the microarray slide containing DNA probes to a simpler platform such as the bottom of an Eppendorf test tube or a 96-well plate, the use of horseradish peroxidase instead of expensive fluorescent dyes, simple protocols which enable large numbers of test sample DNA to be processed more rapidly and economically, and no requirement for dual hybridization including test and reference. However, disadvantages included the numbers of DNA probes that could be printed in comparison to the full microarray glass slide, which were a few hundred instead of several thousand. In addition, this platform is not suitable for detecting gene expression, but only gene presence and absence, due to inclusion of a preamplification step during labeling of the test DNA prior to hybridization.

AMR Detection Using Microarrays

Several groups have used the Alere microarrays to investigate AMR and virulence genes that may be present on mobile genetic elements such as plasmids and transposons and can be coselected. Characterization of enteric pathogens and commensals such as field and clinical isolates of both human and animal origin is important in understanding the dissemination these genes by zoonotic bacteria that may ultimately affect human health and therapeutics. This application was first developed by the Animal and Plant Health Agency (formerly the Veterinary Laboratories Agency) in collaboration with Alere through Identibac for determining virulence genes present in *E. coli* to distinguish between pathogenic and commensal *E. coli* (43), but later it was applied for detecting AMR genes in *E. coli* and *Salmonella* from food animals, as well as from human clinical isolates in several studies (44–50). Examples of the use of these microarrays include the characterization of animal isolates collected through surveillance studies, which revealed that AMR genes in *Salmonella* and *E. coli* may be present on different mobile genetic elements such as plasmids and *Salmonella* genomic island 1 (48, 51).

The Identibac microarrays have been used to determine the presence of AMR genes in both aerobic and anaerobic Gram-negative bacteria isolated from healthy human feces in two longitudinal studies performed with a cohort of healthy human subjects in the United Kingdom and Sweden over 1 year following a course of antibiotic treatment, as part of a European Commission 7th Framework Program project called Antiresdev (45, 52–54). Analysis of isolates from feces purified pre-antibiotic treatment indicated widespread presence of AMR genes in healthy individuals aged between 20 and 60, from both the Swedish and United Kingdom groups, with certain AMR isolates persisting over time. Following treatment, an effect was seen in individuals treated only with amoxicillin, in which a temporary increase in the number of isolates harboring the beta-lactamase gene *bla*TEM was seen in the treated group but not in the placebo group. No other antibiotic had such a profound effect (45, 53). Examination of anaerobic gut microbiota for mobile genetic elements associated with aerobes, to determine if the former acts as an AMR "sink," indicated that these genes were uncommon in anaerobes and that only the *sul* gene, encoding resistance to sulfonamide, was present in *Bacteroides*; however, the genetic element which may be associated with the *sul* gene could not be determined (54). It would be of interest to perform a similar type of study on farms, to determine if certain AMR bacteria and genes persist on farms over time and the possible risk to food safety associated with such persistence.

Another application of the Identibac AMR microarrays has been to look at the resistome of the microbiota, i.e., the AMR gene content present in the whole microbial population rather than in individual isolates, using human DNA. In a study by Card et al. (55), the resistomes present in the oral and fecal microbiota of humans from five countries were compared using the microarray. The results showed the prevalence of AMR genes associated with anaerobic bacteria such as *tetX* and *ermB*, as well as *bla*TEM and *sul2*, which are common in aerobic bacteria, present in the majority of samples. Interestingly, a functionally based screening approach which was also undertaken by the authors to determine the genetic context of the AMR genes such as *bla*TEM and *sul2* that were detected in the microbiota only recovered chromosomal genes. This is probably because AMR genes such as *bla*TEM and *sul2* are present in sufficient abundance to be detected by microarray, which employs a linear multiplex amplification process, but are nevertheless not present in high enough abundance to be present in the bacterial artificial chromosome functional libraries that were prepared. It was therefore indicated that microarrays may be more suited to detect AMR genes present on mobile genetic elements which may be present in low copy numbers than a functional genomic approach, although microarrays may be less sensitive than next-generation sequencing methods, in which the depth of sequencing can be very high (see "Whole Genome Sequence Analysis to Detect AMR"). Although in the above study the human oral and fecal microbiota was used, this technique can be applied to any sample, including from farm animals and the environment.

Other microarrays that have been described for determining AMR include those based on glass slides, which for example, have been applied for the characterization of virulence and AMR genes present in *Salmonella* strains of human clinical relevance (56). There are also arrays in which AMR genes associated with both Gram-positive and Gram-negative bacteria are present. One such array was developed for diagnostic and surveillance activities and was used to test *S. aureus* isolates recovered from milk samples from dairy farms in Quebec, Canada (57). Other organizations, including Alere, have also developed microarrays for detection of virulence and AMR in *S. aureus* isolates recovered from hospital, community, and farm settings (58–64). These arrays are still widely used in both human and veterinary settings.

WGS ANALYSIS TO DETECT AMR

Like PCR and microarrays, WGS has the potential to detect genetic determinants (genes and mutations) conferring AMR (65, 66). The main advantage of using WGS for this purpose is the ability to cover many different targets at the same time and to subtype specific gene variants. The current WGS technology and methodology for analysis offers similar results as well as some of the same shortcomings as PCR (followed by Sanger sequencing of amplicons) and microarrays, which will be described and discussed in detail below. However, as opposed to microarrays, WGS also offers the possibility to rapidly add new target sequences to the analysis database as well as the ability to perform fast *in silico* reanalysis on already sequenced isolates (26).

Current Technologies and Bioinformatic Tools

Sequencing platforms

WGS data are generated on highly sophisticated sequencing platforms, which produce large amounts of sequence data compared to the traditional Sanger sequencing technology. Today, the most common platforms for high-throughput sequencing of bacterial genomes are Illumina and Ion Torrent machines, which perform what has been called second-generation or next-generation sequencing (as opposed to traditional Sanger sequencing). Common to these machines is that the output consists of relatively short reads (100 to 400 bp, depending on the technology), which in most cases are shorter than the genes conferring resistance to antimicrobial agents. Also, the rate of randomly occurring, as well as methodology-based, sequencing errors on single reads originating from the next-generation sequencing technology is relatively high compared to errors encountered with traditional Sanger sequencing. To overcome this problem, a huge surplus (termed *x-fold coverage*) of short-read data is produced for each genome and used for error correction by majority calling. This surplus of (overlapping) short-read data can be either mapped onto known references (reference assembly) or used to build larger fragments (*de novo* assembly) of sequence data (so-called *contigs*), which are combined to constitute the draft genome of the isolate (67–69).

An important prerequisite to detect the presence of any relevant gene, including genes conferring resistance to antimicrobial agents, is that the quality and quantity of short reads are large enough to ensure that a given gene is being correctly detected by downstream analysis to avoid false-negative results. Due to the potentially high sensitivity of the analysis methods used for WGS, another important consideration is to ensure that the WGS data do not contain any traces of contaminant DNA, because this can lead to false-positive results. Unfortunately, low levels of DNA from intraspecies contamination can be very difficult to detect, and good laboratory practice when preparing the DNA and sequencing libraries, in combination with extensive use of appropriate negative controls, is often the best way to avoid, or at least minimize, contamination issues (70). When WGS data from an isolate are found free of contaminants and obtained with a sufficiently high quality and quantity to ensure that relevant genetic information is unlikely to be missing, they can then be used to search for genetic determinants related to AMR (67; http://www.phgfoundation.org/file/16848/).

Bioinformatic approaches for using WGS data to detect genetic determinants related to AMR

Extracting the relevant information to detect genetic determinants related to decreased antimicrobial susceptibility from WGS data is far from being a simple task. The main challenges are (i) to obtain comprehensive databases containing the relevant DNA or protein sequence targets and (ii) to apply appropriate bioinformatic methodologies to accurately extract the relevant information from WGS data based on these target databases. Target databases for WGS analysis are in principle not much different from primer lists directed toward certain PCR targets or DNA probes attached to microarrays as described in the sections above, even though WGS databases have the potential to contain more targets than most PCR or microarray systems. In many instances, a clear correlation between a specific resistance phenotype and a given (resistance) gene exists and has been well-characterized by another method, e.g., PCR or microarray, which makes it relatively easy to include these genes in a target database. However, the task of building a comprehensive database covering all possible genetic variations for a given AMR phenotype is far more complicated. As mentioned above, many genetic mechanisms can be accountable for the AMR phenotype, and a plethora of genetic mechanisms are responsible for resistance phenotypes to different antimicrobial agents. For many of these mechanisms it is difficult to generate simplified *in silico* decision rules for prediction of their corresponding resistance phenotype, especially because some bacterial species employ specialized mechanisms, which may not even be well-characterized or generally applicable beyond a given species (71). Therefore, many of the bioinformatic tools to detect genetic determinants con-

ferring reduced antimicrobial susceptibility are based on target databases containing well-defined genes or specific single point mutations, where a strong correlation between the genetic determinant and a given phenotype exists and can be extracted from either published peer-reviewed articles or from pre-existing archives such as the Antibiotic Resistance Gene Database (ARDB) (https://ardb.cbcb.umd.edu/). A disadvantage of such target databases is that they are based on *a priori* data and are therefore not suitable for detecting completely new genes families, novel genes, or new point mutations. Also, a constant curation of the target database is required to maintain updates when new genes are published.

Because comprehensive knowledge of genetic determinants for AMRs varies among bacterial species, the sensitivity of gene-based target databases can be too low to be applied for WGS as a first-line decision tool for treatment due to the risk of false-negative results. To unveil the current status of employing WGS data for antimicrobial susceptibility testing, EUCAST recently initiated a consultation involving leading experts in the field (71; http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2016/EUCAST_WGS_report_consultation_20160511.pdf). Here, the applicability of WGS for this purpose was thoroughly evaluated in relation to the most common human pathogens, with the main conclusion being that the available published evidence does not currently support the use of WGS inferred susceptibility to guide clinical decision making. However, target databases can still be a valuable tool for local, regional, and global surveillance of AMR, because they offer unprecedented resolution of gene variants, which are difficult to obtain by phenotypic as well as most other genotypic methods. Finally, a WGS-based approach enables fast *in silico* reanalysis of existing data each time the database is updated to ensure backward screening for the presence of new genes or single nucleotide polymorphisms. This approach was successfully used to rapidly reanalyze existing WGS data from Denmark shortly after the first report of the emergence of a mobile colistin resistance gene from China, termed *mcr-1* (24). Here, the *mcr-1* gene was retrieved from GenBank as soon as it became available and was added to the ResFinder database, described below, which was used in combination with the CGE Bacterial Analysis Platform (72) to reanalyze 534 bacterial genomes from humans and 380 bacterial genomes from animal and food samples within 2 days after the release of *mcr-1*, thus enabling detection and reporting of *mcr-1* to the scientific community outside China within 3 weeks of the original publication (26).

The two most common bioinformatic approaches to detect the presence of relevant genes are so-called mapping analysis of raw sequencing reads and BLAST-based analysis of (*de novo* assembled) draft genome contigs against a reference target database (67). In general, the reference mapping approaches using raw reads are more sensitive than BLAST-based analysis of draft genomes when it comes to detecting AMR genes because *de novo* assembly is not required for reference mapping. The lower sensitivity of the BLAST-based approach is most likely to be caused by the inability of *de novo* assembler algorithms to assemble all reads into complete genes, either because the raw read coverage of the particular DNA segment containing the resistance gene is too low for full assembly or because several resistance genes with almost identical sequences are present in the isolate, which will cause most assemblers to split the sequence into separate DNA fragments, thus also splitting the resistance gene (73). Conversely, the higher sensitivity of the mapping algorithms is more prone to produce false-positive results from samples with undetected contamination, because the contaminating DNA may not always be assembled into full-length genes by the *de novo* assemblers and therefore may not be reported by the BLAST alignment, but the algorithm will still be able to map to the target genes, though potentially with lower coverage than correctly mapping reads.

Currently available bioinformatic tools to detect genetic determinants for AMR

The number of freely available bioinformatic tools for detecting genetic determinants for AMR in WGS data is constantly increasing. The tools are available as web services, as standalone programs to be downloaded on a local computer through a graphical user interface, or as command-line tools, which means that the user is required to download the program and run it command-line on a computer running a Unix-based operation system. A selection of the most commonly used, publicly availably tools will be presented below and summarized in Table 1. A more exhaustive list and in-depth presentation and discussion of bioinformatic tools for detecting molecular mechanisms conferring reduced susceptibility to antimicrobial agents has recently been published (74). In addition, some of the target databases, which these tools are using for detection, have been implemented into commercial software solutions such as the CLCbio Genomic Workbench from Qiagen (http://www.qiagen.com) and the Bionumerics Seven software from Applied Math (http://www.applied-maths.com). Below, only the noncommercial solutions

Table 1 Overview of different open-access bioinformatic tools for identification of antimicrobial resistance

Method	Method for gene detection	Database	Reference
ARG-ANNOT	Local BLAST program in Bio-Edit software. Program is downloaded and run on users computer does not work on MAC User selective settings, % identity Analyzes assembled data Analyze without Web interface User sort output results Possible to run with user-created database	FASTA format Three databases: Nucleotide sequences for acquired resistance gene database and corresponding protein database Mutational gene database Do not state when/if the databases are updated	(66)
CARD	Analyzes assembled data Two analysis tools; BLAST and RGI BLAST method BLASTn, BLASTp, BLASTx, tBLASTn, tBLASTx Against CARD protein or nucleotide database RGI (currently only analyze protein sequences) Uses BLAST and curated SNP matrices Possible to download: RGI command-line tool to bulk analyze (Unix) Heatmap BLAST output (excel) ORFs FASTA	FASTA format Four nucleotide databases with corresponding protein databases Resistance genes and mechanisms rRNA mutation genes Mutational genes Wild type genes State on download page when database is updated	(80, 81)
ResFinder	BLASTn User selective settings, % identity and % coverage Program sort output according to user selected thresholds, outputs only best matches. Possible to analyze assembled data or raw reads Possible to download: Results as text Results as tab separated file FASTA with hit in genome FASTA with found resistance genes	FASTA format One database for each antimicrobial class with nucleotide sequences for acquired resistance genes Note file with phenotypic information on resistance genes State on homepage and download page when database is updated	(65)
KmerResistance	Mapping using *K*-mers Analyses raw reads Calculates quality of the raw reads and uses this to set threshold for detected genes Predicts the species of the genome Possible to run as web-server or to download program and run command-line Possible to run with user-created database	Uses the ResFinder database	(73)
SRST2	Mapping using Bowtie2 Analyzes raw reads Threshold for detected genes has been set to 90% identity and 90% coverage Program is downloaded from GitHub and is a command-line tool (Unix) Possible to run with user-created database	Can only handle databases in FASTA format with a specific header format Choose between the ARG-ANNOT database or the ResFinder database, the databases is not regularly updated	(82)

RGI; Resistance Gene Identifier. ORF; Open Reading Frame. SNP; Single Nucleotide Polymorphism.

will be presented and discussed in more detail. However, the numbers and diversity of AMR tools are vast, with many government and research institutions using their own custom-made databases and analytical approaches, some of which may not be publicly available (27, 75–79).

ResFinder

ResFinder (65) is a web server composed of a BLAST-based alignment for detection of acquired AMR genes in assembled WGS data and a curated database in FASTA format containing the resistance genes. The BLAST output is sorted, so only the best hit within a given position in the data, with a 30-bp overlap allowance, is given as a result. This makes it possible to detect the same gene located in multiple positions of the genomic data. ResFinder allows user selection of the minimum percent identity and minimum percent length of the sequence alignment and reports only the best hit for each gene target which meets these parameters. The ideal percent identity and percent length to employ are dependent on the purpose as well as the quality and type of sequence data.

ResFinder allows upload of both preassembled genomes as well as raw data from various sequencing platforms; when raw WGS data is uploaded, it is assembled by Velvet before being analyzed by ResFinder. ResFinder has been included in a web service called the CGE Bacterial Analysis Platform to allow for automated bulk analysis (72).

Because ResFinder detects only acquired genes, it does not detect genetic elements such as chromosomal mutations and multidrug transporters, as well as intrinsic resistance genes. This makes ResFinder well suited for surveillance of AMR in relation to acquired mechanisms but less suited to be used as an alternative for phenotypic antimicrobial sensitivity testing in the clinic.

CARD

The Comprehensive Antibiotic Resistance Database (CARD) is a web service and has two analysis options: BLAST and RGI (Resistance Gene Identifier) (80, 81). The BLAST option performs standard BLAST searches on smaller sequences uploaded by the user (but not whole genomes) against the CARD reference sequences. The RGI supports two detection model types: (i) protein homolog models, which employ BLAST sequence similarity cutoffs to detect AMR genes, and (ii) protein variant models for detection of mutations conferring AMR. Currently, the RGI only analyzes protein sequences, and if assembled contigs are submitted to the tool, the RGI first predicts open reading frames and then analyzes the predicted protein sequences. The RGI is also developed as a command-line tool for bulk analysis of many genomes simultaneously.

ARG-ANNOT

Antibiotic Resistance Gene-Annotation (ARG-ANNOT) (66) uses a local BLAST algorithm in conjunction with the BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), which allows the user to analyze sequences on a local computer without Internet access once the software is installed. Unfortunately, BioEdit does not currently run on Mac OS environments. ARG-ANNOT provides the user with three databases in FASTA format with phenotypic information in the FASTA header, acquired AMR genes with nucleotide or protein sequences, and a mutational gene database. Currently, the ARG-ANNOT system does not detect mutations automatically but outputs the sequence that matches the reference genes so the user is manually able to search from possible mutations. It is also possible for the user to make a custom-made database for specific analysis through ARG-ANNOT.

KmerResistance

KmerResistance (73) is a mapping tool and is available both as a web server and as a command-line tool. KmerResistance performs mapping against the ResFinder gene database by examining the number of co-occurring *k*-mers between the raw sequence data and the database. A *k*-mer is a subsequence of the length *k*.

To detect possible resistance gene contaminants, a novel quality validation estimation of the data has been added to minimize false-positive results. This is done by predicting the bacterial species from the genome data, which gives an estimate of both coverage and depth of the data, and thereby the quality of the data. With this quality measure of the data, the exponential survival function is used to measure the quality of detected genes. Only genes above the quality threshold set by the exponential survival function are given as output, under the assumption that hits to genes with a significantly lower *k*-mer coverage than the *k*-mer hits to the chromosome originate from contamination as described above.

SRST2

Short Read Sequence Typing for Bacterial Pathogens (SRST2) (82) is a command-line tool based on the mapping tool Bowtie2 (83). SRST2 maps raw sequence reads directly against an input database of preference, for example, resistance genes. SRST2 also enables further analysis of the identified genes, such as mutations com-

pared to reference sequence. The authors have set the threshold of SRST2 to 90% identity and 90% coverage.

In addition to the above-mentioned programs, other programs and methods for detecting AMR genes, not only for single isolates but also for metagenomic samples, are available online for free download, most of them as command-line tools. In the future, comparison of results obtained using the same WGS data set but different programs or tools will be useful to understand the benefits and possible shortcomings of each and to determine whether under certain circumstances some tools will provide more accurate results than others.

Detection of antimicrobial determinants in complex samples

The tools mentioned above are best suited for the detection of AMR determinants in WGS data from single (pure) isolates, even though they may to some extent also be applicable for complex samples containing whole bacterial communities. However, more dedicated tools for the analysis of DNA samples originating from whole bacterial communities (often called the microbiome) do exist, even though they are still in their infancy. Such microbiome DNA samples are often referred to as whole-community metagenomic samples and should not be confused with traditional metagenomic samples, in which only species-relevant targets such as 16S DNA, are sequenced and used to estimate species distributions in complex samples. The latter is now often referred to as microbial community profiling to avoid confusion with metagenomic sequence analysis. These methods will be able to cover not only bacteria and AMR, but also other pathogens such as viruses, fungi, and parasites. Additionally, part of the sample can also be tested for chemicals/residues in parallel, thus increasing the amount of information about the sample. Analysis of whole-community metagenomic samples can be useful especially for AMR surveillance, but other applications such as rapid investigation of clinical samples have also been demonstrated (84–86). As a proof-of-concept for whole-community metagenomics in AMR surveillance, shotgun Illumina sequencing of toilet waste from 18 international airplane flights arriving at Copenhagen Airport, Denmark, was performed and mapped against the ResFinder database using the online tool MGmapper (https://cge.cbs.dtu.dk/services/MGmapper/), which builds on a combination of the Burrows-Wheeler Aligner (BWA) algorithm and SAMtools software packages (87, 88). An average of 0.06% of the reads from the samples were assigned to (known) resistance genes, with genes encoding resistance to tetracycline and macrolide and beta-lactam resistance genes as the most abundant in the samples (89).

An initial challenge is to ensure that a representative sampling is performed and then to ensure that the DNA purification method is able to extract DNA from all species present at an equal ratio, especially if the downstream analysis includes quantification and comparison of (resistance) gene distributions for AMR surveillance. Knudsen and Bergmark recently evaluated eight commercial kits for extracting DNA from three microbiome sample types and found large variation in species composition obtained between the different kits (90). This makes it virtually impossible to compare microbiome data across studies unless the same methodology has been used. DNA extracted from whole-community metagenomic samples covers not only species-related information but all available DNA information present in the sample, including that originating from the host, the environment, and bacteria of interest (74).

The amount and variation of DNA can be extremely high in extractions from whole-community samples, and it is often economically unrealistic to sequence more than a subsample of this, even on the large-capacity second-generation sequencing platforms. However, even if samples are sequenced at high depth it can become time-consuming to process the data generated due to computational requirements. Therefore, it is rare to obtain all available sequence information present in the sample, which makes it difficult to perform *de novo* assembly prior to analysis for determinants related to reduced susceptibility to antimicrobial agents. Hence, detection of AMR determinants in DNA from complex communities often relies on mapping of raw sequencing reads to gene databases using mapping approaches similar to those mentioned above and is therefore dependent on the quality of these databases. Furthermore, genes such as AMR genes may be harbored by organisms that are of low prevalence in the microbiota and may be difficult to detect unless DNA extracted from metagenomics samples is sequenced to a high depth, which currently may not be economically viable beyond specific purposes such as research. However, as next-generation sequencing technologies advance, there is no doubt that these difficulties will be overcome.

MALDI-TOF MS IN RELATION TO AMR

MALDI-TOF MS is a powerful analytical tool which has only recently been introduced in many clinical laboratories. In short, MALDI-TOF MS is a technique used to analyze biomolecules such as DNA, carbohydrates, proteins, and peptides by their ability to become ionized and enter gas phase and then measuring their time of flight. Here, the mass/charge (m/z) ratio of the

resulting molecular fragments is analyzed to produce a molecular signature. Analysis can be made directly on biological samples of single organisms in standardized or complex matrices including blood and urine, and each spectrum can then be compared to commercial databases containing, e.g., species-specific spectral information, which has proved useful especially for species identification of microorganisms (91). Furthermore, MALDI-TOF MS offers the possibility to detect specific proteins or enzymes as well as smaller biomolecules such as antimicrobial agents and their degradation products (92). The main explanation for the rapid implementation of MALDI-TOF MS in clinical microbiology is probably its ability to generate rapid and relatively reliable results with a high throughput at a low cost. However, considerable cost is required to purchase and maintain the equipment, as is the case with most instrumentation, but once the equipment is acquired, processing each sample is relatively inexpensive. The practical applications of MALDI-TOF MS in relation to detecting AMR can generally be divided into one of three categories, described in detail below.

Species Identification and Targeted Antimicrobial Treatment

One of the most common applications of MALDI-TOF MS in the clinical microbial laboratory is to perform organism (bacterial species) identification. Application of this method is typically based on the detection of highly abundant proteins in a mass range between 2 and 20 kDa by computing their m/z values (91, 93). The same level of information can be obtained as from classical culture-based species identification methods, but with MALDI-TOF MS, where results are generated much more rapidly than by culture, hours or even days can be saved in initiating the correct treatment. This can have a direct impact on the clinical outcome as shown by Kumar et al., where the survival rate of septic shock patients decreased by 7.6% for each hour of delay in antimicrobial administration, for the first 6 hours (94). Therefore, an obvious benefit of rapid organism identification using MALDI-TOF MS is to utilize this information to perform targeted antimicrobial treatment based on *a priori* knowledge of expected treatment outcomes for each bacterial pathogen or to conduct proper antimicrobial stewardship if no pathogen can be detected in a given sample (95, 96).

Direct Detection of AMR Determinants

A large proportion of AMR determinants are proteins, so it is in principle possible to detect these, or proteolytic fragments of these, directly in the molecular signature from the MALDI-TOF MS, thus providing an on-the-fly resistance profile (97). An initial requirement for this detection is that the resistance genes are actually expressed, which can be an issue in relation to the inducible resistance systems. Initial attempts, however, have not found support for this approach. A study by Schaumann et al., analyzing mass spectra from protein extracts of ESBL-producing and non-ESBL-producing bacteria at the m/z range of 2,000 to 20,000, failed to obtain reliable discrimination between the two populations (98). An alternative approach is to collect spectra from uninduced and induced (by a given antimicrobial agent) cultures and then to perform spectral analysis of these to identify induced peaks in the data (99). A substantial obstacle for both of these methods is that many thousands of resistance protein variants exist, thus making it very difficult to develop specific databases covering all variants, which would need considerable effort and have to be done over time. In addition, signal shielding from nonresistance protein can occur, thus masking the relevant protein signals. This can to some extent be minimized if spectra from protein extracts rather than complete cell extracts are studied and by focusing on a small subset of resistance protein groups such as enzymes involved in resistance to critically important antimicrobial agents such as third-generation cephalosporins and carbapenems (100).

Detection of Antimicrobial Biomolecules and their Degradation Products

Because direct detection of resistance proteins by MALDI-TOF MS has not yet proven to be feasible to implement at the clinical laboratory, an alternative approach, in which the specific antimicrobial biomolecules and their degradation products resulting from the enzymatic activity are detected by MALDI-TOF MS, has been suggested (100). This approach is targeted to specific enzyme-drug combinations and is again mostly relevant in relation to the subgroup of critically important drugs, which are known to be subjected to enzymatic degradation. Therefore, most studies have examined the feasibility of this approach in relation to carbapenem resistance, as a result of carbapenemase activity, and have shown very high sensitivity and specificity (101, 102). However, one drawback of this approach in the clinical laboratory is that detection of carbapenems such as imipenem and meropenem, and their enzymatic degradation products, relies on a preincubation step to allow time for the degradation process to occur. Another drawback is that the analysis has to be performed at a shorter m/z range (0 to 700 m/z)

than that used for species identification because these molecules are relatively small (103). Implementation at a clinical laboratory as a routine analysis is therefore impeded by the need to reset the MALDI-TOF MS machine several times daily or by investing in two identical machines: one for each purpose (Dennis S. Hansen, unpublished data). A final drawback is the lack of enzymatic inactivation for several of the clinically important antimicrobial agents such as methicillin-resistant *S. aureus* and vancomycin-resistant enterococci, which renders this approach impossible.

DISCUSSION

This article has demonstrated that molecular methods for characterizing AMR genes are not only important because they provide insight to the possible mechanism of resistance, but are also increasingly becoming commonplace. For example, methods such as PCR are routinely used by laboratories worldwide both for surveillance and for research and are still irreplaceable in terms of cost and throughput compared with other molecular methods. However, methods such as WGS, which are increasingly being used for molecular characterization of AMR determinants and provide a more comprehensive picture of all AMR genes that may be present in an isolate, could supersede PCR in the future if the cost of WGS continues to fall and if analytical processes are further simplified. WGS provides an advantage over methods such as microarray because once isolates are sequenced, the data are in theory available in perpetuity and can be interrogated infinitely with new genes or data sets. In comparison, microarrays, once performed using a set of genes, cannot be interrogated again for new genes. Designing and printing new primers and probes for genes *in silico* to update microarrays is not an inconsiderable bioinformatic effort. Any new probe and primer not only have to be specific for that gene, but they also cannot cross-react with any other, and they have to have melting temperatures similar to all other existing probes present on the microarray. In addition, the cost of printing new arrays with new primers and probes often means that these additions are performed as batch updates, so *ad hoc* immediate response to new outbreaks, such as the global *mcr-1* outbreak response, is difficult to perform.

However, the rise of WGS and its application in routine AMR surveillance poses the problem of implementation and harmonization of this methodology globally. With some resource-limited countries still struggling to implement simple molecular methods such as PCR in routine surveillance activities, it is unlikely that they will acquire the funds or infrastructure required to perform WGS and ensuing bioinformatic analysis of the data. This will probably affect mitigation and control of AMR, because AMR is a global issue, with new variants likely to arise in different areas of the world but being able to disseminate worldwide due to international trade of food and animals and extensive human travel.

However, with the introduction of WGS, researchers and primary investigators can now have an unprecedented resolution of sequence information, which shifts the workload from running the assay to analyzing the WGS data. Also, for PCR, RT-PCR, and microarrays, results are in most cases either negative or positive, depending on a given cutoff criterion. For instance, a PCR product with the expected size is interpreted as a positive result, even though it may not be 100% identical at the nucleotide level to the result from the positive control. When analyzing WGS data, if nucleotide variations in the genes of interest are reported, the investigator has to decide (or prove) if the phenotype associated with the reference gene is also applicable to the new variant present in the WGS data. For surveillance and infection control purposes, this may not be of primary concern, but if WGS results are used to guide clinical treatment, matters may be different and require further work to gain experimental proof.

Detection of genetic determinants conferring reduced susceptibility to antimicrobial agents requires application of validated analytical methods and target databases for these genotypic-phenotypic correlations to be fully useful. Combining the right bioinformatic and biological competences often requires interdisciplinary collaboration, where construction of the software algorithms and target databases are thoroughly documented and validated by the use of well-documented test sets. Furthermore, it is important to ensure that all genetic mechanisms are correctly and comprehensively assigned to particular resistance phenotypes, which are then added to target databases. This is a complex task which requires in-depth knowledge of AMR genes and includes periodic updating and curation of the databases. In addition, a common nomenclature for genes belonging to the same family, and for reporting the results from various analytical tools, is needed if not only WGS data, but also results of WGS data analysis, are to be shared beyond the local setting. Finally, appropriate quality assurance testing protocols to test the quality of all steps of WGS and WGS analysis will need to be developed and implemented routinely in the future. This is an important criterion to ensure that WGS and data interpretation performed from different laboratories are

comparable. Similar quality assurance testing protocols have been implemented for a number of phenotypic and molecular tests of isolates by reference laboratories across Europe, e.g., antimicrobial susceptibility testing for *Enterobacteriaceae* and variable-number tandem repeat testing for *Salmonella*.

Another important point for consideration of the use of genotypic data for detecting resistance, as discussed by Anjum (9), is that new variants may be overlooked by PCR, microarrays, and WGS, because these detect the presence of genes using primers, probes, and sequences based on prior knowledge. Therefore, as demonstrated by the *mcr-1* gene (24), resistance to even critical antibiotics may be overlooked if only molecular methods are used. Therefore, it is important to continue to perform both methods in reference laboratories, but care must be taken in correlating genotypic and phenotypic data, as already mentioned, because there may be redundancy, i.e., more than one gene or genetic mechanism that can result in the same phenotype. For instance, continuing with the colistin example, Anjum et al. (27) showed that colistin-resistant *E. coli* and *Salmonella* isolates could harbor both the *mcr-1* gene and single nucleotide polymorphisms in several chromosomal genes associated with colistin resistance that would account for the colistin-resistance phenotype seen in these isolates. Until recently, when only chromosomal changes were known to be associated with colistin resistance, detection of single nucleotide polymorphism variants in relevant chromosomal genes was sufficient to declare colistin resistance to be present in *Enterobacteriaceae*; researchers did not look further. Because these chromosomal mutations are rarely transferable in nature, it was therefore deemed safe to use colistin for animal husbandry. However, identification of a transferable plasmid-borne colistin-resistance gene which has been shown to be prevalent worldwide has been a game changer. It has resulted in the recommendation for limited use of colistin in animal husbandry by competent authorities such as the European Medicines Agency (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/05/WC500207233.pdf). The impact and risk of the presence of the *mcr-1* transferable plasmid in *Enterobacteriaceae* is still being assessed in many countries, including in China, where it was first detected and may have been present for longer than previously known (104).

MALDI-TOF MS is a relatively new method which holds promise and could become more commonplace in the future for AMR characterization, but this requires further exploration and validation of data. An important point for consideration when using this method is the upfront cost of buying MALDI-TOF MS equipment solely for AMR diagnostics, which at current prices may not be cost-effective. It may, however, be cost-effective for use in laboratories where the machine is already available for other purposes. For example, MALDI-TOF MS is used routinely in hospitals and other reference laboratory settings in many countries for rapid identification of bacteria isolated from clinical specimens. If any AMR are present in these isolates that could also be accurately characterized using MALDI-TOF, then it would no doubt aid both rapid clinical diagnosis and treatment of bacterial infections. MALDI-TOF also has the added advantage that it is a "phenotypic" method so in principle should be able to detect new and variant forms of proteins or enzymes that may be responsible for AMRs attributed to bacterial isolates.

This article has undertaken to review some of the most common and popular molecular methods that have been or are currently used by researchers and reference laboratories working with AMR. This review is not an exhaustive list of all approaches available but aims to provide readers, especially those new to the field, with some ideas and examples of work being implemented in this area, and some of the possible pitfalls. Because the field of AMR is an area of growing importance, it is expected that other molecular methods, which are not included in this review, may come to prominence in the future. Microbial community profiling and metagenomic sequence analyses are some of the rapidly developing methods which will probably be used for AMR gene detection in the future, although based on current popularity and convenience, the use of PCR and single-isolate WGS seems irreplaceable.

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References

1. **Chan KG.** 2016. Whole-genome sequencing in the prediction of antimicrobial resistance. *Expert Rev Anti Infect Ther* **14:**617–619.
2. **Hollenbeck BL, Rice LB.** 2012. Intrinsic and acquired resistance mechanisms in *Enterococcus*. *Virulence* **3:** 421–433.
3. **Cox G, Wright GD.** 2013. Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *Int J Med Microbiol* **303:**287–292.
4. **Schlessinger D.** 1988. Failure of aminoglycoside antibiotics to kill anaerobic, low-pH, and resistant cultures. *Clin Microbiol Rev* **1:**54–59.
5. **Goodwin A, Kersulyte D, Sisson G, Veldhuyzen van Zanten SJ, Berg DE, Hoffman PS.** 1998. Metronidazole

resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol Microbiol* **28:**383–393.

6. **Huovinen P.** 2001. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis* **32:**1608–1614.
7. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* **67:**1597–1606.
8. **Boyce JM, Medeiros AA, Papa EF, O'Gara CJ.** 1990. Induction of beta-lactamase and methicillin resistance in unusual strains of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **25:**73–81.
9. **Anjum MF.** 2015. Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. *Future Microbiol* **10:**317–320.
10. **Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:**487–491.
11. **Arya M, Shergill IS, Williamson M, Gommersall L, Arya N, Patel HR.** 2005. Basic principles of real-time quantitative PCR. *Expert Rev Mol Diagn* **5:**209–219.
12. **Anjum MF, Lemma F, Cork DJ, Meunier D, Murphy N, North SE, Woodford N, Haines J, Randall LP.** 2013. Isolation and detection of extended spectrum β-lactamase (ESBL)-producing enterobacteriaceae from meat using chromogenic agars and isothermal loop-mediated amplification (LAMP) assays. *J Food Sci* **78:** M1892–M1898.
13. **Glais L, Jacquot E.** 2015. Detection and characterization of viral species/subspecies using isothermal recombinase polymerase amplification (RPA) assays. *Methods Mol Biol* **1302:**207–225.
14. **Abdullahi UF, Naim R, Taib WRW, Saleh A, Muazu A, Aliyu S, Baig AA.** 2015. Loop-mediated isothermal amplification (LAMP), an innovation in gene amplification: bridging the gap in molecular diagnostics; a review. *Indian J Sci Technol* **8:**1–12.
15. **Dallenne C, Da Costa A, Decré D, Favier C, Arlet G.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother* **65:**490–495.
16. **Solanki R, Vanjari L, Subramanian S, B A, E N, Lakshmi V.** 2014. Comparative evaluation of multiplex PCR and routine laboratory phenotypic methods for detection of carbapenemases among Gram negative bacilli. *J Clin Diagn Res* **8:**DC23–DC26.
17. **Poirel L, Walsh TR, Cuvillier V, Nordmann P.** 2011. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* **70:**119–123.
18. **Shen Z, Qu W, Wang W, Lu Y, Wu Y, Li Z, Hang X, Wang X, Zhao D, Zhang C.** 2010. MPprimer: a program for reliable multiplex PCR primer design. *BMC Bioinformatics* **11:**143.
19. **Schwartz T, Kohnen W, Jansen B, Obst U.** 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol Ecol* **43:**325–335.
20. **Lévesque C, Piché L, Larose C, Roy PH.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* **39:**185–191.
21. **Chagas TP, Alves RM, Vallim DC, Seki LM, Campos LC, Asensi MD.** 2011. Diversity of genotypes in CTX-M-producing *Klebsiella pneumoniae* isolated in different hospitals in Brazil. *Braz J Infect Dis* **15:**420–425.
22. **Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM.** 2005. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* **56:**115–121.
23. **Mulvey MR, Bryce E, Boyd DA, Ofner-Agostini M, Land AM, Simor AE, Paton S.** 2005. Molecular characterization of cefoxitin-resistant *Escherichia coli* from Canadian hospitals. *Antimicrob Agents Chemother* **49:** 358–365.
24. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J.** 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* **16:**161–168.
25. **Haenni M, Poirel L, Kieffer N, Châtre P, Saras E, Métayer V, Dumoulin R, Nordmann P, Madec JY.** 2016. Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids. *Lancet Infect Dis* **16:**281–282.
26. **Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas RS, Cavaco LM, Hansen DS, Aarestrup FM, Skov RL.** 2015. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill* **20:**20.
27. **Anjum MF, Duggett NA, AbuOun M, Randall L, Nunez-Garcia J, Ellis RJ, Rogers J, Horton R, Brena C, Williamson S, Martelli F, Davies R, Teale C.** 2016. Colistin resistance in *Salmonella* and *Escherichia coli* isolates from a pig farm in Great Britain. *J Antimicrob Chemother* **71:**2306–2313.
28. **Duggett NA, Sayers E, AbuOun M, Ellis RJ, Nunez-Garcia J, Randall L, Horton R, Rogers J, Martelli F, Smith RP, Brena C, Williamson S, Kirchner M, Davies R, Crook D, Evans S, Teale C, Anjum MF.** 2017. Occurrence and characterization of *mcr-1*-harbouring *Escherichia coli* isolated from pigs in Great Britain from 2013 to 2015. *J Antimicrob Chemother* **72:**691–695.
29. **Nijhuis RH, Veldman KT, Schelfaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, Gooskens J.** 2016. Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay. *J Antimicrob Chemother* **71:** 2344–2346.
30. **Veldman K, van Essen-Zandbergen A, Rapallini M, Wit B, Heymans R, van Pelt W, Mevius D.** 2016. Location

of colistin resistance gene *mcr-1* in *Enterobacteriaceae* from livestock and meat. *J Antimicrob Chemother* **71:** 2340–2342.

31. **Figueiredo R, Card RM, Nunez J, Pomba C, Mendonça N, Anjum MF, Da Silva GJ.** 2016. Detection of an *mcr-1*-encoding plasmid mediating colistin resistance in *Salmonella enterica* from retail meat in Portugal. *J Antimicrob Chemother* **71:**2338–2340.
32. **Strommenger B, Kettlitz C, Werner G, Witte W.** 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J Clin Microbiol* **41:**4089–4094.
33. **Chung Y, Kim TS, Min YG, Hong YJ, Park JS, Hwang SM, Song KH, Kim ES, Park KU.** 2016. Usefulness of multiplex real-time PCR for simultaneous pathogen detection and resistance profiling of staphylococcal bacteremia. **2016:**6913860.
34. **Fang H, Ataker F, Hedin G, Dornbusch K.** 2008. Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J Clin Microbiol* **46:**707–712.
35. **Randall LP, Lemma F, Rogers JP, Cheney TE, Powell LF, Teale CJ.** 2014. Prevalence of extended-spectrum-β-lactamase-producing *Escherichia coli* from pigs at slaughter in the UK in 2013. *J Antimicrob Chemother* **69:**2947–2950.
36. **García-Fernández S, Morosini MI, Marco F, Gijón D, Vergara A, Vila J, Ruiz-Garbajosa P, Cantón R.** 2015. Evaluation of the eazyplex SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical *Enterobacteriaceae* isolates recovered at two Spanish hospitals. *J Antimicrob Chemother* **70:**1047–1050.
37. **Kirchner M, Lemma F, Randall L, Anjum MF.** 2017. Loop-mediated isothermal amplification (LAMP) for extended spectrum β-lactamase gene detection in poultry carcase. *Vet Rec* **181:**119.
38. **Carter B, Wu G, Woodward MJ, Anjum MF.** 2008. A process for analysis of microarray comparative genomics hybridisation studies for bacterial genomes. *BMC Genomics* **9:**53.
39. **Yu X, Susa M, Knabbe C, Schmid RD, Bachmann TT.** 2004. Development and validation of a diagnostic DNA microarray to detect quinolone-resistant *Escherichia coli* among clinical isolates. *J Clin Microbiol* **42:**4083–4091.
40. **Barl T, Dobrindt U, Yu X, Katcoff DJ, Sompolinsky D, Bonacorsi S, Hacker J, Bachmann TT.** 2008. Genotyping DNA chip for the simultaneous assessment of antibiotic resistance and pathogenic potential of extra-intestinal pathogenic *Escherichia coli*. *Int J Antimicrob Agents* **32:**272–277.
41. **Call DR, Bakko MK, Krug MJ, Roberts MC.** 2003. Identifying antimicrobial resistance genes with DNA microarrays. *Antimicrob Agents Chemother* **47:**3290–3295.
42. **Aarts HJM, Guerra B, Malorny B.** 2006. Molecular methods for detection of antimicrobial resistance, p 37–48. *In* Aarestrup FM (ed), *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
43. **Anjum MF, Mafura M, Slickers P, Ballmer K, Kuhnert P, Woodward MJ, Ehricht R.** 2007. Pathotyping *Escherichia coli* by using miniaturized DNA microarrays. *Appl Environ Microbiol* **73:**5692–5697.
44. **Card R, Zhang J, Das P, Cook C, Woodford N, Anjum MF.** 2013. Evaluation of an expanded microarray for detecting antibiotic resistance genes in a broad range of Gram-negative bacterial pathogens. *Antimicrob Agents Chemother* **57:**458–465.
45. **Card RM, Mafura M, Hunt T, Kirchner M, Weile J, Rashid MU, Weintraub A, Nord CE, Anjum MF.** 2015. Impact of ciprofloxacin and clindamycin administration on Gram-negative bacteria isolated from healthy volunteers and characterization of the resistance genes they harbor. *Antimicrob Agents Chemother* **59:**4410–4416.
46. **Mendonça N, Figueiredo R, Mendes C, Card RM, Anjum MF, da Silva GJ.** 2016. Microarray evaluation of antimicrobial resistance and virulence of *Escherichia coli* isolates from Portuguese poultry. *Antibiotics (Basel)* **5:**5.
47. **Szmolka A, Fortini D, Villa L, Carattoli A, Anjum MF, Nagy B.** 2011. First report on IncN plasmid-mediated quinolone resistance gene qnrS1 in porcine *Escherichia coli* in Europe. *Microb Drug Resist* **17:**567–573.
48. **Batchelor M, Hopkins KL, Liebana E, Slickers P, Ehricht R, Mafura M, Aarestrup F, Mevius D, Clifton-Hadley FA, Woodward MJ, Davies RH, Threlfall EJ, Anjum MF.** 2008. Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. *Int J Antimicrob Agents* **31:**440–451.
49. **Szmolka A, Anjum MF, La Ragione RM, Kaszanyitzky EJ, Nagy B.** 2012. Microarray based comparative genotyping of gentamicin resistant *Escherichia coli* strains from food animals and humans. *Vet Microbiol* **156:** 110–118.
50. **Olowe OA, Choudhary S, Schierack P, Wieler LH, Makanjuola OB, Olayemi AB, Anjum M.** 2013. Pathotyping bla CTX-M *Escherichia coli* from Nigeria. *Eur J Microbiol Immunol (Bp)* **3:**120–125.
51. **Anjum MF, Choudhary S, Morrison V, Snow LC, Mafura M, Slickers P, Ehricht R, Woodward MJ.** 2011. Identifying antimicrobial resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. *J Antimicrob Chemother* **66:** 550–559.
52. **Kirchner M, Abuoun M, Mafura M, Bagnall M, Hunt T, Thomas C, Weile J, Anjum MF.** 2013. Cefotaxime resistant *Escherichia coli* collected from a healthy volunteer; characterisation and the effect of plasmid loss. *PLoS One* **8:**e84142.
53. **Kirchner M, Mafura M, Hunt T, Abu-Oun M, Nunez-Garcia J, Hu Y, Weile J, Coates A, Card R, Anjum MF.** 2014. Antimicrobial resistance characteristics and fitness of Gram-negative fecal bacteria from volunteers treated with minocycline or amoxicillin. *Front Microbiol* **5:**722.
54. **Kirchner M, Mafura M, Hunt T, Card R, Anjum MF.** 2013. Antibiotic resistance gene profiling of faecal and

oral anaerobes collected during an antibiotic challenge trial. *Anaerobe* **23:**20–22.

55. **Card RM, Warburton PJ, MacLaren N, Mullany P, Allan E, Anjum MF.** 2014. Application of microarray and functional-based screening methods for the detection of antimicrobial resistance genes in the microbiomes of healthy humans. *PLoS One* **9:**e86428.
56. **Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A, Peters T, Svensson L, Madajczak G, Litrup E, Imre A, Herrera-Leon S, Mevius D, Newell DG, Malorny B.** 2010. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. *Foodborne Pathog Dis* **7:**523–535.
57. **Garneau P, Labrecque O, Maynard C, Messier S, Masson L, Archambault M, Harel J.** 2010. Use of a bacterial antimicrobial resistance gene microarray for the identification of resistant *Staphylococcus aureus*. *Zoonoses Public Health* **57**(Suppl 1)**:**94–99.
58. **Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L.** 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J Antimicrob Chemother* **65:**1145–1154.
59. **El-Adawy H, Ahmed M, Hotzel H, Monecke S, Schulz J, Hartung J, Ehricht R, Neubauer H, Hafez HM.** 2016. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from healthy turkeys and broilers using DNA microarrays. *Front Microbiol* **7:**2019.
60. **McManus BA, Coleman DC, Deasy EC, Brennan GI, O'Connell B, Monecke S, Ehricht R, Leggett B, Leonard N, Shore AC.** 2015. Comparative genotypes, staphylococcal cassette chromosome mec (SCCmec) genes and antimicrobial resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from infections in humans and companion animals. *PLoS One* **10:**e0138079.
61. **Nimmo GR, Steen JA, Monecke S, Ehricht R, Slickers P, Thomas JC, Appleton S, Goering RV, Robinson DA, Coombs GW.** 2015. ST2249-MRSA-III: a second major recombinant methicillin-resistant *Staphylococcus aureus* clone causing healthcare infection in the 1970s. *Clin Microbiol Infect* **21:**444–450.
62. **Schlotter K, Huber-Schlenstedt R, Gangl A, Hotzel H, Monecke S, Müller E, Reißig A, Proft S, Ehricht R.** 2014. Multiple cases of methicillin-resistant CC130 *Staphylococcus aureus* harboring *mecC* in milk and swab samples from a Bavarian dairy herd. *J Dairy Sci* **97:**2782–2788.
63. **Piccinini R, Tassi R, Daprà V, Pilla R, Fenner J, Carter B, Anjum MF.** 2012. Study of *Staphylococcus aureus* collected at slaughter from dairy cows with chronic mastitis. *J Dairy Res* **79:**249–255.
64. **Pilla R, Castiglioni V, Gelain ME, Scanziani E, Lorenzi V, Anjum M, Piccinini R.** 2012. Long-term study of MRSA ST1, t127 mastitis in a dairy cow. *Vet Rec* **170:**312.
65. **Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV.** 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* **67:**2640–2644.
66. **Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain JM.** 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother* **58:**212–220.
67. **Kwong JC, McCallum N, Sintchenko V, Howden BP.** 2015. Whole genome sequencing in clinical and public health microbiology. *Pathology* **47:**199–210.
68. **Padmanabhan R, Mishra AK, Raoult D, Fournier PE.** 2013. Genomics and metagenomics in medical microbiology. *J Microbiol Methods* **95:**415–424.
69. **Edwards DJ, Holt KE.** 2013. Beginner's guide to comparative bacterial genome analysis using next-generation sequence data. *Microb Inform Exp* **3:**2.
70. **Gargis AS, Kalman L, Berry MW, Bick DP, Dimmock DP, Hambuch T, Lu F, Lyon E, Voelkerding KV, Zehnbauer BA, Agarwala R, Bennett SF, Chen B, Chin EL, Compton JG, Das S, Farkas DH, Ferber MJ, Funke BH, Furtado MR, Ganova-Raeva LM, Geigenmüller U, Gunselman SJ, Hegde MR, Johnson PL, Kasarskis A, Kulkarni S, Lenk T, Liu CS, Manion M, Manolio TA, Mardis ER, Merker JD, Rajeevan MS, Reese MG, Rehm HL, Simen BB, Yeakley JM, Zook JM, Lubin IM.** 2012. Assuring the quality of next-generation sequencing in clinical laboratory practice. *Nat Biotechnol* **30:**1033–1036.
71. **Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MT, Hopkins KL, Iredell J, Kahlmeter G, Köser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen Ø, Woodford N.** 2017. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. *Clin Microbiol Infect* **23:**2–22.
72. **Thomsen MC, Ahrenfeldt J, Cisneros JL, Jurtz V, Larsen MV, Hasman H, Aarestrup FM, Lund O.** 2016. A bacterial analysis platform: an integrated system for analysing bacterial whole genome sequencing data for clinical diagnostics and surveillance. *PLoS One* **11:**e0157718.
73. **Clausen PT, Zankari E, Aarestrup FM, Lund O.** 2016. Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. *J Antimicrob Chemother* **71:**2484–2488.
74. **McArthur AG, Tsang KK.** 2017. Antimicrobial resistance surveillance in the genomic age. *Ann N Y Acad Sci* **1388:**78–91.
75. **Sharma M, Nunez-Garcia J, Kearns AM, Doumith M, Butaye PR, Argudín MA, Lahuerta-Marin A, Pichon B, AbuOun M, Rogers J, Ellis RJ, Teale C, Anjum MF.** 2016. Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) clonal complex (CC) 398 isolated from UK animals belong to European lineages. *Front Microbiol* **7:**1741.
76. **Doumith M, Godbole G, Ashton P, Larkin L, Dallman T, Day M, Day M, Muller-Pebody B, Ellington MJ, de**

Pinna E, Johnson AP, Hopkins KL, Woodford N. 2016. Detection of the plasmid-mediated *mcr-1* gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. *J Antimicrob Chemother* **71:**2300–2305.

77. **Garvey MI, Pichon B, Bradley CW, Moiemen NS, Oppenheim B, Kearns AM.** 2016. Improved understanding of an outbreak of meticillin-resistant *Staphylococcus aureus* in a regional burns centre via whole-genome sequencing. *J Hosp Infect* **94:**401–404.
78. **Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, Johnson JR, Walker AS, Peto TE, Crook DW.** 2013. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. *J Antimicrob Chemother* **68:**2234–2244.
79. **Bradley P, Gordon NC, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, de Cesare M, Piazza P, Votintseva AA, Golubchik T, Wilson DJ, Wyllie DH, Diel R, Niemann S, Feuerriegel S, Kohl TA, Ismail N, Omar SV, Smith EG, Buck D, McVean G, Walker AS, Peto TE, Crook DW, Iqbal Z.** 2015. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. *Nat Commun* **6:**10063.
80. **McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD.** 2013. The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother* **57:**3348–3357.
81. **McArthur AG, Wright GD.** 2015. Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. *Curr Opin Microbiol* **27:**45–50.
82. **Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, Zobel J, Holt KE.** 2014. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med* **6:**90.
83. **Langmead B, Salzberg SL.** 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9:**357–359.
84. **Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup FM.** 2014. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol* **52:**139–146.
85. **Schmieder R, Edwards R.** 2012. Insights into antibiotic resistance through metagenomic approaches. *Future Microbiol* **7:**73–89.
86. **Wang Z, Zhang XX, Huang K, Miao Y, Shi P, Liu B, Long C, Li A.** 2013. Metagenomic profiling of antibiotic resistance genes and mobile genetic elements in a tannery wastewater treatment plant. *PLoS One* **8:**e76079.
87. **Li H, Durbin R.** 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26:**589–595.
88. **Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup.** 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25:**2078–2079.
89. **Nordahl Petersen T, Rasmussen S, Hasman H, Carøe C, Bælum J, Schultz AC, Bergmark L, Svendsen CA, Lund O, Sicheritz-Pontén T, Aarestrup FM.** 2015. Metagenomic analysis of toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance. *Sci Rep* **5:**11444.
90. **Knudsen BE, Bergmark L.** 2016. Impact of sample type and DNA isolation procedure on genomic inference of microbiome composition. *mSystems* **1:**e00095-16.
91. **Murray PR.** 2012. What is new in clinical microbiology-microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. *J Mol Diagn* **14:**419–423.
92. **Hrabák J, Chudáčková E, Papagiannitsis CC.** 2014. Detection of carbapenemases in *Enterobacteriaceae*: a challenge for diagnostic microbiological laboratories. *Clin Microbiol Infect* **20:**839–853.
93. **Panda A, Kurapati S, Samantaray JC, Srinivasan A, Khalil S.** 2014. MALDI-TOF mass spectrometry proteomic based identification of clinical bacterial isolates. *Indian J Med Res* **140:**770–777.
94. **Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M.** 2006. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* **34:**1589–1596.
95. **Frickmann H, Masanta WO, Zautner AE.** 2014. Emerging rapid resistance testing methods for clinical microbiology laboratories and their potential impact on patient management. *BioMed Res Int* **2014:**375681.
96. **Bauer KA, Perez KK, Forrest GN, Goff DA.** 2014. Review of rapid diagnostic tests used by antimicrobial stewardship programs. *Clin Infect Dis* **59**(Suppl 3)**:** S134–S145.
97. **Kostrzewa M, Sparbier K, Maier T, Schubert S.** 2013. MALDI-TOF MS: an upcoming tool for rapid detection of antibiotic resistance in microorganisms. *Proteomics Clin Appl* **7:**767–778.
98. **Schaumann R, Knoop N, Genzel GH, Losensky K, Rosenkranz C, Stîngu CS, Schellenberger W, Rodloff AC, Eschrich K.** 2012. A step towards the discrimination of beta-lactamase-producing clinical isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by MALDI-TOF mass spectrometry. *Med Sci Monit* **18:**MT71–MT77.
99. **dos Santos KV, Diniz CG, Veloso LC, de Andrade HM, Giusta MS, Pires SF, Santos AV, Apolônio AC, de Carvalho MA, Farias LM.** 2010. Proteomic analysis of *Escherichia coli* with experimentally induced resistance to piperacillin/tazobactam. *Res Microbiol* **161:**268–275.
100. **Hrabák J, Chudácková E, Walková R.** 2013. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clin Microbiol Rev* **26:**103–114.

101. Burckhardt I, Zimmermann S. 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. *J Clin Microbiol* **49:**3321–3324.

102. Studentova V, Papagiannitsis CC, Izdebski R, Pfeifer Y, Chudackova E, Bergerova T, Gniadkowski M, Hrabak J. 2015. Detection of OXA-48-type carbapenemase-producing *Enterobacteriaceae* in diagnostic laboratories can be enhanced by addition of bicarbonates to cultivation media or reaction buffers. *Folia Microbiol (Praha)* **60:**119–129.

103. Chong PM, McCorrister SJ, Unger MS, Boyd DA, Mulvey MR, Westmacott GR. 2015. MALDI-TOF MS detection of carbapenemase activity in clinical isolates of *Enterobacteriaceae* spp., *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* compared against the Carba-NP assay. *J Microbiol Methods* **111:** 21–23.

104. Wang Y, Tian GB, Zhang R, Shen Y, Tyrrell JM, Huang X, Zhou H, Lei L, Li HY, Doi Y, Fang Y, Ren H, Zhong LL, Shen Z, Zeng KJ, Wang S, Liu JH, Wu C, Walsh TR, Shen J. 2017. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. *Lancet Infect Dis* **17:**390–399.

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Mechanisms of Bacterial Resistance to Antimicrobial Agents

4

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INTRODUCTION

With regard to their structures and functions, antimicrobial agents represent a highly diverse group of low-molecular-weight substances which interfere with bacterial growth, resulting in either a timely limited growth inhibition (bacteriostatic effect) or the killing of the bacteria (bactericidal effect). For more than 60 years, antimicrobial agents have been used to control bacterial infections in humans, animals, and plants. Nowadays, antimicrobial agents are among the most frequently used therapeutics in human and veterinary medicine (1, 2). In the early days of antimicrobial chemotherapy, antimicrobial resistance was not considered as an important problem, since the numbers of resistant strains were low and a large number of new highly effective antimicrobial agents of different classes were detected. These early antimicrobial agents represented products of the metabolic pathways of soil bacteria (e.g., *Streptomyces*, *Bacillus*) or fungi (e.g., *Penicillium*, *Cephalosporium*, *Pleurotus*) (Table 1) and provided their producers with a selective advantage in the fight for resources and the colonization of ecological niches (3). This in turn forced the susceptible bacteria living in close contact with the antimicrobial producers to develop and/or refine mechanisms to circumvent the inhibitory effects of antimicrobial agents. As a consequence, the origins of bacterial resistance to antimicrobial agents can be assumed to be in a time long before the clinical use of these substances. With the elucidation of the chemical structure of the antimicrobial agents, which commonly followed soon after their detection, it was possible not only to produce antimicrobial agents synthetically in larger amounts at lower costs, but also to introduce modifications that altered the pharmacological properties of these substances and occasionally also extended their spectrum of activity.

The increased selective pressure imposed by the widespread use of antimicrobial agents since the 1950s has distinctly accelerated the development and the spread of bacterial resistance to antimicrobial agents. In most cases, it took not longer than three to five years after the introduction of an antimicrobial agent into clinical use until the first resistant target bacteria occurred (1). This is particularly true for broad-spectrum antimicrobial agents, such as tetracyclines, aminoglycosides, macrolides, and β-lactams, which have been used for multiple purposes in human and veterinary medicine, horticulture, and/or aquaculture. In contrast, this time span was extended to ≥15 years for narrow-spectrum agents, such as glycopeptides, which were used at distinctly lower quantities and only for specific applications. Multiple studies have also revealed that resistance to completely synthetic antimicrobial agents, such as sulfonamides, trimethoprim, fluoroquinolones, and oxazolidinones, can develop quickly (4–7). These observations underline the enormous flexibility of the bacteria to cope with less favorable environmental conditions by constantly exploring new ways to survive in the presence of antimicrobial agents.

This chapter summarizes the latest information on resistance mechanisms and the mobile elements involved. It is a completely revised and updated version of the chapter that was published in 2006 (8).

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Table 1 Origins of antimicrobial agents

Class	Antimicrobial agent	Producing organisms	Year(s) of isolation/ description
β-Lactam antibiotics	Natural penicillins	*Penicillium notatum*, *Penicillium chrysogenum*	1929, 1940
	Cephalosporin C	*Cephalosporium acremonium*	1945, 1953
	Imipenem	*Streptomyces cattleya*	1976
	Aztreonam	*Gluconobacter* spp., *Chromobacterium violaceum*	1981
Glycopeptides	Vancomycin	*Amycolatopsis orientalis*	mid-1950s
	Teicoplanin, avoparcin	*Amycolatopsis coloradensis* subsp. *labeda*	1975
Macrolides	Erythromycin	*Streptomyces erythreus*	1952
	Spiramycin	*Streptomyces ambofaciens*	1955
Lincosamides	Lincomycin	*Streptomyces lincolnensis*	1963
Streptogramins	Streptogramin A+B	*Streptomyces diastaticus*	1953
	Virginiamycin A+B	*Streptomyces virginiae*	1955
Tetracyclines	Chlortetracycline	*Streptomyces aureofaciens*	1948
	Oxytetracycline	*Streptomyces rimosus*	1950
Phenicols	Chloramphenicol	*Streptomyces venezuelae*	1947
Aminoglycosides	Streptomycin	*Streptomyces griseus*	1943
	Neomycin	*Streptomyces fradiae*	1949
	Kanamycin	*Streptomyces kanamyceticus*	1957
	Gentamicin	*Micromonospora purpura*	1963
	Tobramycin	*Streptomyces tenebrarius*	1967
Aminocyclitols	Spectinomycin	*Streptomyces spectabilis*	1961
Pleuromutilins	Pleuromutilin, Tiamulin	*Pleurotus* spp.; synthetic	1951, 1976
Polypeptide antibiotics	Polymyxin B	*Bacillus polymyxa (aerosporus)*	1947
	Polymyxin E (colistin)	*B. polymyxa* var. *colistinus*	1949
	Bacitracin	*Bacillus licheniformis*	1943
Epoxide antibiotics	Fosfomycin	*Streptomyces fradiae, Streptomyces wedmorensis, Pseudomonas syringae*	1969
Pseudomonic acid antibiotics	Mupirocin	*Pseudomonas fluorescens*	1971
Steroid antibiotics	Fusidic acid	*Fusidium coccineum*	1960
Streptothricins	Nourseothricin	*Streptomyces noursei*	1963
Sulfonamides	Prontosil, sulfamethoxazole, etc.	Synthetic	1935
Trimethoprim	Trimethoprim	Synthetic	1956
Quinolones	Nalidixic acid	Synthetic	1962
Fluoroquinolones	Flumequine, enrofloxacin, etc.	Synthetic	1973
Oxazolidinones	Linezolid	Synthetic	1987, 1996

RESISTANCE TO ANTIMICROBIAL AGENTS

Resistance to antimicrobial agents can be divided into two basic types, intrinsic resistance and acquired resistance (1, 3, 8, 9). Intrinsic resistance, also known as primary or innate resistance, describes a status of general insensitivity of bacteria to a specific antimicrobial agent or class of agents. This is commonly due to the lack or the inaccessibility of target structures for certain antimicrobial agents, e.g., resistance to β-lactam antibiotics and glycopeptides in cell wall-free bacteria, such as *Mycoplasma* spp., or vancomycin resistance in Gram-negative bacteria due to the inability of vancomycin to penetrate the outer membrane. It can also be due to the presence of export systems or the production of species-specific inactivating enzymes in certain bacteria, e.g., the AcrAB-TolC system or the production of AmpC β-lactamase in certain *Enterobacteriaceae*. In addition, some bacteria, such as enterococci, can use exogenous folates and are thus not dependent on a functional folate synthesis pathway. As a consequence, they are intrinsically resistant to folate pathway inhibitors, such as trimethoprim and sulfonamides (9). Intrinsic resistance is a genus- or species-specific property of bacteria. In contrast, acquired resistance is a strain-specific property which can be due to the acquisition of foreign resistance genes or mutational modification of chromosomal target genes. Mutations that upregulate the expression of multidrug transporter systems may also fall into this category. Three different basic types of resistance mechanisms can be differentiated: (i) enzymatic

inactivation by either disintegration or chemical modification of the antimicrobials (Table 2), (ii) reduced intracellular accumulation by decreased influx and/or increased efflux of antimicrobials (Table 3), and (iii) modification of the cellular target sites by mutation, chemical modification, or protection of the target sites, but also overexpression of sensitive targets or the replacement of sensitive target structures by alternative resistant ones (Table 4) (1, 3, 8, 9).

The following subsections illustrate that bacterial resistance to antimicrobial agents varies depending on the agents, the bacteria, and the resistance mechanism. Resistance to the same antimicrobial agent can be mediated by different mechanisms. In some cases, the same resistance gene/mechanism is found in a wide variety of bacteria, whereas in other cases, resistance genes or mechanisms appear to be limited to certain bacterial species or genera. The data presented in the following subsections do not focus exclusively on resistance genes and mechanisms so far detected in bacteria of animal origin but also include resistance genes and mechanisms identified in bacteria from humans. For the best possible overview of the mechanisms and genes accounting for resistance to a specific class of antimicrobial agents, all data are presented under the names of the classes of antimicrobial agents.

Resistance to -Lactam Antibiotics

A number of penicillins, alone or in combination with a β-lactamase inhibitor, as well as first- to fourth-generation cephalosporins, are licensed for use in veterinary medicine. No carbapenems or monobactams are currently approved for use in animals. Resistance to β-lactam antibiotics is mainly due to inactivation by β-lactamases (10) and decreased ability to bind to penicillin-binding proteins (PBPs) (11) in both Gram-positive and Gram-negative bacteria, but may also be based on decreased uptake of β-lactams due to perme-

Table 2 Examples of resistance to antimicrobials by **decreased intracellular drug accumulation** (modified from ref. 8)[a,b]

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene
efflux via specific exporters	*mef*(A)	10-TMS efflux system of the major facilitator superfamily	14-, 15-membered macrolides	*Streptococcus*, other Gram+ and Gram– bacteria	T, P, C
	tet(A-E, G, H, I, J, K, L, Z), *tetA*(P), *tet*(30)	12-, 14-TMS efflux system of the major facilitator superfamily	tetracyclines	various Gram+ and Gram– bacteria	P, T, C
	pp-flo, floR, floR$_V$	12 TMS efflux system of the major facilitator superfamily	chloramphenicol, florfenicol	*Photobacterium, Vibrio, Salmonella, Escherichia, Klebsiella*, *Pasteurella*	T, P, C
	cmlA	12 TMS efflux system of the major facilitator superfamily	chloramphenicol	*Pseudomonas, Salmonella, E. coli*	T, P, C
	fexA	14 TMS efflux system of the major facilitator superfamily	chloramphenicol, florfenicol	*Staphylococcus*	T, P, C
	fexB	14 TMS efflux system of the major facilitator superfamily	chloramphenicol, florfenicol	*Enterococcus*	P
efflux via multidrug transporters	*emrE*	4-TMS multidrug efflux protein	tetracyclines, nucleic acid binding compounds	*E. coli*	C
	blt, *nor*A	12-TMS multidrug efflux protein of the major facilitator superfamily	chloramphenicol, fluoroquinolones, nucleic acid binding compounds	*Bacillus, Staphylococcus*	C
	mexB-mexA-oprM, *acrA-acrB-tolC*	multidrug efflux in combination with specific OMP's	chloramphenicol, β-lactams, macrolides, fluoroquinolones, tetracyclines, etc.	*Pseudomonas, E. coli, Salmonella*	C

[a]P = plasmid; T = transposon; GC = gene cassette; C = chromosomal DNA.
[b]TMS = transmembrane segments.

Table 3 Examples of resistance to antimicrobials by enzymatic inactivation[a]

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene[b]
Hydrolytic degradation	*bla*	β-lactamases	β-lactam antibiotics	Various Gram+, Gram–, aerobic, anaerobic bacteria	P, T, GC, C
	ere(A), *ere*(B)	esterases	macrolides	Gram+, Gram– bacteria	P, GC
	vgb(A), *vgb*(B)	lactone hydrolases	streptogramin B antibiotics	*Staphylococcus*, *Enterococcus*	P
Chemical modification	*aac*, *aad (ant)*, *aph*	acetyl-, adenyl-, phosphotransferases	aminoglycosides	Gram+, Gram–, aerobic bacteria	T, GC, P, C
	aad (ant)	adenyltransferases	aminoglycosides/ aminocyclitols	Gram+, Gram–, aerobic bacteria	T, GC, P, C
	catA, *catB*	acetyltransferases	chloramphenicol	Gram+, Gram–, aerobic, anaerobic bacteria	P, T, GC, C
	vat(A-G)	acetyltransferases	streptogramin A antibiotics	*Bacteroides*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Yersinia*	P, C
	mph(A-G)	phosphotransferases	macrolides	Gram+, Gram– bacteria	P, T, C
	lnu(A-P)	nucleotidyltransferases	lincosamides	Gram+, Gram– bacteria	P
	tet(X), *tet*(37), *tet*(56)	oxidoreductases	tetracyclines	Gram– bacteria, unknown, *Legionella*	T, P

[a]Modified from reference 8.
[b]Abbreviations: P, plasmid; T, transposon; GC, gene cassette; C, chromosomal DNA.

ability barriers or increased efflux via multidrug transporter systems (12, 13). Inactivation via β-lactamases is most commonly seen, with a wide range of β-lactamases involved. The evolution of β-lactamases which differ distinctly in their substrate spectra is believed to have occurred in response to the selective pressure imposed by the various β-lactam antibiotics that have been introduced into clinical use during the past decades (14).

Enzymatic inactivation of β-lactam antibiotics is based on the cleavage of the amino bond in the β-lactam ring by β-lactamases (10, 15, 16). At present, more than 1,000 β-lactamases have been described, most of which are variants of known β-lactamases that differ in their substrate spectra or their enzyme stability. Two classification schemes are currently in use. The initial classification scheme was based on the similarities in the amino acid sequences and divided the β-lactamases into two molecular classes, A and B (17), which were later expanded to four molecular classes, A to D (18). The second functional classification of β-lactamases was updated in 2010 by Bush and Jacoby (18) and is done on the basis of their substrate spectra and their susceptibility to β-lactamase inhibitors such as clavulanic acid (18). This system subdivides the β-lactamases into three groups, 1 to 3, with group 1 currently comprising 2 subgroups, group 2 comprising 12 subgroups, and group 3 comprising 2 subgroups.

Group 1 β-lactamases (molecular class C), for example, AmpC, CMY, ACT, DHA, FOX, and MIR, are cephalosporinases that are more active on cephalosporins than benzylpenicillin and are usually not inhibited by clavulanic acid. They are widespread among Gram-negative bacteria. The *ampC* genes are commonly located on the chromosome but may also be found on plasmids. Some of these *ampC* genes are expressed inducibly; others are expressed constitutively (18). Point mutations in the promotor or attenuator region may increase β-lactamase production. Subgroup 1e enzymes are group 1 variants with greater activity against ceftazidime and other oxyimino-β-lactams as a result of amino acid substitutions, insertions, or deletions and include GC1 in *Enterobacter cloacae* and plasmid-mediated CMY-10, CMY-19, and CMY-37. They have been named extended-spectrum AmpC β-lactamases (18).

Group 2 β-lactamases (molecular classes A and D) represent diverse enzymes, most of which are sensitive to inhibition by clavulanic acid. Subgroup 2a (molecular class A) includes enzymes such as BlaZ from staphylococci, which can inactivate only penicillins. Subgroup 2b (molecular class A) comprises broad-spectrum β-lactamases, such as TEM-1, TEM-2, SHV-1, and ROB-1, which can hydrolize penicillins and broad-spectrum cephalosporins. Subgroup 2be represents extended-spectrum β-lactamases (ESBLs; e.g., variants of TEM and SHV families and CTX-M type enzymes), which can also inactivate oxyimino cephalosporins and monobactams. Subgroups 2a, 2b, and 2be enzymes are sensi-

Table 4 Examples of resistance to antimicrobials by **target modification** (modified from ref. 8)[a]

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene
methylation of the target site	*erm*	rRNA methylase	macrolides, lincosamides, streptogramin B compounds	various Gram+ bacteria, *Escherichia*, *Bacteroides*	P, T, C
protection of the target site	*tet*(M, O, P, Q, S, T)	ribosome protective proteins	tetracyclines	various Gram+ and Gram– bacteria	T, P, C
	vga(A)	ribosome protective ABC-F protein	lincosamides, pleuromutilins, streptogramin A-compounds	*Staphylococcus*,	P, T, C
	optrA	ribosome protective ABC-F protein	oxazolidinones, phenicols	*Enterococcus*, *Staphylococcus*	P, C
replacement of a sensitive target by an alternative drug-resistant target	*sul1*, *sul2*, *sul3*	sulfonamide-resistant dihydropteroate synthase	sulfonamides	various Gram– bacteria	P, I
	dfrA, *dfrB*, *dfrD*, *dfrG*, *dfrK*	trimethoprim-resistant dihydrofolate reductase	trimethoprim	various Gram+ and Gram– bacteria	P, GC, T, C
	mecA, *mecC*	penicillin-binding proteins with altered substrate specificity	penicillins, cephalosporins, carbapenems, monobactams	*Staphylococcus*	C
	vanA-E	alternative D-Ala-D-Lac or D-Ala-D-Ser peptidoglycan precursors	glycopeptides	*Enterococcus*, *Staphylococcus*	T, P, C
alteration of the LPS	*mcr-1* to *mcr-5*	phosphoethanolamine transferase	colistin	Enterobacteriaceae	T, P, C
mutational modification of the target site	—	mutation in the gene coding for ribosomal protein S12	streptomycin	several Gram+ and Gram– bacteria	C
	—	mutation in the 16S rRNA	streptomycin	*Mycobacterium*	C
	—	mutation in the 23S rRNA	macrolides	*Mycobacterium*	C
	—	mutation in the 16S rRNA	tetracyclines	*Propionibacterium*	C
	—	mutations in the genes for DNA gyrase and topoisomerase	fluoroquinolones	various Gram+ and Gram– bacteria	C
	—	mutation in the gene for the ribosomal protein L3	tiamulin	*E. coli*	C
mutational modification of regulatory elements	—	mutations in the *marRAB* *soxR* or *acrR* genes	fluoroquinolones	*E. coli*	C

[a]P = plasmid; T = transposon; GC = gene cassette; C = chromosomal DNA, I = integron.

tive to inhibition by clavulanic acid. Due to their wide spectrum of activity, ESBLs are a serious cause of concern (19). Most currently known ESBLs belong to the TEM, SHV, CTX-M, or OXA families of β-lactamases. Less common ESBLs include BEL-1, BES-1, SFO-1, TLA-1, TLA-2, and members of the PER and VEB enzyme families. Details about the structure and function of these ESBLs, their location on mobile elements, their dissemination among bacteria of different species and genera, and information on ESBL detection methods can be found in several reviews (19–21). Moreover, a continuosly updated database which lists the known ESBLs and inhibitor-resistant β-lactamases including TEM, SHV, OXA, CTX-M, CMY, IMP, and VIM types can be found at https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/. The enzymes of subgroup 2br (molecular class A) are also broad-spectrum β-lactamases, such as TEM-30, TEM-31, and SHV-10, which however, are not inhibited by clavulanic acid. Analysis of the β-lactamases of subgroups 2b, 2be, and 2br—in particular, those of the TEM and SHV types—revealed the presence of mutations which either extended

the substrate spectrum or affected the enzyme stability (10, 14, 19). TEM enzymes that exhibit an extended spectrum and increased resistance to clavulanic acid inhibition are organized in subgroup 2ber and are called complex mutant TEM (CMT); these include TEM-50 (CMT-1) and TEM-158 (CMT-9) (18). Subgroup 2c (molecular class A) includes inhibitor-sensitive carbenicillinases such as CARB-3, PSE-1, and RTG, whereas the extended-spectrum carbenicillinase RTG-4 in subgroup 2ce shows enhanced activity against cefepime and cefpirome. The β-lactamases of subgroup 2d (molecular class D) (e.g., OXA-type enzymes) exhibit variable sensitivity to inhibitors and can hydrolyze oxacillin or cloxacillin. The extended spectrum of the enzymes in subgroup 2de (e.g., OXA-11 and OXA-15) is defined by their ability to hydrolize oxacillin or cloxacillin as well as oxyimino β-lactams with a preference for ceftazidime. The subgroup 2df assembles OXA enzymes which are not inhibited by clavulanic acid and show carbapenem-hydrolyzing activities. The genes have been detected on plasmids and in the chromosome of Gram-negative bacteria (18). The β-lactamases of subgroups 2e and 2f represent cephalosporinases (e.g., CepA) or serine-carbapenemases (e.g., SME-1, IMI-1, KPC-2), which are sometimes inhibited by clavulanic acid (18).

While the β-lactamases of groups 1 and 2 have a serine residue in the catalytic center, the β-lactamases of group 3 (molecular class B) hydrolyze β-lactams by divalent cations (Zn^{2+}) and are referred to as metallo-β-lactamases (e.g., IMP-type, VIM-type, and NDM-type enzymes). Subgroup 3a consists of plasmid-encoded metallo-β-lactamases, which require two bound zinc ions for their activity. These enzymes can inactivate all β-lactams except monobactams and are insensitive to clavulanic acid but are inhibited by metal ion chelators such as EDTA. The metallo-β-lactamases in subgroup 3b preferentially hydrolyze carbapenems, especially if only one zinc-binding side is occupied (18). The location of many of the β-lactamase genes (*bla*) on either plasmids, transposons, or gene cassettes favors their dissemination (20–22).

Altered PBPs are often associated with resistance due to decreased binding of β-lactam antibiotics (11). PBPs are transpeptidases which play an important role in cell wall synthesis. They are present in most cell wall-containing bacteria, but they vary from species to species in number, size, amount, and affinity to β-lactam antibiotics (11). The acquisition of a novel PBP, such as the *mecA*-encoded PBP2a, which replaces the original β-lactam-sensitive PBP, is the cause of methicillin resistance in *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, and coagulase-negative staphylococci (23, 24). Methicillin-resistant staphylococci are resistant to virtually all β-lactam antibiotics except cefpirome. The *mecA* gene, which codes for the alternative PBP2a, is part of a genetic element, designated *Staphylococcus* cassette chromosome *mec* (SCC*mec*) (25). So far, 11 SCC*mec* types have been described (26). In 2011, a new *mecA* homologue, *mecC* (formerly called mec_{LGA251}), which is part of a distinct SCC*mec* type (SCC*mec* XI), was identified in methicillin-resistant *S. aureus* (MRSA) (27, 28). The majority of known MRSA-carrying *mecC* belong to clonal complex (CC) 130, but other CCs (CC425, CC1943, CC599, CC49) have also been found to harbor *mecC*. In addition, *mecC* is not restricted to *S. aureus* but has been found in several staphylococcal species including *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, *Staphylococcus stepanovicii*, and *Staphylococcus xylosus* (29–31). In addition to PBP2a, the Fem proteins are involved in expression of methicillin resistance. The FemAB proteins contribute to the formation of the pentaglycine crossbridge, which is a unique staphylococcal cell wall component (32). Inactivation of *femAB* has been found to completely restore susceptibility to β-lactams and other antimicrobial agents in MRSA strains (33). PBPs with low affinity for β-lactams have also been detected in streptococci and enterococci (11). Homologous recombinations in the genes coding for PBPs 1a, 2a, and 2b are assumed to result in mosaic proteins with decreased affinity to β-lactams in *Streptococcus pneumoniae* and *Neisseria* spp.. PBPs, which have a low affinity to β-lactams, have been reported to be overproduced in resistant strains of *Enterococcus faecium* and *Enterococcus faecalis*. It is noteworthy that alterations in PBPs do not necessarily result in complete resistance to all β-lactams but can also lead to elevated MICs of selected β-lactam antibiotics (11).

Reduced uptake of β-lactams is due to decreased outer membrane permeability and/or the lack of certain outer membrane proteins, which serve as entries for β-lactams to the bacterial cell, and has been described in various *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and other bacteria (34–36). In *Escherichia coli* and *Klebsiella pneumoniae*, β-lactam resistance can be based on the decreased expression or the structural alteration of the porins OmpF (37) and OmpK36 (38), by which β-lactams cross the outer membrane. In *P. aeruginosa*, resistance to imipenem has been shown to be based on the loss of the porin OprD (39).

Several multidrug transporters such as the MexAB/OprM and the MexCD/OprJ systems in *P. aeruginosa*, the SmeAB/SmeC system in *Stenotrophomonas maltophilia*, and the AcrAB/TolC system in *Salmonella*

enterica and *E. coli* (40, 41) are known to mediate the export of β-lactam antibiotics.

Resistance to Tetracyclines

Among this family of antimicrobial agents, oxytetracycline, chlortetracycline, and tetracycline have been used in veterinary medicine since the 1950s. More recently, doxycycline has been approved for dogs, cats, and pigs. Up to now, minocycline and glycylcyclines have not been licensed for use in animals. Based on aggregated data from a survey on sales of veterinary antimicrobial agents in 25 European countries, the sales of tetracyclines accounted for 37% of the total sales of veterinary antimicrobial agents in 2011 (42). Thus, it is not surprising that tetracycline resistance has become widespread among bacteria of veterinary importance, including in aquaculture (43–45). Tetracycline resistance is usually due to the acquisition of new genes (46). There are 33 efflux genes, which code for energy-dependent efflux of tetracyclines, 12 ribosomal protection genes, which code for a protein that protects bacterial ribosomes, 13 genes which code for enzymes that modify and inactivate the tetracycline molecule, and 1 gene [*tet*(U)] which specifies tetracycline resistance by an unknown mechanism. The products of different *tet* genes share ≤79% amino acid identity (47). An updated database listing the currently known *tet* genes and their occurrence in various bacteria is available at http://faculty.washington.edu/marilynr/. This website is updated twice each year. New *tet* gene names are approved by Dr. Stuart B. Levy, Tufts University, Boston. Antibiotic resistance genes are not randomly distributed among bacteria. This has been well documented with the distribution of *tet* genes (47–50).

The energy-dependent efflux of tetracyclines is mediated by membrane-associated proteins which exchange a proton for a tetracycline-cation complex (46, 51). These tetracycline resistance efflux proteins are part of the major facilitator superfamily and share amino acid and protein structure similarities with other efflux proteins (12). Of the 33 efflux genes, 14 [*tet*(A) to *tet*(E), *tet*(G), *tet*(H), *tet*(J), *tet*(Y), *tet*(30), *tet*(31), *tet*(35), *tet*(41), *tet*(57)] are found only in Gram-negative genera. The remaining are found in both Gram-negative and Gram-positive genera or in bacteria of unknown source. The *tet*(B) gene has been identified in 33 Gram-negative genera, while the *tet*(L) gene has been identified in a total of 46 genera, including 24 Gram-negative and 22 Gram-positive genera (http://faculty.washington.edu/marilynr/). The Tn*10*-associated *tet*(B) gene codes for a unique efflux protein, which confers resistance to both tetracycline and minocycline but not to the new glycylcyclines (46). All the 32 other efflux proteins confer resistance to tetracycline but not to minocycline or glycylcyclines. Laboratory-derived mutations in the *tet*(A), *tet*(B), *tet*(K), and *tet*(L) genes have led to glycylcycline resistance, suggesting that bacterial resistance to tigecycline may develop over time and with clinical use (46, 52, 53). The *tet* efflux genes code for an approximately 46-kDa membrane-bound efflux protein.

The tetracycline efflux proteins present in Gram-negative bacteria commonly exhibit 12 transmembrane segments (TMSs), and upstream of the structural gene and read in the opposite direction is a specific *tet* repressor gene. Induction of the structural gene is based on the binding of a tetracycline-Mg^{2+} complex to the *tet* repressor protein which, in the absence of tetracycline, blocks transcription of the *tet* structural gene (54). The *tet*(A) (n = 25), *tet*(B) (n = 33), *tet*(C) (n = 16), *tet*(D) (n = 22), *tet*(G) (n = 16), *tet*(H) (n = 12), and *tet*(L) (n = 22) genes are most widespread among Gram-negative bacteria of human and veterinary origin, while *tet*(D) and *tet*(E) are often associated with aquaculture environments and fish (44). Their location on either transposons, such as Tn*1721* [*tet*(A)] (55), Tn*10* [*tet*(B)] (56, 57), or Tn*5706* [*tet*(H)] (58), and plasmids facilitates their spread within the Gram-negative gene pool. The Gram-positive *tet*(K) and *tet*(L) efflux genes are not regulated by repressors and confer resistance to tetracyclines, but not to minocycline. They code for proteins with 14 TMSs and are regulated by translational attenuation, which requires the presence of tetracyclines as inducers for the translation of the *tet* gene transcripts (59). These genes are generally found on small transmissible plasmids, which on occasion become integrated into the chromosome and occasionally may undergo interplasmidic recombination with other resistance plasmids (54, 59–61).

The ribosomal protection genes code for cytoplasmic proteins which protect the ribosomes from the action of tetracycline both *in vitro* and *in vivo* and confer resistance to tetracycline, doxycycline, and minocycline (46, 62). These proteins have sequence similarity to the ribosomal elongation factors EF-G and EF-Tu and are grouped in the translation factor superfamily of GTPases (63). Their interaction with the ribosome causes an allosteric disruption of the primary tetracycline binding site(s), which then leads to the release of the tetracycline from the ribosome. This allows the ribosome to return to its functional normal posttranslocational conformational state, which was altered by the binding of tetracycline. A detailed review of the various experiments conducted to elucidate the mode of

action of these proteins can be found in Connell et al. (63). The ribosomal protection genes are of Gram-positive origin and are found extensively among Gram-positive cocci. However, they have also been found in a number of Gram-negative genera. The first gene of this group, the *tet*(M) gene, has the widest host range of all *tet* genes, with 79 genera, of which 41 are Gram-positive and 38 are Gram-negative (http://faculty.washington.edu/marilynr/). This gene is located on conjugative transposons or integrative and conjugative elements, such as Tn*916* (64, 65). The other commonly found genes of both human and veterinary origin are *tet*(O) (20 Gram-positive, 18 Gram-negative genera), *tet*(Q) (eight Gram-positive, 11 Gram-negative genera), and *tet*(W) (11 Gram-positive, 22 Gram-negative genera). Recent work suggests that mutations within the *tet*(M) gene may confer increased resistance to tigecycline and thus may over time increase resistance to tigecycline in nature (52).

Enzymatic inactivation of tetracycline is mediated by 13 genes found in Gram-negative bacteria, nine of which [*tet*(47) to *tet*(55)] have recently been identified by soil functional metagenomic studies (66). The first inactivating gene described was the *tet*(X) gene (67) (which encodes an NADP-requiring oxidoreductase), which modifies and inactivates the tetracycline molecule in the presence of oxygen but was originally found only in a strict anaerobe, *Bacteroides*, where oxygen is excluded. The *tet*(X) gene has now been identified in 13 Gram-negative genera (http://faculty.washington.edu/marilynr/). This gene confers weak intrinsic resistance to tigecycline. The tigecycline activity can be improved by at least four different amino acid substitutions in the Tet(X) protein to obtain clinically relevant tigecycline resistance levels without loss of activity to other tetracyclines and was thought to be alarming for the future of tigecycline therapy (52). The gene *tet*(37) has been identified from the oral cavity of humans but is unrelated to the *tet*(X) or other genes in this class, and the function of the corresponding enzyme depends on oxygen (68). No bacterial host has been identified which carries *tet*(37). A third gene, *tet*(34), with similarities to the xanthine-guanine phosphoribosyl transferase gene of *Vibrio cholerae*, has also been identified in four Gram-negative genera (69). The recently described gene *tet*(56) has been identified in one Gram-negative genus (66), while the genes *tet*(47) to *tet*(55) have been isolated from grasslands and agricultural soils by functional genomics, where the genes were cloned into *E. coli* and shown to inactiviate tetracycline (66). With this recent study, the number of new genes coding for inactivating enzymes from the environment has greatly increased and may also be found in the future in bacteria of veterinary importance.

The *tet*(U) gene has been identified in the three Gram-positive genera: *Enterococcus*, *Staphylococcus*, and *Streptococcus*. However, it is still not clear if the gene confers tetracycline resistance in any of the bacteria it has been identified in.

A mutation in the 16S rRNA consisting of a single base exchange (1058G → 1058C) has been identified in tetracycline-resistant *Propionibacterium acnes* (70). Position 1058 is located in a region which plays an important role in the termination of peptide chain elongation as well as in the accuracy of translation.

Mutations which alter the permeability of the outer membrane porins and/or LPSs in the outer membrane can also affect resistance to tetracycline. A permeability barrier due to the reduced production of the OmpF porin, by which tetracyclines cross the outer membrane, has been described in *E. coli*. Mutations in the *marRAB* operon, which also regulates OmpF expression, may play a role in this type of tetracycline resistance (13).

Different types of multidrug transporters mediating resistance to tetracycline in addition to resistance to a number of structurally unrelated compounds have been described, for instance, in *E. coli* (EmrE), *S. enterica* (AcrAB/TolC), and *P. aeruginosa* (MexAB/OprM, MexCD/OprJ) (12, 40, 41).

Resistance to Macrolides, Lincosamides, and Streptogramins (MLS)

Several macrolide antibiotics, such as erythromycin, spiramycin, tylosin and tilmicosin, tulathromycin, gamithromycin, and tildipirosin, as well as lincosamide antibiotics, such as clindamycin, lincomycin, and pirlimycin, are approved for use in animals. Since the ban of growth promotors in the European Union, no streptogramin antibiotics are licensed for veterinary use in the European Union, but they may be used in other countries. The 16-membered macrolide antibiotics tylosin and spiramycin were previously used as feed additives for animal growth promotion but remain as therapeutics for veterinary use for the control of bacterial dysentery, respiratory disease, and mastitis. Erythromycin, the first macrolide, was introduced into clinical use over 60 years ago and has good activity against Gram-positive cocci and other Gram-positive bacteria and activity against some Gram-negative bacteria such as *Campylobacter* spp.. *Enterobacteriaceae* and *Pseudomonas* spp. have been considered to be innately nonsusceptible to erythromycin due to multidrug transporters which have 14-membered macrolides as substrates

(71). A number of Gram-negative aerobic, facultatively aerobic, and anaerobic genera carry a variety of acquired macrolide-lincosamide and/or streptogramin resistance genes (http://faculty.washington.edu/marilynr/). The data over the past 20 years clearly show that both Gram-positive and Gram-negative bacteria may become MLS resistant by acquisition of new genes normally associated with mobile elements. Acquired resistance mechanisms include specific efflux pumps, rRNA methylases that reduce binding of the antibiotic to the 50S subunit of the ribosome, or a variety of genes that inactivate the antibiotics (72–77). MLS antibiotics, though chemically distinct, are usually considered together because they share overlapping binding sites on the 50S ribosomal subunit, and a number of resistance genes confer resistance to more than one class of these antibiotics (72–74).

Target site modification occurs by rRNA methylases, which are encoded by *erm* genes. The *erm* genes were the first acquired genes that were identified to confer resistance to macrolides, lincosamides, and streptogramin B (MLS_B) antibiotics (74, 75). These genes are found in Gram-positive, Gram-negative, aerobic, and anaerobic genera. Currently, 43 rRNA methylases have been characterized. Each of these enzymes adds one or two methyl groups to a single adenine (A2058 in *E. coli*) in the 23S rRNA moiety which prevents binding of the antibiotic to the target site and thus confers MLS_B resistance to the host bacterium (http://faculty.washington.edu/marilynr; 74–76). The *erm* genes may be expressed all the time (constitutively) or inducibly via translational attenuation (77). This means that the gene is turned on in the presence of low doses of specific antibiotics (74–77); the type of expression depends on a regulatory region upstream of the *erm* gene and on which the antibiotic is able to cause induction (75, 77). In staphylococci, erythromycin and other 14- and 15-membered macrolides are able to induce *erm* gene expression, whereas 16-membered macrolides, lincosamides, and streptogramin B antibiotics are considered noninducers (77). Laboratory selection of *S. aureus* produced mutants that had structural alterations in the translational attenuator region due to deletions, tandem duplications, point mutations, and the insertion of IS*256* (78–80). Similar mutations have also been detected in naturally occurring strains carrying the *erm* genes (81).

Efflux genes include 21 ATP transporters and 5 major facilitator superfamily transporters. These genes confer a variety of resistance patterns including resistance to carbomycin, erythromycin, lincomycin, oleandomycin, spiramycin, tylosin, streptogramin A, streptogramin B, and pleuromutilins, alone or in varying combinations (http://faculty.washington.edu/marilynr; 71–74, 82). Recent work by Sharkey et al. (83) suggests that Vga(A) and Lsa(A) are ABC-F proteins, which lack transmembrane domains, not confer resistance by active efflux, but instead mediate resistance through ribosome protection (83). Further work is needed to determine if other proteins in these classes represent the same mechanism of resistance. The *vga*(A) and *vga*(B) have G+C contents of 29 to 36% and their gene products share 59% identical amino acids. The *msr*(A) gene confers inducible resistance to 14- and 15-membered macrolides and streptogramin B (MS_B) and is found in staphylococci. The hydrophylic protein made from the *msr*(A) gene contains two ATP-binding motifs characteristic of the ABC proteins (74, 83, 84, 85). The *msr*(A) gene confers lower levels of erythromycin resistance than the rRNA methylases (86). There are two groups of major facilitator superfamily transporters: one group encompasses *lmr*(A) and *lmr*(B), which code for lincomycin-specific efflux pumps, and the second group includes *mef*(A), *mef*(B) and *mef*(C) genes, which code for specific efflux pumps for 14- and 15-membered macrolides. The *mef*(A) gene was first described in the 1990s from *Streptococcus* spp. (87), but more recently it has been shown to be present in old isolates of pathogenic *Neisseria* spp. (88) and is now found in 30 different genera. It was the most common acquired macrolide resistance gene in a collection of 176 randomly collected commensal Gram-negative bacteria (89). Downstream of the *mef*(A) gene is a gene for an ABC protein that has now been shown to independently confer macrolide resistance and has been named *msr*(D) (90). In contrast, the *mef*(B) gene is found in *Escherichia* spp. and the *mef*(C) gene in *Photobacterium* spp. and *Vibrio* spp..

The 28 inactivating enzymes identified so far encode three esterases, two lyases, 16 transferases, and seven phosphorylases (http://faculty.washington.edu/marilynr; 74, 82). The esterases [Ere(A), Ere(B), and Ere(D)] hydrolyze the lactone ring of the macrolides. The esterases have been found in both Gram-negative and Gram-positive bacteria, and their genes are often associated with plasmids, though the *ere*(A) gene has been associated with both class 1 and class 2 integrons (91). The lyase gene, *vgb*(A), has been identified in the genera *Enterococcus* and *Staphylococcus*, while the *vgb*(B) gene has been identified in *Staphylococcus*. These enzymes inactivate quinupristin by opening the lactone ring (92). The newest inactivating enzymes have been identified as transferases which confer resistance by adding an acetyl group to streptogramin A, thereby inactivating the antibiotic. Sixteen genes have been found in

both Gram-positive and/or Gram-negative genera as described below (http://faculty.washington.edu/marilynr; 74, 82). The nine lincosamide nucleotidyltransferases [*lnu* genes] confer resistance to lincosamides but not to macrolides by modification and inactivation of the antibiotic. The *lnu*(A) gene has been identified in five Gram-positive genera, and the *lnu*(B) gene in four Gram-positive genera. The gene *lnu*(C) was identified in *Streptococcus* and *Haemophilus*. The gene *lnu*(E) was found in *Streptococcus* and *Enterococococcus*, while *lnu*(F) was identified in *Aeromonas*, *Comamonas*, *Desulfobacterium*, *Escherichia*, *Leclercia*, *Morganella*, *Proteus*, and *Salmonella*. The gene *lnu*(D) is associated with *Streptococcus*, *lnu*(G) with *Enterococcus*, and *lnu*(H) with *Riemerella*. Furthermore, *lnu*(P) has been identified in *Clostridium*. Seven virginiamycin *O*-acetyltransferases (*vat* genes) have been identified, six of which are associated with mobile elements in *Enterococcus*, *Lactobacillus*, *Staphylococcus*, and/or *Bacteroides*. Each gene was found in only one or two of these genera. In contrast, *vat*(F) is chromosomally encoded in *Yersinia enterocolitica*.

There are seven enzymes, encoded by *mph* genes, which confer resistance by phosphorylation of erythromycin. The *mph*(A) gene is unqiue because it confers resistance to azithromycin, while *mph*(B) and *mph*(C) confer resistance to spiramycin (93). Six phosphorylases, encoded by the genes *mph*(A), *mph*(B), *mph*(D), *mph*(E), *mph*(F), and *mph*(G), have been found exclusively in Gram-negative species. To date, the gene *mph* (A) is found in 11 genera, while *mph*(D) and *mph*(E) are each found in six genera. The *mph*(B) gene is present in four genera, while *mph*(F) is found in *Pseudomonas*. The *mph*(G) gene has been found in the fish pathogens *Photobacterium* and *Vibrio* spp. The *mph* (C) gene, which was originally characterized in *Staphylococcus* spp., has now been identified in a clinical *S. maltophilia* isolate (http://faculty.washington.edu/marilynr/).

Usually, mutational changes that affect the 23S RNA, ribosomal proteins, and/or innate efflux pumps may lead to moderate changes in susceptibility (76, 82). Various mutations have been identified in the 23S rRNA (94). Originally, mutations at either the A2058 or A2059 position (*E. coli* numbering) were found in pathogens that had one or two copies of the 23S rRNA, such as *Mycobacterium* or *Helicobacter* (95). Resistance to tylosin, erythromycin, and clindamycin in *Brachyspira hyodysenteriae* was also associated with an A → T substitution at the nucleotide position homologous with position 2058 of the *E. coli* 23S rRNA gene (96). Variations at positions 2058 and 2059 in the 23S rRNA have also been described in erythromycin-resistant *Streptococcus pyogenes*, *S. pneumoniae*, *Campylobacter coli*, *Campylobacter jejuni*, and *Haemophilus influenzae* (97, 98). An A → G substitution at position 2075 of the 23S rRNA was detected in *C. coli* from poultry and pigs which exhibited high-level erythromycin resistance (98). Mutations in ribosomal proteins L4 and/or L22 have been identified which confer elevated MICs of the newer agent telithromycin and/or of other members of the MLS group. Clinical Gram-positive bacteria have been found with the same mutations as mutants created in laboratories. Missense mutations, deletions, and/or insertions may alter the expression of innate pumps which then may alter resistance to the MLS antibiotics. A detailed discussion can be found in reference 71.

Resistance to Aminoglycosides and Aminocyclitols

Various aminoglycoside antibiotics, including gentamicin, kanamycin, amikacin, neomycin, (dihydro)streptomycin, paromomycin and framycetin, are licensed for use in both human and veterinary medicine. Among the aminocyclitol antibiotics, spectinomycin is approved for use in humans and animals, whereas apramycin is used exclusively in veterinary medicine. The main mechanism of resistance to aminoglycosides and aminocyclitols is enzymatic inactivation (99–102). In addition, reduction of the intracellular concentrations of aminoglycosides and modification of the molecular target can also result in resistance to aminoglycosides (103). Decreased intracellular concentration can result from either reduced drug uptake or from active efflux mechanisms. Chromosomal mutations conferring high-level resistance to streptomycin have also been described (13) and are the main resistance mechanism in mycobacteria.

Enzymatic inactivation of aminoglycosides and aminocyclitols is conferred by any of the three types of modifying enzymes: *N*-acetyltransferases (AACs), *O*-nucleotidyltransferases (also referred to as *O*-adenyltransferases [ANTs]), or *O*-phosphotransferases (APHs) (99–102). Acetyl-coenzyme A serves as a donor of acetyl groups in acetylation reactions at amino groups, while ATP is used for the adenylation and phosphorylation reactions at hydroxyl groups. For each of these three classes of aminoglycoside-modifying enzymes, numerous members are known which differ more or less extensively in their structure. Most modifying enzymes exhibit a narrow substrate spectrum. Several reviews have listed the known enzymes involved in modification of aminoglycosides/aminocyclitols and their molecular relationships (49, 99–102). However, new genes for aminoglycoside/aminocyclitol-inactivating enzymes

or variants of already known ones are constantly being reported. Unfortunately, a continuously updated database for the currently known aminoglycoside/aminocyclitol-inactivating enzymes is not available. Another problem is the lack of an unambiguous nomenclature. There are at least two alternatively used designations for genes coding for the same modifying enzyme: one designation, for example, *aph(3´´)-Ib*, refers to the type of modification (*aph*) and the position where the modification is introduced (*3´´*) and lists the subtype of the gene (*Ib*); the other designation, for example, *strA*, is easier to handle, refers only to the corresponding resistance phenotype (*str* for streptomycin resistance), and indicates the subtype (A).

So far, four classes of AACs are known which acetylate the amino groups at positions 1, 3, 2´, and 6´ (99–103). To date, at least 80 AACs have been identified, most of which vary in their substrate spectra. The vast majority of the AAC enzymes were identified in Gram-negative bacteria. Combined resistance to apramycin and gentamicin is due to the enzyme AAC(3)-IV; the corresponding gene emerged after the introduction of apramycin into veterinary use. It was first detected in *E. coli* and *Salmonella* from animals (104) and was found later in *E. coli* from humans as well (105–107). A gene for a bifunctional enzyme, which displays acetyltransferase AAC(6´) and phosphotransferase APH (2´´) activities, is usually found on Tn*4001*-like transposons, which are widely spread among staphylococci, streptococci, and enterococci (108–111).

To date, five classes of ANTs are categorized depending on the position of adenylation (6, 9, 4´, 2´´, and 3´´) on the aminoglycoside molecule (99–102). The ANT (2´´) and ANT(3´´) enzymes are more frequent among Gram-negative bacteria, whereas the ANT(4´), ANT (6), and ANT(9) enzymes are usually found in Gram-positive bacteria (101). The different ANT enzymes also vary considerably in their substrate spectra. Among the seven phosphotransferases [APH(2´´), APH(3´), APH (3´´), APH(4), APH(6), APH(7´´), and APH(9)] which modify the aminoglycosides at positions 2´´, 3´, 3´´, 4, 6, 7´´, and 9 (101), numerous variants have been identified which confer distinctly different resistance phenotypes. Most *aac*, *ant*, and *aph* genes are located on mobile genetic elements, such as plasmids, transposons, and gene cassettes (99–103, 112, 113).

The gene *apmA* codes for an acetyltransferase, which confers resistance to apramycin and decreased susceptibility to gentamicin. It has been detected on plasmids of variable sizes in MRSA ST398 (114–116). In staphylococci, spectinomycin resistance is mediated by the adenyltransferase genes *spc*, *spd*, and *spw* (117–121).

Multidrug efflux systems, such as MexXY in *P. aeruginosa* and AmrAB in *Burkholderia pseudomallei* (40), or the multidrug transporter AcrD in *E. coli* (122) can export aminoglycosides. The transporter MdfA from *E. coli* (123) has also been reported to mediate the efflux of the aminoglycosides kanamycin, neomycin, and hygromycin A.

Decreased uptake of aminoglycosides may be based on a mutation in lipopolysaccharide (LPS) phosphates or on a change in the charge of the LPS in *E. coli* and *P. aeruginosa*, respectively (124). Since the entry of aminoglycosides across the cytoplasmic membrane is mainly based on the electron transport system, anaerobic bacteria and facultative anaerobic bacteria exhibit relatively high insensitivity to aminoglycosides (13).

Methylation of the ribosomal target (16S rRNA) is responsible for high-level aminoglycoside resistance. It is also an emerging mechanism of great concern in clinically relevant Gram-negative bacteria. The first plasmid-mediated gene identified was the 16S rRNA methylase *armA* (125). To date, nine additional genes that encode methylases have been reported: *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rtmD2*, *rmtE*, *rmtF*, *rmtG*, and *npmA* (126). The *rmt* genes confer resistance to gentamicin and amikacin, whereas *npmA* confers resistance to gentamicin, neomycin, amikacin, and apramycin, but not to streptomycin (127).

Mutations in the gene *rpsL* for the ribosomal protein S12 have been shown to result in high-level streptomycin resistance (128). Single base-pair substitutions at different positions in the gene *rrs*, which encodes 16S rRNA in mycobacteria, have been described to be involved in either streptomycin resistance (129) or resistance to amikacin, kanamycin, gentamicin, tobramycin, and neomycin, but not to streptomycin (130). In *Mycobacterium tuberculosis*, mutations in the gene *rpsL*, which encodes the ribosomal protein S12, can cause high-level streptomycin resistance. Overexpression of the acetyltransferase-encoding gene, *eis*, has mainly been associated with resistance to kanamycin. Mutations in the *gidB* gene, which encodes a 7-methylguanosine methyltransferase, are also associated with resistance to aminoglycosides in mycobacteria. It has been suggested that loss of function of this gene confers resistance (130).

Resistance to Sulfonamides and Trimethoprim

Various sulfonamides, trimethoprim, and combinations of sulfonamides and trimethoprim are licensed for use in humans and animals. There are no restrictions on the use of any of these compounds in food animals. Sulfonamides and trimethoprim are competitive inhibi-

tors of different enzymatic steps in folate metabolism. In this regard, sulfonamides represent structural analogs of *p*-aminobenzoic acid and inhibit the enzyme dihydropteroic acid synthase (DHPS), whereas trimethoprim inhibits the enzyme dihydrofolate reductase (DHFR). Various mechanisms of intrinsic and acquired resistance to sulfonamides and trimethoprim have been described in bacteria (131–135).

Permeability barriers and efflux pumps play a relevant role by either preventing the influx or promoting the efflux of both compounds. Intrinsic resistance to both compounds in *P. aeruginosa* was initially thought to be based on outer membrane impermeability. However, the multidrug exporter system MexAB/OprM was found to be mainly responsible for resistance to sulfonamides and trimethoprim in *P. aeruginosa* (136). For other bacteria, such as *K. pneumoniae* and *Serratia marcescens*, impaired membrane permeability is still considered to play a role in sulfonamide and trimethoprim resistance (132, 133).

Naturally insensitive DHFR enzymes and folate auxotrophy play an important role in intrinsic resistance to sulfonamides and trimethoprim. DHFR enzymes which exhibit low affinity for trimethoprim and thus render their hosts intrinsically resistant to trimethoprim are known to occur in several bacterial genera including *Clostridium*, *Neisseria*, *Brucella*, *Bacteroides*, and *Moraxella* (13). Bacteria such as enterococci and lactobacilli which can utilize exogenous folates also show intrinsic resistance to trimethoprim and sulfonamides.

Mutational or recombinational changes in the target enzymes have been observed in a wide variety of bacteria. Mutations in the chromosomal *dhps* gene that lead to sulfonamide resistance by single amino acid substitutions can be generated under *in vitro* conditions but also occur *in vivo*. Such mutations have been identified in *E. coli*, *S. aureus*, *Staphylococcus haemolyticus*, *C. jejuni*, and *Helicobacter pylori* (132). In *S. pneumoniae*, two amino acid duplications which change the tertiary structure of the DHPS have been found to be responsible for sulfonamide resistance (137). Recombinational events between the naturally occurring gene coding for a susceptible DHPS and that of a horizontally acquired resistant DHPS are believed to account for sulfonamide resistance in *Neisseria meningitidis* (132). Trimethoprim resistance has also been shown to be due to a single amino acid substitution in the DHFR protein in *S. aureus* (138) and *S. pneumoniae* (139). Mutations in the promoter region of chromosomal *dhfr* genes have been described to occur in *E. coli* and resulted in overexpression of the trimethoprim-susceptible DHFR (132). Mutations in both the promoter region and the *dhfr* gene have been identified in trimethoprim-resistant *H. influenzae* (140).

The replacement of sensitive enzymes by resistant enzymes usually causes high-level resistance (131–135). To date, three types of resistant DHPS enzymes encoded by the genes *sul1*, *sul2*, and *sul3* have been described to occur in Gram-negative bacteria (141–144). The gene *sul1* is part of class 1 integrons and thus is often associated with other resistance genes. As part of transposons, such as Tn*21*, and conjugative plasmids, it is spread into various Gram-negative species and genera (141, 143). The *sul2* gene often occurs together with the Tn*5393*-associated streptomycin resistance genes *strA-strB* on conjugative or nonconjugative plasmids (142, 143). The gene *sul3* was originally found on a conjugative plasmid from porcine *E. coli*, where it was flanked by copies of the insertion sequence IS*15/26* (144). Meanwhile, it has also been identified in *E. coli* from humans and animals other than pigs, as well as in *S. enterica* from animal and food sources (144–147).

More than 40 DHFR (*dfr*, formerly also referred to as *dhfr*) genes have been identified. The genes occurring in Gram-negative bacteria are subdivided on the basis of their structure into two major groups, *dfrA* and *dfrB* (148). The 33 *dfrA* genes code for DHFR enzymes of 152 to 189 amino acids (aa), whereas the eight *dfrB*-encoded DHFR enzymes consist of only 78 aa. The *dfrA* genes have been detected more frequently than the *dfrB* genes. Additionally, there are *dfr* gene groups in Gram-positive bacteria that currently consist of only one gene each. The gene *dfrG* codes for an enzyme of 165 aa and has been detected in the chromosome of *S. aureus* (149). In *S. haemolyticus* and *Listeria monocytogenes*, the gene *dfrD*, which codes for an enzyme of 162 aa, has been identified on plasmids (150, 151). The gene for the 163-aa DHFR DfrK was first detected on plasmids in *S. aureus* and linked to the *tet*(L) gene (152). Since then, *dfrK* has been found as part of transposon Tn*559* in the chromosomal DNA of staphylococci and enterococci (153, 154) and on small plasmids from *Staphylococcus hyicus* that confer only trimethoprim resistance (115). In staphylococci, the composite transposon Tn*4003* has been identified on various multiresistance plasmids. Tn*4003* is composed of a central *dfrA* gene (also known as *dfrS1*) bracketed by copies of the insertion sequence IS*257* (155).

Transferable trimethoprim resistance genes have been identified in a wide variety of Gram-negative bacteria; several of these genes are part of plasmids, transposons, or gene cassettes (112, 133, 135, 156) and thus are easily disseminated across species and genus borders.

Several studies showed the relationships between the *dfr* genes (133, 135, 156).

Resistance to Quinolones and Fluoroquinolones

Quinolones and fluoroquinolones are potent inhibitors of bacterial DNA replication. While early quinolones such as nalidixic acid and pipemidic acid have not been used in veterinary medicine, oxolinic acid and flumequine (the first fluorinated quinolone) have been used in food-producing animals, including fish, worldwide (1, 157). Since the first of the newer fluoroquinolones, enrofloxacin, was licensed for use in animals in the late 1980s (158), several other fluoroquinolones have been approved for veterinary use in recent years, including marbofloxacin, orbifloxacin, difloxacin, ibafloxacin, danofloxacin, and pradofloxacin. Two major mechanisms account for resistance to fluoroquinolones: mutations in the genes for DNA topoisomerases and decreased intracellular drug accumulation (159–163). In addition, plasmid-mediated (fluoro)quinolone resistance genes have been described in the past decade (163).

Mutational alteration of the target genes *gyrA* and *gyrB* (coding for the A and B subunits of the DNA gyrase) as well as *parC* and *parE* (coding for A and B subunits of the DNA topoisomerase IV) is frequently seen in (fluoro)quinolone-resistant bacteria. Both enzymes are tetramers consisting of two A and B units. The mutations in *gyrA* are commonly located within a region of ca. 130 bp which is referred to as the "quinolone resistance-determining region" (164). Mutations resulting in changes of Ser-83 (to Tyr, Phe, or Ala) and Asp-87 (to Gly, Asn, or Tyr) have been detected most frequently. In addition, double mutations at both positions and various other mutations have been described in Gram-positive and Gram-negative bacteria of human and veterinary importance (157, 160–162, 165). Stepwise mutations in *gyrA* and *parC* can result in an incremental increase in resistance to quinolones (157). Moreover, various mutations may also have different effects on resistance to the various fluoroquinolones (166). The complex interplay between individual mechanisms may also have different effects on fluoroquinolone resistance (167).

Multidrug efflux systems also conferring fluoroquinolone resistance have been identified in various Gram-positive and Gram-negative bacteria, such as *P. aeruginosa* (MexAB/OprM, MexCD/OprJ), *S. aureus* (NorA), *S. pneumoniae* (PmrA), *Bacillus subtilis* (Blt), *E. coli*, and *S. enterica* (AcrAB/TolC); for reviews see references 162, 168, and 169. Since the basal level of expression of these efflux systems is low, upregulation of their expression is required to confer resistance to fluoroquinolones and other antimicrobials. In *E. coli* the level of production of the AcrAB-TolC efflux system is under the control of several regulatory genes, in particular the global regulatory systems *marRAB* and *soxRS*, but also *acrR* (168–171). Mutations in these regulatory systems may lead to overproduction of the AcrAB-TolC efflux pump and expression of the multidrug resistance phenotype (172, 173). Besides overproduction of the AcrAB-TolC efflux pump, it has been recently shown using macroarrays that *E. coli* strains constitutively expressing *marA* showed altered expression of more than 60 chromosomal genes (174).

Interplay between several resistance mechanisms may lead to high-level resistance to quinolones and to other antibiotics when multidrug efflux pumps and decreased outer membrane permeability are involved (167, 175). For *in vitro* selected quinolone-resistant *E. coli* mutants, it has been shown that first-step quinolone-resistant mutants acquire a *gyrA* mutation. Second-step mutants reproducibly acquire a multidrug resistance phenotype and show enhanced fluoroquinolone efflux. In some third-step mutants, fluoroquinolone efflux is further enhanced and additional topoisomerase mutations are acquired. In clinical *E. coli* isolates from humans and animals, the situation appears to be the same, where high-level fluoroquinolone resistance is reached when mutations at several chromosomal loci are acquired (167, 175). It is noteworthy that inactivation of the AcrAB efflux pump renders resistant *E. coli* strains, including those with target gene mutations, hypersusceptible to fluoroquinolones and certain other unrelated drugs (175). Thus, in the absence of the AcrAB efflux pump, gyrase mutations fail to produce clinically relevant levels of fluoroquinolone resistance (176). The same observation has been made for *P. aeruginosa*, in which deletion of the MexAB-OprM efflux pump, which is the homolog of the AcrAB-TolC efflux pump in this species, resulted in a significant decrease in resistance to fluoroquinolones even for strains carrying target gene mutations (177). In high-level fluoroquinolone-resistant *S. enterica* serovar Typhimurium DT204 strains, carrying multiple target gene mutations in *gyrA*, *gyrB*, and *parC*, inactivation of AcrB or TolC resulted in a 16- to 32-fold decrease of resistance levels to fluoroquinolones (178, 179).

Decreased drug uptake in Gram-negative bacteria is due to the *marRAB*-mediated downregulation of OmpF porin production. OmpF is an important porin for the entry of quinolones and fluoroquinolones into the bacterial cell (180, 181). Moreover, mutations in different gene loci (*cfxB*, *norB*, *nfxB*, *norC*, or *nalB*) are also associated with decreased permeability (182, 183).

Plasmid-mediated quinolone resistance mechanisms have been described in recent years in addition to the aforementioned chromosomal resistance mechanisms (184–186). These plasmid-mediated quinolone resistance mechanisms usually confer low-level (fluoro) quinolone resistance by (i) target protection, (ii) acetylation, and (iii) efflux pumps. The protection of the DNA gyrase is mediated by *qnr* genes. Six *qnr* gene families (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qnrVC*) with multiple alleles per gene have been described and are organized in a database (www.lahey.org/qnrStudies). The *qnr* genes are associated with several mobile genetic elements and located on plasmids of varying sizes and different incompatibility groups (185, 186). The gene *aac(6′)-Ib-cr* codes for an aminoglycoside acetyltransferase, which is able to acetylate the amino nitrogen on the piperazinyl ring of quinolones such as ciprofloxacin and norfloxacin (185, 186). Efflux pumps are encoded by the genes *qepA* and *oqxAB*. The OqxAB efflux pump has a wide substrate specificity and is found not only on plasmids but also in the chromosomal DNA. These plasmid-mediated resistance genes have been found in several Gram-negative bacteria. The plasmid-located gene for an efflux pump *qacBIII* is able to confer decreased susceptibility to ciprofloxacin and norfloxacin in *S. aureus* and is the first plasmid-mediated quinolone resistance gene in Gram-positive bacteria (185, 186).

Resistance to Phenicols

Two members of the phenicols, chloramphenicol and its fluorinated derivative florfenicol, are currently approved for use in animals. The predominant mechanism of chloramphenicol resistance in Gram-positive and Gram-negative bacteria is enzymatic inactivation (187–189). In addition, efflux systems that mediate either resistance to only chloramphenicol or combined resistance to chloramphenicol and florfenicol have also been identified (188). Furthermore, permeability barriers and multidrug transporters play a role in certain Gram-negative bacteria (8, 12, 188, 190). Detailed reviews on the different genes and mechanisms accounting for bacterial resistance to chloramphenicol and florfenicol have been published (48, 188).

Enzymatic inactivation of chloramphenicol is commonly achieved by chloramphenicol acetyltransferases (CATs) which transfer acetyl groups from acetyl-CoA to the C3 position of the chloramphenicol molecule. Subsequent transfer of the acetyl group to the C1 position and transfer of a second acetyl group to C3 results in mono- or diacetylated chloramphenicol derivatives, both of which are unable to inhibit bacterial protein biosynthesis (187–189). Two distinct types of CAT enzymes, which differ in their structures, are known: the classical CATs (type A) and a novel type of CAT (type B) (187, 188). All type A and type B CATs have a trimeric structure composed of three identical monomers. The *cat* gene codes for a CAT monomer, the size of which varies between 207 and 238 aa (type A CATs) and 209 and 212 aa (type B CATs) (188). Using the cutoff as set for the classification of tetracycline and MLS resistance genes (47, 74), 16 classes of *catA* determinants and at least another five classes of *catB* determinants can be differentiated (48). Among the *catA* genes, those formerly referred to as *catI*, *catII*, and *catIII* are most widespread among Gram-negative bacteria (48, 191–193). They are associated with either nonconjugative transposons such as Tn*9* or plasmids. Expression of these *catA* genes is constitutive. Various *catA* genes, indistinguishable from or closely related to those present on the *S. aureus* plasmids pC221, pC223/pSCS7, and pC194 (48, 193–196), have been detected in coagulase-positive and -negative staphylococci, but also in members of the genera *Streptococcus*, *Bacillus*, and *Listeria*, respectively. Expression of these mostly plasmid-borne *catA* genes is inducible by chloramphenicol via translational attenuation (197), whereas the Tn*4451*-borne *catA* genes of *Clostridium* spp. are expressed constitutively (198). The *catB* genes—also referred to as *xat* (xenobiotic acetyltransferase) genes—differ distinctly from the *catA* genes but are related to acetyltransferase genes, such as *vat*(A-E), involved in streptogramin resistance (187). Some of the *catB* genes have been found exclusively on the chromosome of either *Agrobacterium tumefaciens*, *P. aeruginosa*, or *V. cholerae*, whereas others proved to be part of transposons (Tn*2424*, Tn*840*) or plasmid-borne integrons. Studies of the level of *catB*-mediated chloramphenicol resistance revealed a distinctly lower level of chloramphenicol resistance compared to that conferred by type A CATs (187).

In addition to inactivation via CATs, enzymatic inactivation of chloramphenicol can also occur by *O*-phosphorylation or by hydrolytic degradation to *p*-nitrophenylserinol (48). Since these mechanisms have so far only been seen in the chloramphenicol producer *Streptomyces venezuelae* and in a soil metagenome library, they are believed to play a role as self-defense mechanisms (48, 188).

A total of 11 classes of specific exporters which mediate either chloramphenicol or chloramphenicol/florfenicol resistance have been identified (48, 188). Among them, seven classes are represented by 10- to 12-TMS chloramphenicol exporters of soil bacteria of

the genera *Streptomyces*, *Rhodococcus*, and *Corynebacterium* or of bacteria of unknown origin, whereas four classes of 12-TMS exporters were found among Gram-negative bacteria of medical importance (188, 199). Among these latter classes, one class represents the *cmlA* subgroup, and the others represent the *floR* subgroup. The gene *cmlA*, which codes for a chloramphenicol exporter, is a Tn*1696*-associated cassette-borne gene which, however, is inducibly expressed via translational attenuation (200). Genes related to *cmlA* are mainly found in *Enterobacteriaceae* and *Pseudomonas*. Genes related to *floR* have been identified in *Photobacterium*, *Vibrio*, *Klebsiella*, *E. coli*, and various *S. enterica* serovars and in *Pasteurella multocida* as part of the chromosomally located ICE*Pmu1* (48, 201–208). In *Vibrio* and *Salmonella*, the gene *floR* has been detected as part of chromosomal multiresistance gene clusters (207, 209), and in *E. coli*, as part of conjugative and nonconjugative multiresistance plasmids (201, 202). In *S. maltophilia* of porcine origin, a novel *floR* variant, $floR_V$, has been identified as part of a chromosomal genomic island (210). Another class of phenicol exporters is represented by FexA, the first specific chloramphenicol/florfenicol exporter of Gram-positive bacteria (211). The gene *fexA*, located on the transposon Tn*558* (212) from *Staphylococcus lentus*, codes for a 14-TMS exporter of the major facilitator superfamily and is expressed inducibly via translational attenuation. A second phenicol exporter, FexB, which exhibited 56.1% amino acid identity with the FexA protein, has been exclusively identified in enterococci (213).

Multidrug transporter systems that export chloramphenicol have been described to occur in several Gram-negative bacteria, including the systems MexAB/OprM and MexCD/OprJ in *P. aeruginosa*, AcrAB/TolC in *E. coli* and *S. enterica*, CeoAB/OpcM in *Burkholderia cepacia*, and ArpAB/ArpC and TtgAB/TtgC in *Pseudomonas putida* (40, 181).

Permeability barriers based on the reduced expression of the OmpF porin in *S. enterica* serovar Typhi or a major outer membrane protein in *H. influenzae* (188) have also been described to confer chloramphenicol resistance. The *mar* locus which is found in various *Enterobacteriaceae* can contribute to chloramphenicol resistance in two ways: on one hand, it can activate the AcrAB/TolC efflux system, leading to increased efflux of chloramphenicol, and on the other hand, MarA can activate the gene *micF*, whose transcripts represent an antisense RNA that effectively inhibits translation of *ompF* transcripts, which results in a decreased influx of chloramphenicol (180, 181).

Mutations in the major ribosomal protein clusters of *E. coli* and *B. subtilis*, but also mutations in the 23S rRNA of *E. coli*, have been described to mediate chloramphenicol resistance (214).

Resistance to Oxazolidinones

Oxazolidinones are a class of synthetic antibiotics that are highly active against Gram-positive bacteria. Currently, two oxazolidinones, linezolid and tedizolid, are exclusively approved for use in human medicine and are considered last-resort antimicrobial agents for the treatment of infections caused by MRSA, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*.

Initially, point mutations within either the 23S rRNA and/or the genes coding for the ribosomal proteins L3 (*rplC*), L4 (*rplD*), and L22 (*rplV*) were recognized as the main mechanisms of reduced oxazolidinone susceptibility (215–217). Mutations in clinical staphylococcal and enterococcal isolates, including G2247T, T2500A, A2503G, T2504C, G2505A, and G2576T, usually were found in the vicinity of the peptidyltransferase center (217). Mutations in the gene *rplC*, including F147L and A157R, resulted in at least 2-fold increases of the oxazolidinone MICs of laboratory and clinical staphylococci (218). The mutations in the *rplD* gene, which resulted in amino acid exchanges K68N or K68Q, and the insertions $_{71}$GGR$_{72}$, $_{65}$WR$_{66}$, and $_{68}$KG$_{69}$ lead to oxazolidinone resistance in *S. pneumoniae* (219). Little is known about the effects of L22 mutations on linezolid resistance; whether the amino acid exchange A29V in L22 detected in two linezolid-resistant MRSA isolates is responsible for the linezolid resistance in these isolates remains to be investigated (220).

The gene *cfr* from *S. sciuri* was the first transferable oxazolidinone resistance gene (221). Initially described as a novel chloramphenicol/florfenicol resistance gene, the elucidation of the resistance mechanism revealed that *cfr* codes for an rRNA methylase, which confers resistance not only to phenicols but also to lincosamides, oxazolidinones, pleuromutilins, and streptogramin A by methylating the adenine residue at position 2503 in 23S rRNA, which is located in the overlapping ribosomal binding site of these antibiotics (222, 223). The gene *cfr* is mainly located on plasmids in staphylococci and has been distributed across species and genus boundaries. In recent years, it has been detected in both Gram-positive and Gram-negative genera, including *Bacillus*, *Enterococcus*, *Escherichia*, *Jeotgalicoccus*, *Macrococcus*, *Staphylococcus*, *Streptococcus*, and *Proteus* (48, 224, 225). Recently, variants of the *cfr* gene have been identified, including *cfr*(B) in *E. faecium* and

Clostridium difficile (226, 227) and *cfr*(C) in *Campylobacter* and *Clostridium* (228).

An ABC-F protein, which is able to mediate resistance to chloramphenicol and florfenicol as well as the oxazolidinones linezolid and tedizolid, is encoded by the gene *optrA*, which has been identified on a conjugative plasmid in *E. faecalis* (229). The insertion sequence IS*1216E* and the transposon Tn*558* have been identified in the *optrA* flanking regions on plasmids and on the chromosome of enterococci from humans, pigs, and chickens (230). Moreover, the gene *optrA* has also been detected on plasmids and in the chromosomal DNA of porcine *S. sciuri* (231, 232). Since then, this gene has been identified in genomes of clinical isolates of staphylococci, enterococci, and streptococci (233).

Resistance to Glycopeptides

Since the ban of the growth promotor avoparcin in 1996, no glycopeptide antibiotics are approved for use in animals. Glycopeptide antibiotics, such as vancomycin and teicoplanin, act by binding to the D-alanine-D-alanine termini of peptidoglycan precursors, thereby preventing transglycosylation and transpeptidation of the bacterial cell wall (234, 235).

Modification of the target site is the common mechanism of bacterial resistance to glycopeptides. So far, four D-Ala-D-Lac operons (*vanA*, *vanB*, *vanD*, and *vanM*) and five D-Ala-D-Ser operons (*vanC*, *vanE*, *vanG*, *vanL*, and *vanN*) have been described in enterococci (236). They differ in their levels of resistance to vancomycin and teicoplanin (235). In the D-Ala-D-Lac operons, the terminal dipeptide D-alanine-D-alanine is replaced by D-alanine-D-lactate, whereas in the D-Ala-D-Ser operons, it is replaced by D-alanine-D-serine. These replacements reduce the ability of glycopeptides to bind to the peptidoglycan precursors and result in the case of D-lactate in high-level and in the case of D-serine in low-level glycopeptide resistance.

The VanC operon is responsible for the intrinsic resistance of *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens* to glycopeptides (235). Similar to VanC, VanD and VanE, both from *E. faecalis*, have been reported not to be transferable (235). In contrast, the *vanA* and *vanB* operons are associated with transposons which can be located on conjugative and nonconjugative plasmids in enterococci. The VanA phenotype is associated with the nonconjugative transposon Tn*1546*, which contains a total of nine reading frames, five of which are essential for high-level glycopeptide resistance (237). Among these, the two genes *vanR* and *vanS* code for a response regulator protein and a sensor protein, respectively, involved in regulatory processes. Three genes are directly involved in resistance: *vanH*, *vanA*, and *vanX*. The gene *vanH* codes for a cytoplasmatic dehydrogenase that produces D-lactate from pyruvate, whereas the gene *vanX* codes for a D,D-dipeptidase which cleaves the D-alanine-D-alanine, and the gene *vanA* codes for a ligase that joins the remaining D-alanine with D-lactate. While glycopeptide resistance is often found in enterococci (238, 239), transfer studies showed that conjugative transfer of VanA-mediated vancomycin resistance from *E. faecalis* to *S. aureus* is possible under *in vitro* conditions (240). In 2002, the first patients infected with *vanA*-carrying high-level vancomycin-resistant *S. aureus* isolates were detected in the United States (241). Genes homologous to enterococcal glycopeptide resistance genes *vanA* and *vanB* have also been detected among members of the genera *Paenibacillus* and *Rhodococcus* (242). Moreover, a new glycopeptide resistance operon, *vanOHX*, has recently been identified in *Rhodococcus equi* (236).

Impaired membrane permeability renders Gram-negative bacteria intrinsically resistant to glycopeptides, large molecules which can cross the outer membrane only poorly, if at all (235).

Resistance to Pleuromutilins

The pleuromutilins tiamulin and valnemulin are mainly used in veterinary medicine for the control and specific therapy of gastrointestinal and respiratory tract infections in swine and to a lesser extent in poultry and rabbits. Retapamulin is used as an ointment to treat bacterial skin infections in humans. Products for systemic use in humans with infections caused by multidrug-resistant bacteria are currently being developed.

The main target bacteria in veterinary medicine are *B. hyodysenteriae*, *Brachyspira pilosicoli*, *Lawsonia intracellularis*, and *Mycoplasma* spp. Resistance derives from chromosomal mutations in the 23S rRNA and *rplC* genes or mobile resistance genes located on plasmids or transposons, such as the *cfr* genes and certain *vga*, *lsa*, and *sal* genes (243, 244). The mechanism of resistance varies among bacterial species.

In *B. hyodysenteriae*, reduced susceptibility to tiamulin has been associated with point mutations in the V domain of the 23S rRNA gene (positions 2032, 2055, 2447, 2499, 2504, and 2572 in *E. coli* numbering) and/or the ribosomal protein L3 gene (245, 246). Mutation at nucleotide position 2032 appears to be related to pleuromutilin resistance and to decreased susceptibility to lincosamides (246). Tiamulin resistance in *B. hyodysenteriae* develops in a stepwise manner both *in vitro* and *in vivo*, suggesting that multiple mutations

are needed to achieve high levels of resistance. The MICs of valnemulin are generally a few dilution steps lower than those of tiamulin (247). To date, data on the resistance mechanisms of *B. pilosicoli* and *L. intracellularis* are lacking, and data on resistance mechanisms of mycoplasmata are limited. A single mutation of the 23S rRNA gene caused elevated tiamulin and valnemulin MICs in *Mycoplasma gallisepticum,* but combinations of two or three mutations were necessary to produce high levels of resistance to these drugs (248).

Resistance in staphylococci can be due to point mutations in the V domain of 23S rRNA or in the *rplC* gene, encoding the ribosomal protein L3 (249). Transferable resistance in staphylococci can be caused by *vga* genes, encoding ABC transporters or ABC-F proteins, resulting in resistance to pleuromutilins, streptogramin A, and lincosamides. There are several *vga* genes which confer pleuromutilin resistance in addition to lincosamide and streptogramin A resistance: *vga*(A) and its variants, *vga*(C), and *vga*(E) and its variant. All these genes have been found on plasmids and transposons of staphylococci (243). Transferable resistance to five classes of antimicrobials (phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A) in staphylococci is mediated by the gene *cfr*. The *sal*(A) gene from *S. sciuri*, also encoding a putative ABC-F protein, has been shown to mediate combined resistance to lincosamides, pleuromutilins, and streptogramin A antibiotics (244).

E. faecalis is intrinsically resistant to pleuromutilins, streptogramin A antibiotics, and lincosamides by the production of the ABC-F protein Lsa(A). In *E. faecium*, acquired resistance to the above-mentioned antimicrobials is mediated by the gene $eat(A)_V$ which may encode a putative ABC-F protein (83, 250). The enterococcal gene *lsa*(E), which may also code for a putative ABC-F protein (83), has been detected in methicillin-susceptible *S. aureus* and in MRSA of human and animal origin (251).

Resistance to Polypeptide Antibiotics

There are three polypeptide antibiotics that are used in human and/or veterinary medicine: bacitracin, polymyxin B, and colistin (polymyxin E). Bacitracin inhibits cell wall synthesis and is active against Gram-positive bacteria. It used to be used as a growth promoter (252). Since the ban of antimicrobial growth promoters in 2006 in the European Union, it is no longer approved for veterinary use as growth promotor in the EU. In China, colistin has also been banned from use as growth promoter in food animals, as of April 2017. However, it is used for that purpose in other countries, and it is still approved for therapeutic purposes in the European Union and China. Polymyxin B and colistin (polymyxin E) disrupt the outer bacterial cell membrane of certain Gram-negative bacteria, such as most *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter baumannii*, whereas other Gram-negative bacteria, including *Proteus* spp., *Providencia* spp., *Morganella morganii*, *Serratia* spp., *Edwardsiella tarda*, and bacteria of the *B. cepacia* complex exhibit intrinsic resistance to these polypeptide antibiotics. Recently, colistin was included in the WHO list of critically important antibiotics (253). In human medicine, colistin is used as a last-line drug in the treatment of severe infections caused by multiresistant Gram-negative bacteria, whereas it is used in veterinary medicine for the treatment of enteric diseases, mainly in swine and poultry (254). So far, several mechanisms of resistance to polymyxins (polymyxin B and colistin) have been described (255). These include a variety of LPS modifications, such as modifications of lipid A with phosphoethanolamine and 4-amino-4-deoxy-L-arabinose, efflux pumps, the formation of capsules, and overexpression of the outer membrane protein OprH (255). Such resistance is chromosomally encoded, and hence spread entailed either *de novo* emergence or clonal expansion of resistant isolates.

The alteration of the LPS on its lipid A moiety is the primary mechanism of resistance to polycationic polymyxins. 4-Amino-4-deoxy-L-arabinose (L-Ara4N) or phosphoethanolamine is added to lipid A by enzymes such as ArnT and EptA, resulting in a decrease of the negative charge of the LPS, thereby lowering the affinity of the positively charged polymyxins to the outer membrane. The genes *arnT* (part of the operon *arnBCADTEF*) and *eptA*, which code for those enzymes, are controlled by chromosomally encoded two-component regulatory systems, such as PmrAB and PhoPQ. Mutations in the operons *pmrAB* and *phoPQ* may lead to an upregulation of *arnT* and *eptA* expression, resulting in polymyxin resistance. This has been described in *E. coli*, *K. pneumoniae*, *S. enterica*, and *P. aeruginosa* (255). Furthermore, mutations in the gene for the negative feedback regulator of PhoPQ, *mgrB*, can activate the *arnBCADTEF* operon in *K. pneumoniae* (256). Other genes coding for phosphoethanolamine transferases are *eptB* in *E. coli*, *eptC* in *C. jejuni*, and *lptA* in *N. meningitidis*. Those phosphoethanolamine transferases add phosphoethanolamine to different positions on lipid A. The main polymyxin resistance mechanism in *A. baumannii* is mutations in the genes of the PmrAB system and the resulting overexpression of *pmrC*, which codes for an EptaA-like phosphoethanolamine transferase. In

P. aeruginosa five two-component regulatory systems (PhoPQ, PmrAB, ParRS, CprRS, ColRS) have been identified, and mutations in the genes for those systems play a role in overexpression of the *arnBCADTEF-ugd* operon (256).

In addition, plasmid-mediated resistance to polymyxins was reported in 2016 (257). The gene *mcr-1* has been identified on a conjugative plasmid in *E. coli* of animal and human origin and codes for a phosphoethanolamine transferase. The *mcr-1* gene was first identified among isolates from China, but since then it has been detected in isolates of various *Enterobacteriaceae* from five continents (254). Furthermore, the gene *mcr-2* has been described in porcine and bovine *E. coli* isolates from Belgium (258). Most recently, another three *mcr* genes, designated *mcr-3* (259), *mcr-4* (260), and *mcr-5* (261), have been identified in *E. coli* and/or *S. enterica*. Several variants of *mcr-1* have been detected in *Enterobacteriaceae* (259), while *mcr-2* variants have been found in *Moraxella* spp. (262). Variants of the gene *mcr-3* have been identified in *Aeromonas* spp. (263, 264).

Polymyxin resistance due to the complete loss of LPS in *A. baumannii* is based on the inactivation of genes such as *lpxA*, *lpxC*, *lpxD*, and *lpsB*, the products of which are involved in LPS biosynthesis (265).

A variety of efflux pumps in several bacterial species have been described to be involved in polymyxin resistance in Gram-negative bacteria. Sensitive antimicrobial peptide proteins, encoded by the *sapABCDF* operon, and the resistance-nodulation-cell division transporter AcrAB-TolC seem to play a role in the susceptibility to polymyxins in *E. coli*, *S. enterica*, and *Proteus mirabilis* (266). The resistance-nodulation-cell division transporter VexB is involved in polymyxin resistance in *V. cholerae* (267). The efflux pump KpnEF, which has been described in isolates of *K. pneumoniae*, belongs to the small multidrug resistance protein family and is part of the Cpx regulon, which regulates capsule synthesis. Resistance to several antibiotics such as colistin, rifampicin, erythromycin, and ceftriaxone is influenced by KpnEF (267). Other efflux pumps of the small multidrug resistance protein family involved in polymyxin resistance have been identified in *B. subtilis* (EbrAB) and in *A. baumannii* (AbeS) (266).

Capsule formation is another mechanism of polymyxin resistance. Capsule polysaccharides limit the interaction of polymyxins with their target sites, thus playing an important role in polymyxin resistance, not only in intrinsically resistant bacteria such as *N. meningitidis* and *C. jejuni*, but also in *K. pneumoniae*, *E. coli*, and *P. aeruginosa* (268, 269). Moreover, anionic bacterial capsule polysaccharides have been shown to neutralize the bactericidal activity of cationic polypeptide antibiotics and antimicrobial peptides (269).

Overexpression of the outer membrane protein OprH contributes to polymyxin resistance in *P. aeruginosa*. OprH is a basic protein that binds to divalent cation-binding sites of LPSs, making these sites unavailable for polymyxins (270).

Resistance to Mupirocin

Mupirocin is a topical antibiotic used mainly for decolonization of MRSA and methicillin-susceptible *S. aureus* in patients and in health care personnel, but also for treatment of local skin and soft tissue infections caused by *S. aureus* and streptococci (271). It is not approved for veterinary use. Mupirocin prevents bacterial protein synthesis by inhibiting the bacterial isoleucyl-tRNA synthetase. Low-level resistance against mupirocin results from point mutations in the native isoleucyl-tRNA synthetase gene, whereas the acquisition of genes coding for alternative isoleucyl-tRNA synthetases leads to high-level resistance. The gene *mupA* (also referred to as *ileS2*) has been identified on conjugative plasmids (272–274), while the gene *mupB* has been found on nonconjugative plasmids in staphylococci (274, 275).

Resistance to Ansamycins

Certain ansamycins, such as rifampicin and rifamycin, are used in veterinary medicine for the treatment of infections of horses caused by *R. equi*. Rifampicin inhibits the bacterial RNA polymerase by interacting with the β-subunit, which is encoded by the gene *rpoB*. Resistance to rifampicin and related compounds is mainly due to point mutations that cause amino acid substitutions in at least one of three rifampicin resistance-determining regions within the *rpoB* gene. It is noteworthy that not all amino acid substitutions have the same effect. In *R. equi*, mutations at different positions within the *rpoB* gene have been shown to correlate with different rifampicin MIC values (276, 277). Mutations of *rpoB* have been described in several bacteria, such as *R. equi* (276, 277), *E. coli* (278), *Mycobacterium* spp. (279), *B. subtilis* (280), *S. aureus* (281), and *S. pseudintermedius* (282).

Furthermore, RNA polymerase binding proteins, such as RbpA in *Streptomyces coelicolor* and DnaA in *E. coli*, play a role in increased insensitivity to rifampicin (283).

Arr enzymes are ADP-ribosyltransferases, which are able to modify rifampicin by ADP-ribosylation and thus inactivate it. *M. smegmatis* carries the *arr* gene in its chromosomal DNA (284), homologues of which

have been identified in the genomes of bacteria such as *S. maltophilia*, *Burkholderia cenocepacia*, and other environmental bacteria (284). The gene *arr-2* has been identified in chromosomal DNA and on various plasmids as part of class 1 integrons or composite transposons in Gram-negative bacteria such as *P. aeruginosa*, *K. pneumoniae*, and *E. coli* (283). An *arr-3* gene was detected in a class 1 integron of *Aeromonas hydrophila* from a koi carp (285).

Other modification mechanisms are glucosylation and phosphorylation in *Nocardia* spp. and phosphorylation in *Bacillus* spp. (283).

Resistance to Fosfomycin

Fosfomycin interferes with the peptidoglycan synthesis of bacteria by inhibiting the enzyme MurA. The irreversible inhibition is due to alkylation of the catalytic cysteine of MurA (286, 287). The exchange of cystein for asparagine is a target alteration, leading to intrinsic fosfomycin resistance in bacteria such as *M. tuberculosis*, *Chlamydia trachomatis*, and *Borrelia burgdorferi* (286, 287). Fosfomycin reaches its target site through GlpT, a glycerol-3-phosphate transporter, or via UhpT, a glucose-6-phosphate transporter. Both substrates induce the expression of their transporter, which is regulated by cAMP. Mutations in the genes coding for GlpT and UhpT or their regulators may lead to defective or inactive transporters, resulting in fosfomycin resistance (286, 287).

Enzymatic inactivation of fosfomycin can be achieved by several fosfomycin-modifying enzymes. The main enzymes described are three types of metalloenzymes (FosA, FosB, and FosX) and two kinases (FomA and FomB). FosA and FosB are thiol transferases, while FosX is a hydrolase. The metalloenzymes open the oxirane ring of fosfomycin and thus render it inactive. FosA enzymes are glutathione-*S*-transferases which use Mn^{2+} and K^{+} as metal cofactors. They add glutathione to the oxirane ring, thereby opening the ring and inactivating fosfomycin (281). Various *fosA* genes have been identified on plasmids or in the chromosomal DNA of Gram-negative bacteria such as *S. marcescens*, *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa* (286, 287). They have been described as parts of transposons such as Tn*2921* or flanked by copies of IS*26* on plasmid pFOS18 (288). The gene *fosC2*, which also codes for a glutathione-*S*-transferase, has been identified on a gene cassette in a class 1 integron on a conjugative plasmid in *E. coli* (289). Other *fos* genes have been described in numerous bacteria, including *fosC* in *Achromobacter denitrificans* and *fosK* in *Acinetobacter soli* (290).

FosB enzymes are bacillithiol-*S*-transferases that use Mg^{2+} as a cofactor. Several *fosB* gene variants have been detected in the chromosomal DNA of Gram-positive bacteria, such as *Staphylococcus epidermidis* (291), *B. subtilis*, *Bacillus anthracis*, and *S. aureus* (286, 287). The gene *fosB3* has been identified on a conjugative plasmid in *E. faecium* (292), while the genes *fosB1*, *fosB5*, and *fosB6* were located on small plasmids in *S. aureus* (293). The gene *fosD* is related to *fosB* and has been found in avian *Staphylococcus rostri* (294).

FosX enzymes are Mn^{2+}-dependent epoxide hydrolases, which use water to break the oxirane ring (286, 287). Variants of the gene *fosX* have been detected in the chromosomal DNA of *Clostridium botulinum*, *L. monocytogenes*, and *Brucella melitensis* (287). The gene $fosX^{CC}$ was detected as part of a multidrug-resistance genomic island in *C. coli* (295).

FomA and FomB are kinases that originate from the fosfomycin producer *Streptomyces wedmorensis*. They sequentially add phosphates to the phosphonate moiety of fosfomycin by using Mg^{2+} as a cofactor (286). Most likely, these enzymes represent part of the self-defense system of the fosfomycin producer (286). Another such kinase, originally called FosC and found in another fosfomycin producer, *Pseudomonas syringae*, is an ortholog of FomA (296).

Resistance to Fusidic Acid

Fusidic acid is a steroidal compound which was isolated from *Fusidium coccineum*. It exhibits antimicrobial activitiy against Gram-positive bacteria, such as staphylococci and *Corynebacterium* spp., as well as the Gram-negative *Neisseria gonorrhoeae*, *N. meningitidis*, and *Moraxella catarrhalis* (297). Fusidic acid is mainly used topically to treat skin infections caused by staphylococci, but it can also be administered systemically. Fusidic acid binds to elongation factor G and thus prevents polypeptide chain elongation during protein synthesis (298). Elongation factor G is encoded by the gene *fusA*. Several mutations in the gene *fusA* that cause resistance to fusidic acid have been described in *S. aureus*, with L461K being the most prevalent (298). Protection of the target site and subsequent fusid acid resistance is conferred by the protein FusB, which prevents the interaction of fusidic acid with elongation factor G (299). The gene *fusB* has been identified on the widespread plasmid pUB101 in *S. aureus* (300). A *fusB*-related gene, *fusF*, has recently been identified in *Staphylococcus cohnii* (301). In contrast, the genes *fusC* and *fusD*, which also confer fusidic acid resistance by target protection, have been detected as part of a chimeric SCC*mec*IV-SCC_{476} element in the chromo-

somal DNA of *S. aureus* (302) and in the chromosomal DNA of *S. saprophyticus* (303), respectively.

Resistance to Streptothricins

Streptothricins are antibiotics that consist of a streptolidine ring, a glucosamine, and a polylysine side chain. One of them, nourseothricin, was used as an antimicrobial feed additive in industrial animal farming in the former East Germany (304). Several *sat* genes have been identified which mediate streptothricin resistance by enzymatic inactivation via acetylation. In Gram-negative bacteria, particularly in *Enterobacteriaceae*, *sat* or *sat2* genes are usually located on gene cassettes in class 1 or class 2 integrons (305, 306). In staphylococci, the *sat4* gene is part of Tn*5405* and, as such, is commonly detected in staphylococci that also harbor *aphA3* and *aadE*. Furthermore, this gene has been detected in canine and feline *S. pseudintermedius* (307–309) and in MRSA of CC8 (ST254) from horses in Germany (310).

Resistance to Substances with Antimicrobial Activity Formerly Used as Growth Promoters

A number of substances with antimicrobial activity have been licensed as growth promoters for livestock. In the European Union, all growth promoters with antimicrobial activity were banned or withdrawn by 2006.

The mechanisms of resistance to the macrolides tylosin and spiramycin, the streptogramin virginiamycin, and the glycopeptide avoparcin were described in the sections "Resistance to Macrolides, Lincosamides, and Streptogramins (MLS)" and "Resistance to Glycopeptides." Hence, a brief summary of resistance to the remaining classes of growth promoters is given below. Two reviews (252, 311) are recommended for detailed insight into the various aspects of the use of growth promoters.

Bacitracin resistance was first described in the producer organism *Bacillus licheniformis*, in which an ABC transporter system, BcrABC, acts as a self-defense system by exporting the antibiotic from the producer cell (312). In *B. subtilis*, two independent but complementary-acting resistance mechanisms have been detected: an ABC transporter, YtsCD, that mediates the efflux of bacitracin, and a protein designated YwoA, which is believed to compete with bacitracin for the dephosphorylation of the C55-isoprenyl pyrophosphate (313). In *E. coli*, the gene *bacA*, which codes for an undecaprenyl pyrophosphate phosphatase, may account for bacitracin resistance (314). An ABC transporter, also termed BcrAB, that mediates bacitracin resistance was identified on a conjugative plasmid in *E. faecalis* (315).

Avilamycin resistance in the producer organism *Streptomyces viridochromogenes* Tü57 is based on the activity of an ABC transporter and two rRNA methyltransferases (316). In *E. faecalis* and *E. faecium*, resistance to avilamycin was initially described to be due to variations in the ribosomal protein L16 (317). Later, an rRNA methyltransferase, EmtA, which confers high-level resistance to avilamycin and evernimicin, was identified (318). Another two methylases—AviRa, which methylates 23S rRNA at the guanosine 2535 base, and AviRb, which methylates the uridine 2479 ribose—have been shown to confer avilamycin resistance (319). In addition, mutations at specific positions in the 23S rRNA also give rise to avilamycin resistance (320). Flavophospholipol (also known as flavomycin or bambermycin) has been reported to have a "plasmid-curing effect" on multiresistant *E. coli* under experimental conditions *in vitro* and *in vivo* (321). Cross-resistance to other antimicrobials has not been observed (252). Moreover, no genes or mutations conferring flavophospholipol resistance have been observed, to date.

Ionophores, such as salinomycin-Na and monensin-Na, are mainly used for the prevention of infections with parasites, such as *Eimeria* spp. (coccidiosis), *Plasmodium* spp., and *Giardia* spp. (252). Resistance or decreased susceptibility has been described in *S. hyicus*, coagulase-negative staphylococci from cattle and from *E. faecium* and *E. faecalis* from poultry and pigs. Genes or mutations accounting for acquired resistance to ionophores have not yet been described (252). Resistance to quinoxalines, such as carbadox and olaquindox, has been reported. An early study identified carbadox resistance to be associated with a conjugative multiresistance plasmid in *E. coli* (322). More than 20 years later, the genes *oqxA* and *oqxB*, which are responsible for olaqindox resistance, were cloned from a conjugative plasmid in *E. coli* (323). The corresponding gene products are homologous to several resistance-nodulation-cell-division family efflux systems and use TolC as the outer membrane component. Interestingly, the OqxAB-TolC system also mediates resistance to chloramphenicol and ethidium bromide (323).

CONCLUSION

The development of antimicrobial resistance—by either mutations, development of new resistance genes, or the acquisition of resistance genes already present in other bacteria—is a complex process that involves various mechanisms. Numerous resistance genes specifying different resistance mechanisms have been identified in various bacteria. The speed of resistance development

differs with regard to the bacteria involved, the selective pressure imposed by the use of antimicrobial agents, and the availability and transferability of resistance genes in the gene pools accessible to the bacteria. These basic facts apply to resistance development in bacteria from humans as well as in bacteria from animals. The loss of acquired resistance properties is often a cumbersome process which is influenced mainly by selective pressure, but also by the colocation of the resistance genes on multiresistance plasmids or in the chromosomal DNA and the organization of the resistance genes in multiresistance gene clusters or integron structures. When organized in resistance gene clusters or integrons, loss of resistance genes may not be expected even in the absence of direct selective pressure. Because we know that the use of every antimicrobial substance can select for resistant bacteria, prudent use of antimicrobial agents is strongly recommended in both human and veterinary medicine, but particularly in food animal production to retain the efficacy of antimicrobial agents for the control of bacterial infections in animals.

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References

1. **Schwarz S, Chaslus-Dancla E.** 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Vet Res* **32:**201–225.
2. **Schwarz S, Kehrenberg C, Walsh TR.** 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *Int J Antimicrob Agents* **17:**431–437.
3. **Schwarz S, Noble WC.** 1999. Aspects of bacterial resistance to antimicrobial agents used in veterinary dermatological practice. *Vet Dermatol* **10:**163–176.
4. **Fiebelkorn KR, Crawford SA, Jorgensen JH.** 2005. Mutations in *folP* associated with elevated sulfonamide MICs for *Neisseria meningitidis* clinical isolates from five continents. *Antimicrob Agents Chemother* **49:**536–540.
5. **Datta N, Hedges RW.** 1972. Trimethoprim resistance conferred by W plasmids in *Enterobacteriaceae*. *J Gen Microbiol* **72:**349–355.
6. **Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP.** 1991. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J Antimicrob Chemother* **27:**199–208.
7. **Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, Moellering RC Jr, Ferraro MJ.** 2001. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet* **358:**207–208.
8. **Schwarz S, Cloeckaert A, Roberts MC.** 2006. Mechanisms and spread of bacterial resistance to antimicrobial agents, p 73–98. *In* Aarestrup FM (ed), *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
9. **Schwarz S, Loeffler A, Kadlec K.** 2017. Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. *Vet Dermatol* **28:**82–e19.
10. **Livermore DM.** 1995. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* **8:**557–584.
11. **Georgopapadakou NH.** 1993. Penicillin-binding proteins and bacterial resistance to β-lactams. *Antimicrob Agents Chemother* **37:**2045–2053.
12. **Paulsen IT, Brown MH, Skurray RA.** 1996. Proton-dependent multidrug efflux systems. *Microbiol Rev* **60:** 575–608.
13. **Quintiliani R Jr, Sahm DF, Courvalin P.** 1999. Mechanisms of resistance to antimicrobial agents, p 1505–1525. *In* Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (ed), *Manual of Clinical Microbiology*, 7th ed. ASM Press, Washington, DC.
14. **Petrosino J, Cantu C III, Palzkill T.** 1998. β-Lactamases: protein evolution in real time. *Trends Microbiol* **6:**323–327.
15. **Bush K.** 2001. New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* **32:**1085–1089.
16. **Bush K, Jacoby GA, Medeiros AA.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* **39:**1211–1233.
17. **Ambler RP.** 1980. The structure of β-lactamases. *Philos Trans R Soc Lond B Biol Sci* **289:**321–331.
18. **Bush K, Jacoby GA.** 2010. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* **54:**969–976.
19. **Bradford PA.** 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* **14:**933–951.
20. **Bonnet R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* **48:**1–14.
21. **Brolund A, Sandegren L.** 2016. Characterization of ESBL disseminating plasmids. *Infect Dis (Lond)* **48:**18–25.
22. **Weldhagen GF.** 2004. Integrons and β-lactamases: a novel perspective on resistance. *Int J Antimicrob Agents* **23:**556–562.
23. **Gilmore KS, Gilmore MS, Sahm DF.** 2002. Methicillin resistance in *Staphylococcus aureus*, p 331–354. *In* Lewis K, Salyers AA, Taber HW, Wax RG (ed), *Bacterial Resistance to Antimicrobials*. Marcel Dekker, New York, NY.
24. **Hackbarth CJ, Chambers HF.** 1989. Methicillin-resistant staphylococci: genetics and mechanisms of resistance. *Antimicrob Agents Chemother* **33:**991–994.
25. **Katayama Y, Ito T, Hiramatsu K.** 2000. A new class of genetic element, *Staphylococcus* cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **44:**1549–1555.

26. **http://www.sccmec.org/Pages/SCC_TypesEN.html**
27. **García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA.** 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis* **11:**595–603.
28. **Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC.** 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **55:**3765–3773.
29. **Loncaric I, Kübber-Heiss A, Posautz A, Stalder GL, Hoffmann D, Rosengarten R, Walzer C.** 2013. Characterization of methicillin-resistant *Staphylococcus* spp. carrying the *mecC* gene, isolated from wildlife. *J Antimicrob Chemother* **68:**2222–2225.
30. **Harrison EM, Paterson GK, Holden MT, Morgan FJ, Larsen AR, Petersen A, Leroy S, De Vliegher S, Perreten V, Fox LK, Lam TJ, Sampimon OC, Zadoks RN, Peacock SJ, Parkhill J, Holmes MA.** 2013. A *Staphylococcus xylosus* isolate with a new *mecC* allotype. *Antimicrob Agents Chemother* **57:**1524–1528.
31. **Małyszko I, Schwarz S, Hauschild T.** 2014. Detection of a new *mecC* allotype, *mecC2*, in methicillin-resistant *Staphylococcus saprophyticus*. *J Antimicrob Chemother* **69:**2003–2005.
32. **Ehlert K.** 1999. Methicillin-resistance in *Staphylococcus aureus*: molecular basis, novel targets and antibiotic therapy. *Curr Pharm Des* **5:**45–55.
33. **Ling B, Berger-Bächi B.** 1998. Increased overall antibiotic susceptibility in *Staphylococcus aureus femAB* null mutants. *Antimicrob Agents Chemother* **42:**936–938.
34. **Charrel RN, Pagès J-M, De Micco P, Mallea M.** 1996. Prevalence of outer membrane porin alteration in β-lactam-antibiotic-resistant *Enterobacter aerogenes*. *Antimicrob Agents Chemother* **40:**2854–2858.
35. **Hopkins JM, Towner KJ.** 1990. Enhanced resistance to cefotaxime and imipenem associated with outer membrane protein alterations in *Enterobacter aerogenes*. *J Antimicrob Chemother* **25:**49–55.
36. **Mitsuyama J, Hiruma R, Yamaguchi A, Sawai T.** 1987. Identification of porins in outer membrane of *Proteus*, *Morganella*, and *Providencia* spp. and their role in outer membrane permeation of β-lactams. *Antimicrob Agents Chemother* **31:**379–384.
37. **Simonet V, Malléa M, Pagès J-M.** 2000. Substitutions in the eyelet region disrupt cefepime diffusion through the *Escherichia coli* OmpF channel. *Antimicrob Agents Chemother* **44:**311–315.
38. **Martínez-Martínez L, Hernández-Allés S, Albertí S, Tomás JM, Benedi VJ, Jacoby GA.** 1996. *In vivo* selection of porin-deficient mutants of *Klebsiella pneumoniae* with increased resistance to cefoxitin and expanded-spectrum-cephalosporins. *Antimicrob Agents Chemother* **40:**342–348.
39. **Wolter DJ, Hanson ND, Lister PD.** 2004. Insertional inactivation of *oprD* in clinical isolates of *Pseudomonas aeruginosa* leading to carbapenem resistance. *FEMS Microbiol Lett* **236:**137–143.
40. **Poole K.** 2002. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms, p 273–298. *In* Paulsen IT, Lewis K (ed), *Microbial Multidrug Efflux*. Horizon Scientific Press, Wymondham, United Kingdom.
41. **Putman M, van Veen HW, Konings WN.** 2000. Molecular properties of bacterial multidrug transporters. *Microbiol Mol Biol Rev* **64:**672–693.
42. **Grave K, Torren-Edo J, Muller A, Greko C, Moulin G, Mackay D, Fuchs K, Laurier L, Iliev D, Pokludova L, Genakritis M, Jacobsen E, Kurvits K, Kivilahti-Mantyla K, Wallmann J, Kovacs J, Lenharthsson JM, Beechinor JG, Perrella A, Mičule G, Zymantaite U, Meijering A, Prokopiak D, Ponte MH, Svetlin A, Hederova J, Madero CM, Girma K, Eckford S, ESVAC Group.** 2014. Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. *J Antimicrob Chemother* **69:**2284–2291.
43. **Casas C, Anderson EC, Ojo KK, Keith I, Whelan D, Rainnie D, Roberts MC.** 2005. Characterization of pRAS1-like plasmids from atypical North American psychrophilic *Aeromonas salmonicida*. *FEMS Microbiol Lett* **242:**59–63.
44. **DePaola A, Roberts MC.** 1995. Class D and E tetracycline resistance determinants in Gram-negative catfish pond bacteria. *Mol Cell Probes* **9:**311–313.
45. **Miranda CD, Kehrenberg C, Ulep C, Schwarz S, Roberts MC.** 2003. Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. *Antimicrob Agents Chemother* **47:**883–888.
46. **Chopra I, Roberts M.** 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* **65:**232–260.
47. **Levy SB, McMurry LM, Barbosa TM, Burdett V, Courvalin P, Hillen W, Roberts MC, Rood JI, Taylor DE.** 1999. Nomenclature for new tetracycline resistance determinants. *Antimicrob Agents Chemother* **43:**1523–1524.
48. **Roberts MC, Schwarz S.** 2016. Tetracycline and phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans. *J Environ Qual* **45:**576–592.
49. **Roberts MC, Schwarz S, Aarts HJ.** 2012. Erratum: acquired antibiotic resistance genes: an overview. *Front Microbiol* **3:**384.
50. **Roberts MC, No D, Kuchmiy E, Miranda CD.** 2015. Tetracycline resistance gene *tet*(39) identified in three new genera of bacteria isolated in 1999 from Chilean salmon farms. *J Antimicrob Chemother* **70:**619–621.
51. **Speer BS, Shoemaker NB, Salyers AA.** 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin Microbiol Rev* **5:**387–399.

52. **Linkevicius M, Sandegren L, Andersson DI.** 2015. Potential of tetracycline resistance proteins to evolve tigecycline resistance. *Antimicrob Agents Chemother* **60:** 789–796.
53. **Fiedler S, Bender JK, Klare I, Halbedel S, Grohmann E, Szewzyk U, Werner G.** 2016. Tigecycline resistance in clinical isolates of *Enterococcus faecium* is mediated by an upregulation of plasmid-encoded tetracycline determinants *tet*(L) and *tet*(M). *J Antimicrob Chemother* **71:** 871–881.
54. **Roberts MC.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev* **19:** 1–24.
55. **Allmeier H, Cresnar B, Greck M, Schmitt R.** 1992. Complete nucleotide sequence of Tn*1721*: gene organization and a novel gene product with features of a chemotaxis protein. *Gene* **111:**11–20.
56. **Chalmers R, Sewitz S, Lipkow K, Crellin P.** 2000. Complete nucleotide sequence of Tn*10*. *J Bacteriol* **182:** 2970–2972.
57. **Lawley TD, Burland V, Taylor DE.** 2000. Analysis of the complete nucleotide sequence of the tetracycline-resistance transposon Tn*10*. *Plasmid* **43:**235–239.
58. **Kehrenberg C, Werckenthin C, Schwarz S.** 1998. Tn*5706*, a transposon-like element from *Pasteurella multocida* mediating tetracycline resistance. *Antimicrob Agents Chemother* **42:**2116–2118.
59. **Projan SJ, Kornblum J, Moghazeh SL, Edelman I, Gennaro ML, Novick RP.** 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. *Mol Gen Genet* **199:**452–464.
60. **Schwarz S, Noble WC.** 1994. Tetracycline resistance genes in staphylococci from the skin of pigs. *J Appl Bacteriol* **76:**320–326.
61. **Werckenthin C, Schwarz S, Roberts MC.** 1996. Integration of pT181-like tetracycline resistance plasmids into large staphylococcal plasmids involves IS*257*. *Antimicrob Agents Chemother* **40:**2542–2544.
62. **Taylor DE, Chau A.** 1996. Tetracycline resistance mediated by ribosomal protection. *Antimicrob Agents Chemother* **40:**1–5.
63. **Connell SR, Tracz DM, Nierhaus KH, Taylor DE.** 2003. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother* **47:**3675–3681.
64. **Flannagan SE, Zitzow LA, Su YA, Clewell DB.** 1994. Nucleotide sequence of the 18-kb conjugative transposon Tn*916* from *Enterococcus faecalis*. *Plasmid* **32:** 350–354.
65. **Salyers AA, Shoemaker NB, Stevens AM, Li L-Y.** 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. *Microbiol Rev* **59:** 579–590.
66. **Forsberg KJ, Patel S, Wencewicz TA, Dantas G.** 2015. The tetracycline destructases: A novel family of tetracycline-inactivating enzymes. *Chem Biol* **22:**888–897.
67. **Speer BS, Bedzyk L, Salyers AA.** 1991. Evidence that a novel tetracycline resistance gene found on two *Bacteroides* transposons encodes an NADP-requiring oxidoreductase. *J Bacteriol* **173:**176–183.
68. **Diaz-Torres ML, McNab R, Spratt DA, Villedieu A, Hunt N, Wilson M, Mullany P.** 2003. Novel tetracycline resistance determinant from the oral metagenome. *Antimicrob Agents Chemother* **47:**1430–1432.
69. **Nonaka L, Suzuki S.** 2002. New Mg^{2+}-dependent oxytetracycline resistance determinant tet 34 in *Vibrio* isolates from marine fish intestinal contents. *Antimicrob Agents Chemother* **46:**1550–1552.
70. **Ross JI, Eady EA, Cove JH, Cunliffe WJ.** 1998. 16S rRNA mutation associated with tetracycline resistance in a Gram-positive bacterium. *Antimicrob Agents Chemother* **42:**1702–1705.
71. **Sutcliffe JA, Leclercq R.** 2003. Mechanisms of resistance to macrolides, lincosamides and ketolides, p 281–317. *In* Schonfeld W, Kirst HA (ed), *Macrolide Antibiotics*. Birkhauser Verlag, Basel, Switzerland.
72. **Schwarz S, Shen J, Kadlec K, Wang Y, Brenner Michael G, Feßler AT, Vester B.** 2016. Lincosamides, streptogramins, phenicols, and pleuromutilins: mode of action and mechanisms of resistance. *Cold Spring Harb Perspect Med* **6:**a027037.
73. **Fyfe C, Grossman TH, Kerstein K, Sutcliffe J.** 2016. Resistance to macrolide antibiotics in public health pathogens. *Cold Spring Harb Perspect Med* **6:**a025395.
74. **Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H.** 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* **43:**2823–2830.
75. **Leclercq R, Courvalin P.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother* **35:**1267–1272.
76. **Weisblum B.** 1995. Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother* **39:** 577–585.
77. **Weisblum B.** 1995. Insights into erythromycin action from studies of its activity as inducer of resistance. *Antimicrob Agents Chemother* **39:**797–805.
78. **Schmitz F-J, Petridou J, Jagusch H, Astfalk N, Scheuring S, Schwarz S.** 2002. Molecular characterization of ketolide-resistant *erm*(A)-carrying *Staphylococcus aureus* isolates selected *in vitro* by telithromycin, ABT-773, quinupristin and clindamycin. *J Antimicrob Chemother* **49:**611–617.
79. **Schmitz F-J, Petridou J, Astfalk N, Köhrer K, Scheuring S, Schwarz S.** 2002. Molecular analysis of constitutively expressed *erm*(C) genes selected *in vitro* by incubation in the presence of the noninducers quinupristin, telithromycin, or ABT-773. *Microb Drug Resist* **8:**171–177.
80. **Lüthje P, Schwarz S.** 2007. Molecular analysis of constitutively expressed *erm*(C) genes selected *in vitro* in the presence of the non-inducers pirlimycin, spiramycin and tylosin. *J Antimicrob Chemother* **59:**97–101.
81. **Werckenthin C, Schwarz S, Westh H.** 1999. Structural alterations in the translational attenuator of constitutively

expressed ermC genes. *Antimicrob Agents Chemother* **43:**1681–1685.

82. **Leclercq R, Courvalin P.** 1991. Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. *Antimicrob Agents Chemother* **35:**1273–1276.
83. **Sharkey LK, Edwards TA, O'Neill AJ.** 2016. ABC-F proteins mediate antibiotic resistance through ribosomal protection. *MBio* **7:**e01975.
84. **Ross JI, Eady EA, Cove JH, Cunliffe WJ, Baumberg S, Wootton JC.** 1990. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Mol Microbiol* **4:** 1207–1214.
85. **Reynolds E, Ross JI, Cove JH.** 2003. *Msr*(A) and related macrolide/streptogramin resistance determinants: incomplete transporters? *Int J Antimicrob Agents* **22:** 228–236.
86. **Lüthje P, Schwarz S.** 2006. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. *J Antimicrob Chemother* **57:**966–969.
87. **Clancy J, Petitpas J, Dib-Hajj F, Yuan W, Cronan M, Kamath AV, Bergeron J, Retsema JA.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. *Mol Microbiol* **22:**867–879.
88. **Cousin S Jr, Whittington WL, Roberts MC.** 2003. Acquired macrolide resistance genes in pathogenic *Neisseria* spp. isolated between 1940 and 1987. *Antimicrob Agents Chemother* **47:**3877–3880.
89. **Ojo KK, Ulep C, Van Kirk N, Luis H, Bernardo M, Leitao J, Roberts MC.** 2004. The *mef*(A) gene predominates among seven macrolide resistant genes identified in 13 Gram-negative genera from healthy Portuguese children. *Antimicrob Agents Chemother* **48:** 3451–3456.
90. **Daly MM, Doktor S, Flamm R, Shortridge D.** 2004. Characterization and prevalence of MefA, MefE, and the associated *msr*(D) gene in *Streptococcus pneumoniae* clinical isolates. *J Clin Microbiol* **42:**3570–3574.
91. **Plante I, Centrón D, Roy PH.** 2003. An integron cassette encoding erythromycin esterase, *ere*(A), from *Providencia stuartii*. *J Antimicrob Chemother* **51:**787–790.
92. **Lipka M, Filipek R, Bochtler M.** 2008. Crystal structure and mechanism of the *Staphylococcus cohnii* virginiamycin B lyase (Vgb). *Biochemistry* **47:**4257–4265.
93. **Chesneau O, Tsvetkova K, Courvalin P.** 2007. Resistance phenotypes conferred by macrolide phosphotransferases. *FEMS Microbiol Lett* **269:**317–322.
94. **Vester B, Douthwaite S.** 2001. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob Agents Chemother* **45:**1–12.
95. **Meier A, Kirschner P, Springer B, Steingrube VA, Brown BA, Wallace RJ Jr, Böttger EC.** 1994. Identification of mutations in 23S rRNA gene of clarithromycin-resistant *Mycobacterium intracellulare*. *Antimicrob Agents Chemother* **38:**381–384.
96. **Karlsson M, Fellström C, Heldtander MU, Johansson KE, Franklin A.** 1999. Genetic basis of macrolide and lincosamide resistance in *Brachyspira* (*Serpulina*) *hyodysenteriae*. *FEMS Microbiol Lett* **172:**255–260.
97. **Haanperä M, Huovinen P, Jalava J.** 2005. Detection and quantification of macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene by pyrosequencing. *Antimicrob Agents Chemother* **49:**457–460.
98. **Harrow SA, Gilpin BJ, Klena JD.** 2004. Characterization of erythromycin resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from pig offal in New Zealand. *J Appl Microbiol* **97:**141–148.
99. **Mingeot-Leclercq M-P, Glupczynski Y, Tulkens PM.** 1999. Aminoglycosides: activity and resistance. *Antimicrob Agents Chemother* **43:**727–737.
100. **Shaw KJ, Rather PN, Hare RS, Miller GH.** 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* **57:**138–163.
101. **Ramirez MS, Tolmasky ME.** 2010. Aminoglycoside modifying enzymes. *Drug Resist Updat* **13:**151–171.
102. **Wright GD.** 1999. Aminoglycoside-modifying enzymes. *Curr Opin Microbiol* **2:**499–503.
103. **Davies J, Wright GD.** 1997. Bacterial resistance to aminoglycoside antibiotics. *Trends Microbiol* **5:**234–240.
104. **Hedges RW, Shannon KP.** 1984. Resistance to apramycin in *Escherichia coli* isolated from animals: detection of a novel aminoglycoside-modifying enzyme. *J Gen Microbiol* **130:**473–482.
105. **Chaslus-Dancla E, Glupcznski Y, Gerbaud G, Lagorce M, Lafont JP, Courvalin P.** 1989. Detection of apramycin resistant *Enterobacteriaceae* in hospital isolates. *FEMS Microbiol Lett* **52:**261–265.
106. **Chaslus-Dancla E, Pohl P, Meurisse M, Marin M, Lafont JP.** 1991. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. *Antimicrob Agents Chemother* **35:**590–593.
107. **Johnson AP, Burns L, Woodford N, Threlfall EJ, Naidoo J, Cooke EM, George RC.** 1994. Gentamicin resistance in clinical isolates of *Escherichia coli* encoded by genes of veterinary origin. *J Med Microbiol* **40:**221–226.
108. **Lyon BR, Skurray R.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* **51:** 88–134.
109. **Rouch DA, Byrne ME, Kong YC, Skurray RA.** 1987. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn*4001* from *Staphylococcus aureus*: expression and nucleotide sequence analysis. *J Gen Microbiol* **133:**3039–3052.
110. **Lange CC, Werckenthin C, Schwarz S.** 2003. Molecular analysis of the plasmid-borne *aacA/aphD* resistance gene region of coagulase-negative staphylococci from chickens. *J Antimicrob Chemother* **51:**1397–1401.
111. **Leelaporn A, Yodkamol K, Waywa D, Pattanachaiwit S.** 2008. A novel structure of Tn*4001*-truncated element, type V, in clinical enterococcal isolates and multiplex PCR for detecting aminoglycoside resistance genes. *Int J Antimicrob Agents* **31:**250–254.

112. Recchia GD, Hall RM. 1995. Gene cassettes: a new class of mobile element. *Microbiology* **141:**3015–3027.

113. Sandvang D, Aarestrup FM. 2000. Characterization of aminoglycoside resistance genes and class 1 integrons in porcine and bovine gentamicin-resistant *Escherichia coli*. *Microb Drug Resist* **6:**19–27.

114. Feßler AT, Kadlec K, Schwarz S. 2011. Novel apramycin resistance gene *apmA* in bovine and porcine methicillin-resistant *Staphylococcus aureus* ST398 isolates. *Antimicrob Agents Chemother* **55:**373–375.

115. Kadlec K, Feßler AT, Couto N, Pomba CF, Schwarz S. 2012. Unusual small plasmids carrying the novel resistance genes *dfrK* or *apmA* isolated from methicillin-resistant or -susceptible staphylococci. *J Antimicrob Chemother* **67:**2342–2345.

116. Feßler AT, Zhao Q, Schoenfelder S, Kadlec K, Brenner Michael G, Wang Y, Ziebuhr W, Shen J, Schwarz S. 2017. Complete sequence of a plasmid from a bovine methicillin-resistant *Staphylococcus aureus* harbouring a novel *ica*-like gene cluster in addition to antimicrobial and heavy metal resistance genes. *Vet Microbiol* **200:** 95–100.

117. Murphy E. 1985. Nucleotide sequence of a spectinomycin adenyltransferase AAD(9) determinant from *Staphylococcus aureus* and its relationship to AAD(3´´) (9). *Mol Gen Genet* **200:**33–39.

118. Wendlandt S, Li B, Lozano C, Ma Z, Torres C, Schwarz S. 2013. Identification of the novel spectinomycin resistance gene *spw* in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* of human and animal origin. *J Antimicrob Chemother* **68:**1679–1680.

119. Jamrozy DM, Coldham NG, Butaye P, Fielder MD. 2014. Identification of a novel plasmid-associated spectinomycin adenyltransferase gene *spd* in methicillin-resistant *Staphylococcus aureus* ST398 isolated from animal and human sources. *J Antimicrob Chemother* **69:**1193–1196.

120. Wendlandt S, Feßler AT, Kadlec K, van Duijkeren E, Schwarz S. 2014. Identification of the novel spectinomycin resistance gene *spd* in a different plasmid background among methicillin-resistant *Staphylococcus aureus* CC398 and methicillin-susceptible *S. aureus* ST433. *J Antimicrob Chemother* **69:**2000–2003.

121. Wendlandt S, Kadlec K, Schwarz S. 2015. Four novel plasmids from *Staphylococcus hyicus* and CoNS that carry a variant of the spectinomycin resistance gene *spd*. *J Antimicrob Chemother* **70:**948–949.

122. Rosenberg EY, Ma D, Nikaido H. 2000. AcrD of *Escherichia coli* is an aminoglycoside efflux pump. *J Bacteriol* **182:**1754–1756.

123. Edgar R, Bibi E. 1997. MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. *J Bacteriol* **179:**2274–2280.

124. Salyers AA, Whitt DD. 1994. *Bacterial Pathogenesis: a Molecular Approach*. ASM Press, Washington, DC.

125. Galimand M, Courvalin P, Lambert T. 2003. Plasmid-mediated high-level resistance to aminoglycosides in *Enterobacteriaceae* due to 16S rRNA methylation. *Antimicrob Agents Chemother* **47:**2565–2571.

126. Potron A, Poirel L, Nordmann P. 2015. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents* **45:**568–585.

127. Wachino J, Arakawa Y. 2012. Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: an update. *Drug Resist Updat* **15:**133–148.

128. Meier A, Sander P, Schaper KJ, Scholz M, Böttger EC. 1996. Correlation of molecular resistance mechanisms and phenotypic resistance levels in streptomycin-resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **40:**2452–2454.

129. Prammananan T, Sander P, Brown BA, Frischkorn K, Onyi GO, Zhang Y, Böttger EC, Wallace RJ Jr. 1998. A single 16S ribosomal RNA substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in *Mycobacterium abscessus* and *Mycobacterium chelonae*. *J Infect Dis* **177:**1573–1581.

130. Cohen KA, Bishai WR, Pym AS. 2014. Molecular basis of drug resistance in *Mycobacterium tuberculosis*. *Microbiol Spectr* **2:**.

131. Elwell LP, Fling ME. 1989. Resistance to trimethoprim, p 249–290. *In* Bryan LE (ed), *Microbial Resistance to Drugs*. Springer Verlag, Berlin, Germany.

132. Huovinen P. 2001. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis* **32:**1608–1614.

133. Huovinen P, Sundström L, Swedberg G, Sköld O. 1995. Trimethoprim and sulfonamide resistance. *Antimicrob Agents Chemother* **39:**279–289.

134. Sköld O. 2000. Sulfonamide resistance: mechanisms and trends. *Drug Resist Updat* **3:**155–160.

135. Sköld O. 2001. Resistance to trimethoprim and sulfonamides. *Vet Res* **32:**261–273.

136. Köhler T, Kok M, Michea-Hamzehpour M, Plesiat P, Gotoh N, Nishino T, Curty LK, Pechere J-C. 1996. Multidrug efflux in intrinsic resistance to trimethoprim and sulfamethoxazole in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **40:**2288–2290.

137. Padayachee T, Klugman KP. 1999. Novel expansions of the gene encoding dihydropteroate synthase in trimethoprim-sulfamethoxazole-resistant *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* **43:**2225–2230.

138. Dale GE, Broger C, D'Arcy A, Hartman PG, DeHoogt R, Jolidon S, Kompis I, Labhardt AM, Langen H, Locher H, Page MG, Stüber D, Then RL, Wipf B, Oefner C. 1997. A single amino acid substitution in *Staphylococcus aureus* dihydrofolate reductase determines trimethoprim resistance. *J Mol Biol* **266:**23–30.

139. Pikis A, Donkersloot JA, Rodriguez WJ, Keith JM. 1998. A conservative amino acid mutation in the chromosome-encoded dihydrofolate reductase confers trimethoprim resistance in *Streptococcus pneumoniae*. *J Infect Dis* **178:**700–706.

140. de Groot R, Sluijter M, de Bruyn A, Campos J, Goessens WHF, Smith AL, Hermans PWM. 1996. Genetic characterization of trimethoprim resistance in *Haemophilus influenzae*. *Antimicrob Agents Chemother* **40:**2131–2136.

141. **Sundström L, Rådström P, Swedberg G, Sköld O.** 1988. Site-specific recombination promotes linkage between trimethoprim- and sulfonamide resistance genes. Sequence characterization of *dhfrV* and *sulI* and a recombination active locus of Tn*21*. *Mol Gen Genet* **213:** 191–201.

142. **Rådström P, Swedberg G.** 1988. RSF1010 and a conjugative plasmid contain *sul*II, one of two known genes for plasmid-borne sulfonamide resistance dihydropteroate synthase. *Antimicrob Agents Chemother* **32:**1684–1692.

143. **Swedberg G, Sköld O.** 1980. Characterization of different plasmid-borne dihydropteroate synthases mediating bacterial resistance to sulfonamides. *J Bacteriol* **142:** 1–7.

144. **Perreten V, Boerlin P.** 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrob Agents Chemother* **47:**1169–1172.

145. **Grape M, Sundström L, Kronvall G.** 2003. Sulphonamide resistance gene *sul3* found in *Escherichia coli* isolates from human sources. *J Antimicrob Chemother* **52:**1022–1024.

146. **Guerra B, Junker E, Helmuth R.** 2004. Incidence of the recently described sulfonamide resistance gene *sul3* among German *Salmonella enterica* strains isolated from livestock and food. *Antimicrob Agents Chemother* **48:**2712–2715.

147. **Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, Helmuth R.** 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. *J Antimicrob Chemother* **52:**489–492.

148. **Pattishall KH, Acar J, Burchall JJ, Goldstein FW, Harvey RJ.** 1977. Two distinct types of trimethoprim-resistant dihydrofolate reductase specified by R-plasmids of different compatibility groups. *J Biol Chem* **252:**2319–2323.

149. **Sekiguchi J, Tharavichitkul P, Miyoshi-Akiyama T, Chupia V, Fujino T, Araake M, Irie A, Morita K, Kuratsuji T, Kirikae T.** 2005. Cloning and characterization of a novel trimethoprim-resistant dihydrofolate reductase from a nosocomial isolate of *Staphylococcus aureus* CM.S2 (IMCJ1454). *Antimicrob Agents Chemother* **49:**3948–3951.

150. **Dale GE, Langen H, Page MG, Then RL, Stüber D.** 1995. Cloning and characterization of a novel, plasmid-encoded trimethoprim-resistant dihydrofolate reductase from *Staphylococcus haemolyticus* MUR313. *Antimicrob Agents Chemother* **39:**1920–1924.

151. **Charpentier E, Courvalin P.** 1997. Emergence of the trimethoprim resistance gene *dfrD* in *Listeria monocytogenes* BM4293. *Antimicrob Agents Chemother* **41:** 1134–1136.

152. **Kadlec K, Schwarz S.** 2009. Identification of a novel trimethoprim resistance gene, *dfrK*, in a methicillin-resistant *Staphylococcus aureus* ST398 strain and its physical linkage to the tetracycline resistance gene *tet* (L). *Antimicrob Agents Chemother* **53:**776–778.

153. **Kadlec K, Schwarz S.** 2010. Identification of the novel *dfrK*-carrying transposon Tn*559* in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* **54:**3475–3477.

154. **López M, Kadlec K, Schwarz S, Torres C.** 2012. First detection of the staphylococcal trimethoprim resistance gene *dfrK* and the *dfrK*-carrying transposon Tn*559* in enterococci. *Microb Drug Resist* **18:**13–18.

155. **Rouch DA, Messerotti LJ, Loo LSL, Jackson CA, Skurray RA.** 1989. Trimethoprim resistance transposon Tn*4003* from *Staphylococcus aureus* encodes genes for a dihydrofolate reductase and thymidylate synthetase flanked by three copies of IS*257*. *Mol Microbiol* **3:**161–175.

156. **Kehrenberg C, Schwarz S.** 2005. *dfrA20*, a novel trimethoprim resistance gene from *Pasteurella multocida*. *Antimicrob Agents Chemother* **49:**414–417.

157. **Webber M, Piddock LJV.** 2001. Quinolone resistance in *Escherichia coli*. *Vet Res* **32:**275–284.

158. **Bager F, Helmuth R.** 2001. Epidemiology of quinolone resistance in *Salmonella*. *Vet Res* **32:**285–290.

159. **Drlica K, Zhao X.** 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev* **61:** 377–392.

160. **Everett MJ, Piddock LJV.** 1998. Mechanisms of resistance to fluoroquinolones, p 259–296. *In* Kuhlmann J, Dalhoff A, Zeiler H-J (ed), *Quinolone Antibacterials*. Springer Verlag, Berlin, Germany.

161. **Hooper DC.** 1999. Mechanisms of fluoroquinolone resistance. *Drug Resist Updat* **2:**38–55.

162. **Ruiz J.** 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother* **51:**1109–1117.

163. **Guan X, Xue X, Liu Y, Wang J, Wang Y, Wang J, Wang K, Jiang H, Zhang L, Yang B, Wang N, Pan L.** 2013. Plasmid-mediated quinolone resistance: current knowledge and future perspectives. *J Int Med Res* **41:**20–30.

164. **Yoshida H, Bogaki M, Nakamura M, Nakamura S.** 1990. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrob Agents Chemother* **34:**1271–1272.

165. **Cloeckaert A, Chaslus-Dancla E.** 2001. Mechanisms of quinolone resistance in *Salmonella*. *Vet Res* **32:**291–300.

166. **Jones ME, Sahm DF, Martin N, Scheuring S, Heisig P, Thornsberry C, Köhrer K, Schmitz F-J.** 2000. Prevalence of *gyrA*, *gyrB*, *parC*, and *parE* mutations in clinical isolates of *Streptococcus pneumoniae* with decreased susceptibilities to different fluoroquinolones and originating from worldwide surveillance studies during the 1997-1998 respiratory season. *Antimicrob Agents Chemother* **44:**462–466.

167. **Everett MJ, Jin YF, Ricci V, Piddock LJV.** 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. *Antimicrob Agents Chemother* **40:**2380–2386.

168. Poole K. 2000. Efflux-mediated resistance to fluoroquinolones in Gram-negative bacteria. *Antimicrob Agents Chemother* **44:**2233–2241.

169. Poole K. 2000. Efflux-mediated resistance to fluoroquinolones in Gram-positive bacteria and the mycobacteria. *Antimicrob Agents Chemother* **44:**2595–2599.

170. Alekshun MN, Levy SB. 1999. The *mar* regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends Microbiol* **7:**410–413.

171. Okusu H, Ma D, Nikaido H. 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. *J Bacteriol* **178:**306–308.

172. Olliver A, Vallé M, Chaslus-Dancla E, Cloeckaert A. 2004. Role of an *acrR* mutation in multidrug resistance of *in vitro*-selected fluoroquinolone-resistant mutants of *Salmonella enterica* serovar Typhimurium. *FEMS Microbiol Lett* **238:**267–272.

173. Oethinger M, Podglajen I, Kern WV, Levy SB. 1998. Overexpression of the *marA* or *soxS* regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. *Antimicrob Agents Chemother* **42:**2089–2094.

174. Barbosa TM, Levy SB. 2000. Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. *J Bacteriol* **182:**3467–3474.

175. Lee A, Mao W, Warren MS, Mistry A, Hoshino K, Okumura R, Ishida H, Lomovskaya O. 2000. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *J Bacteriol* **182:**3142–3150.

176. Oethinger M, Kern WV, Jellen-Ritter AS, McMurry LM, Levy SB. 2000. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. *Antimicrob Agents Chemother* **44:**10–13.

177. Lomovskaya O, Lee A, Hoshino K, Ishida H, Mistry A, Warren MS, Boyer E, Chamberland S, Lee VJ. 1999. Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **43:**1340–1346.

178. Baucheron S, Chaslus-Dancla E, Cloeckaert A. 2004. Role of TolC and *parC* mutation in high-level fluoroquinolone resistance in *Salmonella enterica* serotype Typhimurium DT204. *J Antimicrob Chemother* **53:**657–659.

179. Baucheron S, Imberechts H, Chaslus-Dancla E, Cloeckaert A. 2002. The AcrB multidrug transporter plays a major role in high-level fluoroquinolone resistance in *Salmonella enterica* serovar Typhimurium phage type DT204. *Microb Drug Resist* **8:**281–289.

180. Cohen SP, McMurry LM, Levy SB. 1988. *marA* locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. *J Bacteriol* **170:**5416–5422.

181. McMurry LM, George AM, Levy SB. 1994. Active efflux of chloramphenicol in susceptible *Escherichia coli* strains and in multiple-antibiotic-resistant (Mar) mutants. *Antimicrob Agents Chemother* **38:**542–546.

182. Hooper DC, Wolfson JS, Bozza MA, Ng EY. 1992. Genetics and regulation of outer membrane protein expression by quinolone resistance loci nfxB, nfxC, and cfxB. *Antimicrob Agents Chemother* **36:**1151–1154.

183. Juárez-Verdayes MA, Parra-Ortega B, Hernández-Rodríguez C, Betanzos-Cabrera G, Rodríguez-Martínez S, Cancino-Diaz ME, Cancino-Diaz JC. 2012. Identification and expression of *nor* efflux family genes in *Staphylococcus epidermidis* that act against gatifloxacin. *Microb Pathog* **52:**318–325.

184. Tran JH, Jacoby GA. 2002. Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci USA* **99:**5638–5642.

185. Jacoby GA, Strahilevitz J, Hooper DC. 2014. Plasmid-mediated quinolone resistance. *Microbiol Spectrum* **2:** PLAS-0006-2013

186. Rodríguez-Martínez JM, Machuca J, Cano ME, Calvo J, Martínez-Martínez L, Pascual A. 2016. Plasmid-mediated quinolone resistance: two decades on. *Drug Resist Updat* **29:**13–29.

187. Murray IA, Shaw WV. 1997. *O*-Acetyltransferases for chloramphenicol and other natural products. *Antimicrob Agents Chemother* **41:**1–6.

188. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* **28:** 519–542.

189. Shaw WV. 1983. Chloramphenicol acetyltransferase: enzymology and molecular biology. *CRC Crit Rev Biochem* **14:**1–46.

190. Alekshun MN, Levy SB. 2000. Bacterial drug resistance: response to survival threats, p 323–366. *In* Storz G, Hengge-Aronis R (ed), *Bacterial Stress Responses*. ASM Press, Washington, DC.

191. Alton NK, Vapnek D. 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn*9*. *Nature* **282:**864–869.

192. Murray IA, Hawkins AR, Keyte JW, Shaw WV. 1988. Nucleotide sequence analysis and overexpression of the gene encoding a type III chloramphenicol acetyltransferase. *Biochem J* **252:**173–179.

193. Murray IA, Martinez-Suarez JV, Close TJ, Shaw WV. 1990. Nucleotide sequences of genes encoding the type II chloramphenicol acetyltransferases of *Escherichia coli* and *Haemophilus influenzae*, which are sensitive to inhibition by thiol-reactive reagents. *Biochem J* **272:**505–510.

194. Brenner DG, Shaw WV. 1985. The use of synthetic oligonucleotides with universal templates for rapid DNA sequencing: results with staphylococcal replicon pC221. *EMBO J* **4:**561–568.

195. Horinouchi S, Weisblum B. 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. *J Bacteriol* **150:** 815–825.

196. **Schwarz S, Cardoso M.** 1991. Nucleotide sequence and phylogeny of a chloramphenicol acetyltransferase encoded by the plasmid pSCS7 from *Staphylococcus aureus*. *Antimicrob Agents Chemother* **35:**1551–1556.

197. **Lovett PS.** 1990. Translational attenuation as the regulator of inducible *cat* genes. *J Bacteriol* **172:**1–6.

198. **Bannam TL, Rood JI.** 1991. Relationship between the *Clostridium perfringens catQ* gene product and chloramphenicol acetyltransferases from other bacteria. *Antimicrob Agents Chemother* **35:**471–476.

199. **Lang KS, Anderson JM, Schwarz S, Williamson L, Handelsman J, Singer RS.** 2010. Novel florfenicol and chloramphenicol resistance gene discovered in Alaskan soil by using functional metagenomics. *Appl Environ Microbiol* **76:**5321–5326.

200. **Stokes HW, Hall RM.** 1991. Sequence analysis of the inducible chloramphenicol resistance determinant in the Tn*1696* integron suggests regulation by translational attenuation. *Plasmid* **26:**10–19.

201. **Cloeckaert A, Baucheron S, Chaslus-Dancla E.** 2001. Nonenzymatic chloramphenicol resistance mediated by IncC plasmid R55 is encoded by a *floR* gene variant. *Antimicrob Agents Chemother* **45:**2381–2382.

202. **Cloeckaert A, Baucheron S, Flaujac G, Schwarz S, Kehrenberg C, Martel JL, Chaslus-Dancla E.** 2000. Plasmid-mediated florfenicol resistance encoded by the *floR* gene in *Escherichia coli* isolated from cattle. *Antimicrob Agents Chemother* **44:**2858–2860.

203. **Hochhut B, Lotfi Y, Mazel D, Faruque SM, Woodgate R, Waldor MK.** 2001. Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. *Antimicrob Agents Chemother* **45:**2991–3000.

204. **Kehrenberg C, Schwarz S.** 2005. Plasmid-borne florfenicol resistance in *Pasteurella multocida*. *J Antimicrob Chemother* **55:**773–775.

205. **Keyes K, Hudson C, Maurer JJ, Thayer S, White DG, Lee MD.** 2000. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. *Antimicrob Agents Chemother* **44:**421–424.

206. **Kim E, Aoki T.** 1996. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. *Microbiol Immunol* **40:**665–669.

207. **White DG, Hudson C, Maurer JJ, Ayers S, Zhao S, Lee MD, Bolton L, Foley T, Sherwood J.** 2000. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. *J Clin Microbiol* **38:**4593–4598.

208. **Michael GB, Kadlec K, Sweeney MT, Brzuszkiewicz E, Liesegang H, Daniel R, Murray RW, Watts JL, Schwarz S.** 2012. ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: analysis of the regions that comprise 12 antimicrobial resistance genes. *J Antimicrob Chemother* **67:**84–90.

209. **Hall RM.** 2010. *Salmonella* genomic islands and antibiotic resistance in *Salmonella enterica*. *Future Microbiol* **5:**1525–1538.

210. **He T, Shen J, Schwarz S, Wu C, Wang Y.** 2015. Characterization of a genomic island in *Stenotrophomonas maltophilia* that carries a novel *floR* gene variant. *J Antimicrob Chemother* **70:**1031–1036.

211. **Kehrenberg C, Schwarz S.** 2004. *fexA*, a novel *Staphylococcus lentus* gene encoding resistance to florfenicol and chloramphenicol. *Antimicrob Agents Chemother* **48:**615–618.

212. **Kehrenberg C, Schwarz S.** 2005. Florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn*558*. *Antimicrob Agents Chemother* **49:**813–815.

213. **Liu H, Wang Y, Wu C, Schwarz S, Shen Z, Jeon B, Ding S, Zhang Q, Shen J.** 2012. A novel phenicol exporter gene, *fexB*, found in enterococci of animal origin. *J Antimicrob Chemother* **67:**322–325.

214. **Ettayebi M, Prasad SM, Morgan EA.** 1985. Chloramphenicol-erythromycin resistance mutations in a 23S rRNA gene of *Escherichia coli*. *J Bacteriol* **162:**551–557.

215. **Long KS, Vester B.** 2012. Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrob Agents Chemother* **56:**603–612.

216. **Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN.** 2010. Assessment of linezolid resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. *J Antimicrob Chemother* **65:**2329–2335.

217. **Shaw KJ, Barbachyn MR.** 2011. The oxazolidinones: past, present, and future. *Ann N Y Acad Sci* **1241:**48–70.

218. **Locke JB, Hilgers M, Shaw KJ.** 2009. Mutations in ribosomal protein L3 are associated with oxazolidinone resistance in staphylococci of clinical origin. *Antimicrob Agents Chemother* **53:**5275–5278.

219. **Wolter N, Smith AM, Farrell DJ, Schaffner W, Moore M, Whitney CG, Jorgensen JH, Klugman KP.** 2005. Novel mechanism of resistance to oxazolidinones, macrolides, and chloramphenicol in ribosomal protein L4 of the pneumococcus. *Antimicrob Agents Chemother* **49:**3554–3557.

220. **Shore AC, Lazaris A, Kinnevey PM, Brennan OM, Brennan GI, O'Connell B, Feßler AT, Schwarz S, Coleman DC.** 2016. First report of *cfr*-carrying plasmids in the pandemic sequence type 22 methicillin-resistant *Staphylococcus aureus* staphylococcal cassette chromosome *mec* type IV clone. *Antimicrob Agents Chemother* **60:**3007–3015.

221. **Schwarz S, Werckenthin C, Kehrenberg C.** 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrob Agents Chemother* **44:**2530–2533.

222. **Kehrenberg C, Schwarz S, Jacobsen L, Hansen LH, Vester B.** 2005. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. *Mol Microbiol* **57:**1064–1073.

223. **Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B.** 2006. The Cfr rRNA methyltransferase

confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* **50:**2500–2505.

224. Shen J, Wang Y, Schwarz S. 2013. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* **68:**1697–1706.

225. Wang Y, Li D, Song L, Liu Y, He T, Liu H, Wu C, Schwarz S, Shen J. 2013. First report of the multiresistance gene *cfr* in *Streptococcus suis*. *Antimicrob Agents Chemother* **57:**4061–4063.

226. Hansen LH, Vester B. 2015. A *cfr*-like gene from *Clostridium difficile* confers multiple antibiotic resistance by the same mechanism as the *cfr* gene. *Antimicrob Agents Chemother* **59:**5841–5843.

227. Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, Farrell DJ, Mendes RE. 2015. Detection of a new *cfr*-like gene, *cfr*(B), in *Enterococcus faecium* isolates recovered from human specimens in the United States as part of the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother* **59:**6256–6261.

228. Tang Y, Dai L, Sahin O, Wu Z, Liu M, Zhang Q. 2017. Emergence of a plasmid-borne multidrug resistance gene *cfr*(C) in foodborne pathogen *Campylobacter*. *J Antimicrob Chemother* **72:**1581–1588.

229. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Feßler AT, Wu C, Yu H, Deng X, Xia X, Shen J. 2015. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* **70:**2182–2190.

230. He T, Shen Y, Schwarz S, Cai J, Lv Y, Li J, Feßler AT, Zhang R, Wu C, Shen J, Wang Y. 2016. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. *J Antimicrob Chemother* **71:**1466–1473.

231. Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Feßler AT, Zhang R, Wu C, Shen J. 2016. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. *J Antimicrob Chemother* **71:**1474–1478.

232. Fan R, Li D, Wang Y, He T, Feßler AT, Schwarz S, Wu C. 2016. Presence of the *optrA* gene in methicillin-resistant *Staphylococcus sciuri* of porcine origin. *Antimicrob Agents Chemother* **60:**7200–7205.

233. Huang J, Chen L, Wu Z, Wang L. 2017. Retrospective analysis of genome sequences revealed the wide dissemination of *optrA* in Gram-positive bacteria. *J Antimicrob Chemother* **72:**614–616.

234. Arthur M, Reynolds P, Courvalin P. 1996. Glycopeptide resistance in enterococci. *Trends Microbiol* **4:**401–407.

235. Walsh C. 2003. *Antibiotics: Actions, Origins, Resistance*. ASM Press, Washington, DC.

236. Gudeta DD, Moodley A, Bortolaia V, Guardabassi L. 2014. *vanO*, a new glycopeptide resistance operon in environmental *Rhodococcus equi* isolates. *Antimicrob Agents Chemother* **58:**1768–1770.

237. Arthur M, Molinas C, Depardieu F, Courvalin P. 1993. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* **175:**117–127.

238. Aarestrup FM. 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Microb Drug Resist* **1:**255–257.

239. Klare I, Heier H, Claus H, Reissbrodt R, Witte W. 1995. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. *FEMS Microbiol Lett* **125:**165–171.

240. Noble WC, Virani Z, Cree RG. 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* **72:**195–198.

241. Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC. 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* **302:**1569–1571.

242. Guardabassi L, Christensen H, Hasman H, Dalsgaard A. 2004. Members of the genera *Paenibacillus* and *Rhodococcus* harbor genes homologous to enterococcal glycopeptide resistance genes *vanA* and *vanB*. *Antimicrob Agents Chemother* **48:**4915–4918.

243. Wendlandt S, Shen J, Kadlec K, Wang Y, Li B, Zhang WJ, Feßler AT, Wu C, Schwarz S. 2015. Multidrug resistance genes in staphylococci from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. *Trends Microbiol* **23:**44–54.

244. Wendlandt S, Kadlec K, Feßler AT, Schwarz S. 2015. Identification of ABC transporter genes conferring combined pleuromutilin-lincosamide-streptogramin A resistance in bovine methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *Vet Microbiol* **177:**353–358.

245. Pringle M, Poehlsgaard J, Vester B, Long KS. 2004. Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in *Brachyspira* spp. isolates. *Mol Microbiol* **54:**1295–1306.

246. Hidalgo Á, Carvajal A, Vester B, Pringle M, Naharro G, Rubio P. 2011. Trends towards lower antimicrobial susceptibility and characterization of acquired resistance among clinical isolates of *Brachyspira hyodysenteriae* in Spain. *Antimicrob Agents Chemother* **55:**3330–3337.

247. Pringle M, Landén A, Unnerstad HE, Molander B, Bengtsson B. 2012. Antimicrobial susceptibility of porcine *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* isolated in Sweden between 1990 and 2010. *Acta Vet Scand* **54:**54.

248. Li BB, Shen JZ, Cao XY, Wang Y, Dai L, Huang SY, Wu CM. 2010. Mutations in 23S rRNA gene associated with decreased susceptibility to tiamulin and

valnemulin in *Mycoplasma gallisepticum*. *FEMS Microbiol Lett* **308:**144–149.

249. van Duijkeren E, Greko C, Pringle M, Baptiste KE, Catry B, Jukes H, Moreno MA, Pomba MC, Pyörälä S, Rantala M, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Torren-Edo J, Törneke K. 2014. Pleuromutilins: use in food-producing animals in the European Union, development of resistance and impact on human and animal health. *J Antimicrob Chemother* **69:**2022–2031.

250. Isnard C, Malbruny B, Leclercq R, Cattoir V. 2013. Genetic basis for *in vitro* and *in vivo* resistance to lincosamides, streptogramins A, and pleuromutilins (LS_AP phenotype) in *Enterococcus faecium*. *Antimicrob Agents Chemother* **57:**4463–4469.

251. Wendlandt S, Lozano C, Kadlec K, Gómez-Sanz E, Zarazaga M, Torres C, Schwarz S. 2013. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **68:**473–475.

252. Butaye P, Devriese LA, Haesebrouck F. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *Clin Microbiol Rev* **16:**175–188.

253. WHO. 2017. Critically important antimicrobials for human medicine. 5th revision. http://who.int/foodsafety/publications/antimicrobials-fifth/en/.

254. Schwarz S, Johnson AP. 2016. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother* **71:**2066–2070.

255. Olaitan AO, Morand S, Rolain JM. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* **5:**643.

256. Jeannot K, Bolard A, Plésiat P. 2017. Resistance to polymyxins in Gram-negative organisms. *Int J Antimicrob Agents* **49:**526–535.

257. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* **16:**161–168.

258. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* **21:**pii=30280. http://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2016.21.27.30280.

259. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBio* **8:**e00543-17.

260. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF. 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill* **22:**30589. http://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2017.22.31.30589.

261. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. *J Antimicrob Chemother* **72:**3317–3324.

262. AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, Randall LP, Lemma F, Crook DW, Teale C, Smith RP, Anjum MF. 2017. *mcr-1* and *mcr-2* variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015. *J Antimicrob Chemother* **72:**2745–2749.

263. Ling Z, Yin W, Li H, Zhang Q, Wang X, Wang Z, Ke Y, Wang Y, Shen J. 2017. Chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat. *Antimicrob Agents Chemother* **61:**e01272-17. Epub ahead of print.

264. Eichhorn I, Feudi C, Wang Y, Kaspar H, Feßler AT, Lübke-Becker A, Michael GB, Shen J, Schwarz S. 2018. Identification of novel variants of the colistin resistance gene *mcr-3* in *Aeromonas* spp. from the national resistance monitoring program GERM-Vet and from diagnostic submissions. *J Antimicrob Chemother*.

265. Vila-Farrés X, Ferrer-Navarro M, Callarisa AE, Martí S, Espinal P, Gupta S, Rolain JM, Giralt E, Vila J. 2015. Loss of LPS is involved in the virulence and resistance to colistin of colistin-resistant *Acinetobacter nosocomialis* mutants selected *in vitro*. *J Antimicrob Chemother* **70:**2981–2986.

266. Baron S, Hadjadj L, Rolain JM, Olaitan AO. 2016. Molecular mechanisms of polymyxin resistance: knowns and unknowns. *Int J Antimicrob Agents* **48:**583–591.

267. Bina XR, Provenzano D, Nguyen N, Bina JE. 2008. *Vibrio cholerae* RND family efflux systems are required for antimicrobial resistance, optimal virulence factor production, and colonization of the infant mouse small intestine. *Infect Immun* **76:**3595–3605.

268. Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, Bengoechea JA. 2004. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect Immun* **72:**7107–7114.

269. Llobet E, Tomás JM, Bengoechea JA. 2008. Capsule polysaccharide is a bacterial decoy for antimicrobial peptides. *Microbiology* **154:**3877–3886.

270. Young ML, Bains M, Bell A, Hancock RE. 1992. Role of *Pseudomonas aeruginosa* outer membrane protein OprH in polymyxin and gentamicin resistance: isolation of an OprH-deficient mutant by gene replacement techniques. *Antimicrob Agents Chemother* **36:**2566–2568.

271. Hetem DJ, Bonten MJ. 2013. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. *J Hosp Infect* **85:**249–256.

272. Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS. 1994. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob Agents Chemother* **38:**1205–1208.

273. **Needham C, Rahman M, Dyke KG, Noble WC.** 1994. An investigation of plasmids from *Staphylococcus aureus* that mediate resistance to mupirocin and tetracycline. *Microbiology* **140:**2577–2583.

274. **Poovelikunnel T, Gethin G, Humphreys H.** 2015. Mupirocin resistance: clinical implications and potential alternatives for the eradication of MRSA. *J Antimicrob Chemother* **70:**2681–2692.

275. **Seah C, Alexander DC, Louie L, Simor A, Low DE, Longtin J, Melano RG.** 2012. MupB, a new high-level mupirocin resistance mechanism in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **56:**1916–1920.

276. **Fines M, Pronost S, Maillard K, Taouji S, Leclercq R.** 2001. Characterization of mutations in the *rpoB* gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. *J Clin Microbiol* **39:**2784–2787.

277. **Riesenberg A, Feßler AT, Erol E, Prenger-Berninghoff E, Stamm I, Böse R, Heusinger A, Klarmann D, Werckenthin C, Schwarz S.** 2014. MICs of 32 antimicrobial agents for *Rhodococcus equi* isolates of animal origin. *J Antimicrob Chemother* **69:**1045–1049.

278. **Severinov K, Soushko M, Goldfarb A, Nikiforov V.** 1993. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the β subunit of *Escherichia coli* RNA polymerase. *J Biol Chem* **268:**14820–14825.

279. **Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T.** 1993. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* **341:**647–650.

280. **Perkins AE, Nicholson WL.** 2008. Uncovering new metabolic capabilities of *Bacillus subtilis* using phenotype profiling of rifampin-resistant *rpoB* mutants. *J Bacteriol* **190:**807–814.

281. **Li J, Feßler AT, Jiang N, Fan R, Wang Y, Wu C, Shen J, Schwarz S.** 2016. Molecular basis of rifampicin resistance in multiresistant porcine livestock-associated MRSA. *J Antimicrob Chemother* **71:**3313–3315.

282. **Kadlec K, van Duijkeren E, Wagenaar JA, Schwarz S.** 2011. Molecular basis of rifampicin resistance in methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. *J Antimicrob Chemother* **66:**1236–1242.

283. **Tupin A, Gualtieri M, Roquet-Banères F, Morichaud Z, Brodolin K, Leonetti JP.** 2010. Resistance to rifampicin: at the crossroads between ecological, genomic and medical concerns. *Int J Antimicrob Agents* **35:**519–523.

284. **Baysarowich J, Koteva K, Hughes DW, Ejim L, Griffiths E, Zhang K, Junop M, Wright GD.** 2008. Rifamycin antibiotic resistance by ADP-ribosylation: structure and diversity of Arr. *Proc Natl Acad Sci USA* **105:**4886–4891.

285. **Kadlec K, von Czapiewski E, Kaspar H, Wallmann J, Michael GB, Steinacker U, Schwarz S.** 2011. Molecular basis of sulfonamide and trimethoprim resistance in fish-pathogenic *Aeromonas* isolates. *Appl Environ Microbiol* **77:**7147–7150.

286. **Silver LL.** 2017. Fosfomycin: mechanism and resistance. *Cold Spring Harb Perspect Med* **7:**a025262.

287. **Castañeda-García A, Blázquez J, Rodríguez-Rojas A.** 2013. Molecular mechanisms and clinical impact of acquired and intrinsic fosfomycin resistance. *Antibiotics (Basel)* **2:**217–236.

288. **Jiang Y, Shen P, Wei Z, Liu L, He F, Shi K, Wang Y, Wang H, Yu Y.** 2015. Dissemination of a clone carrying a *fosA3*-harbouring plasmid mediates high fosfomycin resistance rate of KPC-producing *Klebsiella pneumoniae* in China. *Int J Antimicrob Agents* **45:**66–70.

289. **Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y.** 2010. Prevalence of fosfomycin resistance among CTX-M-producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. *Antimicrob Agents Chemother* **54:**3061–3064.

290. **Kitanaka H, Wachino J, Jin W, Yokoyama S, Sasano MA, Hori M, Yamada K, Kimura K, Arakawa Y.** 2014. Novel integron-mediated fosfomycin resistance gene *fosK*. *Antimicrob Agents Chemother* **58:**4978–4979.

291. **Zilhao R, Courvalin P.** 1990. Nucleotide sequence of the *fosB* gene conferring fosfomycin resistance in *Staphylococcus epidermidis*. *FEMS Microbiol Lett* **56:**267–272.

292. **Xu X, Chen C, Lin D, Guo Q, Hu F, Zhu D, Li G, Wang M.** 2013. The fosfomycin resistance gene *fosB3* is located on a transferable, extrachromosomal circular intermediate in clinical *Enterococcus faecium* isolates. *PLoS One* **8:**e78106.

293. **Fu Z, Liu Y, Chen C, Guo Y, Ma Y, Yang Y, Hu F, Xu X, Wang M.** 2016. Characterization of fosfomycin resistance gene, *fosB*, in methicillin-resistant *Staphylococcus aureus* isolates. *PLoS One* **11:**e0154829.

294. **He T, Wang Y, Schwarz S, Zhao Q, Shen J, Wu C.** 2014. Genetic environment of the multi-resistance gene *cfr* in methicillin-resistant coagulase-negative staphylococci from chickens, ducks, and pigs in China. *Int J Med Microbiol* **304:**257–261.

295. **Wang Y, Yao H, Deng F, Liu D, Zhang Y, Shen Z.** 2015. Identification of a novel $fosX^{CC}$ gene conferring fosfomycin resistance in *Campylobacter*. *J Antimicrob Chemother* **70:**1261–1263.

296. **Kim SY, Ju K-S, Metcalf WW, Evans BS, Kuzuyama T, van der Donk WA.** 2012. Different biosynthetic pathways to fosfomycin in *Pseudomonas syringae* and *Streptomyces* species. *Antimicrob Agents Chemother* **56:**4175–4183.

297. **Biedenbach DJ, Rhomberg PR, Mendes RE, Jones RN.** 2010. Spectrum of activity, mutation rates, synergistic interactions, and the effects of pH and serum proteins for fusidic acid (CEM-102). *Diagn Microbiol Infect Dis* **66:**301–307.

298. **Farrell DJ, Castanheira M, Chopra I.** 2011. Characterization of global patterns and the genetics of fusidic acid resistance. *Clin Infect Dis* **52**(Suppl 7)**:**S487–S492.

299. **Lannergård J, Norström T, Hughes D.** 2009. Genetic determinants of resistance to fusidic acid among clinical bacteremia isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **53:**2059–2065.

300. **O'Brien FG, Price C, Grubb WB, Gustafson JE.** 2002. Genetic characterization of the fusidic acid and cadmium

resistance determinants of *Staphylococcus aureus* plasmid pUB101. *J Antimicrob Chemother* **50:**313–321.

301. Chen HJ, Hung WC, Lin YT, Tsai JC, Chiu HC, Hsueh PR, Teng LJ. 2015. A novel fusidic acid resistance determinant, *fusF*, in *Staphylococcus cohnii*. *J Antimicrob Chemother* **70:**416–419.

302. Baines SL, Howden BP, Heffernan H, Stinear TP, Carter GP, Seemann T, Kwong JC, Ritchie SR, Williamson DA. 2016. Rapid emergence and evolution of *Staphylococcus aureus* clones harboring *fusC*-containing Staphylococcal Cassette Chromosome elements. *Antimicrob Agents Chemother* **60:**2359–2365.

303. O'Neill AJ, McLaws F, Kahlmeter G, Henriksen AS, Chopra I. 2007. Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrob Agents Chemother* **51:**1737–1740.

304. Werner G, Hildebrandt B, Witte W. 2001. Aminoglycoside-streptothricin resistance gene cluster *aadE-sat4-aphA-3* disseminated among multiresistant isolates of *Enterococcus faecium*. *Antimicrob Agents Chemother* **45:**3267–3269.

305. Kadlec K, Schwarz S. 2008. Analysis and distribution of class 1 and class 2 integrons and associated gene cassettes among *Escherichia coli* isolates from swine, horses, cats and dogs collected in the BfT-GermVet monitoring study. *J Antimicrob Chemother* **62:**469–473.

306. Ahmed AM, Shimamoto T. 2004. A plasmid-encoded class 1 integron carrying *sat*, a putative phosphoserine phosphatase gene and *aadA2* from enterotoxigenic *Escherichia coli* O159 isolated in Japan. *FEMS Microbiol Lett* **235:**243–248.

307. Boerlin P, Burnens AP, Frey J, Kuhnert P, Nicolet J. 2001. Molecular epidemiology and genetic linkage of macrolide and aminoglycoside resistance in *Staphylococcus intermedius* of canine origin. *Vet Microbiol* **79:**155–169.

308. Kadlec K, Schwarz S, Perreten V, Andersson UG, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L. 2010. Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *J Antimicrob Chemother* **65:**1826–1828.

309. Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J Antimicrob Chemother* **65:**1145–1154.

310. Walther B, Monecke S, Ruscher C, Friedrich AW, Ehricht R, Slickers P, Soba A, Wleklinski CG, Wieler LH, Lübke-Becker A. 2009. Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **47:**704–710.

311. Aarestrup FM. 2000. Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS* Suppl. 101**:**1–8.

312. Podlesek Z, Comino A, Herzog-Velikonja B, Zgur-Bertok D, Komel R, Grabnar M. 1995. *Bacillus licheniformis* bacitracin-resistance ABC transporter: relationship to mammalian multidrug resistance. *Mol Microbiol* **16:**969–976.

313. Bernard R, Joseph P, Guiseppi A, Chippaux M, Denizot F. 2003. YtsCD and YwoA, two independent systems that confer bacitracin resistance to *Bacillus subtilis*. *FEMS Microbiol Lett* **228:**93–97.

314. El Ghachi M, Bouhss A, Blanot D, Mengin-Lecreulx D. 2004. The *bacA* gene of *Escherichia coli* encodes an undecaprenyl pyrophosphate phosphatase activity. *J Biol Chem* **279:**30106–30113.

315. Manson JM, Keis S, Smith JM, Cook GM. 2004. Acquired bacitracin resistance in *Enterococcus faecalis* is mediated by an ABC transporter and a novel regulatory protein, BcrR. *Antimicrob Agents Chemother* **48:**3743–3748.

316. Weitnauer G, Gaisser S, Trefzer A, Stockert S, Westrich L, Quiros LM, Mendez C, Salas JA, Bechthold A. 2001. An ATP-binding cassette transporter and two rRNA methyltransferases are involved in resistance to avilamycin in the producer organism *Streptomyces viridochromogenes* Tü57. *Antimicrob Agents Chemother* **45:**690–695.

317. Aarestrup FM, Jensen LB. 2000. Presence of variations in ribosomal protein L16 corresponding to susceptibility of enterococci to oligosaccharides (avilamycin and evernimicin). *Antimicrob Agents Chemother* **44:**3425–3427.

318. Mann PA, Xiong L, Mankin AS, Chau AS, Mendrick CA, Najarian DJ, Cramer CA, Loebenberg D, Coates E, Murgolo NJ, Aarestrup FM, Goering RV, Black TA, Hare RS, McNicholas PM. 2001. EmtA, a rRNA methyltransferase conferring high-level evernimicin resistance. *Mol Microbiol* **41:**1349–1356.

319. Treede I, Jakobsen L, Kirpekar F, Vester B, Weitnauer G, Bechthold A, Douthwaite S. 2003. The avilamycin resistance determinants AviRa and AviRb methylate 23S rRNA at the guanosine 2535 base and the uridine 2479 ribose. *Mol Microbiol* **49:**309–318.

320. Kofoed CB, Vester B. 2002. Interaction of avilamycin with ribosomes and resistance caused by mutations in 23S rRNA. *Antimicrob Agents Chemother* **46:**3339–3342.

321. van den Bogaard AE, Hazen M, Hoyer M, Oostenbach P, Stobberingh EE. 2002. Effects of flavophospholipol on resistance in fecal *Escherichia coli* and enterococci of fattening pigs. *Antimicrob Agents Chemother* **46:**110–118.

322. Ohmae K, Yonezawa S, Terakado N. 1981. R plasmid with carbadox resistance from *Escherichia coli* of porcine origin. *Antimicrob Agents Chemother* **19:**86–90.

323. Hansen LH, Johannesen E, Burmølle M, Sørensen AH, Sørensen SJ. 2004. Plasmid-encoded multidrug efflux pump conferring resistance to olaquindox in *Escherichia coli*. *Antimicrob Agents Chemother* **48:**3332–3337.

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Resistance to Metals Used in Agricultural Production

5

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INTRODUCTION

Metal compounds are widely used in livestock production because they are necessary supplements and play a very important role as essential trace elements that are part of the nutritional requirements of most animal species. However, copper and zinc compounds are also added to feed in larger concentrations for achieving additional beneficial effects. Therefore, we will focus this chapter on copper and zinc; their use and indications; the concerns related to the selection of resistance, toxicity, and environmental pollution and policies; and the alternatives and new developments regarding their use. Compounds derived from the nonessential metal arsenic, such as roxarsone, which have been used in livestock for feed supplementation in some countries around the world, will not be discussed in great detail in this chapter.

USE OF METAL COMPOUNDS IN AGRICULTURAL PRODUCTION: REQUIREMENTS AND RECOMMENDATIONS

Copper

Copper is an essential trace element that is necessary for the human body because it has major functions regarding fetal growth and early postnatal development, hemoglobin synthesis, maturation of the connective tissues, proper nerve function and bone development, and inflammatory processes. Therefore, most animal species are required to have copper in their feed and exhibit copper deficiency symptoms if copper is insufficient. Copper is also expected to interact with other compounds in the feed (phytates, fiber fraction, zinc, iron, calcium, ascorbic acid, molybdenum, and sulfur), and therefore the concentrations added to feed are increased to ensure that the necessary amount is available for absorption by the target animal species. This is also dependent on the animal's intestinal tract, and these interactions influence the absorption, as extensively reviewed by Suttle (1).

An excess of copper can also be detrimental and lead to toxicity manifested as anemia and liver dysfunction. Release of copper from the liver may lead to vascular hemolysis and death. The toxicity of copper depends on speciation, with Cu(I) being more toxic then Cu(II). Certain animal species such as sheep and young calves are sensitive to excess copper (2). Copper might also exert a selection pressure on the gut microbiota and influence the composition of the gut flora. Furthermore, the use of therapeutic concentrations might select for resistance, which is addressed later in this chapter. Requirements for copper in animal feed depend on the target species, the age group and the feed composition (2).

Copper is contained in a number of plants and seeds in varying concentrations and may become concentrated with the use of copper compounds in agriculture, but the availability of copper in feed is not well known. For food-producing animals and small pet animals such as rabbits, the copper background levels in complete feeds are rather low and in the range of 5 (salmon feed) to 15 (rabbit feed) mg Cu/kg feed, and higher values are observed in feed for dairy cows, containing about 20 mg Cu/kg feed. Supplementation of animal feed is common practice, and the current limits for supplemen-

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tation (3) were revised by the European Union in 2016 (Table 1) (4).

Copper additives

The list of copper compounds authorized for copper supplementation in feed is extensive and includes cupric acetate, monohydrate; basic cupric carbonate, monohydrate; cupric chloride, dihydrate; cupric methionate; cupric oxide; cupric sulfate, pentahydrate; cupric chelate of amino acid hydrate; copper lysine sulfate; cupric chelate of glycine hydrate; copper chelate of hydroxy analogue of methionine; dicopper chloride trihydroxide; and copper bislysinate. These authorized compounds fall under European Economic Community (EEC) number E4 (3). Most copper-containing compounds show bioavailability similar to copper sulfate for all animal species. However, cupric oxide and cupric carbonate (and cuprous iodide in poultry) showed a lower and more variable relative bioavailability (4).

Therapeutic use of copper

Copper may be prescribed for copper deficiency and is normally administered orally using premixes in feed, or it may be administrated using injectable solutions. Additionally, copper has antimicrobial properties, and copper sulfate may be used in footbaths as a fungicide for the control of foot-rot in cattle and sheep (5 to 10% solution) (5). Copper has also been used as a growth promoter, even though the efficacy of this practice has not been very well demonstrated. Growth promotion might depend on the feed antagonists present and relate to improved appetite or digestibility in pigs and poultry (1).

Zinc

Similar to copper, zinc is also an essential trace element and therefore is necessary to comply with the nutritional requirements of livestock. Zinc is part of 10% of all proteins and contributes to their tertiary structure and the function of enzymes, and therefore its role is essential in diverse organs and systems. For example, zinc is needed for glucose and lipid metabolism, cell proliferation, embryogenesis, and systems related to the nervous and immune systems.

Symptoms of zinc deficiency can be observed in most animal species (6). Known examples of this are

Table 1 New proposed maximum limits of copper in complete animal feed[b]

Target species, animal category	R[a]	1.5 × R	Background	NPMC[b]	CAMC[b]
Chickens for fattening, reared for laying	8	12	10	25	25
Laying hens, breeder hens	8	12	10	25	25
Turkeys for fattening, 0–8 weeks of age	10	15	10	25	25
Turkeys for fattening, from 8 weeks of age onward	6	9	10	25	25
Other poultry	8	12	10	25	25
Piglets, weaned	8	12	10	25	170
Pigs for fattening	6	9	10	25	25
Sows	10	15	10	25	25
Calves, milk replacer	10	15	5	15[c]	15
Cattle for fattening	8.8[b]	13.2	10	30[d]	35
Dairy cows	8.8–13.2[b]	13.2–19.8	10	30	35
Sheep	7[b]	10.6	10	15[e]	15
Goats	7–22[b]	10.6–33	10	35	25
Horses	8.8[b]	13.2	10	25	25
Rabbits	5	7.5	10	25	25
Salmonids	5	7.5	10	25	25
Other fish	8	12	10	25	25
Crustaceans				50	50
Dogs	12	15.6[f]	10	25	25
Cats	10	13[f]	10	25	25

[a]R, requirement.
[b]NPMC, newly proposed total maximum contents of copper in complete feed (4); CAMC, currently authorized maximum content of copper in complete feed (3).
[c]Because copper in milk-based diets shows a high bioavailability and does not contain substantial amounts of copper antagonists, the background level (5 mg/kg) is not added to the allowance (15 mg/kg).
[d]Considering cattle feeding on pasture, a potentially high amount of copper antagonists in forage is taken into account.
[e]NPMC is limited by the Mineral Tolerance of Animals (MTL); dietary concentrations above 15 mg/kg could provoke chronic copper poisoning (CCP).
[f]No requirement data available; the allowance (12 and 10 mg/kg for dogs and cats, respectively) is therefore only multiplied by 1.3 to consider different bioavailability of copper supplements.
[g]Adjusted from dry matter to complete feed with 88% dry matter.
[h]Source: reference 4.

paraqueratosis in swine (7) and growth deficiencies in feathering and skeletal development in poultry (8). Zinc is an essential trace element, and zinc deficiency might be related to malabsorption of this metal compound. Similarly, Friesian cows and bull terrier dogs have been shown to have genetic syndromes related to zinc malabsorption (9, 10).

Most of the absorption of zinc occurs in the small intestine, and feed components such as phytates may hamper the absorption in monogastric species, while low molecular weight binding ligands such as citrate, picolinate, EDTA, and amino acids such as histidine and glutamate were shown to increase the absorption of zinc (11).

Zinc is mainly metabolized in the liver, and the main zinc deposit is in bone. Measurement of zinc in bone may be used to determine zinc status or utilization, while measurement of zinc in hair may be used for diagnostic purposes. Zinc concentrations in plasma are not reliable because zinc binds extensively to proteins.

Since zinc is mainly excreted in feces, it is the variable zinc excretion that together with absorption maintains zinc homeostasis in the body. These processes also influence the seepage of zinc from manure into the environment. Zinc absorption and bioavailability are influenced by the feed composition; not only the plant component but also high concentrations of other metals such as copper, nickel, and iron may lead to zinc deficiency. Therefore, formulations of feed need to take into account other feed components that might interfere with zinc absorption and bioavailability.

Toxicity to zinc is low in general, and tolerance to high levels of zinc has been observed. However, ruminants, especially young and pregnant animals, are more susceptible to the effects of excess zinc compared to pigs or poultry. Zinc toxicity has been observed in steers and heifers, but tolerance seems to be higher in dairy cows. Sheep may develop symptoms of excess zinc in their diet, manifesting as symptoms ranging from weakness and jaundice to abortions and stillbirth. Horses, especially young and pregnant animals, are among the most sensitive to excess zinc in the diet and react with lameness and osteochondrosis. Pigs are among the most zinc-tolerant animal species, and it has been shown that even though they can exhibit symptoms of toxicity, it depends on the exposure time to excess zinc diet and the relation of other components of the diet such as calcium. Therefore, in some countries, high zinc concentrations are allowed for therapeutic purposes (12).

Zinc is naturally occurring in agricultural produce, but the levels vary greatly. The content of zinc in natural products depends on the zinc concentration in the soil and the conditions that might influence zinc uptake by plants. Some zinc may also be added due to deposition on plants, soil contamination, and processing. Similar to copper, the available concentrations in raw materials vary and are relatively low in relation to the animal requirements. Therefore, zinc supplementation to animal feed is common (12). However, supplementation has been at relatively high levels, and several organizations have indicated that these levels could be lowered to reduce the impact on environment (12, 13).

For requirements in different animal species and age groups as well as current and proposed supplementation levels, please consult the data in Table 2 (12).

Zinc additives

For supplementation of feed, a number of additives are utilized, including zinc oxide, zinc chloride monohydrate, zinc sulfate monohydrate, zinc sulfate heptahydrate, zinc chelate of amino acids hydrate, zinc acetate dihydrate, and zinc lactate trihydrate, which fall under EEC number E6 (3). In regard to therapeutic use, the products used are similar but require a prescription if regulation permits. In the European Union, this usage has been allowed in some countries but it will be discontinued because the Committee for Veterinary Medicine Products at the European Medicines Agency has recommended that market authorizations be withdrawn for existing products, and new products will be refused largely due to environmental risks and acknowledgement of the risk for of coselection (14).

Therapeutic use of zinc

Zinc may be used in some countries under prescription for treatment and metaphylaxis of diarrheal disease in young animals such as postweaning piglets. Zinc supplements are normally given in the form of premixes used in feed or drinking water. The most relevant zinc forms for this practice are zinc oxide, zinc chloride, and zinc sulfate. For example, in Denmark and Belgium, veterinarians have until recently prescribed zinc oxide supplementation of up to 2,500 ppm for weaning pigs for up to 14 days after weaning, which is much more than the maximum concentration allowed in pig feed—250 ppm in the European Union, with the usual concentrations ranging between 50 and 125 ppm (15–17). Similarly, in other countries around the world, the therapeutic use of zinc is relatively widespread (18), and doses for therapeutic use are between 1,000 and 3,000 ppm for up to 2 to 3 weeks (19). This is contradictory to the fact that these high doses given over a long period may cause toxicity. The use of therapeutic concentrations of zinc has been considered beneficial for the

Table 2 New proposed maximum limits of zinc in complete feed[f]

Target species, animal category	R[a]	1.5 × R	Background	NPMC[b]	CAMC[c]
Chickens for fattening, reared for laying	40–50	60–75	30	100	150
Laying hens, breeder hens	45	67.5	30	100	150
Turkeys for fattening, 0–8 weeks of age	70	105	30	120	150
Turkeys for fattening, from 8 weeks of age onward	50	75	30	120	150
Other poultry	40, 60, 60	80	30	100	150
Piglet below 11 kg weight	100	150	30	150	150
Piglet, weaned above 11 kg weight	80	120	30	150	150
Pigs for fattening	60	90	30	100	150
Sows	50–100	75–150	30	150	150
Calves, milk replacer	40	60	30	100	200
Cattle for fattening	35[d]	53	30	100	150
Dairy cows, dairy heifer	44[d]	66	30	100	150
Sheep	40[d]	60	30	100	150
Goats	31[d]	47	30	100	150
Horses	44[d]	66	30	100	150
Rabbits	70	105	50	150	150
Salmonids	50	75	60	150	200
Other fish	20	30	60	100	200
Dogs		100[e]	70	150	250
Cats		75[e]	70	150	250

[a]R, requirement.
[b]NPMC, newly proposed total maximum contents of zinc in complete feed (13).
[c]CAMC, currently authorized maximum content of zinc in complete feed (3).
[d]Adjusted from dry matter to complete feed with 88% dry matter.
[e]Allowance, taken as 1.5 times the requirement.
[f]Source: reference 13.

reduction and prevention of postweaning diarrhea in weaners and postweaning scouring (20). However, the growth promotion effects are not well understood (12).

According to the risk assessments released at the end of 2016, the benefits of the therapeutic use of zinc compounds in animal production do not outweigh the environmental risks, and additionally, there is an acknowledged risk for coselection of antibiotic resistance; therefore the Committee for Veterinary Medicine Products has recommended the withdrawal of existing market authorizations for veterinary products containing zinc and refusal of approval for new formulations. This recommendation has been backed by the EU Commission, and phase-out is expected in the coming years. Therefore, such products will not be available for therapeutic purposes in the European Union member states in the future (14).

Other Metallic Compounds for Therapeutic Purposes

Arsenic is a metalloid compound that is naturally present in rocks but is present in low amounts in soil and water. The presence of arsenic in the environment may also occur due to human industrial activity (mining, burning nonferrous metals, burning fossil fuel, or use in fertilizers, pesticides, insecticides, etc.). Unlike copper and zinc, arsenic is not thought to be an essential trace element, and therefore supplementation in feed is not considered for nutritional purposes. Arsenic and inorganic arsenic compounds are considered toxic and carcinogenic, while other arsenic-derived compounds are considered either "possibly carcinogenic to humans" or "not classifiable as to their carcinogenicity to humans" (21). However, there have been reports of beneficial effects in cancer therapy, and therefore arsenic might have some unexpected beneficial effects (22). Additionally, exposure to inorganic arsenic in drinking water has been linked to a reduction in breast cancer (23).

Organic arsenical compounds are active against coccidia and other parasites, so they have been used in poultry production to reduce coccidial infection and promote growth. However, even though roxarsone, arsanilic acid, and nitarsone are organic compounds and are thought to be less toxic, it was noticed that their usage led to the increased occurrence of inorganic arsenic residues in liver detected by improved analytical methods, and they have therefore been under scrutiny. These compounds were widely used in the United States and have only recently been banned, starting with a ban on roxarsone, carbasone, and arsanilic acid in 2013, which

was followed by a ban on the use of nitarsone for histomoniasis in turkeys in 2015. However, the widespread use and subsequent exposure to arsenic in humans was determined through a significant association between arsenic content in urine and poultry intake in a study performed in the United States between 2003 and 2010 (24). Because these compounds are now banned in the United States and the European Union, it is important to focus on other places in the world where this type of supplementation is still ongoing (25, 26), both to avoid consumption of arsenic by humans and to avoid pollution of the environment around farms (27).

Effect of Supplementation on Microflora and Organs

Copper

It has been generally assumed that copper does not affect the normal bacterial flora, but an extensive literature study found that in piglets and growing pigs, low copper concentrations (<50 mg/kg feed) affected the microbiota in the gastrointestinal tract. Similarly, 100 to 250 mg/kg copper sulfate was found to significantly change the microbial community structure. In poultry (broilers), supplementation with copper, even at low concentrations (<50 mg/kg feed), appeared to affect the microbiota in the gastrointestinal tract. In particular, the *Clostridia* population seemed to be affected even at low concentrations. At higher concentrations (>200 mg/kg feed), inorganic or organic bound copper also appeared to affect the population of lactobacilli and coliforms in broilers (28). Furthermore, the reduction of the pH of the gizzard content may cause severe gizzard erosion (4).

The risk assessments made for copper salt supplements (including copper carbonate) in relation to human consumption have concluded that a maximum residue level is not needed because it seems unlikely that copper salts, when used according to the specifications in veterinary medicine settings, are likely to cause harm in humans through consumption of residues contained in food of animal origin (29).

Zinc

The main consensus regarding zinc supplementation is that it does not appear to have major consequences on the microflora and organs. However, recent studies demonstrated that the effect observed might be due to the regulation of factors related to the immune response leading to a reduction of stem cell factor expression in the small intestine and causing a reduction in the number of mast cells and histamine release. These findings may have important implications for the prevention of weaning-associated diarrhea in piglets (30). However, a number of studies suggest that changes in the stress response may also indicate a more profound effect of therapeutic zinc supplementation that might affect the immune response (31).

The risk assessments made for zinc supplements in relation to human consumption have concluded that a maximum residue level is not needed because it seems unlikely that zinc salts, when used according to the specifications in veterinary medicine settings, are likely to cause harm in humans through consumption of residues contained in food of animal origin (32).

Usage Data and Distribution

High amounts of zinc additives are used for medical purposes in piglet production in Europe. As an example: European pig production was 248 million heads in 2008 (EURO-25 in 2008); 30% of these were produced in Denmark and Belgium. A percentage piglets were most likely given feed containing 2.5 g Zn/kg in the first 14 days after weaning. If daily feed consumption is 0.4 kg in the first week and 0.5 kg in the second week, the zinc consumed amounts to approximately 1,312 tons per year (13).

A similar scenario can be found in the United States. Here, as in Europe, zinc is fed to newly weaned pigs at high dietary levels of 2.0 to 3.0 g/kg feed (19). Assuming a mean of 2.5 g/kg and the same feed intake during the first 14 days after weaning as stated above for Europe, the 113.8 million pigs raised for slaughter in 2016 (33) would have consumed approximately 1,800 tons of zinc.

Taking Denmark as an example of a major pig-producing country using zinc by prescription, it was observed that the consumption of zinc oxide prescribed by Danish veterinarians has increased over the past 10 years, from ~150 tons in 2005 to ~500 tons in 2014. In this time period, the total consumption of zinc oxide tripled, while pig production increased by only 14% (15, 20). Another example of a European country using zinc oxide for therapeutic purposes is Belgium, where the use of zinc oxide in therapeutic doses in piglets administered for 2 weeks after weaning has been allowed only since September 2013. In Belgium, zinc oxide has become an alternative for colistin, which was previously used in weaned piglets. The data for 2014 and 2015 show a substantial increase in the use of this metal compound, with 81,964 kg and 87,199 kg of zinc oxide, respectively, consumed in Belgium. Simultaneously, the Belgian authorities noticed a decrease in the use of polymyxins (colistin) from 5.8 mg/PCU (population correc-

tion unit, which is defined as a measure of biomass, where 1 PCU is equivalent to 1 kg of biomass of livestock or slaughtered animals) in 2012, when zinc oxide was not available, to 3.3 mg/PCU in 2014, and a further 51% decrease was noticed in 2015 (16, 34). As mentioned above, the market authorizations for zinc products will be withdrawn for use for therapeutic purposes following the decision taken by European Medicines Agency, and therefore we expect a sharp drop in the consumption of this compound in the European Union. Because the use of zinc compounds is not exclusive to Europe and is quite widespread in North America (18) and other regions, some therapeutic use might remain, depending on local regulations. Reducing the amount of zinc to a dietary requirement of 100 mg/kg for newly weaned pigs would reduce the yearly zinc consumption in pig farming by over 90% (35).

It has been shown that high zinc supplementation after weaning until pigs reached 12 kg, followed by high copper supplementation until they reached about 25 kg in body weight was both cost-effective and promoted the best growth. Suggested therapeutic copper levels of 100 to 250 mg/kg are 95 to 98% above the dietary requirement for this age group (19, 35).

Environmental Concerns from Use of Metals in Agriculture: Metal Ecotoxicity

The extensive supplementation of feed with these compounds and the additional practices of therapeutic and/or growth promoter usage are not innocuous. Most of these compounds are excreted by the animals and end up in the environment, where their accumulation can have major consequences (36) because these are not degradable and will increase the concentration in the environment, especially in soils amended with slurry/manure from animal production (37). For example, Denmark, which is one of the largest pig-producing countries in the world and uses metals in animal production, has maintained a national monitoring program of heavy metals in the environment for the last 28 years to better understand the effects of these practices on the environment. The values and analyses published in 2016 indicate that the use of pig slurry has led to a significant increase in the concentrations of copper and zinc in soil (38).

Furthermore, the data show that 45% of the analyzed soils have reached zinc levels above the predicted no-effect concentrations (PNEC), while for copper, only one soil sample was above the PNEC. Continued agricultural use of zinc is expected to increase the levels, and larger areas will have soil exceeding the PNEC. Furthermore, the authors noted that the current situation raises concerns regarding the aquatic environment because the current use of zinc and copper in agriculture might lead to leaching of these metals into water and negatively affect aquatic species (37, 38).

In addition, at the European level, zinc is one of the compounds studied as part of risk assessments regarding the environmental pollution of heavy metals. These reports focus on aspects related to manufacturing of zinc products. A number of studies looking at the accumulation of zinc in soils due to agricultural use are also mentioned, and although these studies are relatively different in scope and methodology, they all agree that zinc will accumulate in soils in a significant manner, and increasing areas will exceed the critical concentrations. However, the time this will take varies and therefore needs to be assessed at the regional level (39). Taking into account these aspects and also acknowledging the risk for coselection, the European authorities have been alerted and are conducting ongoing collection of information for environmental risk assessment of heavy metals as part of veterinary medicinal products. The Committee for Veterinary Medicine Products has therefore concluded that the benefits do not outweigh the risks, and the risk for the environment justifies withdrawing the market authorizations for the therapeutic usage of zinc compounds (34).

Alternatives

As mentioned above, zinc and copper cannot be replaced since they are essential trace elements, but the concentrations can be reduced and adjusted to the essential requirements to avoid overuse. Similarly, in the future, the use of those metals at high concentrations for therapeutic purposes will be discontinued at least in the European Union, not only because of the added pressure leading to selection of bacterial resistance to copper and zinc and subsequently coselection of antibiotic-resistance genes, but mainly because of environmental concerns. Antimicrobials are alternatives for this therapeutic use but are not viable options since there are a number of ongoing efforts to reduce antimicrobial consumption in animals. Replacement should go hand in hand with improvement of management practices to reduce the need for the use of metals or other compounds during the weaning period.

DETECTION OF HEAVY METAL RESISTANCE

In recent years, the identification and/or characterization of strains of bacteria of animal origin that have reduced susceptibility or increased tolerance to heavy

metals has gained a lot of attention, and therefore, an increasing number of studies include heavy metal susceptibility or tolerance testing. The Web of Science database was searched systematically for articles between January 2005 and December 2016 using the search terms "heavy+ metal+ resistance+ animal" and "heavy+ metal+ resistance+ bacteria+ MIC". This resulted in the retrieval of 278 articles, of which only 23 reported MIC data on bacterial isolates of animal origin, including fish. These studies were reviewed and revealed a number of weaknesses, which can impact the quality of the results or the conclusions made. Here, we will highlight the major pitfalls that make comparisons of studies challenging.

Several methods, such as disc diffusion, agar dilution, and microbroth dilution, have been used in metal tolerance assays. Disc diffusion, the use of paper discs impregnated with a specific heavy metal salt concentration placed on agar plates spread with bacteria, was rarely used (only two studies [40, 41]). Agar and microbroth dilution were the most common methods to determine an isolate's MIC to a particular heavy metal. Both methods are based on a dilution series of the heavy metal that is incorporated into either molten agar or liquid broth. The methodology is similar to that used for antibiotic susceptibility testing, where the dilution series is typically 2-fold dilutions rather an exponential serial dilution. While most studies use the lowest concentration at which there is inhibition of visible growth, a few studies use optical density measurements to determine the presence of bacterial growth (42, 43).

As previously described by Hasman et al. (44), the choice of media and the pH of that media are crucial factors that can influence the detection of metal resistance levels. For example, certain components in complex media can sequester free metal ions, reducing the concentration available for the bacteria. Therefore, it is advisable to use standardized media such as Mueller Hinton, which has been extensively validated for use in antibiotic susceptibility testing. Of the 23 studies included in this analysis and described using agar dilution (n = 18), up to six different media were used. While Mueller Hinton agar was the most common, brain heart infusion agar (45), Luria Bertani agar (46), nutrient agar (47, 48), and tryptic soy agar (49, 50) were also used. As previously mentioned, the pH of the medium can influence the detection of metal resistance. For example, pH values above 5.5 can cause zinc sulfate in complex media to precipitate, thereby reducing the concentration of the metal that the bacteria have to encounter. Furthermore, addition of metals into the medium can change the final pH, which can affect bacterial growth. Therefore, it is strongly recommended that after supplementation of the metal, the media is adjusted accordingly to ensure optimal bacterial growth and avoid misleading results. Only seven articles noted that pH adjustments were performed. However, even in studies where adjustments were reported, the pH value was not always consistent; e.g., for $CuSO_4$ in Mueller-Hinton agar, a range of pH values were used by the different studies (pH 7, 7.2, and 7.4) (51–55). It is recommended that when using Mueller Hinton agar or broth, the adjusted pH should be as follows: zinc chloride, pH = 5.5; copper sulfate, pH = 7.2; sodium arsenate, cadmium acetate, and silver nitrate, pH = 7.4 (51, 56).

Studies wanting to categorize bacterial isolates as susceptible or resistant to a particular heavy metal on the basis of their MICs or inhibition zone diameters require approved interpretive criteria. There are no clinical breakpoints for heavy metals. Therefore, epidemiological cutoff values are based on previous studies reporting the MIC distributions for a particular bacterial species. Five studies used a control strain, e.g., the laboratory strain *Escherichia coli* K12 or C600, and strains were considered resistant if the MIC values exceeded that of the control strain (57–61). While this could be a reasonable approach to characterize reduced heavy metal susceptibility among bacterial isolates, the control strain should belong to the same bacterial species as the test isolates. Akinbowale et al. (59) used *E. coli* K12 to describe resistance in *Aeromonas* spp. and *Pseudomonas* spp. While all isolates are Gram negative, there are intrinsic differences between the species that can affect the interpretation of the results.

Increased metal tolerance to zinc and copper has been described in many bacterial species. Initially, these resistant bacterial species were detected among environmental bacteria isolated from areas with high concentrations of heavy metals. Due to the use of heavy metals in food-producing animals and the association of heavy metal resistance with antimicrobial resistance as a result of co-selection, many studies have investigated the levels of reduced susceptibility (Table 3) and the associated genes in a number of bacterial species isolated from food-producing animals (Table 4).

MECHANISMS OF METAL HOMEOSTASIS AND RESISTANCE

Metal homeostasis is a delicate balance of ensuring that a cell's requirement for essential metals is met to ensure proper cell function while at the same time limiting the amount of metal in the cell to avoid toxicity. The

Table 3 Bacteria of animal origin for which copper and zinc resistance has been described

Metal	Bacterial isolate(s)	Animal origin	References
Copper	Actinomycetes	Goat	236
	Aeromonas hydrophilia	Fish including shellfish	48
	Citrobacter spp.	Fish, pigs	47, 144
	Enterococcus faecalis, E. faecium	Chicken, cattle, pigs	56
	Escherichia coli	Chicken, cattle, pigs	56, 144, 145, 155
	Klebsiella pneumoniae	Prawns	237
	Lactobacillus spp.	Pigs, cattle, poultry	238
	Salmonella spp.	Chicken, cattle, pigs	56, 144, 156
	Serratia marcescens	Fish including shellfish	48
	Staphylococcus aureus, *S. hyicus*	Chicken, cattle, pigs	56
	Streptomycetes	Goats	236
Zinc	*A. hydrophilia*	Fish including shellfish	48
	E. faecalis, E. faecium	Chicken, cattle, pigs	56
	E. coli	Chicken, cattle, pigs	56
	K. pneumoniae	Prawns	237
	Lactobacillus sp.	Pigs, cattle, poultry	238
	P. mirabilis	Fish including shellfish	48
	P. aeruginosa	Various	49
	Salmonella spp.	Chicken, cattle, pigs	56
	S. marcescens	Fish including shellfish	48
	S. aureus, *S. hyicus*	Chicken, cattle, pigs	56
	Vibrio cambelli, V. harveyi, V. mimicus, V. ordelli	Fish including shellfish	48

ability to balance the influx and efflux of metals, metal homeostasis, is determined by the presence or absence of metal transport systems, which can depend on an organism's living environment and therefore its exposure to metals.

Copper

As in other soft metals, the bioavailability of copper changed with the appearance of oxygen in the environment during the Great Oxidation Event. Copper became available due to the increased oxidation of copper sulfides and the higher solubility of Cu(II) (62, 63). With life initially evolving under anoxic conditions and the limited availability of copper for cells, prokaryotes utilized a limited number of copper-containing enzymes. Copper homeostasis mechanisms evolved to protect cells from increasing copper availability and its cytotoxicity due to Cu(I) attacking Fe-S clusters and the generation of reactive oxygen species under aerobic conditions (64–66).

Copper uptake

In contrast to eukaryotes, prokaryotes use a small number of copper-containing enzymes that are, with the exception of plastocyanin and caa_3-type cytochrome oxidase, located in the periplasm and cytoplasmic membrane. Therefore, very few specific copper uptake systems have been identified in bacteria. In *Bacillus subtilis*, the protein YcnJ shows homology to CopCD, appearing to be a fusion of the two proteins, and was shown to be involved in copper uptake. Expression of *ycnJ* is regulated by the repressor YcnK (67, 68). While common in the genus *Bacillus*, *ycnJ* does not seem to be present in other members of the *Firmicutes*. *Bacillus cereus* produces a copper-chelator (coproporphyrin III) that might be involved in copper uptake (69).

Gram-negative bacteria face the additional challenge of an outer membrane to take up required nutrients. In *Methylosinus trichosporium*, *E. coli* and *Pseudomonas aeruginosa* porins have been found to play an important role in copper uptake, allowing the metal ion to enter the periplasm and therefore being able to interact with the transporters in the cytoplasmic membrane (70–72). From the periplasm, copper can enter the cell via several mechanisms. ZupT, a member of the ZIP family in *E. coli*, was shown to have a broad substrate spectrum also taking up Cu(II) (73, 74). Since members of this protein family are ubiquitously present, it can be assumed that they can also allow copper uptake in other species as well (75). Similar to YcnJ in *B. subtilis*, some members of the *Proteobacteria* have CopCD, with CopC being a periplasmic copper-binding protein and CopD a cytoplasmic membrane transporter. Overexpression of *copCD* increases the cells' copper sensitiv-

Table 4 Copper- and zinc-resistance genes identified in bacteria from livestock

Heavy metal	Heavy metal-resistance gene	Bacterial species	Animal origin	References
Copper	*pcoA*, *pcoD*	*Salmonella enterica* serovar Mbandaka *S. enterica* serovar Derby *S. enterica* serovar Heidelberg *S. enterica* serovar Typhimurium *S. enterica* serovar Worthington *S. enterica* serovar Rissen *S. enterica* serovar Agona *S. enterica* serovar Senftenberg *S. enterica* serovar London *S. enterica* serovar Ohio *Escherichia coli* *Histophilius somni*	Pigs, cattle, poultry	53, 146, 239–242
	copB	*Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus haemolyticus* *Staphylococcus hominis* *Staphylococcus lentus* *Staphylococcus pasteuri* *Staphylococcus rostri* *Staphylococcus sciuri* *E. coli*	Pigs, cattle, poultry	199, 240, 243
	cueO	*E. coli*	Poultry	240
	cusC	*E. coli*	Poultry	240
	mco	*S. aureus*	Pigs	243
Zinc	*czrC*	Mainly associated with methicillin-resistant staphylococci: *S. aureus* *Staphylococcus hyicus* *Staphylococcus epidermidis* *S. haemolyticus* *S. hominis* *S. lentus*	Pigs, cattle, poultry, meat from these animal species	51, 199–203, 244, 245
	czcD	*S.* Typhimurium *S. enterica* serovar Infantis	Pigs	53
	zntA	*E. coli*	Poultry	240

ity, indicating an uptake system (76). In several Gram-negative bacteria, copper chelators (chalkophore) have been identified, and *Cyanobacteria* utilize siderophores for both copper and iron uptake (70, 77–82).

Chromosomally encoded copper defense systems

Due to the increased bioavailability of copper during Earth's history and the capability of Cu(I) to damage iron-sulfur clusters and catalyze production of reactive oxygen species, organisms had to develop strategies to deal with the toxic effects of copper to cells. While the targets of copper toxicity are mainly restricted to the cytoplasm in Gram-positive bacteria, Gram-negative bacteria face the additional challenge of protecting their periplasm.

Since copper often enters the bacterial cell in an unspecific manner (utilizing other metal-uptake systems), bacteria cannot limit the amount of copper that enters the cytoplasm. Therefore, they had to develop mechanisms to expel excess cytoplasmic copper.

Widespread transporters utilized for copper efflux are members of the P_{1B-1}-ATPase subfamily [Cu(I) transporters] of P_{1B}-ATPases (formerly CPx-ATPases for the CPx-sequence motif in transmembrane helix VI) (83–90). In bacteria, the first copper-transporting ATPases were identified in *Enterococcus hirae* (91–95). *E. hirae* encodes two proteins, CopA and CopB, with

one, CopB, belonging to the "classical" P_{1B-1}-ATPases removing excess Cu(I) from the cytoplasm, while CopA belongs to the FixI/CopA2-like ATPases that are important for proper assembly of membrane-bound Cu-containing enzymes (85, 87, 96). P_{1B}-ATPases have been identified to be involved in copper resistance in several Gram-positive bacteria (97, 98).

Similarly, in Gram-negative bacteria, P_{1B-1}-ATPases have been shown to be involved in expelling excess copper from the cytoplasm. The best characterized are the chromosomally encoded copper homeostasis systems from *E. coli*, including *copA* encoding the P_{1B}-ATPase CopA (99–101). In *E. coli*, *copA* is part of the CueR-regulon. The repressor CueR binds Cu(I) at zeptomolar concentrations and regulates the expression of *copA* and *cueO* (100, 102–105). While CopA in *E. coli* expels Cu(I) from the cytoplasm (106, 107), Cu(I) would now still be in the periplasm to cause damage to the cell. This necessitates CueO, which is a periplasmic multicopper oxidase that has been shown to oxidize Cu(I) into the less toxic Cu(II). CueO also oxidized enterobactin, enabling further sequestration of copper (108–116). The presence of genes encoding homologs of CopA and CueO is widespread among members of the *Enterobacteriaceae*, and this two-pronged approach appears to be a common strategy for copper detoxification (under "normal" aerobic circumstances) (49, 117–121). However, for CueO to be active, the organism requires the presence of oxygen. In the digestive tract of humans and/or animals, limited or no oxygen is available [and Cu(I) is also more stable than in an oxygenated environment]. Therefore, other strategies to keep the periplasm safe from copper toxicity need to be in place (99, 122). In *E. coli*, the CBA transport complex CusCBA is involved in Cu detoxification of the periplasm in the absence of CueO (99, 100, 123). Expression of *cusCFBA* is regulated by the two-component regulatory system CusRS, with CusS being a membrane-bound sensor kinase that detects periplasmic copper and relays the information to the cytoplasmic response regulator CusR, regulating *cusCFBA* expression (124, 125). It was found that in an anaerobic environment, expression was induced at much lower copper concentrations than in the presence of oxygen (100). The CusCBA complex is composed of the resistance nodulation cell division (RND)-protein CusA in the cytoplasmic membrane, the outer membrane factor (OMF) CusC in the outer membrane, and the membrane fusion protein CusB stabilizing the complex. Three molecules of CusA form a complex creating a "vestibule," which forms a funnel to the channel formed by the CusC trimer. Cu(I) enters the funnel in the periplasm and via CusC is expelled into the surrounding medium. Cu(I) transport is energized via the proton gradient across the cytoplasmic membrane (101, 126–131). While CBA transport systems have been described for different types of heavy metals and organic compounds (including antibiotics), the presence of the periplasmic protein CusF is unique to transport systems involved in copper and silver transport (123). CusF could either act as a periplasmic chaperon delivering the metal ion to the CBA transport complex or function as a regulator of the transport (132–139). An alternative system for protection of the periplasm against copper-mediated toxicity under anaerobic conditions can be found in *Salmonella*. Here, the copper tolerance is linked to the presence of the periplasmic protein CueP. Expression of *cueP* in a *cus*-deletion strain of *E. coli* can partially restore its copper resistance under anaerobic conditions (120, 140–143). CueP seems to be confined to members of the genera *Citrobacter* and *Salmonella*.

Copper resistance systems encoded on mobile elements

Genomic islands involved in copper resistance of *Enterobacteria*

A plasmid-bound copper resistance determinant in *Enterobacteria* was first described on plasmid pRJ1004 from *E. coli* (144, 145). Copper resistance encoded on this plasmid is linked to the presence of the *pco* system, which encodes a two-component regulatory system, PcoRS, as well as to the structural proteins involved in copper resistance: PcoA, a periplasmic multicopper oxidase, the outer membrane protein PcoB, the inner membrane protein PcoD, and two periplasmic proteins, PcoC and PcoE (146–149). Expression of *pcoE* is regulated via a chromosomally encoded system, CusRS (124, 149), while expression of *pcoABCD* is regulated by PcoRS (147). Periplasmic PcoE is able to sequester excess copper, giving the cell temporary protection from the toxic effects of copper (149). Periplasmic PcoC also binds copper but is able to transfer it to membrane-bound PcoD to catalyze Cu(I) uptake into the cell. Once in the cytoplasm, Cu(I) is incorporated into the multicopper oxidase PcoA, which is then exported into the periplasm via the twin-arginine translocation pathway. Once in the periplasm, PcoA detoxifies Cu(I) by either oxidation of catechol siderophores and subsequent sequestration of Cu(I) or oxidation of Cu(I) to Cu(II). Cu(II) might be removed from the periplasm via PcoB (Fig. 1) (101, 146, 148, 150, 151). Located adjacent to *pco* on pRJ1004 is a *sil* determinant (150, 151). The *sil* determinant was first identified on plasmid pMG101

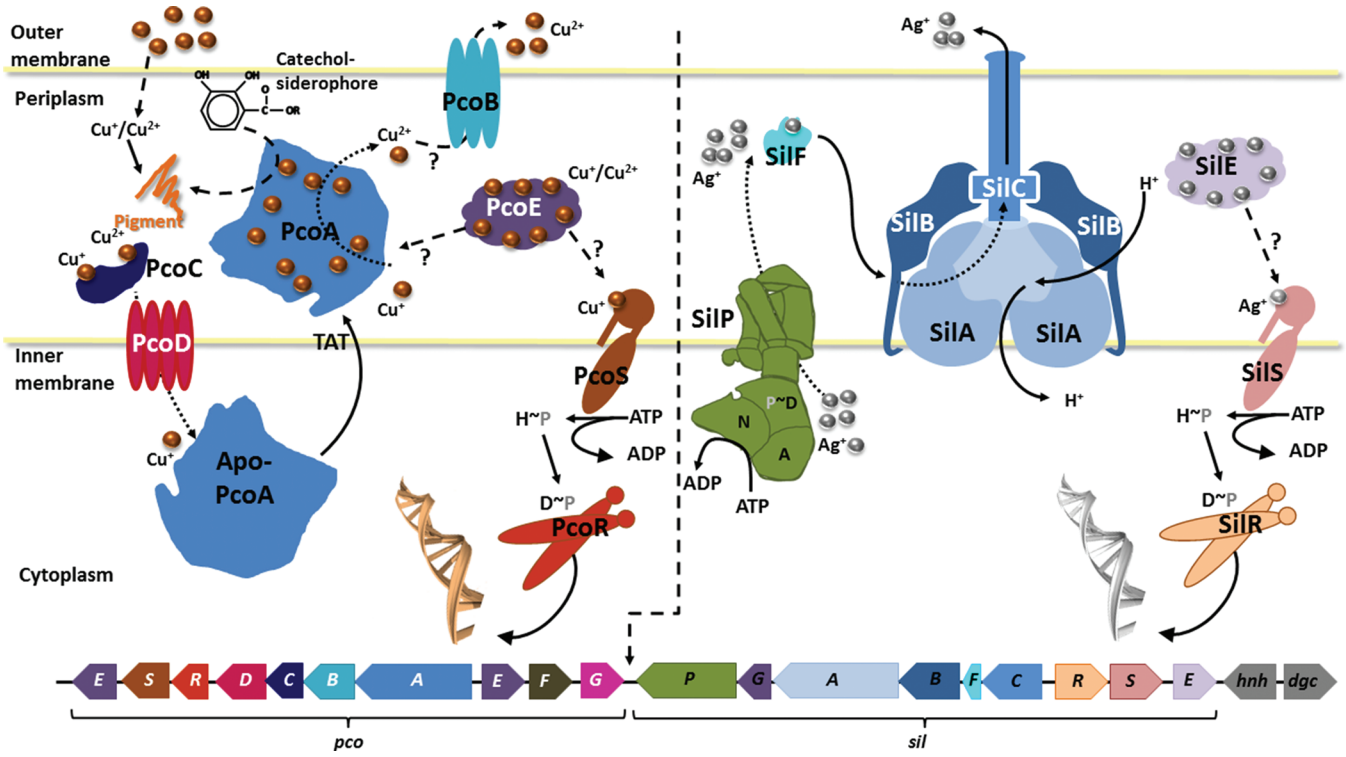


Figure 1 Copper fitness (or pathogenicity) island in *Enterobacteriaceae*. Genes and protein products of the enterobacterial copper fitness island composed of the *pco*- and *sil*-determinants. The genes, including their transcriptional/translational direction, are indicated below the illustration of the proposed or experimentally determined function of the proteins encoded by the *pco/sil* system. Refer to text for details. (Reprinted with permission [171].)

from *Salmonella enterica* serovar Typhimurium and was shown to confer silver resistance (152, 153). Expression of the structural genes of the *sil* determinant is regulated by the two-component regulatory system SilRS. In the presence of silver, *silCFBA* is expressed forming the SilCBA efflux complex (similar to CusCBA), allowing for the export of Ag(I) and Cu(I) from the periplasm. SilF is a periplasmic protein (like CusF) that is able to bind Ag(I) and either shuttles Ag(I) to the transport complex as substrate for export or could act as a regulatory protein of the function of SilCBA. In addition to the RND-dependent transporter SilCBA, the P_{1B-1}-ATPases SilP, the periplasmic protein SilE, and a periplasmic protein, SilG, of unknown function are encoded as part of the *sil* determinant. Like PcoE, SilE is able to bind metal ions [Ag(I) and Cu(I)] and protect the periplasm from short-term metal stress. The P_{1B-1}-ATPase SilP transports silver ions from the cytoplasm into the periplasm, from where they can be removed via SilCBA (150, 151, 153, 154). In contrast to copper, silver ions cannot undergo redox reactions, and therefore oxidation of Ag(I) as a detoxification mechanism is not an option for the cell. However, the entire *sil* resistance determinant also confers resistance to Cu(I). The overall 20-gene cluster *pco/sil* has been referred to as a copper-pathogenicity island (150) or a copper homeostasis and silver resistance island (151) (Fig. 1). The entire gene cluster has been identified in isolates of *E. coli* and *S.* Typhimurium, including *E. coli* O104:H4 from pigs fed a high-copper diet (155, 156).

Analysis of the available completed genome and plasmid sequences of *Enterobacteria* (Table 5) revealed that the *pco/sil* gene cluster is frequently flanked by *Tn*7-like elements, allowing for transfer of the gene cluster (154, 157). Recent sequencing of *E. coli* J53 (pMG101) (NCTC 50110) showed that the *pco/sil* cluster has integrated from pMG101 (152, 153) into the bacterium's genome (154). Interestingly, in some genera (*Citrobacter*, *Kosakonia*, and *Raoultella*), all of the identified *pco/sil* sequences are chromosomally encoded, while in others (*Klyvera* and *Serratia*), the sequences are carried only on plasmids. In most of the genera containing the *pco/sil* determinant, it can be carried on plasmids as well as on the bacterial chromo-

Table 5 Distribution of *pco/sil* and yersiniabactin biosynthesis genes among *Enterobacteriaceae*

Genus[a]	Number of sequences analyzed[b]	Occurrence of copper/silver tolerance determinants				
		pco[c]	*sil*[d]	*pco/sil*[e]	Yersiniabactin synthesis[f]	*pco/silP* and yersiniabactin synthesis[g]
Citrobacter	60 genomes	10	10	10	3	0
	50 plasmids	0	0	0	0	
Cronobacter	29 genomes	1	1	1	0	0
	22 plasmids	6	6	6	0	
Enterobacter	167 genomes	22	28	21	0	0
	121 plasmids	6	7	6	0	
Escherichia	946 genomes	33	31	30	121	18
	901 plasmids	7	11	7	0	
Klebsiella	674 genomes	2	3	2	82	30
	628 plasmids	92	92	91	2	
Kluyvera	6 genomes	0	0	0	0	0
	4 plasmids	1	1	1	0	
Kosakonia	10 genomes	1	1	1	0	0
	4 plasmids	0	0	0	0	
Pantoea	66 genomes	0	0	0	0	0
	61 plasmids	0	1	0	0	
Raoultella	9 genomes	1	1	1	4	1
	8 plasmids	0	0	0	0	
Salmonella	602 genomes	14	13	13	5	0
	369 plasmids	4	6	4	7	
Serratia	64 genomes	0	0	0	0	0
	27 plasmids	2	2	2	0	
Yersinia	192 genomes	0	0	0	33	0
	153 plasmids	0	0	0	0	

[a]Genera of *Enterobacteriaceae* harboring *pco*, *sil*, and/or *ybt*.
[b]Number of completed genomic and plasmid sequences of respective genera available for Microbial Genome BLAST (http://blast.ncbi.nlm.nih.gov; accessed 13 September 2017).
[c]Analysis (blastn) using *pco* from pRJ1004 (GenBank accession number X83541.1 [146]) as query.
[d]Analysis (blastn) using *sil* from pMG101 (GenBank accession number NG_035131.1 -[153]) as query.
[e]Analysis (blastn) using *pco* (GenBank accession number X83541.1; [146]) and *sil* (accession number KC146966.1 [151]) from pRJ1004 as query.
[f]Analysis (tblastn) using Ybt peptide/polyketide synthetase HMWP1 (GenBank accession number AAC69588.1 [246]) as query.
[g]Number of strains harboring *pco/sil* and *ybt* with determinant being located on chromosome and/or plasmid, respectively.

some, including strains that harbor a copy on each location. While in most cases the entire 20-gene cluster *pco/sil* is present, some strains of *Enterobacter*, *Escherichia*, *Klebsiella*, *Pantoea*, and *Salmonella* contain only one of the two resistance determinants, *pco* or *sil* (Table 5).

Another strategy of *Enterobacteriaceae* to protect against the toxic effects of copper is the use of siderophores, especially yersiniabactin (158). The presence of the genes encoding the yersiniabactin synthesis pathway has previously been described as a virulence factor, but its presence in copper-resistant isolates indicates its importance in protection from copper toxicity. Yersiniabactin biosynthesis has been found in strains of *E. coli*, *Salmonella*, *Yersinia pestis*, and *Klebsiella pneumoniae*, including *E. coli* isolates from livestock fed high-copper diets (155, 159–163). Analysis of available completed genome and plasmid sequences of *Enterobacteriaceae* revealed the presence of the yersiniabactin biosynthesis pathways in the genera *Citrobacter* and *Raoultella*, in addition to the previously known genera. While commonly encoded on the bacterial chromosome, in some genera the yersiniabactin biosynthesis genes are located on plasmids as well (Table 5). Some strains of *E. coli* (including strains of enterohemorrhagic *E. coli* O104:H4), *K. pneumoniae*, and *Raoultella* have both the *pco/sil* gene cluster and the yersiniabactin biosynthesis determinant (Table 5).

Genomic islands involved in copper resistance of *Enterococcus*

In addition to Gram-negative bacteria, Gram-positive bacteria of the genus *Enterococcus* have frequently been isolated from livestock and shown to have high copper

resistance (52, 121, 164, 165). Copper resistance in *Enterococcus* has been shown to be plasmid encoded and can be transferred via conjugation (121, 166–169). The first gene that could be linked to the plasmid-encoded copper resistance determinant in *Enterococcus* was *tcrB*. TcrB is a member of the P_{1B}-ATPases of copper transporters, but due to the presence of a histidine-rich cytoplasmic N-terminus, a CPH-motif in transmembrane helix VI, and an MSXST-motif, it would be predicted to belong to the P_{1B-3}-ATPase subfamily utilizing Cu(II) as substrate. TcrB is encoded as part of the *tcrYAZB* operon, which encodes TcrA, an additional P_{1B}-ATPase of the P_{1B-1}-ATPase subfamily with Cu(I) as the transported substrate, a cytoplasmic copper chaperon TcrZ, and TcrY, a copper-dependent regulator (150, 166, 170). Approximately 62% of *Enterococcus* strains that harbor transferrable copper resistance contained not only *tcrYAZB* but also *cueO*. This is in contrast to the strains analyzed in another study, where 22% only had *tcrB* and 16% encoded for CueO (121).

Sequence analysis of copper-resistant *Enterococcus faecalis* showed the presence of *tcrZAYB* and *cueO* as part of a larger gene cluster that also encoded a two-component regulatory system, CusRS, and an additional transcriptional regulator, CopY. Directly adjacent to *copY*, an additional P_{1B-1}-ATPase named CopA is encoded, leading to the prediction of *copA* expression being regulated by CopY. Genes encoding several putative metal-binding proteins possibly serving as metal chaperones in the copper detoxification process are also in close proximity. The genes *cusRS* are directly downstream of *cueO*, suggesting regulation by this two-component regulatory system. Since the P_{1B}-ATPases TcrA, TcrB, and CopA export copper ions from the cytoplasm, regulation of the respective genes by cytoplasmic copper-dependent regulators, CopY and TcrY, seems logical. In contrast, CueO, located just outside the cytoplasmic membrane, oxidizing Cu(I) to the less toxic Cu(II), appears to be regulated by CusRS sensing environmental copper concentrations (Fig. 2) (42, 150, 171). Other strains of *E. faecalis* and *Enterococcus faecium* contain related copper-resistance islands that vary slightly in the presence and arrangement of some of the genes in the copper-resistance island. These changes are probably at least in part due to the large number of putative transposons encoded in this region. BLAST analysis of the *E. faecium* HF50105 genome revealed a similar gene region in 5 of the 149 completed plasmid sequences of members of the genus *Enterococcus*, two belonging to *Enterococcus durans*, two to *E. faecium*, and one to *Enterococcus gallinarum*. No chromosomally encoded copper-resistance island could be identified. Further sequence analysis showed that this gene cluster is only present in the genus *Enterococcus* but not in other *Firmicutes*.

Zinc

Zinc bioavailability has changed drastically in course of Earth's history. The greater bioavailability of zinc after the Great Oxidation Event is reflected in the increased use of zinc in eukaryotes (62, 63, 172). Zinc serves as a structural or catalytic component in hundreds of proteins and is one of the most abundant transition metals in a cell. The total amount of zinc in an *E. coli* cell was determined to be in a range similar to the cellular concentrations of calcium and iron (173). However, free Zn(II) in the cytoplasm is essentially nonexistent, since the Zn(II) regulators ZntR (efflux) and Zur (uptake) respond to free Zn(II) concentrations of 10^{-16} M in *E. coli* (173). This shows that bacteria very tightly control the uptake and efflux of Zn(II) to ensure proper cellular function while avoiding metal toxicity.

Zinc uptake

At least three systems (ABC-transporter, ZIP transporter, and phosphate-bound uptake) have been described to be involved in bacterial zinc uptake. Best understood are the systems involved in zinc uptake of *E. coli*. Under zinc-limiting conditions, *E. coli* utilizes the ABC (ATP-binding cassette) transporter ZnuABC. ZnuA is a zinc-binding protein located in the periplasm. ZnuB spans the cytoplasmic membrane and forms the channel to transport Zn(II) into the cytoplasm. ZnuC is bound to the cytoplasmic site on ZnuB, providing the energy for Zn(II)-transport via ATP hydrolysis (174, 175). Expression of the *znu* operon is regulated by Zur, which binds cytoplasmic Zn(II) and acts as repressor of *znu* by blocking the RNA polymerase from binding to the −10 region of the *znuC* promoter. Half-maximal repression by Zur *in vitro* has been shown to occur at a concentration of 2.0 (± 0.1) × 10^{-16} M free Zn(II), indicating that the presence of any free Zn(II) in the cell effectively turns off Zn(II) uptake by ZnuABC (173, 176–178). While genes encoding ZnuABC homologs have been identified in the genomes of many bacteria, it is absent in the genome of *Cupriavidus metallidurans* CH34, a bacterium originally isolated from a zinc decantation tank (179–181).

Under nonlimiting zinc conditions, *E. coli* utilizes a different uptake system, ZupT, the first identified bacterial member of the ZIP (ZRT-, IRT-like protein [ZRT, zinc-regulated transporter; IRT, iron-regulated transporter]) family of transport proteins (75, 182). Genes encoding ZupT have been identified in members of

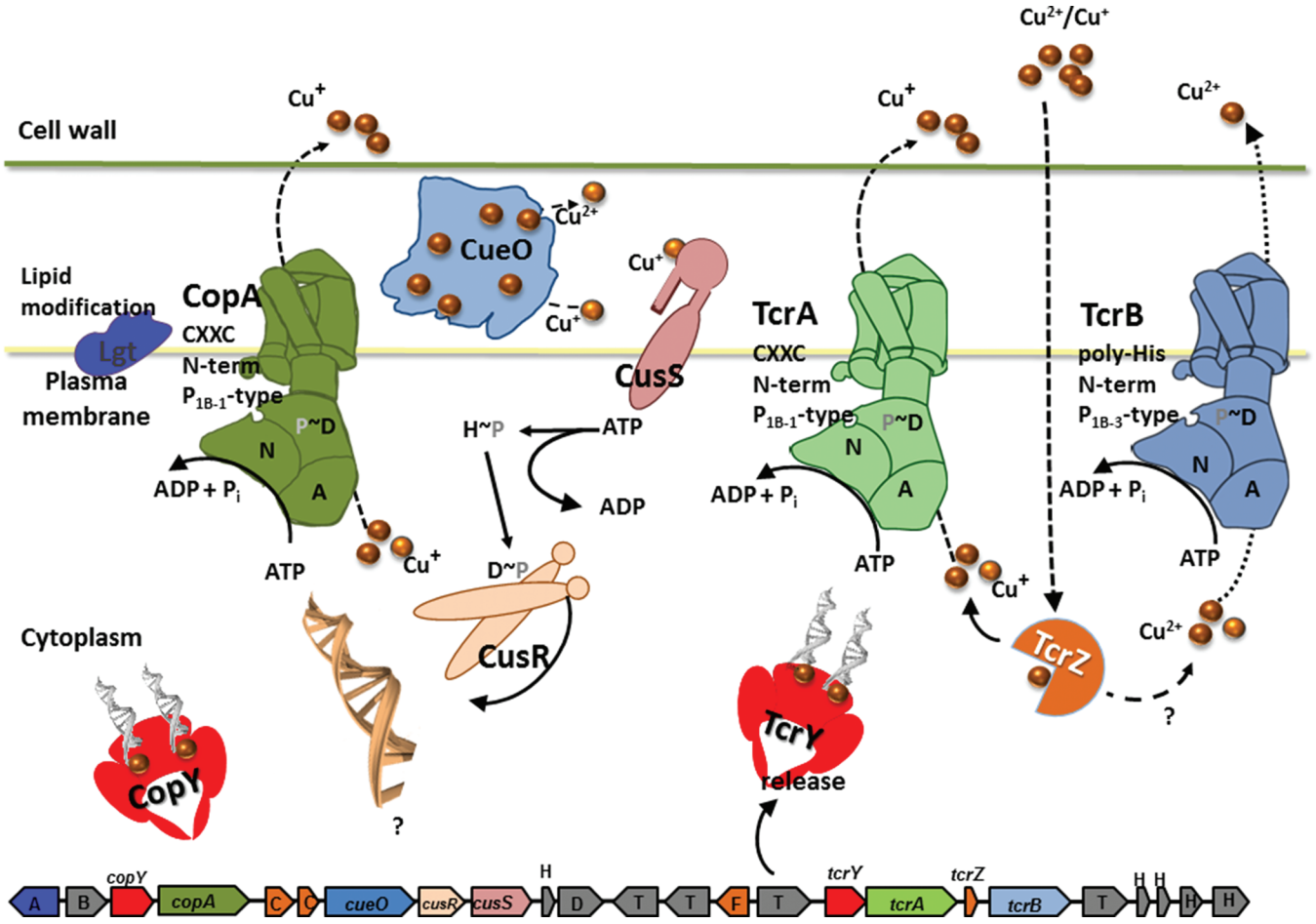


Figure 2 Copper fitness island in *Enterococcus*. Genes and proposed protein products of the copper island in *Enterococcus faecium* HF50105 (GenBank accession number AITS01000024). The genes, including their transcriptional/translational direction, are indicated below the illustration of the proposed function of the proteins. (Refer to text for details.) Adjacent to and separating the genes involved in copper resistance are genes encoding prolipoprotein diacylglyceryl transferase (A), integral membrane protein (B), predicted metal-binding protein/chaperone (C), hypothetical protein (H), transposase (T), and disrupted P-type ATPase (F) that have been identified. (Reprinted with permission [171].)

many bacterial phyla. In *E. coli*, *zupT* is expressed constitutively at a low level, and expression is therefore independent of external zinc availability. In contrast, in *C. metallidurans*, *zupT* expression is regulated by FurC, decreasing the amount of transporter under high zinc conditions and thereby limiting Zn(II) uptake (74, 181). It is not known if or how *zupT* expression is regulated in other microorganisms. While initially described as a zinc uptake system in *E. coli*, ZupT has broad substrate specificity and is also able to take up other metals, including Fe(II), Co(II), and Mn(II). Cadmium and copper also cross the cytoplasmic membrane via ZupT-mediated unspecific uptake (74). Several amino acids have been identified to contribute to ZupT's substrate specificity (73). In uropathogenic *E. coli* as well as *S. enterica*, ZupT plays an important role in bacterial virulence, especially in the absence of *znu* (175, 183).

Under nonlimiting conditions, zinc can also enter *E. coli* as a metal-phosphate complex via PitA (inorganic phosphate uptake system) (184, 185). Magnesium uptake systems have been shown to have a broad substrate spectrum and are able to transport Zn(II) in addition to Mg(II) (180, 186–188). The presence of both zinc uptake systems is very common among bacteria.

Zinc efflux

Zinc resistance in bacteria is facilitated via efflux (189, 190). At least four systems (P_{1B}-type ATPases, CDF

transporters, 2-TM-GxN transporters, and CBA efflux systems) involved in transporting Zn(II) out of the cell have been identified. These systems can be encoded chromosomally or on plasmids.

The most effective transporters that export Zn(II) out of the cell are soft-metal P_{1B}-type ATPases. Members of this family of membrane proteins contain six to eight transmembrane helices and cytoplasmic domains involved in ATP binding and hydrolysis as well as metal-binding domains. Based on structural features and the resulting substrate spectra, several subfamilies can be differentiated. Zn(II) is exported by members of the P_{1B-2}-ATPase subgroup (83, 86). The best-characterized member of this group is ZntA from *E. coli* (191, 192). Members of this subgroup possess eight transmembrane helices, with a conserved CPC motif and Asp as transmembrane metal-binding sites. ZntA has a broad substrate specificity and can also transport Pb(II) and Cd(II), as can other members of this P_{1B-2}-ATPase subgroup. Additionally, other metals such as Co(II), Ni(II), and Cu(II) bind to the transmembrane metal-binding domain, However, when these metals are bound, no ATP hydrolysis or transport takes place (193). Zn(II)-transporting ATPases can be chromosomally encoded, such as ZntA from *E. coli*, or encoded on plasmids, such as CadA from *Staphylococcus aureus* (191, 194, 195). Expression of *zntA* of *E. coli* is regulated by the MerR-like regulator ZntR. In the absence of Zn(II), apo-ZntR binds to the *zntA* promoter, repressing transcription. In the presence of Zn(II) in the cytoplasm, Zn-ZntR acts as a transcriptional activator and allows for transcription of *zntA*. *In vitro* analysis of ZntR affinity to Zn(II) revealed that concentration in the femtomolar range is required for ZntR to bind. However, *in vivo* studies identified ZntR responding to cytoplasmic free Zn(II) concentrations in the nanomolar range (173, 196). On the *E. coli* chromosome, *zntR* and *zntA* are not located in the same gene cluster (197). In contrast, on plasmid pI258 of *S. aureus*, the genes encoding the P_{1B-2}-ATPase and its regulator are organized in an operon, *cadCA*. CadC is a member of the ArsR/SmtB-family and represses transcription in the absence of Zn(II), Cd(II), or Pb(II) (198). P_{1B-2}-ATPases are highly efficient at removing Zn(II) from the cytoplasm, and deletion of the respective genes usually results in Zn(II) sensitivity of the organism (189, 191). Another P_{1B-2}-ATPase of *Staphylococcus* is CzrC, which can be found in some methicillin-resistant strains encoded as part of SCC*mec* (staphylococcal cassette chromosome *mec*) (51, 199, 200). While often associated with SCC*mec*, *czrC* has also been identified in *mecA*-negative strains and on plasmids of *S. aureus* (201). Initially identified in *S. aureus*, *czrC* has also been found in isolates of other *Staphylococcus* species: *S. haemolyticus*, *S. epidermitis*, *S. lentus*, *S. hominis*, and *S. hyicus* (51, 199, 201–204). Out of the 489 completed *Staphylococcus* genomes in the NCBI database (as of 1 November 2017), 15 carry *czrC* (9 *S. aureus*, 1 *S. condimenti*, 3 *S. epidermis*, and 2 *S. simulans*), while the gene is not found on any of the 483 *Staphylococcus* plasmid sequences. While to date, the regulation of *czrC* expression has not yet been studied, it is likely regulated by an ArsR-like regulator encoded directly upstream of *czrC*.

A second family of membrane transporters linked to bacterial zinc export are cation diffusion facilitator (CDF) proteins, which contain six transmembrane helices with a C- and N-terminus located in the cytoplasm. *E. coli* contains two genes encoding CDF proteins. Both transporters, ZitB and FieF, have been shown to be involved in Zn(II) transport (205, 206). While deletion of *zitB* alone does not impact *E. coli*'s ability to handle elevated concentrations of Zn(II), a double mutant defective in both ZitB and the P_{1B-2}-ATPase ZntA exhibits higher zinc sensitivity than a mutant strain defective only in ZntA (205). While no increase in zinc sensitivity was observed after deletion of *fieF*, everted membrane vesicles of *E. coli* GR362 (deficient in ZnuABC, ZupT, ZntA, ZntB, and ZitB) expressing *fieF* accumulated ^{65}Zn(II). This transport was energized via the proton gradient, as has been shown for other CDF proteins (206). Using reporter gene fusion, expression of both genes, *zitB* and *fieF*, was found to be induced by Zn(II) and to a lesser extent by Cd(II). However, later analysis showed that mRNA levels of *zitB* remain constant after addition of Zn(II) and are elevated, but still independent of Zn(II) concentration, in a *zntA* deletion strain (196, 205, 206). It has been suggested that ZitB is involved in maintaining zinc homeostasis under "normal" conditions, while ZntA confers zinc resistance. CDF proteins involved in Zn(II) transport have been identified in many bacterial species. In *S. aureus*, the chromosomally encoded CDF transporter has been named ZntA and is encoded in an operon encoding ZntR in addition to ZntA. ZntA-null mutants were zinc sensitive, and expression of the operon was Zn(II) dependent and regulated by ZntR (207, 208).

First identified in *S.* Typhimurium, ZntB is a member of the 2-TM-GxN family of membrane transporters. This transporter family is widely found in bacteria, and ZntB-like proteins have been identified in many *Proteobacteria*. Deletion of *zntB* rendered *S.* Typhimurium more sensitive to Zn(II) and Cd(II). ZntB has two transmembrane helixes with the ~270-amino acid

N-terminus and the small C-terminus located in the cytoplasm. ZntB forms a pentamer, forming a cytoplasmic funnel. Each soluble domain monomer of ZntB binds three Zn^{2+}, one in the funnel, while full-length ZntB binds four Zn^{2+} (209–213). In *Agrobacterium tumefaciens*, *zntB* is constitutively expressed but does not seem to contribute to the bacterium's metal tolerance (214).

In contrast to the zinc efflux systems described so far, CBA efflux systems are protein complexes composed of a central transporter of the RND family, a membrane fusion protein, and an OMF. CBA efflux systems are involved in the transport of mono- and divalent heavy metals as well as organic compounds. The RND protein has 12 transmembrane helixes and two large periplasmic domains, which interact with the OMF. Both the RND and the OMF function as homotrimers, forming a channel starting in the periplasmic vestibule of the RND and reaching across the outer membrane via the OMF. The membrane fusion protein (hexamer) interacts with the RND protein and the OMF (190, 215, 216).

Of interest here are members of the heavy metal efflux (HME) family. Members of the HME-RND family are involved in the export of heavy metals, and several subfamilies can be differentiated based on sequence motifs and resulting metal substrates. Zn(II) transport has been credited to the HME1 subfamily (217). The best-characterized member of this family is CzcA from *C. metallidurans* CH34. The *czc* gene cluster is encoded on plasmid pMOL30 of *C. metallidurans* CH34 and allows growth in the presence of Co(II), Zn(II), and Cd(II), increasing the MIC by factors of 10, 25, and 100, respectively, compared to the plasmid-free strain AE104 (218). While export of the metal ions from the cytoplasm was assumed and data indicate transport by CzcA across the cytoplasmic membrane, the fact that a CDF protein as well as a P_{1B-4}-ATPase are encoded as part of the *czc* gene cluster in addition to the two-component regulatory system CzcRS, which monitors the metal content within the periplasm, points toward export of the majority of metal ions from the periplasm into the surrounding environment by CzcCBA. As indicated, the CzcCBA system from *C. metallidurans* CH34 is the best-characterized zinc transporting CBA system to date. The structure of CzcCBA has not yet been solved (189, 215, 216, 219–224). The first structure of an RND protein was solved for AcrB, a multidrug transporter from *E. coli* (225, 226). To date, additional structures of RND transporters have been solved, including ZneA from *C. metallidurans* CH34. ZneA was shown to actively transport Zn(II) across the membrane via conformational changes within the three ZneA protomers. ZneA interacts with ZneB (membrane fusion protein) and ZneC (OMF) to form the membrane-spanning transport complex. The structure of ZneB was also shown to undergo conformational changes during export of the metal ions, reminiscent of CusB. However, the involvement of ZneCAB in zinc homeostasis of *C. metallidurans* CH34 is not understood (227, 228). Compared to CDF and P_{1B}-type ATPases, CBA efflux systems are not as frequently present on bacterial genomes in bacterial handling of excess zinc.

Copper and Zinc Resistance Determinants: Link to Bacterial Virulence

In addition to these specific metal resistance determinants, there are numerous other genes that improve survival under elevated copper or zinc concentrations. This overview does not go into detail regarding these rather unspecific genes, but we refer the reader to a recent excellent study as an example (229).

The involvement of zinc and copper in bacterial killing is a recent important finding that is of relevance to understanding potential public health challenges arising from feeding livestock copper and zinc supplements. Professional phagocytes such as macrophages engulf pathogens and destroy them through elevation of Zn^{2+} and Cu^{+} concentrations in the phagosome, along with other mechanisms (oxidative burst, induction of Fe^{2+}, and Mn^{2+} efflux) (230).

However, it is probable that the mechanisms macrophages use to kill infectious agents evolved in protozoa long before multicellular life arose and the need for macrophages appeared (231). This hypothesis was strengthened showing that copper (and probably zinc) poisoning is used in the model amoeba *Dictyostelium discoideum* to kill bacterial prey (232). Bacteria, in turn, were not just inert prey, and some succeeded in bypassing and resisting phagocytic cells by developing a number of strategies to avoid the protozoan killing mechanisms such as counteracting resistance systems specific for Zn and Cu and possibly other antimicrobial metals. This main concept of ongoing evolution driven by protozoan predation could explain the ongoing evolution of pathogens such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and extended spectrum beta-lactamase-producing *E. coli*, all of which pose a very important public health problem (233). Farmed animals such as pigs and poultry receive additional Zn and Cu in their diets due to supplementing elements in compound feed as well as medical remedies. Enteral bacteria in farmed animals have been shown to develop resistance to trace elements such as

Zn and Cu. Resistance to Zn is often linked with resistance to methicillin in staphylococci, and Zn supplementation to animal feed may increase the proportion of multiresistant *E. coli* in the gut. Resistance to Cu in bacteria, enterococci in particular, is often associated with resistance to antimicrobial drugs such as macrolides and glycopeptides (e.g., vancomycin). Since Cu and Zn have an important role in protozoan predation, these metal resistances could make survival of these bacteria much more likely. These strains could then become pathogens after transfer to humans (234, 235).

CONCLUSIONS

Copper and zinc have been widely used in livestock feed both as growth promoters and as necessary supplements. In many countries the use of these metals appears to have increased due to a ban on the use of antibiotics. They are effective growth promoters, but it is not entirely clear how this is achieved. The potential negative impacts include zinc and copper contamination in the environment but more importantly the potential of coselecting antibiotic-resistance genes and possibly generating more pathogenic strains of medically relevant bacteria.

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References

1. **Suttle NF.** 2010. *Mineral Nutrition of Livestock*, 4th ed. CABI Publishing, Wallingford, United Kingdom. p 283–342.
2. **European Commission Scientific Committee for Animal Nutrition (SCAN).** 2003. Opinion of the Scientific Committee for Animal Nutrition on the use of copper in feedingstuffs.
3. **European Commission.** 2003. Commission Regulation (EC) no 1334/2003 of 25 July 2003. Amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements.
4. **EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).** 2016. Revision of the currently authorised maximum copper content in complete feed. *EFSA J* **14:**e04563.
5. **European Medicines Agency (EMA), Committee for Veterinary Medicinal Products (CVMP).** 1998. Copper chloride, copper gluconate, copper heptanoate, copper oxide, copper methionate, copper sulfate and dicopper oxide: Summary Report. EMEA/MRL/431/98-FINAL.
6. **Todd WR, Elvehjem CA, Hart EB.** 1980. Nutrition classics. The American Journal of Physiology. Volume 107, 1934, pages 146–156. "Zinc in the nutrition of the rat" by W.R. Todd, C.A. Elvehjem and E.B. Hart. *Nutr Rev* **38:**151–154.
7. **Tucker HF, Salmon WD.** 1955. Parakeratosis or zinc deficiency disease in the pig. *Proc Soc Exp Biol Med* **88:** 613–616.
8. **O'Dell BL, Newberne PM, Savage JE.** 1958. Significance of dietary zinc for the growing chicken. *J Nutr* **65:** 503–518.
9. **Chesters JK.** 1983. Zinc metabolism in animals: pathology, immunology and genetics. *J Inherit Metab Dis* **6** (Suppl 1)**:**34–38.
10. **Jezyk PF, Haskins ME, MacKay-Smith WE, Patterson DF.** 1986. Lethal acrodermatitis in bull terriers. *J Am Vet Med Assoc* **188:**833–839.
11. **Hambidge KM, Casey CE, Krebs NF.** 1986. Zinc, p 1–137. *In* Mertz W (ed), *Trace Elements in Human and Animal Nutrition*, **vol 2**. Academic Press, San Diego, CA.
12. **European Commission's Scientific Committee for Animal Nutrition (SCAN).** 2003. Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs.
13. **EFSA Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP).** 2014. Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. *EFSA J* **12:**3668.
14. **European Medicines Agency (EMA).** 2016. Committee for Medicinal Products for Veterinary Use (CVMP) meeting 6–8 December 2016. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/events/2015/09/event_detail_001206.jsp&mid=WC0b01ac058004d5c3
15. **DANMAP.** 2014. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark*. DANMAP, Copenhagen, Denmark.
16. **BelVetSAC.** 2015. Belgian Veterinary Surveillance of Antibacterial Consumption National consumption report.
17. **BelVetSAC.** 2014. Belgian Veterinary Surveillance of Antibacterial Consumption National consumption report.
18. **Slifierz M.** 2016. The effects of zinc therapy on the coselection of methicillin-resistance in livestock-associated *Staphylococcus aureus* and the bacterial ecology of the porcine microbiota. PhD thesis. University of Guelph, Guelph, Canada.
19. **Jacela JY, DeRouchey JM, Tokach MD, Goodband RD, Nelssen JL, Renter DG, Dritz SS.** 2010. Feed additives for swine: fact sheets–high dietary levels of copper and zinc for young pigs, and phytase. *J Swine Health Prod* **18:**87–91.
20. **Poulsen HD.** 1995. Zinc oxide for weanling piglets. *Acta Agriculturae Scandinavica* **45:**159–167.
21. **European Food Safety Authority.** 2014. Dietary exposure to inorganic arsenic. *EFSA J* **12:**3597.
22. **Antman KH.** 2001. Introduction: the history of arsenic trioxide in cancer therapy. *Oncologist* **6**(Suppl 2)**:**1–2.
23. **Smith AH, Marshall G, Yuan Y, Steinmaus C, Liaw J, Smith MT, Wood L, Heirich M, Fritzemeier RM, Pegram MD, Ferreccio C.** 2014. Rapid reduction in

breast cancer mortality with inorganic arsenic in drinking water. *EBioMedicine* **1:**58–63.

24. **Nigra AE, Nachman KE, Love DC, Grau-Perez M, Navas-Acien A.** 2017. Poultry consumption and arsenic exposure in the U.S. population. *Environ Health Perspect* **125:**370–377.
25. **Caldas D, Pestana IA, Almeida MG, Henry FC, Salomão MSMB, de Souza CMM.** 2016. Risk of ingesting As, Cd, and Pb in animal products in north Rio de Janeiro state, Brazil. *Chemosphere* **164:**508–515.
26. **Zhang XY, Zhou MY, Li LL, Jiang YJ, Zou XT.** 2017. Effects of arsenic supplementation in feed on laying performance, arsenic retention of eggs and organs, biochemical indices and endocrine hormones. *Br Poult Sci* **58:**63–68.
27. **Xi GF, Zhou SB, Ding HC, Yao CX, Kong JJ.** 2014. Characteristics of arsenic content in the livestock farms' surrounding environment in Shanghai suburbs. *Huan Jing Ke Xue* **35:**1928–1932. (In Chinese.)
28. **Jensen BB.** 2016. Extensive literature search on the 'effects of copper intake levels in the gut microbiota profile of target animals, in particular piglets'. *EFSA Supporting Publications* **13:**1024E-n/a.
29. **European Medicines Agency (EMA), Committee for Veterinary Medicinal Products (CVMP).** 2016. European public MRL assessment report (EPMAR): copper carbonate (all food producing species). EMA/CVMP/758734/2015.
30. **Ou D, Li D, Cao Y, Li X, Yin J, Qiao S, Wu G.** 2007. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. *J Nutr Biochem* **18:**820–826.
31. **Schulte JN, Brockmann GA, Kreuzer-Redmer S.** 2016. Feeding a high dosage of zinc oxide affects suppressor of cytokine gene expression in *Salmonella* Typhimurium infected piglets. *Vet Immunol Immunopathol* **178:**10–13.
32. **European Medicines Agency (EMA), Committee for Veterinary Medicinal Products (CVMP).** 1996. Zinc salts. EMEA/MRL/113/96-FINAL:
33. **United States Department of Agriculture (USDA).** 2017. Livestock slaughter 2016 summary. USDA National Agricultural Statistics Service, Washington, DC.
34. **European Medicines Agency (EMA), European Surveillance of Veterinary Antimicrobial Consumption (ESVAC).** 2016. Sales of veterinary antimicrobial agents in 29 European countries in 2014. EMA/61769/2016.
35. **National Research Council, Committee on Nutrient Requirements of Swine.** 2012. *Nutrient Requirements of Swine*. National Academies Press, Washington, DC.
36. **Stolte J, Tesfai M, Øygarden L, Kværnø S, Keizer J, Verheijen F, Panagos P, Ballabio C, Hessel R (ed).** 2016. *Soil Threats in Europe*. EUR 27607 EN doi: 10.2788/488054. (print); doi:10.2788/828742 (online).
37. **Jensen J, Larsen MM, Bak J.** 2016. National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. *Environ Pollut* **214:**334–340.
38. **Bak JL, Jensen J, Larsen MM.** 2015. Belysning af kobber- og zinkindholdet i jord. Indhold og udvikling i kvadratnettet og måling på udvalgte brugstyper. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 72 s. - Videnskabelig rapport fra DCE - Nationalt Center for Miljø og Energi nr. 159.
39. **Joint Research Center (JRC) European Union.** 2010. Risk assessment report. CAS: 7440-66-6; EINECS No: 231-175-3 ZINC METAL.
40. **Johnson TJ, Siek KE, Johnson SJ, Nolan LK.** 2005. DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic *Escherichia coli* transmissible R plasmid. *Antimicrob Agents Chemother* **49:**4681–4688.
41. **Salem AZM, Ammar H, Lopez S, Gohar YM, González JS.** 2011. Sensitivity of ruminal bacteria isolates of sheep, cattle and buffalo to some heavy metals. *Anim Feed Sci Technol* **163:**143–149.
42. **Chen S, Li X, Sun G, Zhang Y, Su J, Ye J.** 2015. Heavy metal induced antibiotic resistance in bacterium LSJC7. *Int J Mol Sci* **16:**23390–23404.
43. **Henriques I, Tacão M, Leite L, Fidalgo C, Araújo S, Oliveira C, Alves A.** 2016. Co-selection of antibiotic and metal(loid) resistance in Gram-negative epiphytic bacteria from contaminated salt marshes. *Mar Pollut Bull* **109:**427–434.
44. **Hasman H, Franke S, Rensing C.** 2005. Resistance to metals used in agricultural production, p 99–114. *In* Aarestrup FM, Wegener HC (ed), *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
45. **Jacob ME, Fox JT, Nagaraja TG, Drouillard JS, Amachawadi RG, Narayanan SK.** 2010. Effects of feeding elevated concentrations of copper and zinc on the antimicrobial susceptibilities of fecal bacteria in feedlot cattle. *Foodborne Pathog Dis* **7:**643–648.
46. **Bednorz C, Oelgeschläger K, Kinnemann B, Hartmann S, Neumann K, Pieper R, Bethe A, Semmler T, Tedin K, Schierack P, Wieler LH, Guenther S.** 2013. The broader context of antibiotic resistance: zinc feed supplementation of piglets increases the proportion of multi-resistant *Escherichia coli in vivo*. *Int J Med Microbiol* **303:**396–403.
47. **Toroglu S, Dincer S.** 2009. Heavy metal resistances of *Enterobacteriaceae* from Aksu River (Turkey) polluted with different sources. *Asian J Chem* **21:**411–420.
48. **Kumar PA, Joseph B, Patterson J.** 2011. Antibiotic and heavy metal resistance profile of pathogens isolated from infected fish in Tuticorin, south-east coast of India. *Indian J Fish* **58:**121–125.
49. **Deredjian A, Colinon C, Brothier E, Favre-Bonté S, Cournoyer B, Nazaret S.** 2011. Antibiotic and metal resistance among hospital and outdoor strains of *Pseudomonas aeruginosa*. *Res Microbiol* **162:**689–700.
50. **Wei LS, Musa N, Wee W.** 2010. Bacterial flora from a healthy freshwater Asian sea bass (*Lates calcarifer*) fingerling hatchery with emphasis on their antimicrobial and heavy metal resistance pattern. *Vet Arh* **80:**411–420.

51. **Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T, Aarestrup FM.** 2010. Cloning and occurrence of *czrC*, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. *Antimicrob Agents Chemother* **54:** 3605–3608.
52. **Fard RM, Heuzenroeder MW, Barton MD.** 2011. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. *Vet Microbiol* **148:**276–282.
53. **Medardus JJ, Molla BZ, Nicol M, Morrow WM, Rajala-Schultz PJ, Kazwala R, Gebreyes WA.** 2014. In-feed use of heavy metal micronutrients in U.S. swine production systems and its role in persistence of multidrug-resistant salmonellae. *Appl Environ Microbiol* **80:**2317–2325.
54. **Mourão J, Marçal S, Ramos P, Campos J, Machado J, Peixe L, Novais C, Antunes P.** 2016. Tolerance to multiple metal stressors in emerging non-typhoidal MDR *Salmonella* serotypes: a relevant role for copper in anaerobic conditions. *J Antimicrob Chemother* **71:**2147–2157.
55. **Amachawadi RG, Scott HM, Vinasco J, Tokach MD, Dritz SS, Nelssen JL, Nagaraja TG.** 2015. Effects of in-feed copper, chlortetracycline, and tylosin on the prevalence of transferable copper resistance gene, *tcrB*, among fecal enterococci of weaned piglets. *Foodborne Pathog Dis* **12:**670–678.
56. **Aarestrup FM, Hasman H.** 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. *Vet Microbiol* **100:**83–89.
57. **Hacioglu N, Tosunoglu M.** 2014. Determination of antimicrobial and heavy metal resistance profiles of some bacteria isolated from aquatic amphibian and reptile species. *Environ Monit Assess* **186:**407–413.
58. **Fang L, Li X, Li L, Li S, Liao X, Sun J, Liu Y.** 2016. Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids from *E. coli* isolates of food-producing animals. *Sci Rep* **6:**25312.
59. **Akinbowale OL, Peng H, Grant P, Barton MD.** 2007. Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *Int J Antimicrob Agents* **30:**177–182.
60. **He Y, Jin L, Sun F, Hu Q, Chen L.** 2016. Antibiotic and heavy-metal resistance of *Vibrio parahaemolyticus* isolated from fresh shrimps in Shanghai fish markets, China. *Environ Sci Pollut Res Int* **23:**15033–15040.
61. **Hu Q, Chen L.** 2016. Virulence and antibiotic and heavy metal resistance of *Vibrio parahaemolyticus* isolated from crustaceans and shellfish in Shanghai, China. *J Food Prot* **79:**1371–1377.
62. **Williams RJ, Fraústo Da Silva JJ.** 2003. Evolution was chemically constrained. *J Theor Biol* **220:**323–343.
63. **Hong Enriquez RP, Do TN.** 2012. Bioavailability of metal ions and evolutionary adaptation. *Life (Basel)* **2:** 274–285.
64. **Macomber L, Imlay JA.** 2009. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proc Natl Acad Sci USA* **106:**8344–8349.
65. **Dupont CL, Grass G, Rensing C.** 2011. Copper toxicity and the origin of bacterial resistance: new insights and applications. *Metallomics* **3:**1109–1118.
66. **Chillappagari S, Seubert A, Trip H, Kuipers OP, Marahiel MA, Miethke M.** 2010. Copper stress affects iron homeostasis by destabilizing iron-sulfur cluster formation in *Bacillus subtilis*. *J Bacteriol* **192:**2512–2524.
67. **Chillappagari S, Miethke M, Trip H, Kuipers OP, Marahiel MA.** 2009. Copper acquisition is mediated by YcnJ and regulated by YcnK and CsoR in *Bacillus subtilis*. *J Bacteriol* **191:**2362–2370.
68. **Hirooka K, Edahiro T, Kimura K, Fujita Y.** 2012. Direct and indirect regulation of the *ycnKJI* operon involved in copper uptake through two transcriptional repressors, YcnK and CsoR, in *Bacillus subtilis*. *J Bacteriol* **194:**5675–5687.
69. **Yamada-Ankei T, Iwasaki H, Mori T.** 1977. Production of copper coproporphyrin III by *Bacillus cereus*. I. Purification and identification of copper coproporphyrin III. *J Biochem* **81:**835–842.
70. **Balasubramanian R, Kenney GE, Rosenzweig AC.** 2011. Dual pathways for copper uptake by methanotrophic bacteria. *J Biol Chem* **286:**37313–37319.
71. **Teitzel GM, Geddie A, De Long SK, Kirisits MJ, Whiteley M, Parsek MR.** 2006. Survival and growth in the presence of elevated copper: transcriptional profiling of copper-stressed *Pseudomonas aeruginosa*. *J Bacteriol* **188:**7242–7256.
72. **Whiting GC, Rowbury RJ.** 1995. Increased resistance of *Escherichia coli* to acrylic acid and to copper ions after cold-shock. *Lett Appl Microbiol* **20:**240–242.
73. **Taudte N, Grass G.** 2010. Point mutations change specificity and kinetics of metal uptake by ZupT from *Escherichia coli*. *Biometals* **23:**643–656.
74. **Grass G, Franke S, Taudte N, Nies DH, Kucharski LM, Maguire ME, Rensing C.** 2005. The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. *J Bacteriol* **187:**1604–1611.
75. **Guerinot ML.** 2000. The ZIP family of metal transporters. *Biochim Biophys Acta* **1465:**190–198.
76. **Cha JS, Cooksey DA.** 1993. Copper hypersensitivity and uptake in *Pseudomonas syringae* containing cloned components of the copper resistance operon. *Appl Environ Microbiol* **59:**1671–1674.
77. **Fitch MW, Graham DW, Arnold RG, Agarwal SK, Phelps P, Speitel GE Jr, Georgiou G.** 1993. Phenotypic characterization of copper-resistant mutants of *Methylosinus trichosporium* OB3b. *Appl Environ Microbiol* **59:** 2771–2776.
78. **Anttila J, Heinonen P, Nenonen T, Pino A, Iwaï H, Kauppi E, Soliymani R, Baumann M, Saksi J, Suni N, Haltia T.** 2011. Is coproporphyrin III a copper-acquisition compound in *Paracoccus denitrificans*? *Biochim Biophys Acta* **1807:**311–318.
79. **DiSpirito AA, Zahn JA, Graham DW, Kim HJ, Larive CK, Derrick TS, Cox CD, Taylor A.** 1998. Copper-binding compounds from *Methylosinus trichosporium* OB3b. *J Bacteriol* **180:**3606–3613.

80. **Kim HJ, Graham DW, DiSpirito AA, Alterman MA, Galeva N, Larive CK, Asunskis D, Sherwood PM.** 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* **305:**1612–1615.
81. **Zahn JA, DiSpirito AA.** 1996. Membrane-associated methane monooxygenase from *Methylococcus capsulatus* (Bath). *J Bacteriol* **178:**1018–1029.
82. **Nicolaisen K, Hahn A, Valdebenito M, Moslavac S, Samborski A, Maldener I, Wilken C, Valladares A, Flores E, Hantke K, Schleiff E.** 2010. The interplay between siderophore secretion and coupled iron and copper transport in the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120. *Biochim Biophys Acta* **1798:** 2131–2140.
83. **Argüello JM.** 2003. Identification of ion-selectivity determinants in heavy-metal transport P_{1B}-type ATPases. *J Membr Biol* **195:**93–108.
84. **Argüello JM, González-Guerrero M.** 2008. Cu^+ -ATPases brake system. *Structure* **16:**833–834.
85. **Argüello JM, González-Guerrero M, Raimunda D.** 2011. Bacterial transition metal $P_{(1B)}$-ATPases: transport mechanism and roles in virulence. *Biochemistry* **50:** 9940–9949.
86. **Argüello JM, Eren E, González-Guerrero M.** 2007. The structure and function of heavy metal transport P_{1B}-ATPases. *Biometals* **20:**233–248.
87. **Raimunda D, González-Guerrero M, Leeber BW III, Argüello JM.** 2011. The transport mechanism of bacterial Cu^+-ATPases: distinct efflux rates adapted to different function. *Biometals* **24:**467–475.
88. **Rosenzweig AC, Argüello JM.** 2012. Toward a molecular understanding of metal transport by P(1B)-type ATPases. *Curr Top Membr* **69:**113–136.
89. **Solioz M, Odermatt A, Krapf R.** 1994. Copper pumping ATPases: common concepts in bacteria and man. *FEBS Lett* **346:**44–47.
90. **Solioz M, Vulpe C.** 1996. CPx-type ATPases: a class of P-type ATPases that pump heavy metals. *Trends Biochem Sci* **21:**237–241.
91. **Odermatt A, Suter H, Krapf R, Solioz M.** 1992. An ATPase operon involved in copper resistance by *Enterococcus hirae*. *Ann N Y Acad Sci* **671**(1 Ion-Motive AT)**:** 484–486.
92. **Solioz M, Odermatt A.** 1995. Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. *J Biol Chem* **270:**9217–9221.
93. **Solioz M, Stoyanov JV.** 2003. Copper homeostasis in *Enterococcus hirae*. *FEMS Microbiol Rev* **27:**183–195.
94. **Odermatt A, Krapf R, Solioz M.** 1994. Induction of the putative copper ATPases, CopA and CopB, of *Enterococcus hirae* by Ag^+ and Cu^{2+}, and Ag^+ extrusion by CopB. *Biochem Biophys Res Commun* **202:**44–48.
95. **Odermatt A, Solioz M.** 1995. Two trans-acting metalloregulatory proteins controlling expression of the copper-ATPases of *Enterococcus hirae*. *J Biol Chem* **270:**4349–4354.
96. **González-Guerrero M, Raimunda D, Cheng X, Argüello JM.** 2010. Distinct functional roles of homologous Cu^+ efflux ATPases in *Pseudomonas aeruginosa*. *Mol Microbiol* **78:**1246–1258.
97. **Gaballa A, Cao M, Helmann JD.** 2003. Two MerR homologues that affect copper induction of the *Bacillus subtilis copZA* operon. *Microbiology* **149:**3413–3421.
98. **Reyes A, Leiva A, Cambiazo V, Méndez MA, González M.** 2006. Cop-like operon: structure and organization in species of the *Lactobacillale* order. *Biol Res* **39:**87–93.
99. **Grass G, Rensing C.** 2001. Genes involved in copper homeostasis in *Escherichia coli*. *J Bacteriol* **183:**2145–2147.
100. **Outten FW, Huffman DL, Hale JA, O'Halloran TV.** 2001. The independent cue and cus systems confer copper tolerance during aerobic and anaerobic growth in *Escherichia coli*. *J Biol Chem* **276:**30670–30677.
101. **Rensing C, Grass G.** 2003. *Escherichia coli* mechanisms of copper homeostasis in a changing environment. *FEMS Microbiol Rev* **27:**197–213.
102. **Outten FW, Outten CE, Hale J, O'Halloran TV.** 2000. Transcriptional activation of an *Escherichia coli* copper efflux regulon by the chromosomal MerR homologue, cueR. *J Biol Chem* **275:**31024–31029.
103. **Petersen C, Møller LB.** 2000. Control of copper homeostasis in *Escherichia coli* by a P-type ATPase, CopA, and a MerR-like transcriptional activator, CopR. *Gene* **261:** 289–298.
104. **Stoyanov JV, Hobman JL, Brown NL.** 2001. CueR (YbbI) of *Escherichia coli* is a MerR family regulator controlling expression of the copper exporter CopA. *Mol Microbiol* **39:**502–511.
105. **Changela A, Chen K, Xue Y, Holschen J, Outten CE, O'Halloran TV, Mondragón A.** 2003. Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. *Science* **301:**1383–1387.
106. **Rensing C, Fan B, Sharma R, Mitra B, Rosen BP.** 2000. CopA: An *Escherichia coli* Cu(I)-translocating P-type ATPase. *Proc Natl Acad Sci USA* **97:**652–656.
107. **Fan B, Rosen BP.** 2002. Biochemical characterization of CopA, the *Escherichia coli* Cu(I)-translocating P-type ATPase. *J Biol Chem* **277:**46987–46992.
108. **Grass G, Rensing C.** 2001. CueO is a multi-copper oxidase that confers copper tolerance in *Escherichia coli*. *Biochem Biophys Res Commun* **286:**902–908.
109. **Kim C, Lorenz WW, Hoopes JT, Dean JF.** 2001. Oxidation of phenolate siderophores by the multicopper oxidase encoded by the *Escherichia coli yacK* gene. *J Bacteriol* **183:**4866–4875.
110. **Roberts SA, Weichsel A, Grass G, Thakali K, Hazzard JT, Tollin G, Rensing C, Montfort WR.** 2002. Crystal structure and electron transfer kinetics of CueO, a multicopper oxidase required for copper homeostasis in *Escherichia coli*. *Proc Natl Acad Sci USA* **99:**2766–2771.
111. **Roberts SA, Wildner GF, Grass G, Weichsel A, Ambrus A, Rensing C, Montfort WR.** 2003. A labile regulatory copper ion lies near the T1 copper site in the multicopper oxidase CueO. *J Biol Chem* **278:**31958–31963.

112. **Grass G, Thakali K, Klebba PE, Thieme D, Müller A, Wildner GF, Rensing C.** 2004. Linkage between catecholate siderophores and the multicopper oxidase CueO in *Escherichia coli*. *J Bacteriol* **186:**5826–5833.

113. **Singh SK, Grass G, Rensing C, Montfort WR.** 2004. Cuprous oxidase activity of CueO from *Escherichia coli*. *J Bacteriol* **186:**7815–7817.

114. **Sakurai T, Kataoka K.** 2007. Basic and applied features of multicopper oxidases, CueO, bilirubin oxidase, and laccase. *Chem Rec* **7:**220–229.

115. **Djoko KY, Chong LX, Wedd AG, Xiao Z.** 2010. Reaction mechanisms of the multicopper oxidase CueO from *Escherichia coli* support its functional role as a cuprous oxidase. *J Am Chem Soc* **132:**2005–2015.

116. **Singh SK, Roberts SA, McDevitt SF, Weichsel A, Wildner GF, Grass GB, Rensing C, Montfort WR.** 2011. Crystal structures of multicopper oxidase CueO bound to copper(I) and silver(I): functional role of a methionine-rich sequence. *J Biol Chem* **286:**37849–37857.

117. **Lim SY, Joe MH, Song SS, Lee MH, Foster JW, Park YK, Choi SY, Lee IS.** 2002. CuiD is a crucial gene for survival at high copper environment in *Salmonella enterica* serovar Typhimurium. *Mol Cells* **14:**177–184.

118. **Kosman DJ.** 2010. Multicopper oxidases: a workshop on copper coordination chemistry, electron transfer, and metallophysiology. *J Biol Inorg Chem* **15:**15–28.

119. **Rademacher C, Moser R, Lackmann JW, Klinkert B, Narberhaus F, Masepohl B.** 2012. Transcriptional and posttranscriptional events control copper-responsive expression of a *Rhodobacter capsulatus* multicopper oxidase. *J Bacteriol* **194:**1849–1859.

120. **Nies DH, Herzberg M.** 2013. A fresh view of the cell biology of copper in enterobacteria. *Mol Microbiol* **87:**447–454.

121. **Silveira E, Freitas AR, Antunes P, Barros M, Campos J, Coque TM, Peixe L, Novais C.** 2014. Co-transfer of resistance to high concentrations of copper and first-line antibiotics among *Enterococcus* from different origins (humans, animals, the environment and foods) and clonal lineages. *J Antimicrob Chemother* **69:**899–906.

122. **Padilla-Benavides T, George Thompson AM, McEvoy MM, Argüello JM.** 2014. Mechanism of ATPase-mediated Cu^{+} export and delivery to periplasmic chaperones: the interaction of *Escherichia coli* CopA and CusF. *J Biol Chem* **289:**20492–20501.

123. **Franke S, Grass G, Rensing C, Nies DH.** 2003. Molecular analysis of the copper-transporting efflux system CusCFBA of *Escherichia coli*. *J Bacteriol* **185:**3804–3812.

124. **Munson GP, Lam DL, Outten FW, O'Halloran TV.** 2000. Identification of a copper-responsive two-component system on the chromosome of *Escherichia coli* K-12. *J Bacteriol* **182:**5864–5871.

125. **Franke S, Grass G, Nies DH.** 2001. The product of the *ybdE* gene of the *Escherichia coli* chromosome is involved in detoxification of silver ions. *Microbiology* **147:**965–972.

126. **Bagai I, Liu W, Rensing C, Blackburn NJ, McEvoy MM.** 2007. Substrate-linked conformational change in the periplasmic component of a Cu(I)/Ag(I) efflux system. *J Biol Chem* **282:**35695–35702.

127. **Su CC, Yang F, Long F, Reyon D, Routh MD, Kuo DW, Mokhtari AK, Van Ornam JD, Rabe KL, Hoy JA, Lee YJ, Rajashankar KR, Yu EW.** 2009. Crystal structure of the membrane fusion protein CusB from *Escherichia coli*. *J Mol Biol* **393:**342–355.

128. **Long F, Su CC, Zimmermann MT, Boyken SE, Rajashankar KR, Jernigan RL, Yu EW.** 2010. Crystal structures of the CusA efflux pump suggest methionine-mediated metal transport. *Nature* **467:**484–488.

129. **Kulathila R, Kulathila R, Indic M, van den Berg B.** 2011. Crystal structure of *Escherichia coli* CusC, the outer membrane component of a heavy metal efflux pump. *PLoS One* **6:**e15610.

130. **Su CC, Long F, Zimmermann MT, Rajashankar KR, Jernigan RL, Yu EW.** 2011. Crystal structure of the CusBA heavy-metal efflux complex of *Escherichia coli*. *Nature* **470:**558–562.

131. **Kim EH, Nies DH, McEvoy MM, Rensing C.** 2011. Switch or funnel: how RND-type transport systems control periplasmic metal homeostasis. *J Bacteriol* **193:**2381–2387.

132. **Loftin IR, Franke S, Roberts SA, Weichsel A, Héroux A, Montfort WR, Rensing C, McEvoy MM.** 2005. A novel copper-binding fold for the periplasmic copper resistance protein CusF. *Biochemistry* **44:**10533–10540.

133. **Kittleson JT, Loftin IR, Hausrath AC, Engelhardt KP, Rensing C, McEvoy MM.** 2006. Periplasmic metal-resistance protein CusF exhibits high affinity and specificity for both CuI and AgI. *Biochemistry* **45:**11096–11102.

134. **Xue Y, Davis AV, Balakrishnan G, Stasser JP, Staehlin BM, Focia P, Spiro TG, Penner-Hahn JE, O'Halloran TV.** 2008. Cu(I) recognition via cation-pi and methionine interactions in CusF. *Nat Chem Biol* **4:**107–109.

135. **Bagai I, Rensing C, Blackburn NJ, McEvoy MM.** 2008. Direct metal transfer between periplasmic proteins identifies a bacterial copper chaperone. *Biochemistry* **47:**11408–11414.

136. **Bersch B, Derfoufi KM, De Angelis F, Auquier V, Ekendé EN, Mergeay M, Ruysschaert JM, Vandenbussche G.** 2011. Structural and metal binding characterization of the C-terminal metallochaperone domain of membrane fusion protein SilB from *Cupriavidus metallidurans* CH34. *Biochemistry* **50:**2194–2204.

137. **Mealman TD, Bagai I, Singh P, Goodlett DR, Rensing C, Zhou H, Wysocki VH, McEvoy MM.** 2011. Interactions between CusF and CusB identified by NMR spectroscopy and chemical cross-linking coupled to mass spectrometry. *Biochemistry* **50:**2559–2566.

138. **Chacón KN, Mealman TD, McEvoy MM, Blackburn NJ.** 2014. Tracking metal ions through a Cu/Ag efflux pump assigns the functional roles of the periplasmic proteins. *Proc Natl Acad Sci USA* **111:**15373–15378.

139. **Meir A, Natan A, Moskovitz Y, Ruthstein S.** 2015. EPR spectroscopy identifies Met and Lys residues that are essential for the interaction between the CusB N-terminal domain and metallochaperone CusF. *Metallomics* **7:**1163–1172.

140. **Pontel LB, Soncini FC.** 2009. Alternative periplasmic copper-resistance mechanisms in Gram negative bacteria. *Mol Microbiol* **73:**212–225.

141. **Osman D, Waldron KJ, Denton H, Taylor CM, Grant AJ, Mastroeni P, Robinson NJ, Cavet JS.** 2010. Copper homeostasis in *Salmonella* is atypical and copper-CueP is a major periplasmic metal complex. *J Biol Chem* **285:** 25259–25268.

142. **Yun BY, Piao S, Kim YG, Moon HR, Choi EJ, Kim YO, Nam BH, Lee SJ, Ha NC.** 2011. Crystallization and preliminary X-ray crystallographic analysis of *Salmonella* Typhimurium CueP. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **67:**675–677.

143. **Yoon BY, Yeom JH, Kim JS, Um SH, Jo I, Lee K, Kim YH, Ha NC.** 2014. Direct ROS scavenging activity of CueP from *Salmonella enterica* serovar Typhimurium. *Mol Cells* **37:**100–108.

144. **Williams JR, Morgan AG, Rouch DA, Brown NL, Lee BT.** 1993. Copper-resistant enteric bacteria from United Kingdom and Australian piggeries. *Appl Environ Microbiol* **59:**2531–2537.

145. **Tetaz TJ, Luke RK.** 1983. Plasmid-controlled resistance to copper in *Escherichia coli*. *J Bacteriol* **154:**1263–1268.

146. **Brown NL, Barrett SR, Camakaris J, Lee BT, Rouch DA.** 1995. Molecular genetics and transport analysis of the copper-resistance determinant (*pco*) from *Escherichia coli* plasmid pRJ1004. *Mol Microbiol* **17:**1153–1166.

147. **Rouch DA, Brown NL.** 1997. Copper-inducible transcriptional regulation at two promoters in the *Escherichia coli* copper resistance determinant *pco*. *Microbiology* **143:**1191–1202.

148. **Lee SM, Grass G, Rensing C, Barrett SR, Yates CJ, Stoyanov JV, Brown NL.** 2002. The Pco proteins are involved in periplasmic copper handling in *Escherichia coli*. *Biochem Biophys Res Commun* **295:**616–620.

149. **Zimmermann M, Udagedara SR, Sze CM, Ryan TM, Howlett GJ, Xiao Z, Wedd AG.** 2012. PcoE: a metal sponge expressed to the periplasm of copper resistance *Escherichia coli*. Implication of its function role in copper resistance. *J Inorg Biochem* **115:**186–197.

150. **Hao X, Lüthje FL, Qin Y, McDevitt SF, Lutay N, Hobman JL, Asiani K, Soncini FC, German N, Zhang S, Zhu YG, Rensing C.** 2015. Survival in amoeba: a major selection pressure on the presence of bacterial copper and zinc resistance determinants? Identification of a "copper pathogenicity island". *Appl Microbiol Biotechnol* **99:**5817–5824.

151. **Staehlin BM, Gibbons JG, Rokas A, O'Halloran TV, Slot JC.** 2016. Evolution of a heavy metal homeostasis/resistance island reflects increasing copper stress in Enterobacteria. *Genome Biol Evol* **8:**811–826.

152. **McHugh GL, Moellering RC, Hopkins CC, Swartz MN.** 1975. *Salmonella typhimurium* resistant to silver nitrate, chloramphenicol, and ampicillin. *Lancet* **1:**235–240.

153. **Gupta A, Matsui K, Lo JF, Silver S.** 1999. Molecular basis for resistance to silver cations in *Salmonella*. *Nat Med* **5:**183–188.

154. **Randall CP, Gupta A, Jackson N, Busse D, O'Neill AJ.** 2015. Silver resistance in Gram-negative bacteria: a dissection of endogenous and exogenous mechanisms. *J Antimicrob Chemother* **70:**1037–1046.

155. **Lüthje FL, Hasman H, Aarestrup FM, Alwathnani HA, Rensing C.** 2014. Genome sequences of two copper-resistant *Escherichia coli* strains isolated from copper-fed pigs. *Genome Announc* **2:**e01341.

156. **Qin Y, Hasman H, Aarestrup FM, Alwathnani HA, Rensing C.** 2014. Genome sequences of three highly copper-resistant *Salmonella enterica* subsp. I serovar Typhimurium strains isolated from pigs in Denmark. *Genome Announc* **2:**e01334-14.

157. **Peters JE, Fricker AD, Kapili BJ, Petassi MT.** 2014. Heteromeric transposase elements: generators of genomic islands across diverse bacteria. *Mol Microbiol* **93:**1084–1092.

158. **Chaturvedi KS, Henderson JP.** 2014. Pathogenic adaptations to host-derived antibacterial copper. *Front Cell Infect Microbiol* **4:**3.

159. **Schubert S, Dufke S, Sorsa J, Heesemann J.** 2004. A novel integrative and conjugative element (ICE) of *Escherichia coli*: the putative progenitor of the *Yersinia* high-pathogenicity island. *Mol Microbiol* **51:**837–848.

160. **Rakin A, Schneider L, Podladchikova O.** 2012. Hunger for iron: the alternative siderophore iron scavenging systems in highly virulent *Yersinia*. *Front Cell Infect Microbiol* **2:**151/1.

161. **Aviv G, Tsyba K, Steck N, Salmon-Divon M, Cornelius A, Rahav G, Grassl GA, Gal-Mor O.** 2014. A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar Infantis strain. *Environ Microbiol* **16:**977–994.

162. **Chaturvedi KS, Hung CS, Giblin DE, Urushidani S, Austin AM, Dinauer MC, Henderson JP.** 2014. Cupric yersiniabactin is a virulence-associated superoxide dismutase mimic. *ACS Chem Biol* **9:**551–561.

163. **Fodah RA, Scott JB, Tam HH, Yan P, Pfeffer TL, Bundschuh R, Warawa JM.** 2014. Correlation of *Klebsiella pneumoniae* comparative genetic analyses with virulence profiles in a murine respiratory disease model. *PLoS One* **9:**e107394.

164. **Freitas AR, Coque TM, Novais C, Hammerum AM, Lester CH, Zervos MJ, Donabedian S, Jensen LB, Francia MV, Baquero F, Peixe L.** 2011. Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn1546 on indistinguishable plasmids. *J Clin Microbiol* **49:**925–931.

165. **Zhang S, Wang D, Wang Y, Hasman H, Aarestrup FM, Alwathnani HA, Zhu YG, Rensing C.** 2015. Genome sequences of copper resistant and sensitive *Enterococcus faecalis* strains isolated from copper-fed pigs in Denmark. *Stand Genomic Sci* **10:**35-015-0021-1.

166. **Hasman H, Aarestrup FM.** 2002. *tcrB*, a gene conferring transferable copper resistance in *Enterococcus faecium*: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. *Antimicrob Agents Chemother* **46:**1410–1416.

167. **Amachawadi RG, Shelton NW, Jacob ME, Shi X, Narayanan SK, Zurek L, Dritz SS, Nelssen JL, Tokach MD, Nagaraja TG.** 2010. Occurrence of *tcrB*, a transferable copper resistance gene, in fecal enterococci of swine. *Foodborne Pathog Dis* **7:**1089–1097.

168. **Amachawadi RG, Scott HM, Alvarado CA, Mainini TR, Vinasco J, Drouillard JS, Nagaraja TG.** 2013. Occurrence of the transferable copper resistance gene *tcrB* among fecal enterococci of U.S. feedlot cattle fed copper-supplemented diets. *Appl Environ Microbiol* **79:** 4369–4375.

169. **Pasquaroli S, Di Cesare A, Vignaroli C, Conti G, Citterio B, Biavasco F.** 2014. Erythromycin- and copper-resistant *Enterococcus hirae* from marine sediment and co-transfer of *erm(B)* and *tcrB* to human *Enterococcus faecalis*. *Diagn Microbiol Infect Dis* **80:**26–28.

170. **Hasman H.** 2005. The *tcrB* gene is part of the *tcrYAZB* operon conferring copper resistance in *Enterococcus faecium* and *Enterococcus faecalis*. *Microbiology* **151:** 3019–3025.

171. **Rensing C, Alwathnani HA, McDevitt SF.** 2016. The copper metallome in prokaryotic cells, p 161–173. *In* de Bruijn FJ (ed), *Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria*. John Wiley & Sons, Inc., Hoboken, NJ.

172. **Hedges SB, Chen H, Kumar S, Wang DY, Thompson AS, Watanabe H.** 2001. A genomic timescale for the origin of eukaryotes. *BMC Evol Biol* **1:**4.

173. **Outten CE, O'Halloran TV.** 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science* **292:**2488–2492.

174. **Patzer SI, Hantke K.** 1998. The ZnuABC high-affinity zinc uptake system and its regulator Zur in *Escherichia coli*. *Mol Microbiol* **28:**1199–1210.

175. **Sabri M, Houle S, Dozois CM.** 2009. Roles of the extraintestinal pathogenic *Escherichia coli* ZnuACB and ZupT zinc transporters during urinary tract infection. *Infect Immun* **77:**1155–1164.

176. **Patzer SI, Hantke K.** 2000. The zinc-responsive regulator Zur and its control of the *znu* gene cluster encoding the ZnuABC zinc uptake system in *Escherichia coli*. *J Biol Chem* **275:**24321–24332.

177. **Sigdel TK, Easton JA, Crowder MW.** 2006. Transcriptional response of *Escherichia coli* to TPEN. **188:** 6709–6713.

178. **Gilston BA, Wang S, Marcus MD, Canalizo-Hernández MA, Swindell EP, Xue Y, Mondragón A, O'Halloran TV.** 2014. Structural and mechanistic basis of zinc regulation across the *E. coli* Zur regulon. *PLoS Biol* **12:** e1001987.

179. **Mergeay M, Nies D, Schlegel HG, Gerits J, Charles P, Van Gijsegem F.** 1985. *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. *J Bacteriol* **162:**328–334.

180. **Kirsten A, Herzberg M, Voigt A, Seravalli J, Grass G, Scherer J, Nies DH.** 2011. Contributions of five secondary metal uptake systems to metal homeostasis of *Cupriavidus metallidurans* CH34. *J Bacteriol* **193:** 4652–4663.

181. **Herzberg M, Bauer L, Nies DH.** 2014. Deletion of the *zupT* gene for a zinc importer influences zinc pools in *Cupriavidus metallidurans* CH34. *Metallomics* **6:**421–436.

182. **Grass G, Wong MD, Rosen BP, Smith RL, Rensing C.** 2002. ZupT is a Zn(II) uptake system in *Escherichia coli*. *J Bacteriol* **184:**864–866.

183. **Cerasi M, Liu JZ, Ammendola S, Poe AJ, Petrarca P, Pesciaroli M, Pasquali P, Raffatellu M, Battistoni A.** 2014. The ZupT transporter plays an important role in zinc homeostasis and contributes to *Salmonella enterica* virulence. *Metallomics* **6:**845–853.

184. **Jackson RJ, Binet MR, Lee LJ, Ma R, Graham AI, McLeod CW, Poole RK.** 2008. Expression of the PitA phosphate/metal transporter of *Escherichia coli* is responsive to zinc and inorganic phosphate levels. *FEMS Microbiol Lett* **289:**219–224.

185. **Beard SJ, Hashim R, Wu G, Binet MR, Hughes MN, Poole RK.** 2000. Evidence for the transport of zinc (II) ions via the pit inorganic phosphate transport system in *Escherichia coli*. *FEMS Microbiol Lett* **184:** 231–235.

186. **Webb M.** 1970. Interrelationships between the utilization of magnesium and the uptake of other bivalent cations by bacteria. *Biochim Biophys Acta* **222:**428–439.

187. **Krom BP, Huttinga H, Warner JB, Lolkema JS.** 2002. Impact of the Mg(2+)-citrate transporter CitM on heavy metal toxicity in *Bacillus subtilis*. *Arch Microbiol* **178:** 370–375.

188. **Knoop V, Groth-Malonek M, Gebert M, Eifler K, Weyand K.** 2005. Transport of magnesium and other divalent cations: evolution of the 2-TM-GxN proteins in the MIT superfamily. *Mol Genet Genomics* **274:**205–216.

189. **Legatzki A, Grass G, Anton A, Rensing C, Nies DH.** 2003. Interplay of the Czc system and two P-type ATPases in conferring metal resistance to *Ralstonia metallidurans*. *J Bacteriol* **185:**4354–4361.

190. **Nies DH.** 2007. How cells control zinc homeostasis. *Science* **317:**1695–1696.

191. **Rensing C, Mitra B, Rosen BP.** 1998. A Zn(II)-translocating P-type ATPase from *Proteus mirabilis*. *Biochem Cell Biol* **76:**787–790.

192. **Rensing C, Ghosh M, Rosen BP.** 1999. Families of soft-metal-ion-transporting ATPases. *J Bacteriol* **181:**5891–5897.

193. **Sharma R, Rensing C, Rosen BP, Mitra B.** 2000. The ATP hydrolytic activity of purified ZntA, a Pb(II)/Cd (II)/Zn(II)-translocating ATPase from *Escherichia coli*. *J Biol Chem* **275:**3873–3878.

194. **Smith K, Novick RP.** 1972. Genetic studies on plasmid-linked cadmium resistance in *Staphylococcus aureus*. *J Bacteriol* **112:**761–772.

195. **Nucifora G, Chu L, Misra TK, Silver S.** 1989. Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cadA* gene results from a cadmium-efflux ATPase. *Proc Natl Acad Sci USA* **86:**3544–3548.

196. **Wang D, Hosteen O, Fierke CA.** 2012. ZntR-mediated transcription of *zntA* responds to nanomolar intracellular free zinc. *J Inorg Biochem* **111:**173–181.

197. **Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y.** 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277:**1453–1462.

198. **Yoon KP, Silver S.** 1991. A second gene in the *Staphylococcus aureus cadA* cadmium resistance determinant of plasmid pI258. *J Bacteriol* **173:**7636–7642.

199. **Argudín MA, Butaye P.** 2016. Dissemination of metal resistance genes among animal methicillin-resistant coagulase-negative staphylococci. *Res Vet Sci* **105:**192–194.

200. **Slifierz MJ, Park J, Friendship RM, Weese JS.** 2014. Zinc-resistance gene CzrC identified in methicillin-resistant *Staphylococcus hyicus* isolated from pigs with exudative epidermitis. *Can Vet J* **55:**489–490.

201. **Vandendriessche S, Vanderhaeghen W, Larsen J, de Mendonça R, Hallin M, Butaye P, Hermans K, Haesebrouck F, Denis O.** 2014. High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCCmec remnant DNA in MSSA CC398. *J Antimicrob Chemother* **69:**355–362.

202. **Vandendriessche S, Vanderhaeghen W, Soares FV, Hallin M, Catry B, Hermans K, Butaye P, Haesebrouck F, Struelens MJ, Denis O.** 2013. Prevalence, risk factors and genetic diversity of methicillin-resistant *Staphylococcus aureus* carried by humans and animals across livestock production sectors. *J Antimicrob Chemother* **68:**1510–1516.

203. **Agersø Y, Hasman H, Cavaco LM, Pedersen K, Aarestrup FM.** 2012. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. *Vet Microbiol* **157:**246–250.

204. **Nair R, Thapaliya D, Su Y, Smith TC.** 2014. Resistance to zinc and cadmium in *Staphylococcus aureus* of human and animal origin. *Infect Control Hosp Epidemiol* **35**(Suppl 3)**:**S32–S39.

205. **Grass G, Fan B, Rosen BP, Franke S, Nies DH, Rensing C.** 2001. ZitB (YbgR), a member of the cation diffusion facilitator family, is an additional zinc transporter in *Escherichia coli*. *J Bacteriol* **183:**4664–4667.

206. **Grass G, Otto M, Fricke B, Haney CJ, Rensing C, Nies DH, Munkelt D.** 2005. FieF (YiiP) from *Escherichia coli* mediates decreased cellular accumulation of iron and relieves iron stress. *Arch Microbiol* **183:**9–18.

207. **Xiong A, Jayaswal RK.** 1998. Molecular characterization of a chromosomal determinant conferring resistance to zinc and cobalt ions in *Staphylococcus aureus*. *J Bacteriol* **180:**4024–4029.

208. **Singh VK, Xiong A, Usgaard TR, Chakrabarti S, Deora R, Misra TK, Jayaswal RK.** 1999. ZntR is an autoregulatory protein and negatively regulates the chromosomal zinc resistance operon *znt* of *Staphylococcus aureus*. *Mol Microbiol* **33:**200–207.

209. **Van Pham ST, Engman H, Dahlgren LG, Cornvik T, Eshaghi S.** 2010. A systematic approach to isolate mono-disperse membrane proteins: purification of zinc transporter ZntB. *Protein Expr Purif* **72:**48–54.

210. **Papp-Wallace KM, Maguire ME.** 2007. Bacterial homologs of eukaryotic membrane proteins: the 2-TM-GxN family of Mg(2+) transporters. *Mol Membr Biol* **24:**351–356.

211. **Caldwell AM, Smith RL.** 2003. Membrane topology of the ZntB efflux system of *Salmonella enterica* serovar Typhimurium. *J Bacteriol* **185:**374–376.

212. **Wan Q, Ahmad MF, Fairman J, Gorzelle B, de la Fuente M, Dealwis C, Maguire ME.** 2011. X-ray crystallography and isothermal titration calorimetry studies of the *Salmonella* zinc transporter ZntB. *Structure* **19:**700–710.

213. **Worlock AJ, Smith RL.** 2002. ZntB is a novel Zn^{2+} transporter in *Salmonella enterica* serovar Typhimurium. *J Bacteriol* **184:**4369–4373.

214. **Chaoprasid P, Nookabkaew S, Sukchawalit R, Mongkolsuk S.** 2015. Roles of *Agrobacterium tumefaciens* C58 ZntA and ZntB and the transcriptional regulator ZntR in controlling $Cd^{2+}/Zn^{2+}/Co^{2+}$ resistance and the peroxide stress response. *Microbiology* **161:**1730–1740.

215. **Nies DH, Silver S.** 1995. Ion efflux systems involved in bacterial metal resistances. *J Ind Microbiol* **14:**186–199.

216. **Legatzki A, Franke S, Lucke S, Hoffmann T, Anton A, Neumann D, Nies DH.** 2003. First step towards a quantitative model describing Czc-mediated heavy metal resistance in *Ralstonia metallidurans*. *Biodegradation* **14:**153–168.

217. **Nies DH.** 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiol Rev* **27:**313–339.

218. **Nies D, Mergeay M, Friedrich B, Schlegel HG.** 1987. Cloning of plasmid genes encoding resistance to cadmium, zinc, and cobalt in *Alcaligenes eutrophus* CH34. *J Bacteriol* **169:**4865–4868.

219. **Saier MH Jr, Tam R, Reizer A, Reizer J.** 1994. Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. *Mol Microbiol* **11:**841–847.

220. **Diels L, Dong Q, van der Lelie D, Baeyens W, Mergeay M.** 1995. The *czc* operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. *J Ind Microbiol* **14:**142–153.

221. **Grosse C, Anton A, Hoffmann T, Franke S, Schleuder G, Nies DH.** 2004. Identification of a regulatory pathway that controls the heavy-metal resistance system Czc via promoter *czcNp* in *Ralstonia metallidurans*. *Arch Microbiol* **182:**109–118.

222. **Zoropogui A, Gambarelli S, Covès J.** 2008. CzcE from *Cupriavidus metallidurans* CH34 is a copper-binding protein. *Biochem Biophys Res Commun* **365:**735–739.

223. **Scherer J, Nies DH.** 2009. CzcP is a novel efflux system contributing to transition metal resistance in *Cupriavidus metallidurans* CH34. *Mol Microbiol* **73:** 601–621.

224. **von Rozycki T, Nies DH.** 2009. *Cupriavidus metallidurans*: evolution of a metal-resistant bacterium. *Antonie van Leeuwenhoek* **96:**115–139.

225. **Symmons MF, Bokma E, Koronakis E, Hughes C, Koronakis V.** 2009. The assembled structure of a complete tripartite bacterial multidrug efflux pump. *Proc Natl Acad Sci USA* **106:**7173–7178.

226. **Murakami S, Nakashima R, Yamashita E, Matsumoto T, Yamaguchi A.** 2006. Crystal structures of a multidrug transporter reveal a functionally rotating mechanism. *Nature* **443:**173–179.

227. **De Angelis F, Lee JK, O'Connell JD III, Miercke LJ, Verschueren KH, Srinivasan V, Bauvois C, Govaerts C, Robbins RA, Ruysschaert JM, Stroud RM, Vandenbussche G.** 2010. Metal-induced conformational changes in ZneB suggest an active role of membrane fusion proteins in efflux resistance systems. *Proc Natl Acad Sci USA* **107:**11038–11043.

228. **Pak JE, Ekendé EN, Kifle EG, O'Connell JD III, De Angelis F, Tessema MB, Derfoufi KM, Robles-Colmenares Y, Robbins RA, Goormaghtigh E, Vandenbussche G, Stroud RM.** 2013. Structures of intermediate transport states of ZneA, a Zn(II)/proton antiporter. *Proc Natl Acad Sci USA* **110:**18484–18489.

229. **Vaccaro BJ, Lancaster WA, Thorgersen MP, Zane GM, Younkin AD, Kazakov AE, Wetmore KM, Deutschbauer A, Arkin AP, Novichkov PS, Wall JD, Adams MW.** 2016. Novel metal cation resistance systems from mutant fitness analysis of denitrifying *Pseudomonas stutzeri*. *Appl Environ Microbiol* **82:**6046–6056.

230. **Djoko KY, Ong CL, Walker MJ, McEwan AG.** 2015. The role of copper and zinc toxicity in innate immune defense against bacterial pathogens. *J Biol Chem* **290:** 18954–18961.

231. **German N, Doyscher D, Rensing C.** 2013. Bacterial killing in macrophages and amoeba: do they all use a brass dagger? *Future Microbiol* **8:**1257–1264.

232. **Hao X, Lüthje F, Rønn R, German NA, Li X, Huang F, Kisaka J, Huffman D, Alwathnani HA, Zhu YG, Rensing C.** 2016. A role for copper in protozoan grazing: two billion years selecting for bacterial copper resistance. *Mol Microbiol* **102:**628–641.

233. **Poole K.** 2017. At the nexus of antibiotics and metals: the impact of Cu and Zn on antibiotic activity and resistance. *Trends Microbiol* **25:**820–832.

234. **Molmeret M, Horn M, Wagner M, Santic M, Abu Kwaik Y.** 2005. Amoebae as training grounds for intracellular bacterial pathogens. *Appl Environ Microbiol* **71:**20–28.

235. **Chandrangsu P, Rensing C, Helmann JD.** 2017. Metal homeostasis and resistance in bacteria. *Nat Rev Microbiol* **15:**338–350.

236. **Tan H, Deng Z, Cao L.** 2009. Isolation and characterization of actinomycetes from healthy goat faeces. *Lett Appl Microbiol* **49:**248–253.

237. **Choudhury P, Kumar R.** 1998. Multidrug- and metal-resistant strains of *Klebsiella pneumoniae* isolated from *Penaeus monodon* of the coastal waters of deltaic Sundarban. *Can J Microbiol* **44:**186–189.

238. **Dutta GN, Devriese LA.** 1981. Sensitivity and resistance to growth promoting agents in animal lactobacilli. *J Appl Bacteriol* **51:**283–288.

239. **Siddaramappa S, Challacombe JF, Duncan AJ, Gillaspy AF, Carson M, Gipson J, Orvis J, Zaitshik J, Barnes G, Bruce D, Chertkov O, Detter JC, Han CS, Tapia R, Thompson LS, Dyer DW, Inzana TJ.** 2011. Horizontal gene transfer in *Histophilus somni* and its role in the evolution of pathogenic strain 2336, as determined by comparative genomic analyses. *BMC Genomics* **12:**570.

240. **Deus D, Krischek C, Pfeifer Y, Sharifi AR, Fiegen U, Reich F, Klein G, Kehrenberg C.** 2017. Comparative analysis of the susceptibility to biocides and heavy metals of extended-spectrum β-lactamase-producing *Escherichia coli* isolates of human and avian origin, Germany. *Diagn Microbiol Infect Dis* **88:**88–92.

241. **Agga GE, Scott HM, Amachawadi RG, Nagaraja TG, Vinasco J, Bai J, Norby B, Renter DG, Dritz SS, Nelssen JL, Tokach MD.** 2014. Effects of chlortetracycline and copper supplementation on antimicrobial resistance of fecal *Escherichia coli* from weaned pigs. *Prev Vet Med* **114:**231–246.

242. **Campos J, Cristino L, Peixe L, Antunes P.** 2016. MCR-1 in multidrug-resistant and copper-tolerant clinically relevant *Salmonella* 1,4,[5],12:i:- and *S.* Rissen clones in Portugal, 2011 to 2015. *Euro Surveill* **21:**30270.

243. **Gómez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, Schwarz S.** 2013. Novel erm(T)-carrying multiresistance plasmids from porcine and human isolates of methicillin-resistant *Staphylococcus aureus* ST398 that also harbor cadmium and copper resistance determinants. *Antimicrob Agents Chemother* **57:**3275–3282.

244. **Argudín MA, Lauzat B, Kraushaar B, Alba P, Agerso Y, Cavaco L, Butaye P, Porrero MC, Battisti A, Tenhagen BA, Fetsch A, Guerra B.** 2016. Heavy metal and disinfectant resistance genes among livestock-associated methicillin-resistant *Staphylococcus aureus* isolates. *Vet Microbiol* **191:**88–95.

245. **Cavaco LM, Hasman H, Aarestrup FM, Wagenaar JA, Graveland H, Veldman K, Mevius D, Fetsch A, Tenhagen B-A, Concepcion Porrero M, Dominguez L, Granier SA, Jouy E, Butaye P, Kaszanyitzky E, Dán A, Zmudzki J, Battisti A, Franco A, Schwarz S, Gutierrez M, Weese JS, Cui S, Pomba C, Members of MRSA-CG.** 2011. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* **150:**344–348.

246. **Gehring AM, DeMoll E, Fetherston JD, Mori I, Mayhew GF, Blattner FR, Walsh CT, Perry RD.** 1998. Iron acquisition in plague: modular logic in enzymatic biogenesis of yersiniabactin by *Yersinia pestis*. *Chem Biol* **5:**573–586.

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Resistance of Bacteria to Biocides

6

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BIOCIDE USAGE

Chemical biocides have been used for centuries for making water and foodstuff safe to consume, for treating wounds, and for preserving materials since well before the discovery of microorganisms. Today chemical biocides are heavily used in a wide range of applications and environments including the consumer product, water, wastewater, and food industries; goods manufacturing; the pharmaceutical industry; the health care and veterinary sectors; and the oil and gas industries (1). This wide range of applications reflects the versatility of biocide products for environmental disinfection, product preservation, and antisepsis (2). In Europe it is difficult to estimate the quantity of chemical biocides that are used in products or imported (1), although in 2006 the market for biocides was estimated to be €10 billion to €11 billion (1). It is, however, clear that the usage of chemical biocides is continuing to increase, particularly in consumer products. This increased usage may be partly due to consumers' increased awareness of microbial contamination and infection. The rise in antibiotic resistance in bacteria might also have impacted on the usage of biocides, at least in the health care and veterinary settings (3). Widespread media coverage of issues of hospital cleanliness and "superbugs" have also contributed to better-informed customers, providing better marketing arguments for manufacturers and distributors of biocidal products (3). Alongside a better-informed public, the global increase in antimicrobial resistance in bacteria is forcing decision makers to tackle this growing issue. One of the recommended interventions is better hygiene and control of bacteria on surfaces in health care settings but also in animal husbandry (4).

In health care settings biocides are heavily used for the disinfection of environmental surfaces and medical devices and for antisepsis. The growing number of studies highlighting the presence, and at times persistence, of bacterial pathogens, including multidrug-resistant ones, on surfaces despite the use of decontamination (5–14) acknowledges that microorganisms can survive on surfaces and be transmitted to patients, staff, and inanimate objects (15), thus finally emphasizing the importance of controlling the microbial burden on surfaces. This newly found appreciation for controlling microbial pathogens on surfaces has led to an explosion of surface disinfection products and their marketing (16–18), contributing to a higher concentration of biocides eventually released in the environment.

The ability of biocides or biocidal products to decrease the microbial bioburden on surfaces is also highly relevant in animal husbandry, farm buildings, barns, equipment, and vehicles, where their use should contribute to reducing the spread of pathogens. This also includes their use to prevent infectious outbreaks from spreading from farms; for example, large quantities of biocides are being sprayed in the environment and on vehicles in an attempt to decrease the spread of animal viral diseases (19). The heavy use of biocidal products where heavy soiling is present, in particular, their use on vehicle wheels and undercarriages, deserves better scrutiny of its efficacy in preventing potential outbreaks.

The use of biocidal products also includes the disinfection of various environmental surfaces, antibiofouling, the preservation of building materials, and water and wastewater treatment. Biocides play an important role as food preservatives and for controlling microbial contaminants that may enter the food chain during food production. As mentioned previously, one growing area for biocide manufacturers is consumer products, including the preservation of cosmetics, but more recently, personal care products, household products, and textiles.

In Europe, the incorporation of biocides in products and the use of chemical biocides in general is heavily regulated (20), with the consequence that fewer bio-

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cides are available for manufacturers to use. This restriction on the number and type of chemical biocides available for manufacturers has, however, not reduced the number of biocidal products and biocide applications. On the contrary, awareness of the role of microorganisms in contamination, infection, or the production of odors, together with the growing threat of bacterial resistance to chemotherapeutic antibiotics, has resulted in the biocidal product market expanding. In Europe, the amounts of chemical biocides used per application is difficult to measure (1). Chemical biocides used in diverse applications eventually find their way to the environment (1). For example, high concentrations of triclosan have been found in river and wastewater effluents (1.4 to 40,000 ng/liter in surface water, up to 85,000 ng/liter in wastewater, and up to 133,000 μg/kg in biosolids from wastewater treatment plants) (20–23). There should be little doubt that chemical biocides even at a low concentration (i.e., sub-MIC level) will exert a selective pressure on microorganisms (18, 24–26), which should be monitored where biocidal products are heavily used (18, 27). The increase in the use of biocides and biocidal products might aggravate the possible link between biocide usage and emergence of antimicrobial resistance in bacteria (1, 3, 18, 28), although there is no doubt that overall biocide usage has brought immense benefit to human and animal health (1–3, 29).

This article explores reports of bacterial resistance to biocides and our current knowledge of the mechanisms of bacterial resistance. It also reflects on the effect of biocides' interactions with bacteria that may lead to a change in susceptibility to antimicrobials. This article does not cover bacterial biofilms.

BIOCIDE RESISTANCE: A QUESTION OF DEFINITIONS

One of the main issues when dealing with bacterial resistance is the definition of "resistance." This definition is linked with the test protocols to measure resistance, and these protocols are described later. There are many definitions of resistance to biocides, some of which describe only a small decrease in susceptibility (18, 28, 31–34). This contrasts with the definition of bacterial resistance to chemotherapeutic antibiotics, which reflects clinical resistance. With biocides the terms "resistance," "tolerance," "decreased susceptibility," "reduced susceptibility," "insusceptibility," and "acquired reduced susceptibility" are used. Such diversity in terms reflects a lack of consensus within the scientific community and is contributing to a degree of confusion in our understanding of bacterial resistance to biocides. From a practical point of view, a bacterium surviving in a biocidal product is resistant to that product, whatever the concentration of biocide is in the product.

Many papers have used the term "reduced susceptibility," which is based on the measurement of the MIC or the minimum bactericidal concentration. A biocide or biocide product at its in-use concentration may, however, still be effective (18, 35). One of the main difficulties is to determine what fold-difference in MIC or minimum bactericidal concentration reflects a change that will be significant in practice, i.e., a decrease in biocide effectiveness. This is likely to be biocide/biocidal product dependent.

From an academic perspective, other definitions of bacterial resistance have been used: (i) a bacterial strain that is not killed by a biocide concentration to which the majority of the bacterial species are susceptible and (ii) bacterial cells in a culture that survive biocide exposure that kills the majority of the bacterial population in that culture. This latest definition has been used mainly to identify specific mechanisms of biocide resistance in bacteria following stepwise exposure to a specific biocide.

Empirically, bacterial resistance to biocides has been labeled as intrinsic, a natural property of the bacterium, or acquired, following the acquisition of resistance genes or following mutations (36). These definitions still hold true, although the concept of transient resistance, following the expression of a mechanism(s) in response to a direct selective pressure, recognizes that the effect of a biocide on a bacterium may be more complex and short-lived as long as the biocide, exerting a selective pressure, is present (24, 25).

What appears to be more of a concern is the ability of a bacterium to become clinically resistant to an antibiotic(s) following exposure to a biocide/biocidal product. Such cross-resistance has been raised by the European Commission following reports from the Scientific Committee on Emerging and Newly Identified Health Risks (1, 37) and the Scientific Committee on Consumer Safety (38). The Biocidal Product Regulation (20), which regulates the commercialization of biocidal products on the European market, now mentions the potential issue of bacterial resistance and cross-resistance following biocide application. In the United States, the Federal Drug Administration (FDA) recently proposed several rules based on the concern about bacterial resistance linked to the use of certain chemical biocides (39). Demonstrating that a chemical biocide or a biocidal product will not give rise to resistance in bacteria is a question not only of definition but also of methodology.

OCCURRENCE OF BACTERIAL RESISTANCE TO BIOCIDES

Bacterial resistance to biocides and biocidal products has now been well documented in the literature, although examples are often anecdotal where a specific product was investigated. Biocides are a very diverse group of chemicals (1). Surprisingly, bacterial resistance has been studied with only a few biocides. For biocidal products, the formulation will help and hopefully optimize the delivery of the biocide(s) and/or negate some undesirable effects such as corrosiveness of surfaces, pungent smell, poor stability, or toxicity. Components of the formulations may also have a profound effect on biocide efficacy, either increasing or, on occasion, decreasing efficacy. In the peer-reviewed literature, formulations have rarely been studied in the past, although recently, several studies concerned the effect of formulated biocides on bactericidal efficacy (3, 18, 40, 41). Bacterial resistance has been investigated *in vitro* against several chemical classes, including phenolics (e.g., triclosan) (42–49), cationic biocides (e.g., chlorhexidine, quaternary ammonium compounds, particularly cetylpyridinium chloride, and benzalkonium chloride) (50–56), isothiazolinones (57), and more reactive biocides such as iodophors (58), alkylating agents (e.g., glutaraldehyde) (59–64), and several oxidizing compounds (65–68). Studies often differed in their methodology, rendering the comparison of results difficult (1, 18). Using realistic *in vitro* protocols to generate bacteria resistant to a specific biocide is not straightforward either (69). Investigations can generally be divided into four categories:

1. *In vitro* testing of bacterial resistance to a specific biocide, often involving training the bacteria to survive increasing concentrations of a biocide (46–48, 57, 69–73)
2. Studies reporting the isolation of environmental isolates resistant to specific biocides. These investigations principally concern environmental bacterial isolates from, for example, health care settings, manufacturing, and slaughterhouses and include biocides such as glutaraldehyde (59–62, 74), chlorine dioxide (65), chlorhexidine (75–79), triclosan (48, 80), quaternary ammonium compounds (79, 81–83), alcohol, and iodine (75)
3. Studies reporting the contamination of biocidal products principally used in health care settings and possible associations with infection outbreaks and pseudo-outbreaks (74, 84–89)
4. *In situ* studies reporting the impact on bacterial resistance of using specific biocidal products (90–93)

One criticism of *in vitro* studies is that they might not reflect the way bacteria encounter a biocide/biocidal product in practice (3, 69). For example, the use of stepwise training, i.e., the passaging of bacteria in increasing concentrations of a biocide, does not reflect conditions *in situ*. These studies have, however, yielded many insights on bacterial resistance mechanisms (46–48, 57, 69–71, 94). Another issue is that the development and nature of resistance to a biocide depend on the bacterial isolates investigated. Ciusa and colleagues (95) reported that bacterial strains from standard culture collection were not necessarily appropriate to study mechanisms of resistance to triclosan because they did not reflect the level and type of mutations observed with clinical isolates when exposed to bisphenol. This study also highlighted that valuable information is being learned through the study of large numbers of isolates (in this study 1,388 *Staphylococcus aureus* isolates were used) and questioned studies reporting the use of a single isolate (95).

The study of environmental isolates rather than standard culture collection strains yields important and probably more relevant information in terms of expressed mechanisms of resistance. Such investigations reiterate that bacteria can express multiple mechanisms at the same time and that some mechanisms responsible for a stable biocide-resistant phenotype are still unknown. Martin et al. (96) described a vegetative *Bacillus subtilis* endoscope washer isolate with stable resistance to the in-use concentration of chlorine dioxide and hydrogen peroxide, but also to peracetic acid (96, 97). Although this isolate is a good biofilm producer, the mechanisms responsible for the observed level of resistance to these oxidizing agents have not all been identified (96). Other studies that have isolated bacteria from environments where antimicrobials are heavily used identified a decrease in biocide susceptibility (81, 82, 95, 98) in some but not all isolates when compared to counterpart bacteria from standard culture collection (95, 98).

Studies reporting bacterial growth in biocidal products and subsequent infections or pseudo-infections have been very helpful in identifying the risks associated with some products and practices (89). Reported incidents often result from the inappropriate application of a product or the inappropriate preparation of a product, including the use of contaminated tap water, topping up of stock solutions, use of diluted products or inappropriate dilution, and inappropriate storage conditions. Some microorganisms, notably *Pseudomonas* spp., *Burkholderia* spp., and atypical mycobacteria can, however, contaminate the stock solution of a prod-

uct because of their intrinsic resistance to the product (89). The preconceived idea that bacterial resistance occurs more readily in less reactive biocides such as phenolics (e.g., triclosan) and cationic biocides (e.g., chlorhexidine) rather than reactive ones such as alkylating and oxidizing agents does not hold true. For example, there have been many studies on atypical mycobacterial (*Mycobacterium chelonae*) resistance to 2% glutaraldehyde, which is used for the high-level disinfection of medical devices (59–64). It was speculated that these bacteria arose from a decrease in the effective concentration of glutaraldehyde (i.e., <2%) (60). Fisher et al. (99) reported the presence of glutaraldehyde-resistant atypical mycobacteria associated with endoscope reprocessing systems. Outbreaks of *M. chelonae* linked to endoscope reprocessing using glutaraldehyde have been described since 1991 (100). The more recent nosocomial outbreaks of *Mycobacterium abscessus* subsp. *massiliense* in Brazil, however, identified an isolate that was resistant to both 2% glutaraldehyde and first-line antimycobacterial antibiotics, highlighting the existence of cross-resistance mechanisms that remain to be identified (74).

Studies of the effect of biocidal product applications on emerging bacterial resistance in the community or health care settings remain scarce. These studies usually highlight the difficulty in data interpretation, notably in relation to the definition of "bacterial resistance." The few *in situ* studies nevertheless provide interesting insight on the long-term usage of selected biocidal products. Two studies from Cole and colleagues failed to show any cross-resistance between antibiotics and antibacterial wash products (91, 92). Likewise, Aiello et al. (90) failed to show any statistically significant correlation between the use of triclosan-containing product and reduced susceptibility to antibiotics. A study of benzalkonium chloride-containing product usage in households, however, found a correlation between elevated QAC MIC and bacterial resistance to antibiotics (93).

There should be no doubt that bacteria have a great ability to survive biocide exposure and that the inappropriate use or preparation of biocidal products can result in bacterial resistance. The reporting of cross-resistance between biocides and unrelated chemicals such as chemotherapeutic antibiotics is increasing as scientists focus more on this possibility.

MECHANISMS OF BACTERIAL RESISTANCE

Biocides and biocidal products induce stress on the bacterial cell. In response, a bacterium expresses several mechanisms to prevent the detrimental effect caused by a biocide. These mechanisms aim to decrease the biocide concentration sufficiently that it is no longer damaging to the bacterial cells and include the ability of the bacterium to repair damages. If damage cannot be repaired efficiently or worsens, for example, because of high metabolic activity, the bacterial cell will be committed to a lethal pathway (Table 1). By some accounts that the maintenance of the cytoplasmic pH is key in that pathway (101, 102). Overall, our understanding of the bacterial mechanisms in place to decrease the susceptibility of a bacterium to biocides has improved, but they remain poorly studied. There is no doubt that bacteria have a plethora of mechanisms at their disposal and that often several mechanisms contribute together to the observed resistance phenotype. Our understanding of the effect of biocide interaction with bacteria and especially the stress response effect on gene expression remains poor. Examples given in the literature are often anecdotal. Understanding and measuring the expression of mechanisms following a biocide or biocidal product interaction with a bacterium has become

Table 1 Levels of biocide interactions with a bacterial cell

Exposure	Interactions	Types of damage	Events
Short exposure	Disruption of the transmembrane PMF leading to an uncoupling of oxidative phosphorylation and inhibition of active transport across the membrane		Reversible
	Inhibition of respiration or catabolic/anabolic reactions		
Prolonged exposure	Disruption of metabolic processes		Reversible
	Disruption of replication		
	Loss of membrane integrity resulting in leakage of essential intracellular constituents (K^+, inorganic phosphate, pentoses, nucleotides and nucleosides, proteins)	Imbalance of pHi	Irreversible
	Coagulation of intracellular materials	Commitment to cell death (autocidal pathway)	
	Lysis	Cell death	

important because it underlies the principle of the observed transient phenotypic changes in bacteria and the cross-resistance mechanisms between antimicrobials.

Mechanisms that Decrease the Concentration of Antimicrobials in Bacteria

Bacteria can use several mechanisms to decrease the lethal or inhibitory concentration of a biocide. Biocides have multiple target sites against the bacterial structure and as such they are often regarded as nonspecific. The sum of the damage caused to multiple target sites and the importance of the target sites defines whether the interaction will lead to a lethal or inhibitory effect (Table 1) (3, 101–105). Decreasing a damaging concentration of a biocide/biocidal product will enable the target bacteria to survive. It should be recognized that a low concentration (sub- MIC) of a biocide will affect the bacteria and, notably, trigger mechanisms to further decrease the biocide concentration. It is now well established in *in vitro* laboratory experiments but also in practice that a low concentration of a biocide will give rise to bacteria that are less susceptible to the biocide, enabling at times the survival of the bacteria in products (60, 89, 96).

Furthermore, biocides are used in complex formulations (i.e., the biocidal product) in practice, yet the effect of a biocidal product on bacterial resistance is not often tested (3, 18, 41, 76, 81). Excipients such as surfactants, chelators, and wetting agents may have a direct effect on the bacterial cell structure and increase the efficacy of a biocide. Arguably, there is sometimes incompatibility between a biocide and an excipient, effectively reducing the bactericidal activity of the product.

Reducing biocide penetration

The effect of bacterial cell structure to prevent or reduce the penetration of antimicrobials has been well established, notably with bacterial endospores (106), Gram-negative bacteria, and mycobacteria (103, 104). The presence of the lipopolysaccharide layer in Gram-negative bacteria has been well documented for its role in decreasing the activity of several membrane active agents such as quaternary ammonium compounds and biguanides. Evidence of the role of lipopolysaccharide in decreasing the activity of a membrane active agent has often been indirect with the use of permeabilizing agents such as chelators and the use of bacterial protoplasts (103, 105, 107, 108). Genetic alterations of the bacterial membrane with, for example, transposon mutagenesis have also provided some important information on biocide/bacterial cell interactions (109). In mycobacteria, in the presence of mycolic acid associated with the arabinogalactan/arabinomannan cell wall, the lipid-rich outer cell wall is responsible for the lack of penetration of many antimicrobials (61, 104, 110–113). Likewise, porins have been shown to play an important role in the activity of glutaraldehyde and *ortho*-phthalaldehyde in mycobacteria (114). Reducing the expression of porins has been associated with reduced biocide and antibiotic efficacy (115, 116). Changes in bacterial cell membrane and cell wall composition following biocide exposure have been associated with a reduction in biocide activity (94, 115–120). Membrane alterations include membrane protein composition (57, 115, 121, 122), fatty acids (115, 123–127), and phospholipid content (128). A change in membrane potential has also been associated with a decrease in biocide susceptibility in *Pseudomonas aeruginosa* (129).

Efflux pumps

Efflux pumps, which are widespread in bacteria, contribute to decreasing the concentration of antimicrobials that penetrate the bacterial cells. The effect of active efflux on antimicrobial activity has been particularly well documented in *S. aureus* (130–139), *P. aeruginosa* (140–145), *Escherichia coli* (46, 82, 94, 146–149), *Salmonella enterica* serovar Typhimurium (150, 151), and *Acinetobacter baumannii* (116, 152). Five main classes of efflux pumps have been reported (Fig. 1) (153; 160): the drug/metabolite transporter superfamily, the major facilitator superfamily, the ATP-binding cassette family, the resistance-nodulation-division family, and the multidrug and toxic compound extrusion family.

The ability of efflux pumps alone to confer resistance to biocides/biocidal products is questionable, and it is likely that efflux pumps are part of several mechanisms used by a bacterium to survive biocide/biocidal product exposure (3, 83, 155). Some studies investigating triclosan claimed, however, that efflux was responsible for high-level resistance to the bisphenol (142, 144). Studies of bacterial isolates from environments where antimicrobials are heavily used, notably biguanides and QAC, have identified a high prevalence of efflux genes (e.g., *qacA/B*, *norA*, *nor B*, *smr*) in isolates that showed a decreased susceptibility to biocides (77–79, 82, 135).

Efflux can be induced by some antimicrobials (153, 156, 161). The expression of an efflux pump can increase following antimicrobial exposure, not necessarily by inducing the efflux pumps but by affecting global gene regulators, notably *marA* and *soxS* (46, 162). The effect of triclosan on bacteria has been particularly well studied with regard to efflux (46, 49, 140–142, 163, 164). In *S. enterica* serovar Typhimurium, overexpression of efflux results in decreased anti-

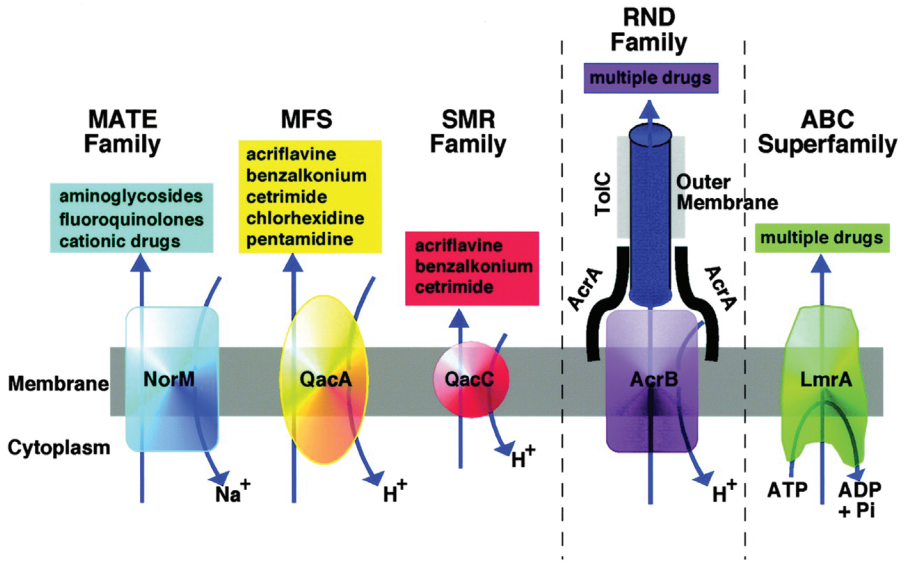


Figure 1 Diagrammatic comparison of the five families of efflux pumps (reproduced from reference 153). MATE, multidrug and toxic compound extrusion; MFS, major facilitator superfamily; SMR, •••; RND, resistance-nodulation-division; ABC, ATP-binding cassette.

microbial susceptibility (162–165). Overexpression of efflux pumps resulting in decreased biocide efficacy has also been described in *Stenotrophomonas maltophilia* with the overexpression of SmedEF (166); in *E. coli* with the overexpression of *acrAB*, *marA*, or *soxS* (46, 49, 162); and in *Campylobacter jejuni* overexpressing CmeB (167). The extent of efflux pumps and their role in bacteria are continuously evolving in the literature. Triggering overexpression of efflux in bacteria following biocide exposure is a concern that is debated later in this article.

Enzymatic degradation

Some bacteria can produce enzymes that degrade biocides. The presence of catalase and superoxide dismutase, for example, has been shown to decrease bacterial susceptibility to oxidizing agents (66, 168). The production of enzymes alone conferring resistance to a biocide is, however, doubtful. This would suggest that enzymatic activity is high and that enzymes are not themselves affected by the biocide. It is more likely that the production of detoxifying enzymes contributes to the battery of mechanisms available to the bacteria to survive biocide injuries (96).

Other examples of enzymatic activity conferring decreased susceptibility to a biocide include the parabens (169, 170), aldehydes (171), and metallic ions. In the latter case the ions are reduced to the inactive metal (34).

Physiological and Metabolic Changes

Bacterial metabolism can be associated with antimicrobial efficacy in that bacteria with a high metabolism are more susceptible to antimicrobials than those with no metabolic activity (172). Exposure of a bacteria to a physical or chemical process, such as a biocide/biocidal product, results in a mixed population of dead, injured, and uninjured bacteria. In the food industry, the recovery of injured bacteria is considered essential (173). This is not so when the efficacy of a biocide treatment is measured. Standard efficacy tests do not consider the effect of the recovery media and incubation conditions post-biocide treatment. The impact of resuscitated injured bacteria following treatment has been exemplified by the dual use of traditional plate counting on a rich nonselective recovery media such as tryptone soy agar and the use of the Bioscreen microbial growth analyzer, which measures bacterial growth in liquid (174). The ability of a bacterium to repair injuries is likely to play an important role when resistance is considered. As shown in Table 1, initial damage caused by a biocide is reversible. In practice, where incubation conditions posttreatment favor recovery from injury, repairs can be visualized with an extended lag phase (173). Biocide exposure has, however, been linked to a decreased growth rate and extended lag phase in bacteria (172, 175–177) because of a direct action of the biocide on the bacterial cells, although in many studies the ability of bacteria to repair injuries was not considered. Change

in metabolic pathways has been particularly well exemplified with *S. enterica* exposure to triclosan. The bisphenol at a low concentration has been shown to target specifically the enoyl acyl carrier reductase in bacteria, which affects fatty acid lipid synthesis in the target bacteria (47, 177–179). Webber et al. (180) showed that *S. enterica* could alter its metabolic pathway to produce pyruvate and fatty acids following triclosan exposure. This change was part of a "triclosan resistance network" involving the expression of distinct mechanisms (180). Curiao and colleagues reported similar findings, evoking multiple pathways in the adaptation of *S. enterica* to triclosan and other biocides such as chlorhexidine and benzalkonium chloride (181).

Codling and colleagues (109) showed that in *Serratia marcescens*, the disruption of biosynthetic and metabolic pathways of the bacterium increased bacterial susceptibility to a QAC. A change in metabolic processes following exposure to biocides has also been observed in other bacteria, including *S. aureus* (182) and *P. aeruginosa* (183). The full impact of a change in metabolic pathways on decreasing biocide/biocidal product efficacy has not been assessed, nor has the reproducibility of such a change when exposed to specific antimicrobials. At present, such observations have been bacteria/biocide specific.

Mutations

Mutations in bacteria are by nature random but can be driven by the continuous presence of a selective pressure, notably the presence of antimicrobials. Although it is widely recognized that the presence of chemotherapeutic antibiotics will drive target site mutations, there are far fewer examples with biocides. Mutations resulting from a biocide exposure have been mainly described with triclosan in several bacteria (43, 178, 180, 184–190). Mutations are linked to the use of triclosan at a low concentration and concern the enoyl-acyl reductase carrier protein (47, 179, 186, 191–193). In Salmonella biocide exposure resulted in mutations included de-repression of multidrug efflux pump AcrAB-TolC, and *rpoA*, which controls the RNA polymerase α-subunit (190). Interestingly, the investigation of 1,388 *S. aureus* clinical isolates' response to triclosan exposure identified several mutations that were similar among the isolates (Fig. 2) but not comparable to those observed with the standard culture collection strain (95). Recent observations questioned the choice of a culture collection strain to study biocide resistance. Standard strains have been widely used in biocide resistance studies with the comparability of results between studies in mind. From Ciusa et al. (95), it appears that standard strains and clinical isolates do not behave the same way. Stepwise training protocols that rely on passaging bacteria in increasing concentrations of biocides have yielded bacteria with decreased susceptibility or resistance to a given biocide but may be criticized for not reflecting real-world conditions (3). Many *in vitro* studies have investigated genetic changes in standard culture collection strains following biocide exposure. The work of Ciusa et al. (95) addresses the appropriateness of this approach and would favor the use of en-

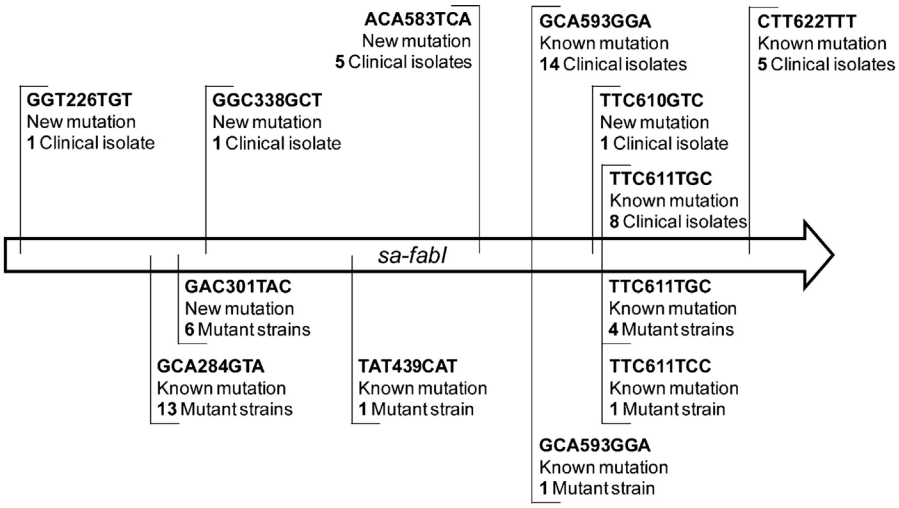


Figure 2 Schematic map of mutations in the *Staphylococcus aureus fabI* (*sa-fabI*) and *Staphylococcus haemolyticus fabI* (*sh-fabI*) genes. Mutations in *sa-fabI* are reported on a schematic map. Mutations detected in clinical isolates are mapped above the sequence, while mutations selected *in vitro* are shown below the sequence. (Reproduced from reference 95.)

vironmental isolates that have been exposed to a biocide/biocidal product.

INDUCTION OF GENE EXPRESSION CONFERRING BACTERIAL RESISTANCE

Biocide exposure even at a low concentration produces a stress on the target bacteria, even if the bacteria are intrinsically resistant to the biocide. The bacterial response to the stress will lead to a change in gene expression (24, 109, 116, 155, 162–167, 181, 194–196), particularly that of regulatory genes (46, 49, 68, 149, 162, 180, 197). The concentration of a biocide that is available to interact with the target bacteria is thus paramount (2, 3, 105, 198, 199), since a low, nonlethal concentration will not kill the bacterium but will undoubtedly produce a stress response. An indication of stress response is given by investigating the bacterial growth curve in the presence of a biocide at different concentrations. Increased lag phase or decreases in bacterial doubling time are indicators of a bacteria/biocide interaction and may reflect the induction/expression of mechanisms enabling the bacteria to decrease the toxicity of the biocide (28, 72, 172) and, as mentioned, allow earlier repair of injuries. Some bacterial mechanisms that play a role in decreasing the susceptibility to biocides are controlled by global regulators such as *soxS* and *marA* (46, 49, 146, 162). Antibiotic resistance mechanisms are also controlled by the same regulators (168, 200), which leads to the concern that biocide exposure can trigger antibiotic resistance. The induction of gene expression of global regulators leading to the expression of several mechanisms in bacterial resistance might not, however, be particularly problematic for the use of biocidal products since such expression might be transient. Some studies have shown that a decrease in bacterial susceptibility to biocides and sometimes to antibiotics was transient and only observed in the presence of the biocide (24, 25, 155).

As mentioned earlier, the efficient repair of sublethal injuries may play an important role in bacterial survival of biocide exposure. However, bacteria's ability to repair damage following biocide exposure has received little attention (195, 201, 202). In *E. coli* polyhexamethylene biguanide alters the expression of several genes, notably *rhs*, involved in repairing nucleic acid (202). The involvement of effective DNA repair mechanisms has been proposed to explain the high-level resistance of an environmental isolate of *B. subtilis* to several oxidizing agents (96). Efficient DNA repair mechanisms enable *Deinococcus radiodurans* to survive ionizing and UV radiation and exposure to chemicals that damage nucleic acid (203). In *Lactobacillus pentosus*, strains that have adapted to sublethal concentrations of antimicrobials overexpressed ribosomal proteins and glutamyl tRNA synthetase, which was interpreted as a response to damaged proteins directly caused by the antimicrobial exposure (195).

CROSS-RESISTANCE

Exposure to a biocide/biocidal product can lead to a stress response involving the expression of global gene regulators and ultimately the expression of nonspecific mechanisms enabling bacterial survival (116, 155, 156, 162, 181, 190, 200, 204–211). The link between biocide usage and antibiotic resistance has led to many discussions with conflicting evidence; some studies support a link, while others fail to identify any cross-resistance (1, 24, 25, 48, 73, 78, 79, 81, 82, 90–93, 98, 196, 212–220). Where cross-resistance between biocide exposure and antibiotic resistance was identified, suggested common resistance mechanisms included overexpression of efflux (18, 82, 153, 156, 161), changes in bacterial cell wall permeability (115, 117), and changes in bacterial metabolism (180). Differences in protocols to (i) grow test bacteria, (ii) expose test bacteria to the biocide/biocidal product, and (iii) measure resistance to biocides and antibiotics contribute to differences in reported observations of the biocide's effect on antibiotic resistance (18). Although the evidence is mainly *in vitro* based, the few *in situ* studies conducted also reported conflicting information about the association between the usage of biocidal products at home and an increase in antibiotic resistance among environmental isolates (89–93). It is worth noting, however, the study from Duarte and colleagues (74) reporting a post-surgical outbreak of a *M. abscessus* subsp. *massiliense*-resistant clone resistant to 2% glutaraldehyde and resistant to frontline antimycobacterial antibiotics.

There should be no doubt that bacteria have the capacity to express mechanisms that will lead to decreased susceptibility to both biocides/biocidal products and chemotherapeutic antibiotics. The question remains as to how commonly cross- resistance occurs in practice and what triggers emerging resistance in the first place. For example, efflux can easily be triggered in bacteria, not only by biocides but by a wide range of stimuli, such as spices and essential oil. (221).

MEASURING BACTERIAL RESISTANCE

One of the most important aspects of biocide/biocidal product resistance is how to measure bacterial resis-

tance and cross-resistance. This has become even more pressing with the publication in Europe of the Biocidal Product Regulation (20), which asks manufacturers to demonstrate that their biocidal product will not cause emerging bacterial resistance. Likewise, in North America the FDA (39) issued a final rule on the safety and effectiveness of antibacterial soaps, effectively banning the use of certain biocides for that application. Rules concerning benzalkonium chloride, benzethonium chloride, and chloroxylenol, biocides that are commonly used in several products, deferred. One major issue for manufacturers is that neither the Biocidal Product Regulation nor the FDA indicate what appropriate tests should be conducted to demonstrate the safety of biocidal products where bacterial resistance is concerned.

MIC determination has often been used as a marker for resistance (18, 28, 69–71, 183, 219), although the validity of MIC to measure bacterial resistance has been questioned (1, 3, 18) mainly since in practice, biocides are often used at concentrations exceeding the MIC (120) and biocides are used as part of a formulation whose ingredients will impact on product efficacy (3, 18, 76). MIC could, however, be used as a trend indicator (3, 18, 25, 28, 76, 198, 199, 222, 223). It is thus unfortunate that some studies measure an increase in bacterial resistance in terms of MIC (28, 224). Other studies have used a prevalue biocide concentration above which the environmental isolates were considered to be resistant. For example, Lavilla Lerma and colleagues used a threshold biocide concentration of 0.025 μg/ml at which any bacterial growth was considered bacterial resistance to the biocide (81). Furthermore, resistance has sometimes been defined as a small increase (e.g., 2-fold) in MIC. This definition remains questionable, especially when using a standard protocol such as a broth microdilution method (225) because a 2-fold change might only reflect a 1-dilution difference. The determination of changes in minimum biocidal concentration might be more appropriate, because this indicates a change in the lethal effect of the biocide (18, 223). Some studies have looked at a change in inactivation kinetics. Such protocols, although very useful because they determine the ability of a biocide/biocidal product to kill target bacteria over time, are very cumbersome and time-consuming and would not be able to be used routinely (3, 18).

The determination of a change in the susceptibility profile to chemotherapeutic antibiosis is somewhat easier to perform because the protocols used can follow well-established standards that provide clear guidance but also breakpoints for selected bacteria/antibiotics (226, 227). It is, however, clear that the clinical significance should be reported rather than reporting a mere change in the antibiotic zone of inhibition.

Measuring a change in the susceptibility profile to determine a prediction of the risk associated with biocidal product usage is acceptable if the exposure of the target bacterium to the biocidal product is realistic, i.e., if it reflects *in situ* exposure of bacteria with the biocidal products, encompassing dilution of the product upon usage if necessary, extended contact time for residual activity, etc. (18, 25). The test bacterial inoculum preparation needs to be strictly controlled to ensure reproducibility of the assay. When a significant change (here significant means a ≥10-fold change) in susceptibility profile is recorded (25, 222), the nature of this change, whether transient or permanent, needs to be established (18, 25, 223).

A protocol to predict the change in susceptibility profile of target bacteria following exposure to a biocide/biocidal product has been proposed (18). The use of such a protocol established the effect of various biocidal products on *S. enterica* (223), *E. coli*, and *S. aureus* (25). In these studies, triclosan was used as a positive control (25, 223). Triclosan is the most studied biocide in terms of interaction with bacteria. Studies have repeatedly show amended bacterial susceptibility profile to triclosan and antibiotics following exposure to the bisphenol. (23, 25, 228).

CONCLUSIONS

Biocidal products are useful compounds to control microbial contamination and kill pathogens. A biocidal concentration that will not kill the target bacteria will, regardless of the method of application, cause a stress response, which will lead to the expression of mechanisms that enable bacterial survival (3, 18, 28, 173). The concentration of biocide available to interact with the target bacteria is thus paramount. In the veterinary field, the presence of organic matter at the point of the biocidal product application, contributes to reduce the efficacy of the product, and therefore, cleaning the animate or inanimate surface prior to use of the biocidal product should be indicated but might not be practical.

Biocidal products are heavily used for veterinary applications, notably in animal husbandry, disinfection of udders in dairy animals, and in fish farming (1). Despite an increasing use of biocidal products, information related to the occurrence of bacterial resistance in these environments remains scarce (55, 73, 150, 229, 230). Nevertheless, with the growing knowledge and evidence of bacterial resistance in environments where biocides/biocidal products are heavily used, environ-

mental surveillance has been timidly proposed to study the potential spread and occurrence of resistant bacteria (27, 73, 77).

With an increase in biocidal product usage, emerging bacterial resistance is possible, but to date, the risk associated with biocidal product usage has not been measured, mainly because of the lack of standard protocols. The only protocol to date has not yet been widely used against a small number of bacteria (18, 25, 223). The reproducibility of the data obtained may also depend on the bacterial species investigated (24). Such a predictive protocol also relies on using appropriate test parameters that reflect the biocidal product usage in practice (25, 223). Overall, investigating the biocidal effect on bacterial resistance should be welcome because it provides a better understanding of the biocide-bacteria interactions and should contribute to the development of more performant and safer biocidal products. This is particularly pertinent with the increased usage of biocidal products and the usage conditions in animal husbandry.

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References

1. **Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).** 2009. The antibiotic resistance effect of biocides. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf. Accessed January 2017.
2. **Maillard J-Y.** 2005. Usage of antimicrobial biocides and products in the healthcare environment: efficacy, policies, management and perceived problems. *Ther Clin Risk Manag* **1:**340–370.
3. **Maillard J-Y, Denyer SP.** 2009. Emerging bacterial resistance following biocide exposure: should we be concerned? *Chim Oggi* **27:**26–28.
4. **O'Neill J.** 2016. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance*. HM Government, London, United Kingdom.
5. **Otter JA, Yezli S, French GL.** 2011. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol* **32:** 687–699.
6. **Lawley TD, Clare S, Deakin LJ, Goulding D, Yen JL, Raisen C, Brandt C, Lovell J, Cooke F, Clark TG, Dougan G.** 2010. Use of purified *Clostridium difficile* spores to facilitate evaluation of health care disinfection regimens. *Appl Environ Microbiol* **76:**6895–6900.
7. **Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL.** 2008. Norwalk virus: how infectious is it? *J Med Virol* **80:**1468–1476.
8. **Boyce JM, Potter-Bynoe G, Chenevert C, King T.** 1997. Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: possible infection control implications. *Infect Control Hosp Epidemiol* **18:** 622–627.
9. **Bhalla A, Pultz NJ, Gries DM, Ray AJ, Eckstein EC, Aron DC, Donskey CJ.** 2004. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. *Infect Control Hosp Epidemiol* **25:**164–167.
10. **Vonberg RP, Kuijper EJ, Wilcox MH, Barbut F, Tüll P, Gastmeier P, van den Broek PJ, Colville A, Coignard B, Daha T, Debast S, Duerden BI, van den Hof S, van der Kooi T, Maarleveld HJ, Nagy E, Notermans DW, O'Driscoll J, Patel B, Stone S, Wiuff C, European C difficile-Infection Control Group, European Centre for Disease Prevention and Control (ECDC).** 2008. Infection control measures to limit the spread of *Clostridium difficile*. *Clin Microbiol Infect* **14**(Suppl 5)**:**2–20.
11. **Kramer A, Schwebke I, Kampf G.** 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* **6:**130–138.
12. **Fawley WN, Wilcox MH.** 2001. Molecular epidemiology of endemic *Clostridium difficile* infection. *Epidemiol Infect* **126:**343–350.
13. **Talon D.** 1999. The role of the hospital environment in the epidemiology of multi-resistant bacteria. *J Hosp Infect* **43:**13–17.
14. **Hota B.** 2004. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* **39:**1182–1189.
15. **Cheeseman KE, Denyer SP, Hosein IK, Williams GJ, Maillard J-Y.** 2009. Evaluation of the bactericidal efficacy of three different alcohol hand rubs against 57 clinical isolates of *S. aureus*. *J Hosp Infect* **72:**319–325.
16. **Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard J-Y.** 2009. Limitations of the efficacy of surface disinfection in the healthcare setting. *Infect Control Hosp Epidemiol* **30:**570–573.
17. **Siani H, Cooper C, Maillard J-Y.** 2011. Efficacy of "sporicidal" wipes against *Clostridium difficile*. *Am J Infect Control* **39:**212–218.
18. **Maillard J-Y, Bloomfield S, Coelho JR, Collier P, Cookson B, Fanning S, Hill A, Hartemann P, McBain AJ, Oggioni M, Sattar S, Schweizer HP, Threlfall J.** 2013. Does microbicide use in consumer products promote antimicrobial resistance? A critical review and recommendations for a cohesive approach to risk assessment. *Microb Drug Resist* **19:**344–354.
19. **Department for Environment, Food & Rural Affairs.** 2012. Controlling disease in farm animals. https://www.gov.uk/guidance/controlling-disease-in-farm-animals. Accessed January 2017.
20. **Pedrouzo M, Borrull F, Marcé RM, Pocurull E.** 2009. Ultra-high-performance liquid chromatography-tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters. *J Chromatogr A* **1216:**6994–7000.
21. **Kumar KS, Priya SM, Peck AM, Sajwan KS.** 2010. Mass loadings of triclosan and triclocarbon from four wastewater treatment plants to three rivers and landfill

in Savannah, Georgia, USA. *Arch Environ Contam Toxicol* **58:**275–285.

22. **Wilson B, Chen RF, Cantwell M, Gontz A, Zhu J, Olsen CR.** 2009. The partitioning of triclosan between aqueous and particulate bound phases in the Hudson River Estuary. *Mar Pollut Bull* **59:**207–212.
23. **Scientific Committee on Consumer Safety.** 2010. Opinion on triclosan antimicrobial resistance. http://ec.europa.eu/health//sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_054.pdf. Accessed January 2017.
24. **Knapp L, Rushton L, Stapleton H, Sass A, Stewart S, Amezquita A, McClure P, Mahenthiralingam E, Maillard J-Y.** 2013. The effect of cationic microbicide exposure against *Burkholderia cepacia* complex (Bcc); the use of *Burkholderia lata* strain 383 as a model bacterium. *J Appl Microbiol* **115:**1117–1126.
25. **Wesgate R, Grasha P, Maillard J-Y.** 2016. Use of a predictive protocol to measure the antimicrobial resistance risks associated with biocidal product usage. *Am J Infect Control* **44:**458–464.
26. **Oggioni MR, Furi L, Coelho JR, Maillard JY, Martínez JL.** 2013. Recent advances in the potential interconnection between antimicrobial resistance to biocides and antibiotics. *Expert Rev Anti Infect Ther* **11:**363–366.
27. **Cookson B.** 2005. Clinical significance of emergence of bacterial antimicrobial resistance in the hospital environment. *J Appl Microbiol* **99:**989–996.
28. **Maillard J-Y.** 2007. Bacterial resistance to biocides in the healthcare environment: should it be of genuine concern? *J Hosp Infect* **65**(Suppl 2)**:**60–72.
29. **Siani H, Maillard J-Y.** 2015. Best practice in healthcare environment decontamination. *Eur J Clin Microbiol Infect Dis* **34:**1–11.
30. **Chapman JS.** 1998. Characterizing bacterial resistance to preservatives and disinfectants. *Int Biodeter Biodeg* **41:**241–245.
31. **Chapman JS, Diehl MA, Fearnside KB.** 1998. Preservative tolerance and resistance. *Int J Cosmet Sci* **20:**31–39.
32. **Hammond SA, Morgan JR, Russell AD.** 1987. Comparative susceptibility of hospital isolates of Gram-negative bacteria to antiseptics and disinfectants. *J Hosp Infect* **9:**255–264.
33. **Russell AD.** 2003. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect Dis* **3:**794–803.
34. **Cloete TE.** 2003. Resistance mechanisms of bacteria to antimicrobial compounds. *Int Biodeter Biodegrad* **51:** 277–282.
35. **Dettenkofer M, Wenzler S, Amthor S, Antes G, Motschall E, Daschner FD.** 2004. Does disinfection of environmental surfaces influence nosocomial infection rates? A systematic review. *Am J Infect Control* **32:**84–89.
36. **Poole K.** 2002. Mechanisms of bacterial biocide and antibiotic resistance. *J Appl Microbiol* **92**(Suppl)**:**55S–64S.
37. **Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).** 2010. Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides. http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_028.pdf. Accessed January 2017.
38. **Scientific Committee on Consumer Safety (SCCS).** 2010. Opinion on triclosan antimicrobial resistance. http://ec.europa.eu/health//sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_054.pdf. Accessed January 2017.
39. **U.S. Food and Drug Administration.** 2016. Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm517478.htm. Accessed January 2017.
40. **Lavilla Lerma L, Benomar N, Casado Muñoz MC, Gálvez A, Abriouel H.** 2015. Correlation between antibiotic and biocide resistance in mesophilic and psychrotrophic *Pseudomonas* spp. isolated from slaughterhouse surfaces throughout meat chain production. *Food Microbiol* **51:**33–44.
41. **Cowley NL, Forbes S, Amézquita A, McClure P, Humphreys GJ, McBain AJ.** 2015. Effects of formulation on microbicide potency and mitigation of the development of bacterial insusceptibility. *Appl Environ Microbiol* **81:**7330–7338.
42. **Sasatsu M, Shimizu K, Noguchi N, Kono M.** 1993. Triclosan-resistant *Staphylococcus aureus*. *Lancet* **341:**756.
43. **Heath RJ, Yu YT, Shapiro MA, Olson E, Rock CO.** 1998. Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. *J Biol Chem* **273:**30316–30320.
44. **Bamber AI, Neal TJ.** 1999. An assessment of triclosan susceptibility in methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *J Hosp Infect* **41:**107–109.
45. **Randall LP, Cooles SW, Piddock LJ, Woodward MJ.** 2004. Effect of triclosan or a phenolic farm disinfectant on the selection of antibiotic-resistant *Salmonella enterica*. *J Antimicrob Chemother* **54:**621–627.
46. **McMurry LM, Oethinger M, Levy SB.** 1998. Overexpression of *marA*, *soxS*, or *acrAB* produces resistance to triclosan in laboratory and clinical strains of *Escherichia coli*. *FEMS Microbiol Lett* **166:**305–309.
47. **McMurry LM, McDermott PF, Levy SB.** 1999. Genetic evidence that InhA of *Mycobacterium smegmatis* is a target for triclosan. *Antimicrob Agents Chemother* **43:** 711–713.
48. **Cottell A, Denyer SP, Hanlon GW, Ochs D, Maillard JY.** 2009. Triclosan-tolerant bacteria: changes in susceptibility to antibiotics. *J Hosp Infect* **72:**71–76.
49. **Curiao T, Marchi E, Viti C, Oggioni MR, Baquero F, Martinez JL, Coque TM.** 2015. Polymorphic variation in susceptibility and metabolism of triclosan-resistant mutants of *Escherichia coli* and *Klebsiella pneumoniae* clinical strains obtained after exposure to biocides and antibiotics. *Antimicrob Agents Chemother* **59:**3413–3423.
50. **Adair FW, Geftic SG, Gelzer J.** 1971. Resistance of *Pseudomonas* to quaternary ammonium compounds. II. Cross-resistance characteristics of a mutant of *Pseudomonas aeruginosa*. *Appl Microbiol* **21:**1058–1063.

51. **Russell AD.** 2002. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol* **92**(Suppl)**:**121S–135S.
52. **Chapman JS.** 2003. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeter Biodegrad* **51:**271–276.
53. **Stickler DJ.** 1974. Chlorhexidine resistance in *Proteus mirabilis*. *J Clin Pathol* **27:**284–287.
54. **Gillespie MT, May JW, Skurray RA.** 1986. Plasmid-encoded resistance to acriflavine and quaternary ammonium compounds in methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* **34:**47–51.
55. **Randall LP, Cooles SW, Sayers AR, Woodward MJ.** 2001. Association between cyclohexane resistance in *Salmonella* of different serovars and increased resistance to multiple antibiotics, disinfectants and dyes. *J Med Microbiol* **50:**919–924.
56. **Romão CMCPA, Faria YN, Pereira LR, Asensi MD.** 2005. Susceptibility of clinical isolates of multiresistant *Pseudomonas aeruginosa* to a hospital disinfectant and molecular typing. *Mem Inst Oswaldo Cruz* **100:**541–548.
57. **Winder CL, Al-Adham IS, Abdel Malek SM, Buultjens TE, Horrocks AJ, Collier PJ.** 2000. Outer membrane protein shifts in biocide-resistant *Pseudomonas aeruginosa* PAO1. *J Appl Microbiol* **89:**289–295.
58. **O'Rourke E, Runyan D, O'Leary J, Stern J.** 2003. Contaminated iodophor in the operating room. *Am J Infect Control* **31:**255–256.
59. **Griffiths PA, Babb JR, Bradley CR, Fraise AP.** 1997. Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectors. *J Appl Microbiol* **82:**519–526.
60. **van Klingeren B, Pullen W.** 1993. Glutaraldehyde resistant mycobacteria from endoscope washers. *J Hosp Infect* **25:**147–149.
61. **Manzoor SE, Lambert PA, Griffiths PA, Gill MJ, Fraise AP.** 1999. Reduced glutaraldehyde susceptibility in *Mycobacterium chelonae* associated with altered cell wall polysaccharides. *J Antimicrob Chemother* **43:**759–765.
62. **Fraud S, Maillard J-Y, Russell AD.** 2001. Comparison of the mycobactericidal activity of *ortho*- phthalaldehyde, glutaraldehyde and other dialdehydes by a quantitative suspension test. *J Hosp Infect* **48:**214–221.
63. **Walsh SE, Maillard J-Y, Russell AD, Hann AC.** 2001. Possible mechanisms for the relative efficacies of *ortho*-phthalaldehyde and glutaraldehyde against glutaraldehyde-resistant *Mycobacterium chelonae*. *J Appl Microbiol* **91:**80–92.
64. **Nomura K, Ogawa M, Miyamoto H, Muratani T, Taniguchi H.** 2004. Antibiotic susceptibility of glutaraldehyde-tolerant *Mycobacterium chelonae* from bronchoscope washing machines. *Am J Infect Control* **32:**185–188.
65. **Martin DJH, Denyer SP, McDonnell G, Maillard J-Y.** 2008. Resistance and cross-resistance to oxidising agents of bacterial isolates from endoscope washer disinfectors. *J Hosp Infect* **69:**377–383.
66. **Greenberg JT, Demple B.** 1989. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. *J Bacteriol* **171:**3933–3939.
67. **Greenberg JT, Monach P, Chou JH, Josephy PD, Demple B.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc Natl Acad Sci USA* **87:**6181–6185.
68. **Dukan S, Touati D.** 1996. Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. *J Bacteriol* **178:**6145–6150.
69. **Walsh SE, Maillard J-Y, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG.** 2003. Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. *J Hosp Infect* **55:**98–107.
70. **Tattawasart U, Maillard J-Y, Furr JR, Russell AD.** 1999. Development of resistance to chlorhexidine diacetate and cetylpyridinium chloride in *Pseudomonas stutzeri* and changes in antibiotic susceptibility. *J Hosp Infect* **42:**219–229.
71. **Thomas L, Maillard J-Y, Lambert RJW, Russell AD.** 2000. Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a "residual" concentration. *J Hosp Infect* **46:**297–303.
72. **Thomas L, Russell AD, Maillard J-Y.** 2005. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. *J Appl Microbiol* **98:**533–543.
73. **Molina-González D, Alonso-Calleja C, Alonso-Hernando A, Capita R.** 2014. Effect of sub-lethal concentrations of biocides on the susceptibility to antibiotics of multidrug resistant *Salmonella enterica* strains. *Food Control* **40:**329–334.
74. **Duarte RS, Lourenço MCS, Fonseca LS, Leão SC, Amorim EL, Rocha IL, Coelho FS, Viana-Niero C, Gomes KM, da Silva MG, Lorena NS, Pitombo MB, Ferreira RM, Garcia MH, de Oliveira GP, Lupi O, Vilaça BR, Serradas LR, Chebabo A, Marques EA, Teixeira LM, Dalcolmo M, Senna SG, Sampaio JL.** 2009. Epidemic of postsurgical infections caused by *Mycobacterium massiliense*. *J Clin Microbiol* **47:**2149–2155.
75. **Wisplinghoff H, Schmitt R, Wöhrmann A, Stefanik D, Seifert H.** 2007. Resistance to disinfectants in epidemiologically defined clinical isolates of *Acinetobacter baumannii*. *J Hosp Infect* **66:**174–181.
76. **Bock LJ, Wand ME, Sutton JM.** 2016. Varying activity of chlorhexidine-based disinfectants against *Klebsiella pneumoniae* clinical isolates and adapted strains. *J Hosp Infect* **93:**42–48.
77. **Liu Q, Zhao H, Han L, Shu W, Wu Q, Ni Y.** 2015. Frequency of biocide-resistant genes and susceptibility to chlorhexidine in high-level mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MuH MRSA). *Diagn Microbiol Infect Dis* **82:**278–283.
78. **Hijazi K, Mukhopadhya I, Abbott F, Milne K, Al-Jabri ZJ, Oggioni MR, Gould IM.** 2016. Susceptibility to

chlorhexidine amongst multidrug-resistant clinical isolates of *Staphylococcus epidermidis* from bloodstream infections. *Int J Antimicrob Agents* **48:**86–90.

79. **Conceição T, Coelho C, de Lencastre H, Aires-de-Sousa M.** 2015. High prevalence of biocide resistance determinants in *Staphylococcus aureus* isolates from three African countries. *Antimicrob Agents Chemother* **60:** 678–681.
80. **Lear JC, Maillard J-Y, Dettmar PW, Goddard PA, Russell AD.** 2002. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources. *J Ind Microbiol Biotechnol* **29:**238–242.
81. **Lavilla Lerma L, Benomar N, Gálvez A, Abriouel H.** 2013. Prevalence of bacteria resistant to antibiotics and/or biocides on meat processing plant surfaces throughout meat chain production. *Int J Food Microbiol* **161:**97–106.
82. **Grande Burgos MJ, Fernández Márquez ML, Pérez Pulido R, Gálvez A, Lucas López R.** 2016. Virulence factors and antimicrobial resistance in *Escherichia coli* strains isolated from hen egg shells. *Int J Food Microbiol* **238:**89–95.
83. **Martínez-Suárez JV, Ortiz S, López-Alonso V.** 2016. Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of *Listeria monocytogenes* in food processing environments. *Front Microbiol* **7:**638.
84. **Sanford JP.** 1970. Disinfectants that don't. *Ann Intern Med* **72:**282–283.
85. **Prince J, Ayliffe GAJ.** 1972. In-use testing of disinfectants in hospitals. *J Clin Pathol* **25:**586–589.
86. **Bridges K, Lowbury EJL.** 1977. Drug resistance in relation to use of silver sulphadiazine cream in a burns unit. *J Clin Pathol* **30:**160–164.
87. **Klasen HJ.** 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* **26:**131–138.
88. **Reiss I, Borkhardt A, Füssle R, Sziegoleit A, Gortner L.** 2000. Disinfectant contaminated with *Klebsiella oxytoca* as a source of sepsis in babies. *Lancet* **356:**310.
89. **Weber DJ, Rutala WA, Sickbert-Bennett EE.** 2007. Outbreaks associated with contaminated antiseptics and disinfectants. *Antimicrob Agents Chemother* **51:**4217–4224.
90. **Aiello AE, Marshall B, Levy SB, Della-Latta P, Larson E.** 2004. Relationship between triclosan and susceptibilities of bacteria isolated from hands in the community. *Antimicrob Agents Chemother* **48:**2973–2979.
91. **Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, Newell MS, Wilkins J, Gaber DJ, Wineinger T, Criger DA.** 2003. Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *J Appl Microbiol* **95:**664–676.
92. **Cole EC, Addison RM, Dulaney PD, Leese KE, Madanat HM, Guffey AM.** 2011. Investigation of antibiotic and antibacterial susceptibility and resistance in *Staphylococcus* form the skin of users and non-users of antibacterial wash products in home environments. *Int J Microbiol Res* **3:**90–96.
93. **Carson RT, Larson E, Levy SB, Marshall BM, Aiello AE.** 2008. Use of antibacterial consumer products containing quaternary ammonium compounds and drug resistance in the community. *J Antimicrob Chemother* **62:** 1160–1162.
94. **Alonso-Calleja C, Guerrero-Ramos E, Alonso-Hernando A, Capita R.** 2015. Adaptation and cross-adaptation of *Escherichia coli* ATCC 12806 to several food-grade biocides. *Food Control* **56:**86–94.
95. **Ciusa ML, Furi L, Knight D, Decorosi F, Fondi M, Raggi C, Coelho JR, Aragones L, Moce L, Visa P, Freitas AT, Baldassarri L, Fani R, Viti C, Orefici G, Martinez JL, Morrissey I, Oggioni MR, BIOHYPO Consortium.** 2012. A novel resistance mechanism to triclosan that suggests horizontal gene transfer and demonstrates a potential selective pressure for reduced biocide susceptibility in clinical strains of *Staphylococcus aureus*. *Int J Antimicrob Agents* **40:**210–220.
96. **Martin DJH, Wesgate RL, Denyer SP, McDonnell G, Maillard J-Y.** 2015. *Bacillus subtilis* vegetative isolate surviving chlorine dioxide exposure: an elusive mechanism of resistance. *J Appl Microbiol* **119:**1541–1551.
97. **Bridier A, Le Coq D, del Pilar Sanchez-Vizuete M, Aymerich S, Meylheuc T, Maillard J-Y, Thomas V, Dubois-Brissonnet F, Briandet R.** 2012. Biofilms of a *Bacillus subtilis* endoscope WD isolate that protect *Staphylococcus aureus* from peracetic acid. *PLoS One* **7:**e44506.
98. **Lear JC, Maillard J-Y, Dettmar PW, Goddard PA, Russell AD.** 2006. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources: susceptibility to antibiotics and other biocides. *Int Biodeter Biodegrad* **57:** 51–56.
99. **Fisher CW, Fiorello A, Shaffer D, Jackson M, McDonnell GE.** 2012. Aldehyde-resistant mycobacteria bacteria associated with the use of endoscope reprocessing systems. *Am J Infect Control* **40:**880–882.
100. **Alvarado CJ, Stolz SM, Maki DG, Centers for Disease Control (CDC).** 1991. Nosocomial infection and pseudoinfection from contaminated endoscopes and bronchoscopes—Wisconsin and Missouri. *MMWR Morb Mortal Wkly Rep* **40:**675–678.
101. **Denyer SP, Stewart GSAB.** 1998. Mechanisms of action of disinfectants. *Int Biodeter Biodegrad* **41:**261–268.
102. **Maillard J-Y.** 2002. Bacterial target sites for biocide action. *J Appl Microbiol* **92**(Suppl)**:**16S–27S.
103. **Denyer SP, Maillard J-Y.** 2002. Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. *J Appl Microbiol* **92**(Suppl)**:**35S–45S.
104. **Lambert PA.** 2002. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *J Appl Microbiol* **92**(Suppl)**:**46S–54S.
105. **McDonnell G, Russell AD.** 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* **12:**147–179.
106. **Leggett MJ, Schwarz JS, Burke PA, Mcdonnell G, Denyer SP, Maillard J-Y.** 2015. Resistance to and killing by the sporicidal microbicide peracetic acid. *J Antimicrob Chemother* **70:**773–779.

107. **Munton TJ, Russell AD.** 1970. Effect of glutaraldehyde on protoplasts of *Bacillus megaterium*. *J Gen Microbiol* **63:**367–370.

108. **Ayres HM, Payne DN, Furr JR, Russell AD.** 1998. Effect of permeabilizing agents on antibacterial activity against a simple *Pseudomonas aeruginosa* biofilm. *Lett Appl Microbiol* **27:**79–82.

109. **Codling CE, Jones BV, Mahenthiralingam E, Russell AD, Maillard J-Y.** 2004. Identification of genes involved in the susceptibility of *Serratia marcescens* to polyquaternium-1. *J Antimicrob Chemother* **54:**370–375.

110. **Walsh SE, Maillard J-Y, Russell AD, Hann AC.** 2001. Possible mechanisms for the relative efficacies of *ortho*-phthalaldehyde and glutaraldehyde against glutaraldehyde-resistant *Mycobacterium chelonae*. *J Appl Microbiol* **91:**80–92.

111. **McNeil MR, Brennan PJ.** 1991. Structure, function and biogenesis of the cell envelope of mycobacteria in relation to bacterial physiology, pathogenesis and drug resistance; some thoughts and possibilities arising from recent structural information. *Res Microbiol* **142:**451–463.

112. **Broadley SJ, Jenkins PA, Furr JR, Russell AD.** 1995. Potentiation of the effects of chlorhexidine diacetate and cetylpyridinium chloride on mycobacteria by ethambutol. *J Med Microbiol* **43:**458–460.

113. **Fraud S, Hann AC, Maillard J-Y, Russell AD.** 2003. Effects of ortho-phthalaldehyde, glutaraldehyde and chlorhexidine diacetate on *Mycobacterium chelonae* and *Mycobacterium abscessus* strains with modified permeability. *J Antimicrob Chemother* **51:**575–584.

114. **Svetlíková Z, Skovierová H, Niederweis M, Gaillard J-L, McDonnell G, Jackson M.** 2009. Role of porins in the susceptibility of *Mycobacterium smegmatis* and *Mycobacterium chelonae* to aldehyde-based disinfectants and drugs. *Antimicrob Agents Chemother* **53:** 4015–4018.

115. **Tattawasart U, Maillard JY, Furr JR, Russell AD, Russell AD.** 2000. Outer membrane changes in *Pseudomonas stutzeri* resistant to chlorhexidine diacetate and cetylpyridinium chloride. *Int J Antimicrob Agents* **16:** 233–238.

116. **Fernández-Cuenca F, Tomás M, Caballero-Moyano FJ, Bou G, Martínez-Martínez L, Vila J, Pachón J, Cisneros JM, Rodríguez-Baño J, Pascual Á, Spanish Group of Nosocomial Infections (GEIH) from the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) and the Spanish Network for Research in Infectious Diseases (REIPI), Spanish Group of Nosocomial Infections GEIH from the Spanish Society of Clinical Microbiology and Infectious Diseases SEIMC and the Spanish Network for Research in Infectious Diseases REIPI.** 2015. Reduced susceptibility to biocides in *Acinetobacter baumannii*: association with resistance to antimicrobials, epidemiological behaviour, biological cost and effect on the expression of genes encoding porins and efflux pumps. *J Antimicrob Chemother* **70:** 3222–3229.

117. **Tattawasart U, Hann AC, Maillard J-Y, Furr JR, Russell AD.** 2000. Cytological changes in chlorhexidine-resistant isolates of *Pseudomonas stutzeri*. *J Antimicrob Chemother* **45:**145–152.

118. **Braoudaki M, Hilton AC.** 2005. Mechanisms of resistance in *Salmonella enterica* adapted to erythromycin, benzalkonium chloride and triclosan. *Int J Antimicrob Agents* **25:**31–37.

119. **Pagès JM, James CE, Winterhalter M.** 2008. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol* **6:** 893–903.

120. **Nikaido H.** 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* **67:**593–656.

121. **Gandhi PA, Sawant AD, Wilson LA, Ahearn DG.** 1993. Adaptation and growth of *Serratia marcescens* in contact lens disinfectant solutions containing chlorhexidine gluconate. *Appl Environ Microbiol* **59:**183–188.

122. **Brözel VS, Cloete TE.** 1994. Resistance of *Pseudomonas aeruginosa* to isothiazolone. *J Appl Bacteriol* **76:** 576–582.

123. **Jones MV, Herd TM, Christie HJ.** 1989. Resistance of *Pseudomonas aeruginosa* to amphoteric and quaternary ammonium biocides. *Microbios* **58:**49–61.

124. **Méchin L, Dubois-Brissonnet F, Heyd B, Leveau JY.** 1999. Adaptation of *Pseudomonas aeruginosa* ATCC 15442 to didecyldimethylammonium bromide induces changes in membrane fatty acid composition and in resistance of cells. *J Appl Microbiol* **86:**859–866.

125. **Guérin-Méchin L, Dubois-Brissonnet F, Heyd B, Leveau JY.** 1999. Specific variations of fatty acid composition of *Pseudomonas aeruginosa* ATCC 15442 induced by quaternary ammonium compounds and relation with resistance to bactericidal activity. *J Appl Microbiol* **87:**735–742.

126. **Guérin-Méchin L, Dubois-Brissonnet F, Heyd B, Leveau JY.** 2000. Quaternary ammonium compound stresses induce specific variations in fatty acid composition of *Pseudomonas aeruginosa*. *Int J Food Microbiol* **55:**157–159.

127. **Tkachenko O, Shepard J, Aris VM, Joy A, Bello A, Londono I, Marku J, Soteropoulos P, Peteroy-Kelly MA.** 2007. A triclosan-ciprofloxacin cross-resistant mutant strain of *Staphylococcus aureus* displays an alteration in the expression of several cell membrane structural and functional genes. *Res Microbiol* **158:** 651–658.

128. **Boeris PS, Domenech CE, Lucchesi GI.** 2007. Modification of phospholipid composition in *Pseudomonas putida* A ATCC 12633 induced by contact with tetradecyltrimethylammonium. *J Appl Microbiol* **103:**1048–1054.

129. **Bruinsma GM, Rustema-Abbing M, van der Mei HC, Lakkis C, Busscher HJ.** 2006. Resistance to a polyquaternium-1 lens care solution and isoelectric points of *Pseudomonas aeruginosa* strains. *J Antimicrob Chemother* **57:**764–766.

130. **Lyon BR, Skurray R.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* **51:**88–134.

131. **Tennent JM, Lyon BR, Midgley M, Jones IG, Purewal AS, Skurray RA.** 1989. Physical and biochemical characterization of the *qacA* gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *J Gen Microbiol* **135:**1–10.

132. **Littlejohn TG, Paulsen IT, Gillespie MT, Tennent JM, Midgley M, Jones IG, Purewal AS, Skurray RA.** 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. *FEMS Microbiol Lett* **74:**259–265.

133. **Leelaporn A, Paulsen IT, Tennent JM, Littlejohn TG, Skurray RA.** 1994. Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J Med Microbiol* **40:**214–220.

134. **Heir E, Sundheim G, Holck AL.** 1998. The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance. *FEMS Microbiol Lett* **163:**49–56.

135. **Heir E, Sundheim G, Holck AL.** 1999. The *qacG* gene on plasmid pST94 confers resistance to quaternary ammonium compounds in staphylococci isolated from the food industry. *J Appl Microbiol* **86:**378–388.

136. **Rouch DA, Cram DS, DiBerardino D, Littlejohn TG, Skurray RA.** 1990. Efflux-mediated antiseptic resistance gene *qacA* from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. *Mol Microbiol* **4:**2051–2062.

137. **Huet AA, Raygada JL, Mendiratta K, Seo SM, Kaatz GW.** 2008. Multidrug efflux pump overexpression in *Staphylococcus aureus* after single and multiple *in vitro* exposures to biocides and dyes. *Microbiology* **154:** 3144–3153.

138. **Schindler BD, Kaatz GW.** 2016. Multidrug efflux pumps of Gram-positive bacteria. *Drug Resist Updat* **27:**1–13.

139. **Santos Costa S, Viveiros M, Rosato AE, Melo-Cristino J, Couto I.** 2015. Impact of efflux in the development of multidrug resistance phenotypes in *Staphylococcus aureus*. *BMC Microbiol* **15:**232.

140. **Chuanchuen R, Beinlich K, Hoang TT, Becher A, Karkhoff-Schweizer RR, Schweizer HP.** 2001. Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects nfxB mutants overexpressing MexCD-OprJ. *Antimicrob Agents Chemother* **45:**428–432.

141. **Chuanchuen R, Narasaki CT, Schweizer HP.** 2002. The MexJK efflux pump of *Pseudomonas aeruginosa* requires OprM for antibiotic efflux but not for efflux of triclosan. *J Bacteriol* **184:**5036–5044.

142. **Mima T, Joshi S, Gomez-Escalada M, Schweizer HP.** 2007. Identification and characterization of TriABC-OpmH, a triclosan efflux pump of *Pseudomonas aeruginosa* requiring two membrane fusion proteins. *J Bacteriol* **189:**7600–7609.

143. **Schweizer HP.** 1998. Intrinsic resistance to inhibitors of fatty acid biosynthesis in *Pseudomonas aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. *Antimicrob Agents Chemother* **42:**394–398.

144. **Chuanchuen R, Karkhoff-Schweizer RR, Schweizer HP.** 2003. High-level triclosan resistance in *Pseudomonas aeruginosa* is solely a result of efflux. *Am J Infect Control* **31:**124–127.

145. **Morita Y, Murata T, Mima T, Shiota S, Kuroda T, Mizushima T, Gotoh N, Nishino T, Tsuchiya T.** 2003. Induction of mexCD-oprJ operon for a multidrug efflux pump by disinfectants in wild-type *Pseudomonas aeruginosa* PAO1. *J Antimicrob Chemother* **51:**991–994.

146. **Moken MC, McMurry LM, Levy SB.** 1997. Selection of multiple-antibiotic-resistant (mar) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the *mar* and *acrAB* loci. *Antimicrob Agents Chemother* **41:** 2770–2772.

147. **Nishino K, Yamaguchi A.** 2001. Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. *J Bacteriol* **183:**5803–5812.

148. **Lomovskaya O, Lewis K.** 1992. *Emr*, an *Escherichia coli* locus for multidrug resistance. *Proc Natl Acad Sci USA* **89:**8938–8942.

149. **Davin-Regli A, Bolla JM, James CE, Lavigne JP, Chevalier J, Garnotel E, Molitor A, Pagès JM.** 2008. Membrane permeability and regulation of drug "influx and efflux" in enterobacterial pathogens. *Curr Drug Targets* **9:**750–759.

150. **Randall LP, Cooles SW, Coldham NG, Penuela EG, Mott AC, Woodward MJ, Piddock LJ, Webber MA.** 2007. Commonly used farm disinfectants can select for mutant *Salmonella enterica* serovar Typhimurium with decreased susceptibility to biocides and antibiotics without compromising virulence. *J Antimicrob Chemother* **60:**1273–1280.

151. **Webber MA, Randall LP, Cooles S, Woodward MJ, Piddock LJ.** 2008. Triclosan resistance in *Salmonella enterica* serovar Typhimurium. *J Antimicrob Chemother* **62:**83–91.

152. **Rajamohan G, Srinivasan VB, Gebreyes WA.** 2010. Novel role of *Acinetobacter baumannii* RND efflux transporters in mediating decreased susceptibility to biocides. *J Antimicrob Chemother* **65:**228–232.

153. **Piddock LJ.** 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* **19:**382–402.

154. **Noguchi N, Suwa J, Narui K, Sasatsu M, Ito T, Hiramatsu K, Song JH.** 2005. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qacA/B* and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. *J Med Microbiol* **54:**557–565.

155. **Sánchez MB, Decorosi F, Viti C, Oggioni MR, Martínez JL, Hernández A.** 2015. Predictive studies suggest that the risk for the selection of antibiotic resistance by biocides is likely low in *Stenotrophomonas maltophilia*. *PLoS One* **10:**e0132816.

156. **Poole K.** 2007. Efflux pumps as antimicrobial resistance mechanisms. *Ann Med* **39:**162–176.

157. **Brown MH, Paulsen IT, Skurray RA.** 1999. The multidrug efflux protein NorM is a prototype of a new family of transporters. *Mol Microbiol* **31:**394–395.

158. **Borges-Walmsley MI, Walmsley AR.** 2001. The structure and function of drug pumps. *Trends Microbiol* **9:** 71–79.

159. **Poole K.** 2001. Multidrug resistance in Gram-negative bacteria. *Curr Opin Microbiol* **4:**500–508.

160. **Poole K.** 2002. Outer membranes and efflux: the path to multidrug resistance in Gram-negative bacteria. *Curr Pharm Biotechnol* **3:**77–98.

161. **Buffet-Bataillon S, Tattevin P, Maillard J-Y, Bonnaure-Mallet M, Jolivet-Gougeon A.** 2016. Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria. *Future Microbiol* **11:**81–92.

162. **Bailey AM, Constantinidou C, Ivens A, Garvey MI, Webber MA, Coldham N, Hobman JL, Wain J, Woodward MJ, Piddock LJ.** 2009. Exposure of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to triclosan induces a species-specific response, including drug detoxification. *J Antimicrob Chemother* **64:**973–985.

163. **Randall LP, Cooles SW, Coldham NG, Penuela EG, Mott AC, Woodward MJ, Piddock LJ, Webber MA.** 2007. Commonly used farm disinfectants can select for mutant *Salmonella enterica* serovar Typhimurium with decreased susceptibility to biocides and antibiotics without compromising virulence. *J Antimicrob Chemother* **60:**1273–1280.

164. **Webber MA, Randall LP, Cooles S, Woodward MJ, Piddock LJ.** 2008. Triclosan resistance in *Salmonella enterica* serovar Typhimurium. *J Antimicrob Chemother* **62:**83–91.

165. **Buckley AM, Webber MA, Cooles S, Randall LP, La Ragione RM, Woodward MJ, Piddock LJ.** 2006. The AcrAB-TolC efflux system of *Salmonella enterica* serovar Typhimurium plays a role in pathogenesis. *Cell Microbiol* **8:**847–856.

166. **Sánchez P, Moreno E, Martinez JL.** 2005. The biocide triclosan selects *Stenotrophomonas maltophilia* mutants that overproduce the SmeDEF multidrug efflux pump. *Antimicrob Agents Chemother* **49:**781–782.

167. **Pumbwe L, Randall LP, Woodward MJ, Piddock LJV.** 2004. Expression of the efflux pump genes *cmeB*, *cmeF* and the porin gene *porA* in multiple-antibiotic-resistant *Campylobacter jejuni*. *J Antimicrob Chemother* **54:**341–347.

168. **Demple B.** 1996. Redox signaling and gene control in the *Escherichia coli* soxRS oxidative stress regulon: a review. *Gene* **179:**53–57.

169. **Hutchinson J, Runge W, Mulvey M, Norris G, Yetman M, Valkova N, Villemur R, Lepine F.** 2004. *Burkholderia cepacia* infections associated with intrinsically contaminated ultrasound gel: the role of microbial degradation of parabens. *Infect Control Hosp Epidemiol* **25:**291–296.

170. **Valkova N, Lépine F, Valeanu L, Dupont M, Labrie L, Bisaillon JG, Beaudet R, Shareck F, Villemur R.** 2001. Hydrolysis of 4-hydroxybenzoic acid esters (parabens) and their aerobic transformation into phenol by the resistant *Enterobacter cloacae* strain EM. *Appl Environ Microbiol* **67:**2404–2409.

171. **Kümmerle N, Feucht HH, Kaulfers PM.** 1996. Plasmid-mediated formaldehyde resistance in *Escherichia coli*: characterization of resistance gene. *Antimicrob Agents Chemother* **40:**2276–2279.

172. **Gomez Escalada M, Russell AD, Maillard J-Y, Ochs D.** 2005. Triclosan- bacteria interactions: single or multiple target sites? *Lett Appl Microbiol* **41:**476–481.

173. **Wu VCH.** 2008. A review of microbial injury and recovery methods in food. *Food Microbiol* **25:**735–744.

174. **Lambert RJW, van der Ouderaa M-LH.** 1999. An investigation into the differences between the Bioscreen and the traditional plate count disinfectant test methods. *J Appl Microbiol* **86:**689–694.

175. **Brown MRW, Williams P.** 1985. Influence of substrate limitation and growth phase on sensitivity to antimicrobial agents. *J Antimicrob Chemother* **15**(Suppl A)**:**7–14.

176. **Wright NE, Gilbert P.** 1987. Influence of specific growth rate and nutrient limitation upon the sensitivity of *Escherichia coli* towards chlorhexidine diacetate. *J Appl Bacteriol* **62:**309–314.

177. **Gomez Escalada M, Harwood JL, Maillard J-Y, Ochs D.** 2005. Triclosan inhibition of fatty acid synthesis and its effect on growth of *E. coli* and *Ps. aeruginosa*. *J Antimicrob Chemother* **55:**879–882.

178. **McMurry LM, Oethinger M, Levy SB.** 1998. Triclosan targets lipid synthesis. *Nature* **394:**531–532.

179. **Levy CW, Roujeinikova A, Sedelnikova S, Baker PJ, Stuitje AR, Slabas AR, Rice DW, Rafferty JB.** 1999. Molecular basis of triclosan activity. *Nature* **398:**383–384.

180. **Webber MA, Coldham NG, Woodward MJ, Piddock LJV.** 2008. Proteomic analysis of triclosan resistance in *Salmonella enterica* serovar Typhimurium. *J Antimicrob Chemother* **62:**92–97.

181. **Curiao T, Marchi E, Grandgirard D, León-Sampedro R, Viti C, Leib SL, Baquero F, Oggioni MR, Martinez JL, Coque TM.** 2016. Multiple adaptive routes of *Salmonella enterica* Typhimurium to biocide and antibiotic exposure. *BMC Genomics* **17:**491.

182. **Seaman PF, Ochs D, Day MJ.** 2007. Small-colony variants: a novel mechanism for triclosan resistance in methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **59:**43–50.

183. **Abdel-Malek SM, Al-Adham IS, Winder CL, Buultjens TE, Gartland KM, Collier PJ.** 2002. Antimicrobial susceptibility changes and T-OMP shifts in pyrithione-passaged planktonic cultures of *Pseudomonas aeruginosa* PAO1. *J Appl Microbiol* **92:**729–736.

184. **Parikh SL, Xiao G, Tonge PJ.** 2000. Inhibition of InhA, the enoyl reductase from *Mycobacterium tuberculosis*, by triclosan and isoniazid. *Biochemistry* **39:** 7645–7650.

185. **Chen Y, Pi B, Zhou H, Yu Y, Li L.** 2009. Triclosan resistance in clinical isolates of *Acinetobacter baumannii*. *J Med Microbiol* **58:**1086–1091.

186. **Zhu L, Lin J, Ma J, Cronan JE, Wang H.** 2010. Triclosan resistance of Pseudomonas aeruginosa PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. *Antimicrob Agents Chemother* **54:** 689–698.

187. **Heath RJ, Li J, Roland GE, Rock CO.** 2000. Inhibition of the *Staphylococcus aureus* NADPH-dependent enoyl-acyl carrier protein reductase by triclosan and hexachlorophene. *J Biol Chem* **275:**4654–4659.

188. **Slater-Radosti C, Van Aller G, Greenwood R, Nicholas R, Keller PM, DeWolf WE Jr, Fan F, Payne DJ, Jaworski DD.** 2001. Biochemical and genetic characterization of the action of triclosan on *Staphylococcus aureus*. *J Antimicrob Chemother* **48:**1–6.

189. **Massengo-Tiassé RP, Cronan JE.** 2008. *Vibrio cholerae* FabV defines a new class of enoyl-acyl carrier protein reductase. *J Biol Chem* **283:**1308–1316.

190. **Webber MA, Whitehead RN, Mount M, Loman NJ, Pallen MJ, Piddock LJV.** 2015. Parallel evolutionary pathways to antibiotic resistance selected by biocide exposure. *J Antimicrob Chemother* **70:**2241–2248.

191. **Roujeinikova A, Levy CW, Rowsell S, Sedelnikova S, Baker PJ, Minshull CA, Mistry A, Colls JG, Camble R, Stuitje AR, Slabas AR, Rafferty JB, Pauptit RA, Viner R, Rice DW.** 1999. Crystallographic analysis of triclosan bound to enoyl reductase. *J Mol Biol* **294:**527–535.

192. **Stewart MJ, Parikh S, Xiao G, Tonge PJ, Kisker C.** 1999. Structural basis and mechanism of enoyl reductase inhibition by triclosan. *J Mol Biol* **290:**859–865.

193. **Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, Rock CO.** 1999. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *J Biol Chem* **274:**11110–11114.

194. **McCay PH, Ocampo-Sosa AA, Fleming GTA.** 2010. Effect of subinhibitory concentrations of benzalkonium chloride on the competitiveness of *Pseudomonas aeruginosa* grown in continuous culture. *Microbiology* **156:** 30–38.

195. **Casado Muñoz MC, Benomar N, Ennahar S, Horvatovich P, Lavilla Lerma L, Knapp CW, Gálvez A, Abriouel H.** 2016. Comparative proteomic analysis of a potentially probiotic *Lactobacillus pentosus* MP-10 for the identification of key proteins involved in antibiotic resistance and biocide tolerance. *Int J Food Microbiol* **222:**8–15.

196. **Casado Muñoz MC, Benomar N, Lavilla Lerma L, Knapp CW, Gálvez A, Abriouel H.** 2016. Biocide tolerance, phenotypic and molecular response of lactic acid bacteria isolated from naturally-fermented Aloreña table to different physico-chemical stresses. *Food Microbiol* **60:**1–12.

197. **Jang H-J, Chang MW, Toghrol F, Bentley WE.** 2008. Microarray analysis of toxicogenomic effects of triclosan on *Staphylococcus aureus*. *Appl Microbiol Biotechnol* **78:**695–707.

198. **Cerf O, Carpentier B, Sanders P.** 2010. Tests for determining in-use concentrations of antibiotics and disinfectants are based on entirely different concepts: "resistance" has different meanings. *Int J Food Microbiol* **136:**247–254.

199. **Russell AD, McDonnell G.** 2000. Concentration: a major factor in studying biocidal action. *J Hosp Infect* **44:**1–3.

200. **Koutsolioutsou A, Peña-Llopis S, Demple B.** 2005. Constitutive *soxR* mutations contribute to multiple-antibiotic resistance in clinical *Escherichia coli* isolates. *Antimicrob Agents Chemother* **49:**2746–2752.

201. **Mokgatla RM, Gouws PA, Brözel VS.** 2002. Mechanisms contributing to hypochlorous acid resistance of a *Salmonella* isolate from a poultry-processing plant. *J Appl Microbiol* **92:**566–573.

202. **Allen MJ, White GF, Morby AP.** 2006. The response of *Escherichia coli* to exposure to the biocide polyhexamethylene biguanide. *Microbiology* **152:**989–1000.

203. **Slade D, Radman M.** 2011. Oxidative stress resistance in *Deinococcus radiodurans*. *Microbiol Mol Biol Rev* **75:**133–191.

204. **Daniels C, Ramos JL.** 2009. Adaptive drug resistance mediated by root-nodulation-cell division efflux pumps. *Clin Microbiol Infect* **15**(Suppl 1)**:**32–36.

205. **Maseda H, Hashida Y, Konaka R, Shirai A, Kourai H.** 2009. Mutational upregulation of a resistance-nodulation-cell division-type multidrug efflux pump, SdeAB, upon exposure to a biocide, cetylpyridinium chloride, and antibiotic resistance in *Serratia marcescens*. *Antimicrob Agents Chemother* **53:**5230–5235.

206. **Walsh C, Fanning S.** 2008. Antimicrobial resistance in foodborne pathogens: a cause for concern? *Curr Drug Targets* **9:**808–815.

207. **Li XZ, Nikaido H.** 2009. Efflux-mediated drug resistance in bacteria: an update. *Drugs* **69:**1555–1623.

208. **Oethinger M, Kern WV, Goldman JD, Levy SB.** 1998. Association of organic solvent tolerance and fluoroquinolone resistance in clinical isolates of *Escherichia coli*. *J Antimicrob Chemother* **41:**111–114.

209. **Pomposiello PJ, Bennik MH, Demple B.** 2001. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *J Bacteriol* **183:**3890–3902.

210. **Fraise AP.** 2002. Biocide abuse and antimicrobial resistance: a cause for concern? *J Antimicrob Chemother* **49:** 11–12.

211. **Langsrud S, Sidhu MS, Heir E, Holck AL.** 2003. Bacterial disinfectant resistance: a challenge for the food industry. *Int Biodeter Biodegrad* **51:**283–290.

212. **Braoudaki M, Hilton AC.** 2004. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol* **42:**73–78.

213. **Braoudaki M, Hilton AC.** 2004. Low level of cross-resistance between triclosan and antibiotics in *Escherichia coli* K-12 and *E. coli* O55 compared to *E. coli* O157. *FEMS Microbiol Lett* **235:**305–309.

214. **Gilbert P, McBain AJ.** 2003. Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. *Clin Microbiol Rev* **16:**189–208.

215. **Russell AD.** 2004. Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon. *J Hosp Infect* **57:**97–104.

216. Alonso-Hernando A, Capita R, Prieto M, Alonso-Calleja C. 2009. Comparison of antibiotic resistance patterns in *Listeria monocytogenes* and *Salmonella enterica* strains pre-exposed and exposed to poultry decontaminants. *Food Control* **20:**1108–1111.

217. Weber DJ, Rutala WA. 2006. Use of germicides in the home and the healthcare setting: is there a relationship between germicide use and antibiotic resistance? *Infect Control Hosp Epidemiol* **27:**1107–1119.

218. Pumbwe L, Skilbeck CA, Wexler HM. 2007. Induction of multiple antibiotic resistance in *Bacteroides fragilis* by benzene and benzene-derived active compounds of commonly used analgesics, antiseptics and cleaning agents. *J Antimicrob Chemother* **60:**1288–1297.

219. Lara HH, Ayala-Nunez NV, Turrent LDCI, Padilla CR. 2010. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World J Microbiol Biotechnol* **26:**615–621.

220. Peyrat MB, Soumet C, Maris P, Sanders P. 2008. Phenotypes and genotypes of *Campylobacter* strains isolated after cleaning and disinfection in poultry slaughterhouses. *Vet Microbiol* **128:**313–326.

221. Gilbert P, McBain AJ, Bloomfield SF. 2002. Biocide abuse and antimicrobial resistance: being clear about the issues. *J Antimicrob Chemother* **50:**137–139, author reply 139–140.

222. Lear JC, Maillard J-Y, Dettmar PW, Goddard PA, Russell AD. 2006. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources: susceptibility to antibiotics and other biocides. *Int Biodeter Biodegrad* **57:** 51–56.

223. Knapp L, Amézquita A, McClure P, Stewart S, Maillard J-Y. 2015. Development of a protocol for predicting bacterial resistance to microbicides. *Appl Environ Microbiol* **81:**2652–2659.

224. Sundheim G, Langsrud S, Heir E, Holck AL. 1998. Bacterial resistance to disinfectants containing quaternary ammonium compounds. *Int Biodeter Biodegrad* **41:** 235–239.

225. International Organization for Standardization. 2006. ISO: 20776-1. Clinical laboratory testing and *in vitro* diagnostic test systems: susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices. Part 1. Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. British Standard Institute, London, United Kingdom.

226. European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2014. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0. 2014. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf. Accessed January 2017.

227. Andrews JM, BSAC Working Party on Susceptibility Testing. 2009. BSAC standardized disc susceptibility testing method (version 8). *J Antimicrob Chemother* **64:** 454–489.

228. Saleh S, Haddadin RNS, Baillie S, Collier PJ. 2011. Triclosan: an update. *Lett Appl Microbiol* **52:**87–95.

229. Gradel KO, Randall L, Sayers AR, Davies RH. 2005. Possible associations between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a putative role of *mar*. *Vet Microbiol* **107:** 127–138.

230. Chuanchuen R, Pathanasophon P, Khemtong S, Wannaprasat W, Padungtod P. 2008. Susceptibilities to antimicrobials and disinfectants in *Salmonella* isolates obtained from poultry and swine in Thailand. *J Vet Med Sci* **70:**595–601.

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Antimicrobial Resistance among Staphylococci of Animal Origin

7

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INTRODUCTION

During the past decades, several review articles and book chapters focused on the genetic basis of antimicrobial resistance in staphylococci. Some articles included staphylococci of human and animal origin (1–4), while others focused on either staphylococci of animal origin in general (5, 6) or on specific animal-associated staphylococci such as *Staphylococcus pseudintermedius* (7), bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus*, and canine *S. pseudintermedius* (8) or livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) (9). As inhabitants of the skin or the mucosal surfaces, staphylococci of animal origin do not live in genetic isolation but are in close contact with a wide variety of other bacteria in the same animal host. Under such conditions and given a high bacterial density in the different compartments, genetic material can be exchanged not only between staphylococci in intraspecies and interspecies exchange events, but also between staphylococci and other Gram-positive bacteria in so-called intergenus exchange processes. Previous studies have shown that staphylococci can act either as donors or as recipients of resistance genes in these events (9). In addition, staphylococci may also be transferred to other animals or humans either by direct contact or by contact with excretions of the animals, e.g., by sneezing, coughing, or licking. Moreover, indirect transmission, e.g., via dust, aerosols, or a contaminated environment, may also play a role in the dissemination of staphylococci. A comparison of the resistance genes detected in human staphylococci and in animal staphylococci (6) revealed that both groups of staphylococci share a large number of resistance genes, whereas only comparatively small numbers of resistance genes have been found exclusively in either human or animal staphylococci (Fig. 1).

This article summarizes the latest information on resistance genes and resistance-mediating mutations detected in staphylococci of animal origin. It represents a completely revised and updated version of a review that we published in 2013 (6). Since different detection methods may give different results, preference is given to those genes for which nucleotide sequence data have been deposited in databases, i.e., the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nuccore/) or the European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/) (6, 10, 11). The data presented in this article focus on resistance genes and resistance-mediating mutations detected in staphylococci from healthy and diseased animals, whereas those found in staphylococci—in particular, methicillin-resistant *S. aureus* (MRSA)—from food of animal origin have been excluded because this was the topic of a recent review (12).

RESISTANCE TO β-LACTAM ANTIBIOTICS

Two resistance mechanisms mainly account for β-lactam resistance in staphylococci: (i) enzymatic inactivation by the *blaZ*- or bla_{ARL}-encoded β-lactamases and (ii) target site replacement by the gene products of the *mecA*, *mecB* (formerly $mecA_m$), or *mecC* (formerly $mecA_{LGA251}$) genes (Table 1).

The *blaZ-blaI-blaR1* operon was identified on transposon Tn*552* (13), which has been detected on plas-

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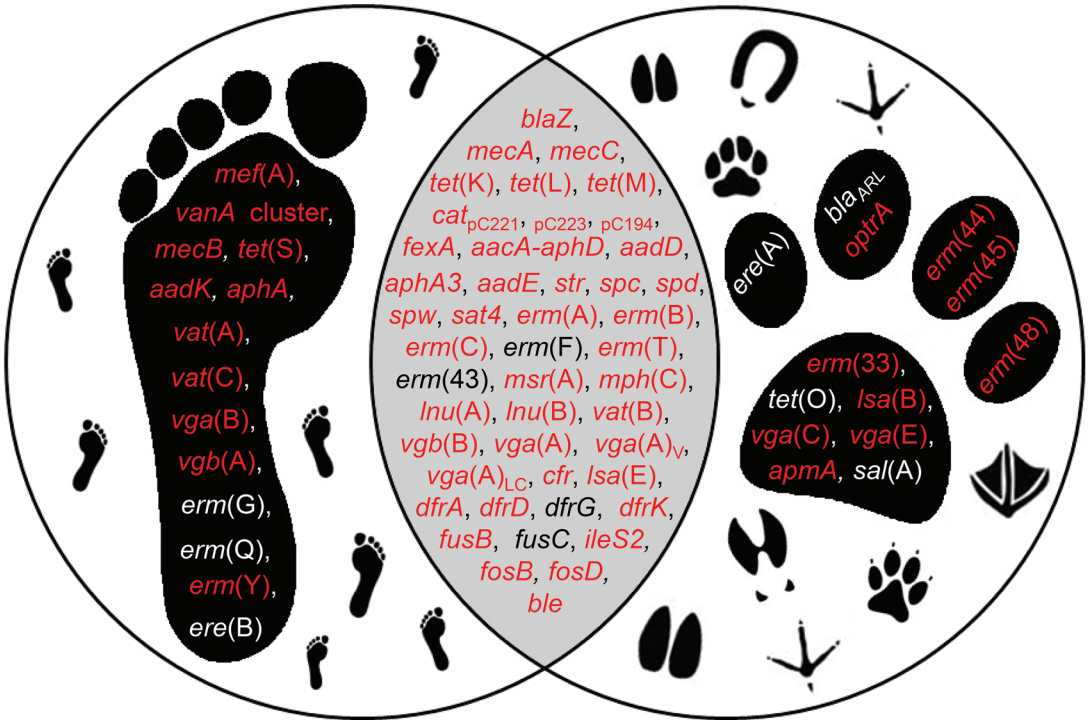


Figure 1 Antimicrobial resistance genes present in staphylococci of only human origin (left), only animal origin (right), and both origins (gray-shaded area in the middle). The genes depicted in red are associated with mobile genetic elements. Please see the text for the function of the different resistance genes. It should be noted that the tetracycline resistance genes *tet*(S) and *tet*(W), both coding for ribosome protective proteins, have recently been identified in staphylococci isolated from retail ground meat (284), and another *tet* gene, *tet*(38), coding for an efflux pump of the major facilitator superfamily, was identified in a *Staphylococcus* isolate of not further specified origin (285). For antimicrobial resistance genes present only in staphylococci of human origin, please see references 1–6.

mids as well as in the chromosomal DNA (1). The *blaZ*-encoded β-lactamase confers resistance to penicillins except isoxazolyl-penicillins. The *blaZ* gene has been detected in *S. aureus*, coagulase-negative staphylococci (CoNS), and *S. hyicus* from cases of bovine mastitis (14–18). The *blaZ* gene has also been described in *S. hyicus* isolates from pigs with exudative epidermitis (19). This gene was also present in *S. aureus* from zoo animals and wildlife (20). Moreover, the *blaZ* gene was also present in most MRSA and methicillin-susceptible (but penicillin/ampicillin-resistant) *S. aureus* from food-producing animals (21–30) and from various European wildlife species (31). Furthermore, the *blaZ* gene was present in staphylococci of canine and feline origin (32), including both methicillin-resistant and methicillin-susceptible *S. pseudintermedius* (33–36) (Table 2).

Whole-genome sequencing of the penicillin-resistant *Staphylococcus arlettae* strain SAN1670 from bovine mastitis milk led to the identification of a novel β-lactamase operon consisting of the β-lactamase-encoding gene bla_{ARL}, the antirepressor-encoding gene $blaR1_{ARL}$, and the repressor-encoding gene $blaI_{ARL}$ (37). The functionality of bla_{ARL} was demonstrated by gene expression in *S. aureus*. The bla_{ARL} operon was located in the chromosomal DNA. It was detected in 10 additional unrelated strains, suggesting that it may be responsible for intrinsic penicillin resistance in *S. arlettae*. A GenBank search revealed more unique potential β-lactamases in *Staphylococcus* species (37).

The *mec* genes code for alternative penicillin-binding proteins with a strongly reduced affinity to virtually all β-lactam antibiotics, except some anti-MRSA cephalosporins, such as ceftobiprole and ceftaroline, which are approved only for use in human medicine. Staphylococci of animal origin expressing the *mecA* gene should be considered resistant to all β-lactam antibiotics approved for veterinary applications. Whether the same is true for isolates carrying *mecC* needs to be confirmed (6). The *mecA* and *mecC* genes are part of a mobile genetic island called the "staphylococcal

cassette chromosome *mec*" (SCC*mec*). Currently, at least 13 major types of SCC*mec* elements plus various subtypes have been described in staphylococci (38–41).

Numerous studies have identified *mecA* genes as part of various SCC*mec* elements in staphylococci of animal origin, and several highly informative reviews have been published (42–50). Moreover, other studies focused on MRSA from pigs (21–23, 51–54), cattle (24, 55–62), sheep (27, 62), goats (62, 63), llamas and alpacas (62), poultry (28–30, 64, 65), horses (25, 66–68), and dogs and cats (69–73) but also from pet animals, including guinea pigs and rabbits and exotic animals such as turtles, parrots, and bats (70). Moreover, *mecA*-mediated methicillin resistance has become rather common in canine and feline *S. pseudintermedius* (34–36, 74–79). Hassler et al. (80) reported the presence of the *mecA* gene in an *S. hyicus* isolate. The *mecA* gene has also been detected in a number of methicillin-resistant CoNS from various animal species, e.g., cattle (81–83), pigs (81, 84, 85), chickens (81, 85, 86), turkeys (81), ducks (81, 85), a goose (81), horses (87–91), dogs and cats (89, 91, 92), as well as sheep and goats (81). In a convenience sample of *S. aureus* (n = 124) from European wildlife, six *mecA*-carrying isolates were recovered, one from a European brown hare and the remaining five from rooks (31) (Table 2). It should be noted that *Staphylococcus sciuri* and *Staphylococcus vitulinus* may carry *mecA* alleles that do not confer β-lactam resistance (93). Thus, the detection of *mecA* in isolates of these species should always be accompanied by phenotypic confirmation of oxacillin resistance.

The *mecC* gene was initially identified in *S. aureus* from humans and cattle (94, 95). Further screening for this gene identified it in *S. aureus* from cattle in France (96), goats in Austria (62), and a cat in Norway (97). A study from the United Kingdom identified it in *S. aureus* isolates from a dog, brown rats, a rabbit, a common seal, sheep, and a chaffinch (98, 99). In Germany, Walther et al. (100) identified it in *S. aureus* from cats, dogs, and a guinea pig. Screening of wildlife identified the *mecC* gene not only in MRSA from a European otter and a European brown hare, but also in a *Staphylococcus stepanovicii* from a Eurasian lynx (101). In another study of European wildlife (31), eight *mecC*-positive MRSA isolates were identified: two from European brown hares, four from hedgehogs, one from a red fox, and one from a fallow deer. In addition, *mecC*-carrying *S. aureus* isolates were also found in wood mice (102), red deer (103), white stork nestlings (104), and an Indian flying fox (20) (Table 2). Although identified in 2011, screening of strain collections revealed that the oldest *mecC*-carrying *S. aureus* isolate dates back to 1975 (105). In recent years, two new *mecC* allotypes have been identified: *mecC1* in *Staphylococcus xylosus* from bovine mastitis (106) and *mecC2* in *Staphylococcus saprophyticus* from a common shrew (107).

Most recently, the *mecB* gene, originally designated *mecA*$_m$, which is part of a methicillin resistance gene complex often associated with transposon Tn*6045* in *Macrococcus caseolyticus*, has been detected on a multiresistance plasmid in a human cefoxitin-resistant *S. aureus* isolate (108).

Moreover, reduced susceptibility to oxacillin was also observed in *mecA*- and *mecC*-negative *S. aureus* isolates from horses, but the resistance mechanism was not determined (68). Reasons for reduced susceptibility to oxacillin might be the overproduction of a β-lactamase, mutations in penicillin-binding proteins as described for *S. aureus* from human clinical cases (109), or decreased expression of *femA* and *femB* genes (110).

RESISTANCE TO TETRACYCLINES

In animal staphylococci, resistance to tetracyclines is often mediated by the genes *tet*(K) and *tet*(L), which code for membrane-associated efflux proteins of the major facilitator superfamily. In addition, the gene *tet*(M), which codes for a ribosome-protective protein, is also found frequently (Table 1) (111).

The Tet(K) and Tet(L) proteins exhibit 14 transmembrane domains and are composed of 459 and 458 amino acids (aa), respectively (111). The analysis of 228 tetracycline-resistant staphylococcal isolates from pigs, cattle, horses, dogs, cats, rabbits, guinea pigs, mink, turkeys, ducks, and pigeons identified the *tet*(K) gene in 110 isolates including 23 *S. hyicus*, 17 *S. xylosus*, 12 *Staphylococcus epidermidis*, 11 *Staphylococcus haemolyticus*, 11 *Staphylococcus lentus*, seven *Staphylococcus warneri*, six *S. sciuri*, six *S. aureus*, five *S. intermedius* group isolates, four *Staphylococcus simulans*, three *Staphylococcus hominis*, two *S. arlettae*, and single isolates of *Staphylococcus capitis*, *Staphylococcus chromogenes*, and *S. saprophyticus* (112). In addition, the *tet*(K) gene was found in combination with the gene *tet*(M) in eight *S. intermedius* group isolates and two *S. lentus* isolates. In contrast, the gene *tet*(L) was detected as the only *tet* gene in 14 isolates which comprised eight *S. sciuri*, two *S. aureus*, two *S. xylosus*, and single *S. lentus* and *S. hyicus* isolates. Furthermore, *tet*(L) was identified together with *tet*(M) in a single *S. aureus* and two *S. intermedius* group isolates but also together with *tet*(O) in a single *S. xylosus* isolate

Table 1 Resistance genes detected in staphylococci of animal origin[a]

Resistance to …	Mechanism	Gene	Localization[b]	Mobile genetic element	Accession no. (or reference if there is no accession no.)
β-Lactams	Enzymatic inactivation (β-lactamase)	*blaZ*	Tn, P, C	Tn*552*	X52734
		bla_{ARL}	C	Unknown	KY363215
	Target site replacement (alternative penicillin binding proteins)	*mecA*	C	SCC*mec*	AB033763
		mecC ($mecA_{LGA251}$)	C	SCC*mec*	FR823292 FR821779
Tetracyclines (except minocycline and glycylcyclines)	Active efflux (major facilitator superfamily)	*tet*(K)	P	pT181	J01764.1 S67449
		tet(L)	P	pKKS25	FN390947
Tetracyclines (including minocycline but excluding glycylcyclines)	Target site protection (ribosome protective protein)	*tet*(M)	Tn, C	Tn*916*	M21136.1
		tet(O)	Unknown	Unknown	112
Nonfluorinated phenicols	Enzymatic inactivation (acetyltransferase)	cat_{pC221}	P	pC221	NC_006977
		cat_{pC223}	P	pC223	NC_005243
		cat_{pC194}	P	pC194	NC_002013
	Active efflux (major facilitator superfamily)	$fexA_V$	Tn, C	Tn*558*	HF679552
All phenicols	Active efflux (major facilitator superfamily)	*fexA*	Tn, P, C	Tn*558*	AM086211.1
Aminoglycosides (gentamicin, kanamycin, tobramycin, amikacin)	Enzymatic inactivation (acetyltransferase and phospotransferase)	*aacA-aphD*	Tn, P, C	Tn*4001*	GU565967
Aminoglycosides (kanamycin, neomycin, tobramycin)	Enzymatic inactivation (adenyltransferase)	*aadD*	P, C	pUB110	M37273.1
				pKKS825	NC_013034.2
Aminoglycosides (kanamycin, neomycin, amikacin)	Enzymatic inactivation (phospotransferase)	*aphA3*	Tn, P, C	Tn*5405*	AB699882.1
Aminoglycosides (streptomycin)	Enzymatic inactivation (adenyltransferase)	*aadE*	Tn, P, C	Tn*5405*	AB699882.1
		str	P	pS194	NC_005564
Aminocyclitols (spectinomycin)	Enzymatic inactivation (adenyltransferase)	*spc*	Tn, P, C	Tn*554*	X03216.1
		spw	P, C	pV7037	JX560992.1
		spd	P	pDJ91S	KC895984
Aminocyclitols/aminoglycosides (apramycin, decreased susceptibility to gentamicin)	Enzymatic inactivation (acetyltransferase)	*apmA*	P	pAFS11	FN806789.3
				pKKS49	HE611647.1
Streptothricins	Enzymatic inactivation (acetyltransferase)	*sat4*	Tn, P	Tn*5405*	AB699882.1
Macrolides. lincosamides, streptogramin B	Target site modification (rRNA methylase)	*erm*(A)	Tn, P, C	Tn*554*	X03216.1
		erm(B)	Tn, P	Tn*917*	FN806789.3
		erm(C)	P	pE194	V01278.1
				pNE131	M12730.1
		erm(F)	Unknown	Unknown	164, 205
		erm(T)	P	pKKS25	FN390947.1
		erm(33)	P	pSCFS1	AJ313523
		erm(43)	C	Unknown	HE650138
		erm(44)	C	Prophage ΦJW4341	HG796218.2
		$erm(44)_v$	C	Unknown	KJ728533.1
		erm(45)	GI	Genomic island	LN680996.1
		erm(48)	P	pJW2311	LT223129

(Continued)

Table 1 *(Continued)*

Resistance to …	Mechanism	Gene	Localization[b]	Mobile genetic element	Accession no. (or reference if there is no accession no.)
Macrolides, streptogramin B	Target site protection (ribosome protective ABC-F protein)	*msr*(A)	C, P	pMS97	NG_048001.1 AB013298.1
Macrolides	Enzymatic inactivation (phospotransferase)	*mph*(C)	C, P	pMS97	AB013298.1
	Enzymatic inactivation (esterase)	*ere*(A)	Unknown	Unknown	194
Lincosamides	Enzymatic inactivation (nucleotidyltransferase)	*lnu*(A)	P	pBMSa1	AY541446 JQ861958.1
		lnu(B)	P, C	pV7037	JX560992.1 JQ861959.1
	Target site protection (ribosome protective ABC-F protein)	*lsa*(B)	P	pSCFS1	KU510528.1
Streptogramin A	Enzymatic inactivation (acetyltransferase)	*vat*(B)	P	pIP1156	U19459.1
Streptogramin B	Enzymatic inactivation (hydrolase)	*vgb*(B)	P	pIP1714	AF015628.1
Lincosamides, pleuromutilines, streptogramin A	Target site protection (ribosome protective ABC-F protein)	*vga*(A)	Tn, P	Tn*5406*	M90056.1 FN806791.1
		$vga(A)_{LC}$	P	p131A	KX712120
		$vga(A)_{V}$	C	Unknown	AF186237
		vga(C)	P	pKKS825	NC_013034.2
		vga(E)	Tn	Tn*6133*	FR772051.1
		$vga(E)_{v}$	P	pSA-7	KF540226.1
		lsa(E)	P, C	pV7037	JX560992.1
		sal(A)	C	Unknown	KC693025.1
All phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A	Target site modification (rRNA methylase)	*cfr*	P, C	pSCFS1	AJ879565.1
Oxazolidinones, all phenicols	Target site protection (ribosome protective ABC-F protein)	*optrA*	P, C	pWo28-3	KT601170.1 KX447572.1
Trimethoprim	Target replacement (trimethoprim-resistant dihydrofolate reductase)	*dfrA* (*dfrS1*)	Tn, P, C	Tn*4003*	GU565967.1
		dfrD	C	Unknown	KX149097.1
		dfrG	C	Unknown	NG_047756.1
		dfrK	Tn, P, C	Tn*559*, pKKS2187	FN390947
Fusidic acid	Target site protection (ribosome protective protein)	*fusB*	P	p11819-97 pUB101	NC_017350.1 NC_005127.1
		fusC	P, C	SCC*mec*	HE980450.1
Mupirocin	Target replacement (mupirocin-insensitive isoleucyl-tRNA synthase)	*ileS2* (*mupA, ileS*)	P	pPR5	HQ625438.1
Bleomycin	Bleomycin binding protein	*ble*	P, C	pUB110	NC_001384.1
Fosfomycin	Fosfomycin thiol transferase	*fosB*	Unknown	Unknown	164
		fosD	P	pJP2	KC989517

[a]Modified from references 6 and 114.
[b]Tn (transposon), P (plasmid), C (chromosome).

Table 2 Examples of the distribution of resistance genes in staphylococci from different animals

Resistance genes	Bacterial species[a]	Animal host species (reference)
blaZ	*S. aureus*	Pig (21–23, 158, 177), cattle (14–17, 24, 55), sheep (27), horse (25, 68, 88), donkey (26), dog (32, 73), cat (32, 73), chicken (28–30), turkey (28, 226), European wildlife species (rook, red fox, marmot, mouflon, harbor porpoise, moose, badger, red deer, roe deer, wild boar, brown hare, vole) (31), naked mole rat (20), red squirrel (20), Eurasian lynx (20)
	SIG	Cat (32, 35, 79), dog (32–34, 36, 38, 79, 160)
	CoNS	Pig (147, 177), cattle (15, 17, 18), horse (88), dog (32), cat (147)
	S. hyicus	Pig (19, 147), cattle (18)
bla_{ARL}	CoNS	Cattle (37)
mecA	*S. aureus*	Pig (21–23, 51–54, 150, 207, 218), cattle (24, 55–62, 163), sheep (27, 62), goat (62, 63), horse (25, 66–68, 74, 90), llama (62), alpaca (62), dog (70–74, 91, 196), cat (69, 70, 73, 74, 196), chicken (28–30, 64, 65), turkey (28, 227), guinea pig (70), rabbit (70), turtle (70), parrot (70), bat (70), European brown hare (31), rook (31), Eurasian lynx (20)
	SIG	Horse (74, 75), donkey (75), dog (34, 36, 74, 75, 78, 79, 92), cat (35, 38, 74–77, 79), parrot (75)
	CoNS	Pig (81, 84, 85, 218), cattle (81–83, 91, 211), sheep (81), goat (81), horse (87–91), dog (89, 91, 92), cat (91), chicken (81, 85, 86, 207), turkey (81), duck (81, 85), goose (81)
	S. hyicus	Pig (80)
mecC ($mecA_{LGA251}$)	*S. aureus*	Cattle (94, 96), sheep (98), cat (97, 100), goat (62), dog (98, 100), brown rat (98), rabbit (98), common seal (98), chaffinch (98, 99), guinea pig (100), European otter (101), European brown hare (31, 101), hedgehog (31), red fox (31), fallow deer (31), wood mouse (102), red deer (103), white stork nestling (104), Indian flying fox (20)
	CoNS	Cattle (106), Eurasian lynx (101), common shrew (107)
tet(K)	*S. aureus*	Pig (21, 23, 160, 177), cattle (24, 112), sheep (27), horse (112), dog (73), cat (73, 112), chicken (28, 29), poultry (186), rabbit (112, 198), rook (31), harbor porpoise (31), wild boar (31), brown hare (31), naked mole rat (20)
	SIG	Dog (34, 36, 38, 78, 79, 117, 160), cat (35, 79)
	CoNS	Pig (118, 120, 147, 177), cattle (164, 211), horse (88, 112), dog (120), cat (112), turkey (112), poultry (186), duck (112), pigeon (112), bank vole (119), common shrew (119, 122), rabbit (112), guinea pig (112)
	S. hyicus	Pig (19, 112, 118, 147), poultry (186)
tet(L)	*S. aureus*	Pig (21, 123–126, 160, 161, 177, 207, 218), cattle (24), chicken (28, 29), turkey (28), white-face whistling duck (20)
	CoNS	Pig (118, 120, 128, 177, 218), cattle (164), dog (120), turkey (112), duck (112), guinea pig (112), rabbit (112), pigeon (112)
	S. hyicus	Pig (19, 23, 118, 147)
tet(M)	*S. aureus*	Pig (21, 22, 160, 177), cattle (24, 55), horse (25), donkey (26), dog (72, 73) chicken (28–30), turkey (28, 227), poultry (186)
	SIG	Dog (33, 34, 36, 38, 78, 79, 112), cat (35, 79, 112, 129), pigeon (112), mink (112)
	CoNS	Pig (118, 120, 177), cattle (164), horse (88), dog (120), cat (130), pigeon (112)
	S. hyicus	Pig (147)
tet(O)	*SIG*	Dog (112)
	CoNS	Mink (112)
cat_{pC221}	*S. aureus*	Cattle (135, 136), rook (31), red squirrel (20)
	SIG	dog (34, 36, 38, 79, 117, 138, 139), cat (35, 79)
	CoNS	cattle (144), horse (88, 142), dog (116, 143), mink (145)
	S. hyicus	Pig (140, 141)
cat_{pC223}	*S. aureus*	Cattle (136)
	CoNS	Horse (88), dog (146), mink (145)
cat_{pC194}	CoNS	Pig (147), dog (143)
$fexA_V$	SIG	Dog (154)
fexA	*S. aureus*	Pig (21, 22, 150, 152, 207), cattle (24, 60), horse (152), marmot (31)
	SIG	Pig (151)

(Continued)

Table 2 *(Continued)*

Resistance genes	Bacterial species[a]	Animal host species (reference)
	CoNS	Pig (151, 153, 167), cattle (148, 152, 164); duck (167), chicken (167)
	S. hyicus	Pig (153)
aacA-aphD	*S. aureus*	Pig (21, 22, 160–162), cattle (24, 163), goat (62), alpaca (62), horse (25, 68, 88, 165), chicken (28, 29, 30), turkey (28), rook (31)
	SIG	Dog (34, 36, 78, 79, 160), cat (35, 79, 160)
	CoNS	Pig (86, 151, 167), cattle (164), horse (88), cat (166), chicken (85, 159, 167), duck (85, 167)
aadD	*S. aureus*	Pig (21, 22, 124), cattle (24, 55, 126), horse (25), dog (73), cat (73), turkey (28), chicken (29, 30) rook (31)
	CoNS	Pig (128), cattle (164), horse (88), duck (167) chicken (167), bird (169)
aphA3	*S. aureus*	Pig (22) cattle (24, 163), horse (25, 68), sheep (27), rook (31)
	SIG	Dog (34, 36, 38, 171), cat (35)
	CoNS	Cattle (164), horse (88)
aadE	*S. aureus*	Pig (161, 162, 207), sheep (27)
	SIG	Dog (34, 36, 38, 171), cat (35, 171)
	CoNS	Pig (169), cattle (164), horse (88)
	S. hyicus	Pig (19, 147)
str	*S. aureus*	Pig (23, 177)
	CoNS	Pig (169, 177), cattle (144), horse (88), dog (169, 208)
	S. hyicus	Pig (141, 176)
spc	*S. aureus*	Pig (21, 23, 207), cattle (24), chicken (28), turkey (28, 227)
	CoNS	Pig (147), cattle (164, 180), cat (147)
	S. hyicus	Pig (147)
spw	*S. aureus*	Pig (161, 181)
	S. hyicus	Pig (147)
spd	*S. aureus*	Pig (182, 183), cattle (182), horse (182), chicken (182, 183), rat (182)
	CoNS	Pig (147)
	S. hyicus	Pig (147)
apmA	*S. aureus*	Pig (184, 185), cattle (126, 164, 184), chicken (30)
	CoNS	Cattle (164)
sat4	*S. aureus*	Horse (25), rook (31)
	CoNS	Cattle (164), horse (88)
	SIG	Dog (34, 36, 38, 171), cat (171)
erm(A)	*S. aureus*	Pig (21–23, 160, 187), cattle (24, 55, 60), horse (25), donkey (26), dog (73), cat (73), dog/cat (187), chicken (28), turkey (28, 29, 227), poultry (186, 190), red fox (31)
	SIG	Dog (187, 188), cat (187), pigeon (189)
	CoNS	Cattle (164, 191), cat (147), poultry (186, 190)
	S. hyicus	Pig (19, 147, 187)
erm(B)	*S. aureus*	Pig (21, 161, 162, 187), cattle (24, 126, 194), dog (187, 188), cat (187), chicken (29), turkey (28, 227), poultry (190), brown hare (31)
	SIG	Dog (33, 34, 36, 38, 74, 79, 171, 172, 188), cat (35, 80, 171), dog/cat (187)
	CoNS	Pig (85, 167), cattle (164, 193, 194), horses (88), chicken (85, 167), poultry (190), turkey (191), duck (85, 167), poultry (190), mink (192)
	S. hyicus	Pig (19, 147, 187, 188)
erm(C)	*S. aureus*	Pig (21–23, 89, 187), cattle (24, 55, 194, 195) horse (25, 68), donkey (26) dog (72, 196), cat (196), dog/cat (187), sheep (27), goat (62), chicken (28–30, 197), turkey (28), poultry (186, 190), rabbits (195, 198), wild boar (31)
	SIG	Dog (78, 117, 188), carrier pigeon (195)
	CoNS	Pig (147, 191, 195), cattle (164, 193, 194), sheep (195), horse (88, 195), dogs (195, 203), cat (195), chicken (85, 167, 195), turkey (191), poultry (190), carrier pigeon (195, 204), bank vole (201), rodents/insectivores (202), parrot (195), guinea pig (195)
	S. hyicus	Pig (19, 147, 187, 195, 199, 200)

(Continued)

Table 2 *(Continued)*

Resistance genes	Bacterial species[a]	Animal host species (reference)
erm(F)	SIG	Dog (205)
	CoNS	Cattle (164), not further specified animal (205)
erm(T)	*S. aureus*	Pig (126), cattle (24), chicken (28–30), turkey (28)
	CoNS	Cattle (164)
erm(33)	*S. aureus*	Pig (207)
	CoNS	Cattle (180, 206)
erm(43)	CoNS	Dog (208), chicken (208)
erm(44)	CoNS	Cattle (209)
erm(45)	CoNS	Cattle (211)
erm(48)	CoNS	Cattle (212)
msr(A)	*S. aureus*	Cattle (194), horse (68), dog/cat (187), poultry (190)
	SIG	Dog/cat (187)
	CoNS	Pig (191), cattle (164, 191, 193, 194), turkey (191), rodents/insectivores (202)
	S. hyicus	Cattle (194)
mph(C)	*S. aureus*	Cattle (194), horse (68), dog/cat (187)
	CoNS	Pig (191), cattle (164, 191, 193, 194, 209), sheep (208), horse (88), turkey (191), chicken (208), rodents/insectivores (202)
ere(A)	*S. aureus*	Cattle (194)
	CoNS	Cattle (194)
lnu(A)	*S. aureus*	Pig (22, 218, 219), cattle (55, 193, 195, 217), turkey (28)
	SIG	Dog (34)
	CoNS	Pig (218), cattle (164, 194, 220)
	S. hyicus	Pig (187), cattle (194)
lnu(B)	*S. aureus*	Pig (161, 162, 207)
	S. hyicus	Pig (147)
lsa(B)	CoNS	Pig (153), cattle (180)
vat(B)	CoNS	Poultry (186)
vgb(B)	CoNS	Poultry (186)
vga(A) + variants	*S. aureus*	Pig (21, 23, 207, 219, 228), cattle (24, 194), dog (73), cat (219, 232), chicken (29), turkey (28)
[*vga*(A)$_V$, *vga*	CoNS	Cattle (164, 194, 230)
(A)$_{LC}$]	*S. hyicus*	Pig (147)
vga(C)	*S. aureus*	Pig (124, 228), cattle (24)
	S. hyicus	Pig (147)
vga(E)	*S. aureus*	Pig (226), cattle (227), turkey (28, 227)
vga(E)$_v$	CoNS	Pig (231)
lsa(E)	*S. aureus*	Pig (161, 162, 207), cattle (230),
	CoNS	cattle (230), dog (232)
	S. hyicus	Pig (147)
sal(A)	CoNS	Cattle (230), cat (232), dog (232)
cfr	*S. aureus*	Pig (22, 150–152, 207, 241), cattle (60), horse (152), marmot (31)
	CoNS	Pig (85, 151, 153, 167), cattle (83, 152, 164, 180, 234), chicken (85, 167), duck (85, 167)
	S. hyicus	Pig (153)
optrA	CoNS	Pig (241–244)
dfrA (*dfrS1*)	*S. aureus*	Pig (22) horse (25, 68, 88), dog (73), rook (31)
	CoNS	Cattle (163), horse (88)
dfrD	*S. aureus*	Pig (22)
	CoNS	Cattle (163), horse (88)
dfrG	*S. aureus*	Pig (21–23, 177), horse (68)
	SIG	Dog (34, 36, 38, 79), cat (35, 79)
	CoNS	Pig (177), cattle (164)

(Continued)

Table 2 *(Continued)*

Resistance genes	Bacterial species[a]	Animal host species (reference)
dfrK	*S. aureus*	Pig (21, 22, 123–126, 218, 245), cattle (24), horse (68), chicken (28–30), turkey (28)
	CoNS	Cattle (164)
	S. hyicus	Pig (185)
fusB	*S. aureus*	Sheep (27)
	CoNS	Cattle (164, 248)
fusC	*S. aureus*	Donkey (26)
	SIG	Dog (33)
ileS2 (*mupA, ileS*)	SIG	Dog (254, 255)
fosB	*S. aureus*	Horse (25), dog (73), cat (73), European wildlife species (brown hare, gray partridge, red fox, marmot, mouflon, wild boar, harbor porpoise, rook, moose, badger, red deer, roe deer, chamois, mute swan, red kite, green woodpecker, common magpie, great tit, whitetailed eagle, tawny owl, golden eagle, bank vole, wild cat) (31)
	CoNS	Cattle (164)
fosD	CoNS	Duck (167)

[a]SIG, *Staphylococcus intermedius* group; CoNS, coagulase-negative staphylococci.

(112). In addition, the *tet*(K) gene has also been identified in methicillin-susceptible *S. aureus* (MSSA) from chickens (28), turkeys (28), and a naked mole rat (20) (Table 2).

The genes *tet*(K) and *tet*(L) are often located on plasmids. The *tet*(K)-carrying plasmids usually display a structure similar to the 4.4-kb plasmid pT181 (113, 114) and rarely carry additional resistance genes. Such plasmids have been identified in staphylococci of animal origin including coagulase-positive (*S. aureus*, *S. intermedius/pseudintermedius*), coagulase-variable (*S. hyicus*), and various CoNS (e.g., *Staphylococcus cohnii*, *S. epidermidis*, *Staphylococcus equorum*, *S. haemolyticus*, *S. saprophyticus*, *S. sciuri*, *S. warneri*, and *S. xylosus*) (112, 115–120). The small *tet*(K)-carrying plasmid pT181 can be integrated via IS*431* into the J1 region of SCC*mec* elements commonly found in LA-MRSA (40, 121) or via IS*257* into larger plasmids (114). The 6,913-bp plasmid pSTE2 from *S. lentus* obtained from a common shrew harbors *tet*(K) and the macrolide-lincosamide-streptogramin B (MLS_B) resistance gene *erm*(C) (122). Detailed structural analysis suggested that plasmid pSTE2 developed from pT181- and pPV141-like ancestor plasmids by cointegrate formation at the staphylococcal recombination site RS_A (122). Plasmids that carry the *tet*(L) gene have been found among *S. aureus* (in particular, among LA-MRSA ST398) (21, 24, 123–126), *S. hyicus*, and CoNS, such as *S. epidermidis*, *S. lentus*, *S. sciuri*, and *S. xylosus*, of animal origin (112, 118) (Table 3). In contrast to *tet*(K)-carrying plasmids, those carrying *tet*(L) range in size between 5.5 and >40 kb and often harbor one or more additional resistance genes (114, 118, 124–128).

In addition, tetracycline resistance via *tet*(M) is also commonly observed in staphylococci of animal origin, whereas *tet*(O) genes have been detected very rarely in staphylococci (112). The *tet*(M) genes are commonly located on conjugative transposons of enterococcal origin, such as Tn*916* or Tn*1545* (111). The *tet*(M) and *tet*(O) gene products represent ribosome protective proteins which mediate resistance to all tetracyclines, including minocycline (Table 1). The analysis of the aforementioned 228 tetracycline-resistant staphylococci obtained from diverse animal sources identified *tet*(M) genes as the sole *tet* genes in 89 *S. intermedius* group isolates (112). Further studies confirmed that tetracycline resistance in *S. intermedius/S. pseudintermedius* was almost exclusively based on the presence of a *tet*(M) gene (7, 34, 35, 79, 112, 129). The *tet*(M) gene was also present in MSSA from chickens and turkeys (28–30). The *tet*(O) gene has so far only been detected in a single *S. pseudintermedius* isolate and in combination with a *tet*(L) gene in a single *S. xylosus* isolate (112). Studies of LA-MRSA from pigs, cattle, and chickens and ducks showed that such strains—regardless of their origin—often carried the genes *tet*(M), *tet*(K), and *tet*(L) in various combinations with two or all three *tet* genes being present in the same isolate (21, 22, 24, 28–30) (Table 2). While *tet*(M) genes are commonly found in the chromosomal DNA of staphylococci, a plasmid-borne *tet*(M) gene as part of a largely truncated Tn*916* was identified on the 28,743-bp multiresistance plasmid pSWS47 from a feline *S. epidermidis* ST5 isolate (130) (Table 3)

In a recent study (108), a *tet*(S) gene, also coding for a ribosome-protective protein, was identified on a

multiresistance plasmid in a human clinical *mecB*-carrying *S. aureus* isolate.

RESISTANCE TO PHENICOLS

Resistance to nonfluorinated (e.g., chloramphenicol) or fluorinated (e.g., florfenicol) phenicols among animal staphylococci is mediated by (i) enzymatic inactivation via chloramphenicol acetyltransferases, (ii) active efflux via exporters of the major facilitator superfamily, (iii) target site modification via the rRNA methylase Cfr (131), or (iv) ribosome protection by the ABC-F protein OptrA.

So far, three types of chloramphenicol acetyltransferases have been identified among staphylococci (Table 1) (3, 114, 131). The corresponding *cat* genes have been named according to the plasmids on which they were first identified: cat_{pC221}, cat_{pC223}, and cat_{pC194} (132–135). These *cat* genes code for Cat monomers of 215, 215, and 216 aa, respectively, which form the trimeric mature Cat enzyme (131). In staphylococci of animal origin, the cat_{pC221} gene has been detected on a wide variety of plasmids in bovine *S. aureus* (136, 137), canine *S. pseudintermedius* (34, 36, 38, 79, 117, 138, 139), porcine *S. hyicus* (140, 141), canine *S. epidermidis* (116), equine (88, 142), canine (143), and bovine *S. sciuri* (144), and mink *S. lentus* (145). Plasmids that carry a cat_{pC223} gene have been detected only in bovine *S. aureus* (136), canine *S. haemolyticus* (146), equine *S. xylosus* (88), and mink *S. lentus* (145). In staphylococci from animals, a cat_{pC194}-like gene has so far been identified on plasmid pSCS34 from a canine *S. sciuri* isolate (143) and in a porcine *S. equorum* isolate (147) (Table 2). It should be noted that all three chloramphenicol acetyltransferases confer resistance to only nonfluorinated phenicols.

In contrast, the 475-aa FexA protein of the major facilitator superfamily, which consists of 14 transmembrane domains, exports not only nonfluorinated phenicols, but also fluorinated phenicols such as florfenicol (Table 1) (148). The *fexA* gene is located on transposon Tn*558* and was first identified in a bovine *S. lentus* isolate (149). This transposon can be located on plasmids (including multiresistance plasmids) (Table 3) and in the chromosomal DNA. The f*exA* gene has also been detected in MRSA from pigs (21, 22, 150–152), cattle (24, 60), and a horse (152) and in *S. aureus* from a marmot (31). This gene has also been identified in various CoNS species including bovine *S. chromogenes*, *S. simulans*, and *S. sciuri* (152) and porcine *S. arlettae*, *S. cohnii*, *S. epidermidis*, *S. equorum*, *S. haemolyticus*, *S. hominis*, *S. hyicus S. lentus*, *S. pseudintermedius*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, *S. warneri*, and *S. xylosus* (151–153) (Table 2). A *fexA* variant, $fexA_V$, which conferred only chloramphenicol, but not florfenicol, resistance was detected in a canine *S. pseudintermedius* isolate from Spain. The $FexA_V$ protein exhibited two amino acid substitutions, Gly33Ala and Ala37Val, both of which seem to be important for substrate recognition. Site-directed mutagenesis that

Table 3 Examples for multiresistance plasmids among staphylococci from animals

Plasmid	Bacterial species	Host species	Size (kb)	Antimicrobial resistance genes	Other resistance genes	Accession no	Reference
pWo28-3	*S. sciuri*	Pig	60.563	*optrA*, *cfr*, *fexA*, *ble*, *aacA-aphD*, *aadD*		KT601170.1	241
pJP2	S. rostri	Duck	~50	*erm*(B), *aacA-aphD*, *fosD*, *ble*, *aadD*, *fexA*, *cfr*		KC989517.1	167
pAFS11	*S. aureus*	Cattle	49.192	*aadD*, *erm*(B), *tet*(L), *dfrK*, *apmA*	*cadD, cadX*, *mco*, *copA*	FN806789.3	126
pSCFS6	*S. warneri*	Pig	~43	*lsa*(B), *cfr*, *fexA*		AM408573.1	153
pV7037	*S. aureus*	Pig	40.971	*aacA-aphD*, *erm*(B), *tet*(L), *aadE*, *spw*, *lsa*(E), *lnu*(B), Δ*blaZ*	*cadD, cadX*,	JX560992.1, HF586889.1	161, 162
pSS-01	*S. cohnii*	Pig	~40	*aacA-aphD*, *fexA*, *cfr*		JF834909.1	151
pJP1-like	*S. lentus*	Chicken	~40	*aacA-aphD*, *ble*, *aadD*, *fexA*, *cfr*		KF129408	167
pSWS47	*S. epidermidis*	Cat	28.743	*vga*(A), *aadD*, *tet*(L), *dfrK*, *tet*(M), *blaZ*		HG380319.1	130
pUR1902	*S. aureus*	Pig	~22	*erm*(T), *tet*(L), *aadD*	*cadD*, *cadX*, *mco*, *copA*	HF583291.1	271
pSCFS1	*S. sciuri*	Cattle	17.108	*cfr*, *lsa*(B), *erm*(33), *spc*		NC_005076.1	180
pKKS825	*S. aureus*	Pig	14.365	*aadD*, *tet*(L), *dfrK*, *vga*(C)		FN377602.2	124
pMSA16	*S. aureus*	Cattle	7.054	*cfr*, *erm*(A)		JQ246438.1	60
pSTS7	*S. epidermidis*	Pig	~5.5	*tet*(L), *aadD*, *ble*		U35229.1	128

reverted the mutated base pairs to those present in the original *fexA* gene restored the chloramphenicol/florfenicol resistance phenotype (154).

Phenicol resistance may also be based on target site modification via the methylase Cfr or on ribosomal protection via the ABC-F protein OptrA. For more on this, please see the section on resistance to oxazolidinones.

RESISTANCE TO AMINOGLYCOSIDES

Resistance to aminoglycosides is based on a number of inactivating enzymes which differ in their specific substrate spectra (Table 1) (155). In staphylococci of animal origin, the following genes for aminoglycoside-modifying enzymes have been detected so far.

The gene *aacA-aphD* [also known as *aac(6´)-Ie–aph(2´´)-Ia*] codes for a bifunctional enzyme of 479 aa that shows acetyltransferase and phosphotransferase activity and confers resistance to gentamicin, kanamycin, tobramycin and—when overexpressed—amikacin (156, 157). This gene is located on transposon Tn*4001*, which consists of a central resistance gene region that is bracketed by two IS*256* elements located in opposite orientations (158). Detailed analysis of *aacA-aphD*-carrying plasmids in avian CoNS isolates, including *S. warneri* and *S. sciuri*, identified truncated Tn*4001* elements in which the terminal IS*256* sequences were in part deleted by the insertion of complete or incomplete copies of IS*257* (159). The *aacA-aphD* gene is widespread in staphylococci of animal origin, including canine and feline *S. pseudintermedius* (34–36, 78, 79, 160), *S. aureus* (mostly MRSA) from pigs (21–23, 160–162), cattle (24, 163, 164), a goat and an alpaca (62), horses (25, 68, 88, 165), chickens and turkeys (28–30), and rooks (31) but also CoNS from dogs (166), pigs (85, 151, 167), poultry (85, 159, 167), and horses (88) (Table 2).

The gene *aadD* [also known as *ant(4´)-Ia*] codes for the 256-aa adenyltransferase, which confers resistance to kanamycin, neomycin, and tobramycin. This gene was initially identified on plasmid pUB110 (168), which is also often integrated in type II SCC*mec* elements. In animal staphylococci, it has been detected—often on plasmids—in LA-MRSA/MSSA from pigs (21, 22, 124), cattle (24, 55), turkeys (28), chickens (29, 30), horses (25), and rooks (31). It has also been identified among CoNS from animals, e.g., in an avian *S. sciuri* isolate (169) and a porcine *S. epidermidis* isolate (128) (Table 2).

The gene *aphA3* [also known as *aph(3´)-IIIa*] codes for a phosphotransferase of 264 aa, which mediates resistance to kanamycin, neomycin, and amikacin (170, 171). In animal staphylococci, this gene was found mainly in canine and feline *S. pseudintermedius* (34–36, 171, 172) but also in MRSA from pigs (22), cattle (24, 163), sheep (27), horses (25, 68), and rooks (31) (Table 2).

The gene *aadE* [also known as *ant(6)-Ia*] encodes a 302-aa adenyltransferase, which confers streptomycin resistance. Both genes, *aadE* and *aphA3*, together with the streptothricin resistance gene *sat4* are part of transposon Tn*5405* (170). The *aadE* gene seems to be the predominant streptomycin resistance gene in canine and feline *S. pseudintermedius* (34–36, 171). It was also detected in porcine *S. hyicus* (19) and *S. sciuri* (169). The *aadE* gene is also part of multiresistance gene clusters of enterococcal origin found in porcine MRSA ST9 and CC398 (147, 161, 162, 173, 174) and porcine *S. hyicus* (147) (Table 2).

The gene *str* [also known as *aad(6)*] encodes a 282-aa adenyltransferase (175), which mediates streptomycin resistance. Among staphylococci of animal origin, this gene has been identified in porcine MRSA (23), canine and porcine *S. sciuri* (169), in porcine *S. hyicus* (141, 176), in porcine *S. aureus* and *S. rostri* (177), and in bovine *S. sciuri* (144) (Table 2).

RESISTANCE TO AMINOCYCLITOLS

Two aminocyclitol antibiotics, spectinomycin and apramycin, are used in veterinary medicine. Spectinomycin resistance in staphylococci is based on adenyltransferases encoded by the genes *spc*, *spw*, and *spd*, whereas a single gene, *apmA*, has been described so far to code for apramycin resistance in staphylococci.

The gene *spc* [also known as *aad(9)-Ia*] encodes a 260-aa adenyltransferase (Table 1) (178). The gene *spc* is part of the transposon Tn*554* and as such is often found in combination with the also Tn*554*-associated MLS_B resistance gene *erm*(A). A PCR assay for the detection of the physical linkage between these two genes has been developed and applied (24, 179). The gene *spc* has been detected in MRSA from pigs (21, 23), cattle (24), and chickens and turkeys (28). Among CoNS, it was identified on a multiresistance plasmid from bovine *S. sciuri* (180) and in feline *S. simulans* (147). Moreover, it was also detected in a single porcine *S. hyicus* isolate (147).

The gene *spw* codes for an adenyltransferase of 269 aa. It was found as part of a multiresistance gene cluster in MRSA ST398 and ST9 isolates from pigs and chickens (161, 162, 174, 181). The Spw protein had only 64.7% identity to the Tn*554*-associated Spc protein (181). In 2014, a third spectinomycin resistance gene, designated *spd*, was detected (182). The Spd protein codes for an adenyltransferase of 259 aa that shared only 45% sequence identity with the Spw pro-

tein. The *spd* gene was initially detected in MRSA ST398 from humans and various animal species (182) and soon thereafter, also in porcine MSSA ST433 (183). Further studies of *S. hyicus* and CoNS from animals identified a variant of the gene *spd* on small plasmids in porcine *S. hyicus*, *S. chromogenes*, and *S. equorum* (147). This gene variant had a 12-bp deletion in the terminal part of the *spd* gene, which, however, had no impact on the high spectinomycin MIC conferred by the gene (147) (Table 2).

The gene *apmA* is the first and so far only apramycin resistance gene in staphylococci (Table 1). It codes for a 274-aa acetyltransferase which confers resistance to apramycin and decreased susceptibility to gentamicin (184). It was detected on large multiresistance plasmids in bovine, porcine, and avian LA-MRSA of CC398 (30, 184) or, in a single case, on a small plasmid that conferred only apramycin resistance in a porcine LA-MRSA of CC398 (185). Argudín and coworkers also found the *apmA* gene in a *S. lentus* isolate from a veal calf (164). Recently, the complete sequence of the *apmA*-carrying plasmid pAFS11 was published and showed that this plasmid carried several antimicrobial resistance genes in combination with cadmium and copper resistance genes and a novel *ica*-like gene cluster (126) (Table 3).

RESISTANCE TO STREPTOTHRICINS

Resistance against streptothricins is mediated by the gene *sat4*, which codes for a 176-aa acetyltransferase protein (Table 1) (170). The *sat4* gene is also part of Tn*5405* and, as such, is commonly detected in staphylococci that also harbor *aphA3* and *aadE*. In staphylococci of animal origin, this gene has been detected in canine and feline *S. pseudintermedius* (34–36, 38, 171, 172), in MRSA CC8 (ST254) from horses in Germany (25), and in MRSA CC1 from rooks in Austria (31). Moreover, it has also been detected in CoNS of bovine (164) and equine origin (88) (Table 2).

RESISTANCE TO MACROLIDES, LINCOSAMIDES, AND STREPTOGRAMINS

Resistance to macrolides, lincosamides, and streptogramins is based on a variety of genes that code for different resistance mechanisms with diverse substrate spectra (Table 1).

Combined Resistance to MLS_B

According to the nomenclature center for macrolide, lincosamide, and streptogramin resistance genes (http://faculty.washington.edu/marilynr/ermweb4.pdf), MLS_B resistance in staphylococci of animal origin is based on the presence of one or more *erm* genes of the classes A, B, C, F, T, 33, 43, 44, 45 and 48 (Table 1). These genes code for methylases that modify the target site A2058 in 23S rRNA and thereby inhibit the binding of MLS_B compounds to the bacterial ribosome.

Of the *erm* genes that have been detected in staphylococci of animal origin, *erm*(A) and *erm*(B) are associated with transposons Tn*554* and Tn*917*/Tn*551*, respectively. Tn*554* has also been frequently found to be integrated into SCC*mec* type II elements. The *erm*(A) gene was found in *S. aureus* (mostly MRSA) from various animal species (21, 22–25, 28, 29, 31, 159, 186, 187) but also in *S. pseudintermedius* from dogs (187, 188) and a pigeon (189). In addition, the *erm*(A) gene was also found in porcine *S. hyicus* (19, 145), feline *S. simulans* (147), bovine *S. equorum*, *S. lentus*, and *S. sciuri* (164), and novobiocin-resistant CoNS from poultry (186). Nawaz and coworkers detected the *erm*(A) gene in all 24 poultry-associated *Staphylococcus* isolates investigated (190). Jaglic et al. identified the gene *erm*(A) in a not further specified CoNS isolate from cattle (191) (Table 2).

The *erm*(B) gene is the most predominant *erm* gene in canine and feline *S. pseudintermedius* (33–36, 38, 78, 171, 172, 187, 188). In addition, the *erm*(B) gene was present in canine *S. aureus* as well as porcine *S. aureus* and *S. hyicus* (19, 21, 147, 161, 162, 187, 188). Moreover, the *erm*(B) gene has also been detected in LA-MRSA from pigs (21), cattle (24), chickens and turkeys (28, 29), and a brown hare (31). It was also found on small plasmids in an *S. lentus* isolate from a mink (192) and in various CoNS from pigs (151). *erm*(B) was also present in *Staphylococcus* spp. from poultry (190). Jaglic et al. detected the gene *erm*(B) in not further specified CoNS isolates from turkeys (191). The *erm*(B) gene was also found in CoNS from pigs (85, 167), cattle (164, 193, 194), horses (88), and chickens and ducks (85, 167) (Table 2).

The gene *erm*(C) is commonly located on small plasmids ranging in size from 2.3 to 4.4 kb that do not carry additional resistance genes. Such plasmids have been identified in *S. aureus* (including MRSA) from pigs (21–23, 89, 187), cattle (24, 55, 194, 195), horses (25), dogs and cats (72, 187, 196), sheep (27), goats (62), poultry (28–30, 186, 190, 197), rabbits (195, 198), and a wild boar (31). The *erm*(C) gene has rarely been detected in canine *S. pseudintermedius* (78, 117, 188) and in avian *S. intermedius* (195), whereas it has been detected in *S. hyicus* (199, 200) and in a wide variety of CoNS, including *S. saprophyticus* and *S. lentus*

from rodents and insectivores (201, 202), canine *S. epidermidis* (195, 203), equine *S. xylosus*, porcine *S. haemolyticus* and *S. equorum*, feline *S. warneri*, ovine *S. sciuri*, *Staphylococcus gallinarum* from a chicken, *S. hominis* from a parrot, *S. capitis* from a guinea pig, and *S. lentus* from carrier pigeons (195, 204). Jaglic and coworkers found the gene *erm*(C) in not further specified CoNS isolates from turkeys and pigs (191) (Table 2).

During a study of the host range of the gene *erm*(F) in bacteria of human and animal origin, this methylase gene was detected in isolates of the *S. intermedius* group but also in CoNS of animal origin, such as *S. haemolyticus*, *S. lentus*, and *S. sciuri* (205). The *erm*(F) gene has also been identified in *S. sciuri* isolates from veal calves (164).

The gene *erm*(T) is often found on large multiresistance plasmids and was first identified in staphylococci in 2010 in a porcine MRSA CC398 isolate (125). Since then, it has been identified in MRSA from cattle (24) and chickens and turkeys (28–30). In most cases, the *erm*(T) gene was plasmid-borne and the corresponding plasmids harbored additional resistance genes (Table 3). The *erm*(T) gene has also been detected in bovine CoNS (164) (Table 2).

The gene *erm*(33) is an *in vivo* recombination product between *erm*(A) and *erm*(C) and has been detected on a the multiresistance plasmid pSCFS1 from bovine *S. sciuri* (180, 206). The *erm*(33) gene has also been detected in porcine MRSA ST9 and ST63 isolates from China (207). The gene *erm*(43) has been detected in the chromosomal DNA of *S. lentus* isolates of human, dog, and chicken origin (208). The gene *erm*(44) was found to be associated with a prophage. It was detected in the chromosomal DNA of an *S. xylosus* isolate from bovine mastitis (209). The *erm*(44) gene and a novel *erm*(44) variant, which showed only 84% aa identity, were found in *S. xylosus* and *S. saprophyticus*, respectively, from aquatic environments (210). The gene *erm*(45) was detected in a genomic island in a *Staphylococcus fleurettii* isolate from bovine milk (211) (Table 2). The gene *erm*(48) was identified by whole-genome sequencing of a *S. xylosus* isolate from bovine mastitis milk. It was located on the nonconjugative 49,273-bp plasmid pJW2311, which also carried the macrolide resistance genes *mph*(C) and *msr*(A) (212).

Combined Resistance to Macrolides and Streptogramin B

The *msr*(A) gene codes for a 488-aa ABC-F protein which confers resistance to macrolides and streptogramin B antibiotics (Table 1) (213). ABC-F proteins are neither fused to transmembrane domains nor are they genetically linked to genes that code for transmembrane proteins in operon structures. Experimental evidence has been published that ABC-F proteins involved in antimicrobial resistance act by protecting the ribosomes from the inhibitory effects of the antimicrobial agents rather than by exporting the respective antimicrobial agents from the bacterial cell (214). The *msr*(A) gene has been detected in *S. aureus* isolates of poultry (190), cattle (194), horse (68), and dog/cat origin (187), in canine *S. pseudintermedius* (187), and in various species of CoNS from pigs (191), cattle (164, 191, 194), turkeys (191), and rodents and insectivores (202). This gene was also found in bovine *S. hyicus* (194) (Table 2).

Resistance to Macrolides Only

The gene *mph*(C) codes for a macrolide phosphotransferase of 299 aa which confers only resistance to macrolides (Table 1) (88, 202, 215). However, it should be noted that *mph*(C) is often linked to *msr*(A) and that the Mph(C) phosphotransferase alone confers only low-level resistance to macrolides (193). The *mph*(C) gene has been detected in a canine *S. aureus* isolate (187). Moreover, *mph*(C) was also detected in CoNS from pigs (191), horses (88), cattle (164, 191, 193, 194, 209), sheep (208), chickens (208), turkeys (191), and rodents and insectivores (202). It should be noted that *mph*(C) gene variants, whose gene products do not confer macrolide resistance, have been identified in *S. lentus*, *S. sciuri*, and *S. cohnii* from animal sources (202) (Table 2).

The gene *ere*(A) codes for a macrolide esterase that confers resistance to macrolides. This gene was identified in *S. aureus* and in CoNS, including *S. chromogenes*, *S. haemolyticus*, *S. epidermidis*, and *S. hyicus*, all from cases of bovine mastitis (194).

Resistance to Lincosamides Only

The gene *lnu*(A) encodes a lincosamide nucleotidyltransferase of 161 aa (Table 1) (216). This gene is often located on small plasmids. The *lnu*(A) gene was identified in *S. aureus*, including MRSA from dairy cattle (217), pigs (22, 218, 219), and a turkey (28) and a methicillin-resistant canine *S. pseudintermedius* isolate (34) but also from various CoNS species, including *S. epidermidis*, *S. chromogenes*, *S. simulans*, *S. haemolyticus*, *S. warneri*, *S. sciuri*, and *S. equorum* of bovine origin (164, 193, 195, 220) and a methicillin-resistant *S. sciuri* isolate of porcine origin (218). Moreover, the *lnu*(A) gene was also detected in porcine and bovine *S. hyicus* isolates (187, 194) (Table 2).

The gene *lnu*(B) codes for a lincosamide nucleotidyltransferase of 267 aa (Table 1) and usually occurs in the genera *Streptococcus* and *Enterococcus* (187). Recently, it has been detected as part of multiresistance gene clusters on a plasmid as well as in the chromosomal DNA of porcine and avian MRSA ST9 isolates from China (161, 162, 173, 174) and porcine *S. hyicus* (147) (Table 2).

The gene *lnu*(E) was originally found in porcine *Streptococcus suis* but was shown to be functionally active after cloning and expression in *S. aureus*. This gene confers only low-level resistance to lincomycin (221).

The plasmid-borne *lsa*(B) gene encodes a 492-aa ABC-F protein which has been reported to confer decreased susceptibility to lincosamides (180, 214) (Table 1). This gene has also been detected in a bovine *S. sciuri* isolate and porcine *S. simulans* and *S. warneri* isolates (153, 180).

Resistance to Streptogramin A Only

Inactivation of streptogramin A antibiotics in staphylococci is mediated by *vat*(A), *vat*(B), or *vat*(C) genes, which code for acetyltransferases of 219, 212, or 212 aa, respectively (3). Among them, only the *vat*(B) gene has been identified in two *S. xylosus* isolates of poultry origin (Table 1) (186). It should be noted that resistance to streptogramin A can also be mediated by *vga*, *lsa* and *sal* genes; for more on this, please see the section on pleuromutilin resistance (Table 2).

Resistance to Streptogramin B Only

The genes *vgb*(A) and *vgb*(B) code for streptogramin B lyases, also known as streptogramin B lactonases or steptogramin B lactone hydrolases, of 299 and 295 aa, respectively (3, 222). The two *vat*(B)-positive *S. xylosus* isolates from poultry also carried a *vgb*(B) gene (186) (Tables 1 and 2).

RESISTANCE TO PLEUROMUTILINS

The ABC-F proteins specified by the genes *vga*(A), *vga*$(A)_{LC}$, *vga*(B), *vga*(C), and *vga*(E) exhibit sizes of 522, 522, 552, 522, and 524 aa, respectively (124, 223–229). All of them mediate resistance to streptogramin A antibiotics, whereas the Vga(C) and Vga(E) proteins as well as certain Vga(A) proteins also confer resistance to lincosamides and pleuromutilins (Table 1) (124, 219, 224–229) by protecting the ribosomes from the inhibitory effects of the corresponding antimicrobial agents (214). In staphylococci of animal origin, *vga*(A) gene variants have been identified not only in *S. aureus* (mostly MRSA) from pigs (21, 23, 228), cattle (24), and chickens and turkeys (28, 29), but also in CoNS from cattle (164, 194, 230) and *S. hyicus* from pigs (147). Moreover, the gene *vga*(C) was detected in MRSA from pigs (125, 228) and cattle (24) as well as in porcine *S. hyicus* (147), whereas the gene *vga*(E) was identified in MRSA from pigs (226), cattle (227), and chickens and turkeys (28, 227). A novel variant of the *vga*(E) gene, which codes for an ABC-F protein of 524 aa, was found on the same 5,585-bp plasmid in independent porcine *S. cohnii* and *S. simulans* isolates (231). The variant gene shared 85.7% nucleotide sequence identity with the original *vga*(E) gene, and the variant protein shared 85.3% amino acid sequence identity with the original Vga(E) protein (Table 2).

The *lsa*(E) gene, which also mediates combined resistance to pleuromutilins, lincosamides, and streptogramin A, has been detected in MRSA from pigs and poultry (161, 162, 173, 207), MRSA from cattle (230), CoNS from cattle (230) and dogs (232), and *S. hyicus* from pigs (147). This gene codes for an ABC-F protein of 494 aa. The *lsa*(E) gene, together with the genes *lnu*(B), *aadE*, and *spw*, forms the core component of a multiresistance gene cluster which most likely originates from *Enterococcus faecalis* (173) (Table 2).

The gene *sal*(A), originally described as a lincosamide-streptogramin A resistance gene (233), has also been shown to confer pleuromutilin resistance (230). This gene codes for an ABC-F protein of 541 aa which is only distantly related to the Vga and Lsa proteins.

A survey of pleuromutilin resistance genes among bovine MRSA and CoNS identified two *S. haemolyticus* and single *S. xylosus*, *S. lentus*, and *S. hominis* isolates as *vga*(A)-positive. Twelve *S. aureus*, two *S. warneri*, and single *S. lentus* and *S. xylosus* isolates carried the *lsa*(E) gene. Moreover, single *S. aureus*, *S. haemolyticus*, *S. xylosus*, and *S. epidermidis* isolates were positive for both genes, *vga*(A) and *lsa*(E). The *sal* (A) gene was found in one *S. sciuri* isolate (230). Although the *sal*(A) gene was believed to be restricted to *S. sciuri*, a recent study of pleuromutilin resistance genes among staphylococci from pets identified the *sal* (A) gene not only in the chromosomal DNA of feline and canine *S. sciuri*, but also in a feline *S. haemolyticus* and in canine *S. epidermidis* and *S. xylosus* isolates (232). Moreover, that study identified the *lsa*(E) gene in a novel variant of the aforementioned multiresistance gene cluster in a canine *S. epidermidis* isolate and a *vga* $(A)_{LC}$ gene on a plasmid in a feline *S. haemolyticus* isolate (232).

Pleuromutilin resistance can also be conferred by the methyltransferase Cfr; for more on this, see the following section on resistance to oxazolidinones.

RESISTANCE TO OXAZOLIDINONES

Oxazolidinones are not approved for veterinary use but may be used in non-food-producing animals via the Animal Medicinal Drug Use Clarification Act of 1994 in the United States or similar regulations in other countries. Only two transferable oxazolidinone resistance genes, *cfr* and *optrA*, are currently known to occur in staphylococci of animal origin.

The gene *cfr* was initially identified on a multiresistance plasmid in a bovine *S. sciuri* isolate (234). It codes for an rRNA methylase of 349 aa, which methylates the adenine at position 2503 in the 23S rRNA (235). Because this adenine residue is located in the overlapping binding region of phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics, methylation of A2503 interferes with the binding and correct positioning of these antimicrobial agents. Thus, Cfr confers a penta-resistance phenotype which includes the aforementioned classes of antimicrobial agents (Table 1) (236). Because oxazolidinones are last-resort antimicrobial agents in human medicine and the gene *cfr* confers transferable oxazolidinone resistance, there is growing interest in the dissemination of this gene (237–239).

In staphylococci of animal origin, the *cfr* gene has been detected in *S. aureus* (including MRSA) from pigs (22, 150–152, 207), cattle (60), a horse (152), and a marmot (31) and in porcine *S. hyicus* (153). The CoNS species in which *cfr* was detected include bovine *S. lentus*, *S. simulans*, and *S. sciuri* (83, 152, 234), porcine *S. arlettae*, *S. cohnii*, *S. haemolyticus*, *S. hyicus*, *S. lentus*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, and *S. warneri* (85, 150–153), chicken *S. cohnii*, *S. lentus*, and *S. sciuri*; and duck *S. arlettae*, *S. rostri*, and *S. sciuri* (85, 167) (Table 2). Although the *cfr* gene is most often located on plasmids in staphylococci (239) (Table 3), it has also been detected to be integrated via insertion sequences into a SCC*mec* cassette in a porcine ST9 MRSA isolate (207).

The *optrA* gene codes for an ABC-F protein of 655 aa, which mediates resistance not only to the oxazolidinones linezolid and tedizolid, but also to fluorinated and nonfluorinated phenicols (240). This gene was recently identified in enterococci from humans and animals (240). Soon thereafter, the *optrA* gene was detected on plasmids and in the chromosomal DNA of porcine, canine, and feline *S. sciuri* isolates (241–244). These plasmids harbored additional resistance genes which may favor coselection and persistence of *optrA* (241–244). Most recently, the *optrA* gene was detected in *S. simulans* from household pigs in rural China (244) (Table 2).

RESISTANCE TO TRIMETHOPRIM

Resistance to trimethoprim is based on any of the four genes *dfrA* (also known as *dfrS1*), *dfrD*, *dfrG*, and *dfrK* (9), which code for trimethoprim-resistant dihydrofolate reductases of 161, 166, 165, and 163 aa, respectively (Table 1).

The Tn*4003*-associated gene *dfrA* is most widespread among staphylococci from humans (1). In staphylococci of animal origin, it has rarely been found. There are few reports about its occurrence in *S. aureus* (mostly MRSA) from pigs (22), horses (25, 68, 88), dogs (73), and rooks (31). The gene *dfrD* has been detected even more rarely in animal staphylococci. There is only a single report, which describes its occurrence together with *dfrA* in a single porcine MRSA isolate (22). The genes *dfrA* and *dfrD* have also been found in CoNS from cattle (164) and horses (88). In contrast, the gene *dfrG* seems to be the predominant *dfr* gene in canine and feline *S. pseudintermedius* (34–36, 79). It has also been detected in MRSA (21–23), MSSA (177), and the coagulase-negative *S. rostri* (177), all from pigs, but also in bovine CoNS (164). The gene *dfrK* was initially found on multiresistance plasmids from porcine MRSA ST398, where it was linked to the tetracycline resistance gene *tet*(L) (124–126) (Table 3). Later, the *dfrK* gene was also identified as part of the transposon Tn*559* in a porcine MSSA CC398 isolate (245). PCR assays have been developed and applied to differentiate between its linkage with *tet*(L) and its location within Tn*559* (24, 179). The *dfrK* gene has been found not only in MRSA from pigs (21–23), cattle (24), horses (68), and chickens and turkeys (28–30), but also in a porcine *S. hyicus* isolate (185) and in CoNS from cattle (164) (Table 2).

RESISTANCE TO FUSIDIC ACID

Resistance to fusidic acid is based on the expression of the genes *fusB* (*far1*) and *fus*C, which code for cytoplasmatic proteins of 213 and 212 aa (246) or on single point mutations in the *fusA* gene (Table 1). It has been shown that the FusB protein binds to the staphylococcal elongation factor G (EF-G) and thereby protects the translation system from inhibition by fusidic acid (247). Fusidic acid resistance in animal staphylococci has rarely been detected. The *fusB* gene has been found in MRSA isolates from sheep (27) and in single isolates of *S. epidermidis*, *S. haemolyticus*, and *S. hominis* from cattle (164, 248). Few reports describe the presence of *fus*C in *S. aureus* from donkeys (26) and in canine *S. pseudintermedius* (33) (Table 2). Loeffler et al. identified six MRSA isolates from cats

and dogs with high MICs of fusidic acid (≥ 512 mg/liter), but no information is available about the corresponding resistance genes (249).

RESISTANCE TO MUPIROCIN

Resistance to mupirocin in staphylococci is commonly due to a mupirocin-insensitive isoleucyl-tRNA synthase of 1,024 aa, encoded by the gene *ileS2*, also known as *ileS* or *mupA* (Table 1) (250, 251). Although a small number of mupirocin-resistant MRSA/MSSA isolates from dogs have been reported (252, 253), no information about the corresponding resistance genes is available. In 2013, the *ileS2* gene was identified in a canine *S. pseudintermedius* isolate (254), where it was located together with the aminoglycoside resistance gene *aacA-aphD* on a conjugative plasmid. In another study, only 1 of 581 *S. pseudintermedius* isolates was resistant to mupirocin and also carried the high-level mupirocin resistance gene *ileS2* on a plasmid (255) (Table 2).

FLUOROQUINOLONE RESISTANCE

Fluoroquinolone resistance in staphylococci is mainly based on mutations that resulted in amino acid substitutions in the quinolone-resistance-determining regions of the topoisomerase genes *gyrA*, *gyrB*, *grlA*, and *grlB*. Such mutations have been described in *S. pseudintermedius* (7, 38). A few studies reported such mutations in naturally occurring *S. aureus* from animals and food of animal origin (256–258). A detailed description of the known mutations in *S. aureus* from animals is given in reference 4. So far, no plasmid-mediated quinolone resistance genes have been detected in staphylococci.

RIFAMPICIN RESISTANCE

Rifampicin interacts with the β subunit of the bacterial RNA polymerase encoded by the *rpoB* gene. While numerous mutations in the *rpoB* gene have been detected in clinical *S. aureus* isolates from humans and laboratory strains, little information is currently available about rifampicin-resistance-mediating mutations in staphylococci from animals (258). Within the *rpoB* gene of porcine MRSA ST9, mutations were found at the following positions: Asp471Tyr, Ala473Glu, His481Asn, and Ser529Leu (258). It became apparent that not all mutations had the same effect on the rifampicin MICs and that occasionally isolates with two or three *rpoB* mutations exhibited higher MICs than isolates with a single *rpoB* mutation (258). A detailed description of the known rifampicin-resistance-mediating mutations in *S. aureus* from animals is given in reference 4. Another study identified *rpoB* mutations in multidrug-resistant *S. pseudintermedius* isolates from dogs (259). In that study, single mutations were detected at positions Gln513Leu, Ala522Asp, His526Arg, His5256Pro, His526Tyr, and Ser531Leu, whereas double mutations included the positions Ser508Asn + Ser509Pro and Ser509Pro + Asp516Asn (259).

MISCELLANEOUS RESISTANCE PROPERTIES

Resistance to bleomycin encoded by the gene *ble* is commonly associated with the aminoglycoside resistance gene *aadD* (168). Both *aadD* and *ble* are located on small plasmids of the type pUB110, which are also integrated into certain types of SCC*mec* cassettes (39) and into larger plasmids (3, 114).

In contrast to the situation in humans, fully vancomycin-resistant staphylococci have so far not been isolated from animals. However, porcine MRSA ST9 isolates from China which displayed reduced vancomycin susceptibility (260) and porcine vancomycin-intermediate MRSA ST398/t9538 isolates from Brazil (261) have been described.

Sulfonamide resistance is known to occur in staphylococci (1, 4). However, in contrast to *Enterobacteriaceae*, no specific sulfonamide resistance genes have been identified in staphylococci so far. In contrast, sulphonamide resistance is believed to result from (i) mutations in the dihydropteroate synthase genes, which change the affinity of the corresponding enzymes to sulfonamides, (ii) overexpression of sulfonamide-susceptible dihydropteroate synthase genes, or (iii) increased production of *p*-aminobenzoic acid (1).

Fosfomycin resistance in staphylococci was found to be encoded by the gene *fosD*, which codes for a 139-aa fosfomycin thiol transferase. This gene was detected on a ca. 50-kb multiresistance plasmid of a *S. rostri* isolate of duck origin. This plasmid also harbored the resistance genes *erm*(B), *aacA-aphD*, *cfr*, *ble*, *aadD*, and *fexA* (169). The fosfomycin resistance gene *fosB* was detected in an *S. epidermidis* isolate from a veal calf (163) and in *S. aureus* from horses (25), dogs (73), cats (73), and European wildlife species (31) (Table 2).

Resistance to heavy metals in staphylococci has been known of for more than 30 years (1). A review on metal resistance (262) provides additional information. Witte et al. (263) investigated *S. aureus* of human and animal origin for its resistance to mercury and cadmium. Mercury resistance was detected in human isolates, but the isolates from cattle, pigs, chicken, and sheep were classified as susceptible. In comparison, cadmium

resistance was detected among isolates from humans and cattle. An analysis of 32 porcine and 20 bovine *S. hyicus* isolates revealed that cadmium resistance was present in 28 and 16 isolates, respectively (264). However, there was no information on the genetic basis of the cadmium resistance. In 2010, Aarestrup et al. (265) found a correlation between methicillin resistance and decreased susceptibility to zinc in porcine LA-MRSA CC398, and subsequently Cavaco et al. (266) identified the cadmium and zinc resistance gene *czrC* within the type V SCC*mec* element. When the *czrC* gene was cloned and transferred in *S. aureus* RN4220, it conferred a 4-fold increase of the zinc chloride MIC and an 8-fold increase of the cadmium MIC (267). The strong correlation of the presence of the *czrC* gene with MRSA CC398 was confirmed when testing porcine and bovine MRSA and MSSA isolates from 10 European countries, Canada, and China (267). In 2011, novel SCC*mec* elements were described to occur in MRSA CC398, which carried numerous metal resistance genes, including *cadDX* (cadmium resistance), *arsRBC* and *arsDARBC* (arsenic resistance), *copB* (copper resistance), and *czr*C (cadmium/zinc resistance) (268). Moreover, resistance to heavy metals was seen among LA-MRSA, and the resistance genes *arsA*, *cadD*, *copB*, and *czrC* were identified (269). In another study, 130 methicillin-resistant CoNS from pigs and veal calves were investigated for the presence of metal resistance genes, and almost half of the isolates carried metal resistance genes (*czrC* 5.4%, *copB* 38.5%, *cadD* 7.7%, *arsA* 26.2%), regardless of their SCC*mec* type (270). Cadmium and copper resistance genes were also detected on multiresistance plasmids from Spanish porcine MRSA isolates. They harbored the functional *cadD*/*cadX* cadmium resistance operon and/or the multicopper oxidase gene *mco* and the ATPase copper transport gene *copA* on plasmids that also carried the antimicrobial resistance genes *aadD* and/or *erm*(T) and *tet*(L) (271). A novel multiresistance plasmid from bovine MRSA ST398 that harbored the antimicrobial resistance genes *aadD*, *tet*(L), *dfrK*, *erm*(B), and *apmA* together with a novel biofilm gene cluster and the metal resistance operons *cadD*/*cadX* and *copA*/*mco* was recently sequenced (126). These examples show that metal resistance and antimicrobial resistance can easily be coselected. In this regard, it is important to know that the increased use of metals in livestock animals, especially zinc in pigs, may coselect for methicillin resistance in staphylococci.

Resistance to biocides is also detected frequently among staphylococci. The reader is referred elsewhere for a review (272) that provides detailed information on the mechanisms of biocide resistance and the genes involved. In staphylococci of animal origin, *qac* genes that confer resistance to quaternary ammonium compounds were identified in staphylococci from cattle (*qacA/B*, *smr*, *qacG*, and *qacJ*) and goats (*qacA/B* and *smr*). In many cases, these genes were located on plasmids (273). Another study identified the genes *qacC/D* among 3/86 non-CC398 MRSA isolates originating from pigs (269). The resistance genes *qacG* and *qacC* were identified in 3/79 and 1/79 porcine CC30 *S. aureus* isolates, respectively (274). A study from Portugal identified the biocide resistance genes *qacG* and *qacJ* in 13/74 and 1/74 MRSA CC398 isolates from pigs, respectively, while 4/74 MRSA isolates (3 CC5 from dogs and humans and 1 CC22 from a dog) had insertions in the −10 motif of the *norA* promoter (275). Slifierz et al. found that MRSA from pigs with benzalkonium MICs of ≥2 mg/liter carried at least one of the biocide resistance genes *qacG*, *qacH*, *qacA/B*, and *smr*, with about one-third of the isolates harboring the combination *qacG-qacH-smr* (276). A study of methicillin-resistant staphylococci from horses identified *S. haemolyticus* and *S. cohnii* subsp. *cohnii* isolates that carried plasmid-borne *qacA* and *sh-fabI* or *qacB* and *qacH*-like genes, respectively (277).

Resistance to antimicrobial peptides has rarely been investigated in staphylococci of animal origin. A study of the *in vitro* activity of human and animal cathelicidins against LA-MRSA revealed that none of the 14 most common antimicrobial resistance genes affected the antimicrobial activity of the cathelicidins (278). In a follow-up study, MICs of *S. aureus* field isolates for the cathelicidins LL-37, mCRAMP, CAP18, BMAP-27, and BMAP-28 in the presence and absence of different efflux pump inhibitors were determined (279). After blocking resistance-nodulation-cell division-type efflux pumps with 1-(1-naphthylmethyl)-piperazine, the MICs for CAP18, but not those for the other cathelicidins tested, were significantly decreased. In good correlation with these data, significantly decreased MICs for CAP18 and BMAP-27 were observed for SecDF knockout mutants. In addition, the MIC values increased again after reintroducing a cloned *secDF* via plasmid complementation. These results indicated involvement of SecDF in reduced efficacy of species-specific cathelicidins against *S. aureus* (279).

RESISTANCE DATA OF ANIMAL STAPHYLOCOCCI

Percentages of resistant staphylococci are presented in numerous publications. However, as described else-

where (280), the results are only comparable when the same antimicrobial susceptibility testing methodology and the same interpretive criteria are used. Moreover, the most reliable results are obtained if the tested isolates were obtained following a defined sampling plan that secures sufficient numbers of isolates and avoids the inclusion of multiple members of the same bacterial clone. As such, the most reliable results are obtained from national monitoring programs. A survey of national monitoring programs in the veterinary sector, however, revealed that staphylococci of animal origin are only rarely part of national monitoring programs (281). Table 4 presents data obtained from the Swedish SVARM (282) and the German Germ-Vet (283) programs. Both programs followed the standards for broth microdilution of the Clinical and Laboratory Standards Institute (CLSI) and CLSI-approved clinical breakpoints for the evaluation of the results. As can be seen from this example, striking differences were seen for the different staphylococcal species from infections of the different animal species. This observation emphasizes the need for detailed analyses that include the staphylococcal species, the animal host, and the site of infection.

CONCLUSIONS AND PERSPECTIVES

The data presented in this chapter show that staphylococci from animals harbor a wide range of resistance genes and resistance-mediating mutations. For most resistance properties, several resistance genes, which account for the same resistance trait, such as the various *erm*, *tet*, or *cat* genes, have been detected in staphylococci. Moreover, staphylococci of animal origin have been shown to simultaneously carry two or three resistance genes that specify the same resistance phenotype. Examples are the carriage of the *tet*(M), *tet*(K), and *tet*(L) genes or the *erm*(A), *erm*(B), *erm*(C), or *erm*(T) genes in various combinations in LA-MRSA from pigs, cattle, and poultry (21, 24, 28–30). Since one of these genes is sufficient to confer tetracycline or MLS_B resistance, respectively, the simultaneous presence of two or three of these *tet* or *erm* genes may be explained by their acquisition at different times and their location on plasmids that carry other resistance genes.

The analysis of the resistance genes present in animal staphylococci has also identified genes that confer resistance to antimicrobial agents, such as florfenicol or apramycin, that are approved for the treatment of infections in livestock other than those caused by staphylococci. However, it needs to be understood that the applied antimicrobial agents not only target the causative agents of an infection, but also put the physiological microbiota in the respective body compartments under selective pressure. As a consequence, staphylococci which live as commensals on the skin or the mucosal surfaces may also acquire resistance genes that allow their survival in the presence of antimicrobial agents such as florfenicol and apramycin. The observation that florfenicol resistance genes such as *fexA* and *cfr* have been detected as the only phenicol resistance genes among MRSA from cattle and pigs might reflect the use of florfenicol for the control of respiratory tract infections in these animal species.

This observation also showed that the use of antimicrobial agents adds to the presence of antimicrobial resistance genes in animal staphylococci. However, there is no linear correlation between antimicrobial use and the presence of resistance properties or resistance genes. In this regard, coselection and persistence of resistance genes in the absence of a direct selective pressure need to be taken into account, especially since many mobile genetic elements carry more than a single antimicrobial resistance gene (1–6) (Table 3). In this regard, genes that confer resistance to heavy metals or disinfectants and that are located on the same multiresistance plasmid or SCC*mec* cassette, may also contribute to the coselection process. Thus, measures such as the ban or the limitation of use of certain antimicrobial agents do not necessarily result in a decrease or loss of resistance properties/genes. To make an educated guess about what to expect from such measures, it is indispensable to know which resistance genes are physically linked to other resistance genes or are part of resistance gene clusters that are commonly cotransferred (Table 3). Hence, detailed analyses of multiresistance plasmids or multiresistance gene clusters in staphylococci further our understanding of processes such as coselection and persistence of resistance.

Finally, the data presented in this article has shown that staphylococci of animal origin do not exist in genetic isolation. Instead, they share resistance genes with staphylococci of human origin but also with other bacteria, such as *Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp., *Lactococcus* spp., and *Lactobacillus* spp. Database searches revealed that some of the resistance genes found in staphylococci of animal origin, such as *aacA-aphD*, *erm*(B), and *tet*(M) are widespread among Gram-positive bacteria, while others, such as *apmA*, *erm*(33), *erm*(43), *lsa*(B), *vga*(C), *vga*(E), and *vgb*(B) have not yet been detected in bacteria other than staphylococci.

In summary, coagulase-positive, -variable, and -negative staphylococci of animal origin harbor a wide

Table 4 Resistance rates to selected antimicrobial agents among staphylococci of animal origin

Antimicrobial agent	Staphylococcal species	Origin	Year	Isolates tested	Resistant isolates (%)	Country	Reference
Penicillin	*S. aureus*	Horses – skin infection	2016	75	15 (20.0)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	3 (1.8)	Sweden	282
	S. felis	Cats – various infections	2016	277	39 (14.1)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	23 (65.7)	Germany	283
	SIG[c]	Dogs – skin/soft tissue infections	2014	59	47 (79.7)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	94 (25.9)	Germany	283
	S. hyicus	Pigs – various infections	2015	39	30 (76.9)	Germany	283
Oxacillin	*S. aureus*	Horses – skin infection	2016	75	0 (0.0)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	0 (0.0)	Sweden	282
	S. felis	Cats – various infections	2016	277	0 (0.0)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	3 (8.6)	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	57	11 (19.3)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	15 (4.1)	Germany	283
	S. hyicus	Pigs – various infections	2015	39	0 (0.0)	Germany	283
Tetracycline	*S. aureus*	Horses – skin infection	2016	75	1 (1.3)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	6 (3.7)	Sweden	282
	S. felis	Cats – various infections	2016	277	2 (0.7)	Sweden	282
	S. aureus	Poultry – various infections	2014	59	23 (65.7)	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	59	23 (39.0)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	53 (14.6)	Germany	283
	S. hyicus	Pigs – various infections	2015	39	15 (38.5)	Germany	283
Erythromycin	*S. aureus*	Horses – skin infection	2016	75	0 (0.0)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	9 (5.5)	Sweden	282
	S. felis	Cats – various infections	2016	277	46 (16.6)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	17 (48.6)	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	59	26 (44.1)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	29 (8.0)	Germany	283
	S. hyicus	Pigs – various infections	2015	39	8 (20.5)	Germany	283
Clindamycin	*S. aureus*	Horses – skin infection	2016	75	2 (2.7)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	12 (7.4)	Sweden	282
	S. felis	Cats – various infections	2016	277	19 (6.9)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	17 (48.6)[a]	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	59	23 (39.0)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	32 (8.8)[a]	Germany	283
	S. hyicus	Pigs – various infections	2015	39	13 (33.3)[a]	Germany	283
Gentamicin	*S. aureus*	Horses – skin infection	2016	75	3 (4.0)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	163	1 (0.6)	Sweden	282
	S. felis	Cats – various infections	2016	277	2 (0.7)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	1 (2.9)	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	59	5 (8.5)	Germany	283
	S. aureus	Cattle – mastitis	2015	363	3 (0.8)	Germany	283
	S. hyicus	Pigs – various infections	2015	39	2 (5.1)	Germany	283
Enrofloxacin	*S. aureus*	Horses – skin infection	2016	35	1 (2.9)	Sweden	282
	S. schleiferi	Dogs – various infections	2016	55	11 (20.0)	Sweden	282
	S. felis	Cats – various infections	2016	97	0 (0.0)	Sweden	282
	S. aureus	Poultry – various infections	2014	35	26 (74.3)[b]	Germany	283
	SIG	Dogs – skin/soft tissue infections	2014	59	9 (15.3)[b]	Germany	283
	S. aureus	Cattle – mastitis	2015	363	28 (7.7)[b]	Germany	283
	S. hyicus	Pigs – various infections	2015	39	7 (17.9)[b]	Germany	283

[a]Isolates showing an MIC of ≥4 mg/liter.
[b]Isolates showing an MIC of ≥1 mg/liter.
[c]SIG, *S. intermedius* group (*S. intermedius*, *S. pseudintermedius*, and *S. delphini*).

range of resistance genes. The analysis of these resistance genes and their genetic environment, including the mobile genetic elements on which resistance genes are located, clearly point toward gene exchange events within the Gram-positive resistance gene pool. Growing interest in staphylococci of animal origin accompanied by a growing number of available whole-genome sequences will surely identify additional resistance genes and resistance-mediating mutations in the future and thereby broaden our knowledge in this field.

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References

1. **Lyon BR, Skurray R.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* **51:** 88–134.
2. **Jensen SO, Lyon BR.** 2009. Genetics of antimicrobial resistance in *Staphylococcus aureus*. *Future Microbiol* **4:** 565–582.
3. **Schwarz S, Feßler AT, Hauschild T, Kehrenberg C, Kadlec K.** 2011. Plasmid-mediated resistance to protein biosynthesis inhibitors in staphylococci. *Ann N Y Acad Sci* **1241:**82–103.
4. **Feßler AT, Li J, Kadlec K, Wang Y, Schwarz S.** 2018. Antimicrobial resistance properties of *Staphylococcus aureus*, p 57–86. *In* Fetsch A (ed), *Staphylococcus aureus – a Foodborne Pathogen: Epidemiology, Detection, Characterization, Prevention and Control*. Academic Press, London, United Kingdom.
5. **Aarestrup FM, Schwarz S.** 2006. Antimicrobial resistance in staphylococci and streptococci of animal origin, p 187–212. *In* Aarestrup FM (ed), *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
6. **Wendlandt S, Feßler AT, Monecke S, Ehricht R, Schwarz S, Kadlec K.** 2013. The diversity of antimicrobial resistance genes among staphylococci of animal origin. *Int J Med Microbiol* **303:**338–349.
7. **Kadlec K, Schwarz S.** 2012. Antimicrobial resistance of *Staphylococcus pseudintermedius*. *Vet Dermatol* **23:** 276–282, e55.
8. **Werckenthin C, Cardoso M, Martel J-L, Schwarz S.** 2001. Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus*, and canine *Staphylococcus intermedius*. *Vet Res* **32:**341–362.
9. **Kadlec K, Feßler AT, Hauschild T, Schwarz S.** 2012. Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* **18:**745–755.
10. **van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ.** 2011. Acquired antibiotic resistance genes: an overview. *Front Microbiol* **2:**203.
11. **Roberts MC, Schwarz S, Aarts HJ.** 2012. Erratum: acquired antibiotic resistance genes: an overview. *Front Microbiol* **3:**384.
12. **Wendlandt S, Schwarz S, Silley P.** 2013. MRSA: a foodborne pathogen? *Annu Rev Food Sci Technol* **4:** 117–139.
13. **Rowland SJ, Dyke KG.** 1989. Characterization of the staphylococcal β-lactamase transposon Tn*552*. *EMBO J* **8:**2761–2773.
14. **Vesterholm-Nielsen M, Olhom Larsen M, Olsen JE, Aarestrup FM.** 1999. Occurrence of the *blaZ* gene in penicillin resistant *Staphylococcus aureus* isolated from bovine mastitis in Denmark. *Acta Vet Scand* **40:**279–286.
15. **Yazdankhah SP, Sørum H, Oppegaard H.** 2000. Comparison of genes involved in penicillin resistance in staphylococci of bovine origin. *Microb Drug Resist* **6:** 29–36.
16. **Haveri M, Suominen S, Rantala L, Honkanen-Buzalski T, Pyörälä S.** 2005. Comparison of phenotypic and genotypic detection of penicillin G resistance of *Staphylococcus aureus* isolated from bovine intramammary infection. *Vet Microbiol* **106:**97–102.
17. **Olsen JE, Christensen H, Aarestrup FM.** 2006. Diversity and evolution of *blaZ* from *Staphylococcus aureus* and coagulase-negative staphylococci. *J Antimicrob Chemother* **57:**450–460.
18. **Sawant AA, Gillespie BE, Oliver SP.** 2009. Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. *Vet Microbiol* **134:** 73–81.
19. **Aarestrup FM, Jensen LB.** 2002. Trends in antimicrobial susceptibility in relation to antimicrobial usage and presence of resistance genes in *Staphylococcus hyicus* isolated from exudative epidermitis in pigs. *Vet Microbiol* **89:**83–94.
20. **Feßler AT, Thomas P, Mühldorfer K, Grobbel M, Brombach J, Eichhorn I, Monecke S, Ehricht S, Schwarz S.** Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from zoo and wild animals. *Vet Microbiol* **218:**98–103.
21. **Kadlec K, Ehricht R, Monecke S, Steinacker U, Kaspar H, Mankertz J, Schwarz S.** 2009. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-

resistant *Staphylococcus aureus* ST398 from diseased swine. *J Antimicrob Chemother* **64:**1156–1164.

22. **Argudín MA, Tenhagen BA, Fetsch A, Sachsenröder J, Käsbohrer A, Schroeter A, Hammerl JA, Hertwig S, Helmuth R, Bräunig J, Mendoza MC, Appel B, Rodicio MR, Guerra B.** 2011. Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. *Appl Environ Microbiol* **77:**3052–3060.
23. **Overesch G, Büttner S, Rossano A, Perreten V.** 2011. The increase of methicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type ST49 in slaughter pigs in Switzerland. *BMC Vet Res* **7:**30.
24. **Feßler A, Scott C, Kadlec K, Ehricht R, Monecke S, Schwarz S.** 2010. Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. *J Antimicrob Chemother* **65:**619–625.
25. **Walther B, Monecke S, Ruscher C, Friedrich AW, Ehricht R, Slickers P, Soba A, Wleklinski CG, Wieler LH, Lübke-Becker A.** 2009. Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **47:** 704–710.
26. **Gharsa H, Ben Sallem R, Ben Slama K, Gómez-Sanz E, Lozano C, Jouini A, Klibi N, Zarazaga M, Boudabous A, Torres C.** 2012. High diversity of genetic lineages and virulence genes in nasal *Staphylococcus aureus* isolates from donkeys destined to food consumption in Tunisia with predominance of the ruminant associated CC133 lineage. *BMC Vet Res* **8:**203.
27. **Gharsa H, Ben Slama K, Lozano C, Gómez-Sanz E, Klibi N, Ben Sallem R, Gómez P, Zarazaga M, Boudabous A, Torres C.** 2012. Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. *Vet Microbiol* **156:**367–373.
28. **Monecke S, Ruppelt A, Wendlandt S, Schwarz S, Slickers P, Ehricht R, Jäckel SC.** 2013. Genotyping of *Staphylococcus aureus* isolates from diseased poultry. *Vet Microbiol* **162:**806–812.
29. **Wendlandt S, Kadlec K, Feßler AT, Monecke S, Ehricht R, van de Giessen AW, Hengeveld PD, Huijsdens X, Schwarz S, van Duijkeren E.** 2013. Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers. *J Antimicrob Chemother* **68:** 2458–2463.
30. **Wendlandt S, Kadlec K, Feßler AT, Mevius D, van Essen-Zandbergen A, Hengeveld PD, Bosch T, Schouls L, Schwarz S, van Duijkeren E.** 2013. Transmission of methicillin-resistant *Staphylococcus aureus* isolates on broiler farms. *Vet Microbiol* **167:**632–637.
31. **Monecke S, Gavier-Widén D, Hotzel H, Peters M, Guenther S, Lazaris A, Loncaric I, Müller E, Reissig A, Ruppelt-Lorz A, Shore AC, Walter B, Coleman DC, Ehricht R.** 2016. Diversity of *Staphylococcus aureus* isolates in European wildlife. *PLoS One* **11:**e0168433.
32. **Malik S, Christensen H, Peng H, Barton MD.** 2007. Presence and diversity of the β-lactamase gene in cat and dog staphylococci. *Vet Microbiol* **123:**162–168.
33. **Norström M, Sunde M, Tharaldsen H, Mørk T, Bergsjø B, Kruse H.** 2009. Antimicrobial resistance in *Staphylococcus pseudintermedius* in the Norwegian dog population. *Microb Drug Resist* **15:***55–59*.
34. **Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L.** 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J Antimicrob Chemother* **65:**1145–1154.
35. **Kadlec K, Schwarz S, Perreten V, Andersson UG, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L.** 2010. Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *J Antimicrob Chemother* **65:**1826–1828.
36. **Gómez-Sanz E, Torres C, Lozano C, Sáenz Y, Zarazaga M.** 2011. Detection and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in healthy dogs in La Rioja, Spain. *Comp Immunol Microbiol Infect Dis* **34:**447–453.
37. **Andreis SN, Perreten V, Schwendener S.** 2017. Novel β-Lactamase bla_{ARL} in *Staphylococcus arlettae*. *MSphere* **2:**e00117-17.
38. **Descloux S, Rossano A, Perreten V.** 2008. Characterization of new staphylococcal cassette chromosome *mec* (SCC*mec*) and topoisomerase genes in fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius*. *J Clin Microbiol* **46:**1818–1823.
39. **Black CC, Solyman SM, Eberlein LC, Bemis DA, Woron AM, Kania SA.** 2009. Identification of a predominant multilocus sequence type, pulsed-field gel electrophoresis cluster, and novel staphylococcal chromosomal cassette in clinical isolates of *mecA*-containing, methicillin-resistant *Staphylococcus pseudintermedius*. *Vet Microbiol* **139:**333–338.
40. **International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC).** 2009. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* **53:**4961–4967.
41. **Wu Z, Li F, Liu D, Xue H, Zhao X.** 2015. Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinase, CcrC2. *Antimicrob Agents Chemother* **59:**7597–7601.
42. **Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, Strommenger B, Walther B, Wieler L, Witte W.** 2010. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *Int J Med Microbiol* **300:**109–117.
43. **Loeffler A, Lloyd DH.** 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiol Infect* **138:**595–605.
44. **Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P.** 2010. Methicillin-resistant *Staphylococcus aureus*

(MRSA) in food production animals. *Epidemiol Infect* **138:**606–625.

45. **Weese JS.** 2010. Methicillin-resistant *Staphylococcus aureus* in animals. *ILAR J* **51:**233–244.
46. **Weese JS, van Duijkeren E.** 2010. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Vet Microbiol* **140:**418–429.
47. **Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA.** 2011. Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. *Int J Med Microbiol* **301:**630–634.
48. **Fitzgerald JR.** 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *Trends Microbiol* **20:**192–198.
49. **Fluit AC.** 2012. Livestock-associated *Staphylococcus aureus*. *Clin Microbiol Infect* **18:**735–744.
50. **Pantosti A.** 2012. Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. *Front Microbiol* **3:**127.
51. **Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M.** 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis* **11:**1965–1966.
52. **de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WD, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW.** 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet Microbiol* **122:**366–372.
53. **van Duijkeren E, Jansen MD, Flemming SC, de Neeling H, Wagenaar JA, Schoormans AH, van Nes A, Fluit AC.** 2007. Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. *Emerg Infect Dis* **13:**1408–1410.
54. **Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Bosch T, Van Duijkeren E.** 2009. Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. *Vet Microbiol* **139:**405–409.
55. **Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R.** 2007. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Vet Microbiol* **125:**128–140.
56. **Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P.** 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Vet Microbiol* **144:**166–171.
57. **Holmes MA, Zadoks RN.** 2011. Methicillin resistant *S. aureus* in human and bovine mastitis. *J Mammary Gland Biol Neoplasia* **16:**373–382.
58. **Spohr M, Rau J, Friedrich A, Klittich G, Fetsch A, Guerra B, Hammerl JA, Tenhagen BA.** 2011. Methicillin-resistant *Staphylococcus aureus* (MRSA) in three dairy herds in southwest Germany. *Zoonoses Public Health* **58:**252–261.
59. **Feßler AT, Olde Riekerink RG, Rothkamp A, Kadlec K, Sampimon OC, Lam TJ, Schwarz S.** 2012. Characterization of methicillin-resistant *Staphylococcus aureus* CC398 obtained from humans and animals on dairy farms. *Vet Microbiol* **160:**77–84.
60. **Wang XM, Zhang WJ, Schwarz S, Yu SY, Liu H, Si W, Zhang RM, Liu S.** 2012. Methicillin-resistant *Staphylococcus aureus* ST9 from a case of bovine mastitis carries the genes *cfr* and *erm*(A) on a small plasmid. *J Antimicrob Chemother* **67:**1287–1289.
61. **Juhász-Kaszanyitzky E, Jánosi S, Somogyi P, Dán A, van der Graaf-van Bloois L, van Duijkeren E, Wagenaar JA.** 2007. MRSA transmission between cows and humans. *Emerg Infect Dis* **13:**630–632.
62. **Schauer B, Krametter-Frötscher R, Knauer F, Ehricht R, Monecke S, Feßler AT, Schwarz S, Grunert T, Spergser J, Loncaric I.** 2018. Diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and New World camelids. *Vet Microbiol* **215:**77–82.
63. **Chu C, Yu C, Lee Y, Su Y.** 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and *sec*-dependent mastitis of dairy goats in Taiwan. *BMC Vet Res* **8:**39.
64. **Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, Struelens M, Devriese LA, Pasmans F, Haesebrouck F.** 2008. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. *Antimicrob Agents Chemother* **52:**3817–3819.
65. **Persoons D, Van Hoorebeke S, Hermans K, Butaye P, de Kruif A, Haesebrouck F, Dewulf J.** 2009. Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerg Infect Dis* **15:**452–453.
66. **Cuny C, Strommenger B, Witte W, Stanek C.** 2008. Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. *Microb Drug Resist* **14:**307–310.
67. **van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM, Multem J, Troelstra A, Fluit AC, van Wamel WJ, Houwers DJ, de Neeling AJ, Wagenaar JA.** 2010. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. *Vet Microbiol* **141:**96–102.
68. **Sieber S, Gerber V, Jandova V, Rossano A, Evison JM, Perreten V.** 2011. Evolution of multidrug-resistant *Staphylococcus aureus* infections in horses and colonized personnel in an equine clinic between 2005 and 2010. *Microb Drug Resist* **17:**471–478.
69. **Sing A, Tuschak C, Hörmansdorfer S.** 2008. Methicillin-resistant *Staphylococcus aureus* in a family and its pet cat. *N Engl J Med* **358:**1200–1201.
70. **Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, Brunnberg L, Lübke-Becker A.** 2008. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Vet Microbiol* **127:**171–178.
71. **Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Schwarz S, Simon D, Nolte I.** 2009. Transmission of methicillin-resistant *Staphylococcus aureus* strains

between humans and dogs: two case reports. *J Antimicrob Chemother* **64:**660–662.

72. **Coelho C, Torres C, Radhouani H, Pinto L, Lozano C, Gómez-Sanz E, Zaragaza M, Igrejas G, Poeta P.** 2011. Molecular detection and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from dogs in Portugal. *Microb Drug Resist* **17:**333–337.
73. **Haenni M, Saras E, Châtre P, Médaille C, Bes M, Madec JY, Laurent F.** 2012. A USA300 variant and other human-related methicillin-resistant *Staphylococcus aureus* strains infecting cats and dogs in France. *J Antimicrob Chemother* **67:**326–329.
74. **Ruscher C, Lübke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B.** 2009. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. *Vet Microbiol* **136:**197–201.
75. **Ruscher C, Lübke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, Stamm I, Kopp P, Wieler LH, Walther B.** 2010. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. *Vet Microbiol* **144:**340–346.
76. **Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Kreienbrock L, Schwarz S, Simon D, Nolte I.** 2011. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. *Vet Microbiol* **150:**191–197.
77. **Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, Schwarz S, Kreienbrock L, Nolte I, Simon D.** 2011. Methicillin-resistant *Staphylococcus pseudintermedius* among cats admitted to a veterinary teaching hospital. *Vet Microbiol* **153:**414–416.
78. **Wang Y, Yang J, Logue CM, Liu K, Cao X, Zhang W, Shen J, Wu C.** 2012. Methicillin-resistant *Staphylococcus pseudintermedius* isolated from canine pyoderma in North China. *J Appl Microbiol* **112:**623–630.
79. **Kadlec K, Weiß S, Wendlandt S, Schwarz S, Tonpitak W.** 2016. Characterization of canine and feline methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from Thailand. *Vet Microbiol* **194:**93–97.
80. **Hassler C, Nitzsche S, Iversen C, Zweifel C, Stephan R.** 2008. Characteristics of *Staphylococcus hyicus* strains isolated from pig carcasses in two different slaughterhouses. *Meat Sci* **80:**505–510.
81. **Zhang Y, Agidi S, LeJeune JT.** 2009. Diversity of staphylococcal cassette chromosome in coagulase-negative staphylococci from animal sources. *J Appl Microbiol* **107:**1375–1383.
82. **Feßler AT, Billerbeck C, Kadlec K, Schwarz S.** 2010. Identification and characterization of methicillin-resistant coagulase-negative staphylococci from bovine mastitis. *J Antimicrob Chemother* **65:**1576–1582.
83. **Vanderhaeghen W, Vandendriessche S, Crombé F, Nemeghaire S, Dispas M, Denis O, Hermans K, Haesebrouck F, Butaye P.** 2013. Characterization of methicillin-resistant non-*Staphylococcus aureus* staphylococci carriage isolates from different bovine populations. *J Antimicrob Chemother* **68:**300–307.
84. **Tulinski P, Fluit AC, Wagenaar JA, Mevius D, van de Vijver L, Duim B.** 2012. Methicillin-resistant coagulase-negative staphylococci on pig farms as a reservoir of heterogeneous staphylococcal cassette chromosome *mec* elements. *Appl Environ Microbiol* **78:**299–304.
85. **Wang Y, He T, Schwarz S, Zhao Q, Shen Z, Wu C, Shen J.** 2013. Multidrug resistance gene *cfr* in methicillin-resistant coagulase-negative staphylococci from chickens, ducks, and pigs in China. *Int J Med Microbiol* **303:** 84–87.
86. **Kawano J, Shimizu A, Saitoh Y, Yagi M, Saito T, Okamoto R.** 1996. Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. *J Clin Microbiol* **34:**2072–2077.
87. **Yasuda R, Kawano J, Matsuo E, Masuda T, Shimizu A, Anzai T, Hashikura S.** 2002. Distribution of *mecA*-harboring staphylococci in healthy mares. *J Vet Med Sci* **64:**821–827.
88. **Schnellmann C, Gerber V, Rossano A, Jaquier V, Panchaud Y, Doherr MG, Thomann A, Straub R, Perreten V.** 2006. Presence of new *mecA* and *mph*(C) variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *J Clin Microbiol* **44:**4444–4454.
89. **Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L.** 2007. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. *Vet Microbiol* **121:**307–315.
90. **De Martino L, Lucido M, Mallardo K, Facello B, Mallardo M, Iovane G, Pagnini U, Tufano MA, Catalanotti P.** 2010. Methicillin-resistant staphylococci isolated from healthy horses and horse personnel in Italy. *J Vet Diagn Invest* **22:**77–82.
91. **van Duijkeren E, Box AT, Heck ME, Wannet WJ, Fluit AC.** 2004. Methicillin-resistant staphylococci isolated from animals. *Vet Microbiol* **103:**91–97.
92. **Kania SA, Williamson NL, Frank LA, Wilkes RP, Jones RD, Bemis DA.** 2004. Methicillin resistance of staphylococci isolated from the skin of dogs with pyoderma. *Am J Vet Res* **65:**1265–1268.
93. **Monecke S, Müller E, Schwarz S, Hotzel H, Ehricht R.** 2012. Rapid microarray-based identification of different *mecA* alleles in staphylococci. *Antimicrob Agents Chemother* **56:**5547–5554.
94. **García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA.** 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis* **11:**595–603.
95. **Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC.** 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal com-

plex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **55:**3765–3773.

96. **Laurent F, Chardon H, Haenni M, Bes M, Reverdy ME, Madec JY, Lagier E, Vandenesch F, Tristan A.** 2012. MRSA harboring *mecA* variant gene *mecC*, France. *Emerg Infect Dis* **18:**1465–1467.
97. **Medhus A, Slettemeås J, Marstein L, Larssen K, Sunde M.** 2013. MRSA with the novel *mecC* gene variant isolated from a cat suffering from chronic conjunctivitis. *J Antimicrob Chemother* **68:**968–969.
98. **Paterson GK, Larsen AR, Robb A, Edwards GE, Pennycott TW, Foster G, Mot D, Hermans K, Baert K, Peacock SJ, Parkhill J, Zadoks RN, Holmes MA.** 2012. The newly described *mecA* homologue, $mecA_{LGA251}$, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. *J Antimicrob Chemother* **67:**2809–2813.
99. **Robb A, Pennycott T, Duncan G, Foster G.** 2013. *Staphylococcus aureus* carrying divergent *mecA* homologue ($mecA_{LGA251}$) isolated from a free-ranging wild bird. *Vet Microbiol* **162:**300–301.
100. **Walther B, Wieler LH, Vincze S, Antão E-M, Brandenburg A, Stamm I, Kopp PA, Kohn B, Semmler T, Lübke-Becker A.** 2012. MRSA variant in companion animals. *Emerg Infect Dis* **18:**2017–2020.
101. **Loncaric I, Kübber-Heiss A, Posautz A, Stalder GL, Hoffmann D, Rosengarten R, Walzer C.** 2013. Characterization of methicillin-resistant *Staphylococcus* spp. carrying the *mecC* gene, isolated from wildlife. *J Antimicrob Chemother* **68:**2222–2225.
102. **Gómez P, González-Barrio D, Benito D, García JT, Viñuela J, Zarazaga M, Ruiz-Fons F, Torres C.** 2014. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in wild small mammals in Spain. *J Antimicrob Chemother* **69:**2061–2064.
103. **Gómez P, Lozano C, González-Barrio D, Zarazaga M, Ruiz-Fons F, Torres C.** 2015. High prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in a semi-extensive red deer (*Cervus elaphus hispanicus*) farm in Southern Spain. *Vet Microbiol* **177:**326–331.
104. **Gómez P, Lozano C, Camacho MC, Lima-Barbero JF, Hernández JM, Zarazaga M, Höfle U, Torres C.** 2016. Detection of MRSA ST3061-t843-*mecC* and ST398-t011-*mecA* in white stork nestlings exposed to human residues. *J Antimicrob Chemother* **71:**53–57.
105. **Paterson GK, Harrison EM, Holmes MA.** 2014. The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol* **22:**42–47.
106. **Harrison EM, Paterson GK, Holden MT, Morgan FJ, Larsen AR, Petersen A, Leroy S, De Vliegher S, Perreten V, Fox LK, Lam TJ, Sampimon OC, Zadoks RN, Peacock SJ, Parkhill J, Holmes MA.** 2013. A *Staphylococcus xylosus* isolate with a new *mecC* allotype. *Antimicrob Agents Chemother* **57:**1524–1528.
107. **Małyszko I, Schwarz S, Hauschild T.** 2014. Detection of a new *mecC* allotype, *mecC2*, in methicillin-resistant *Staphylococcus saprophyticus*. *J Antimicrob Chemother* **69:**2003–2005.
108. **Becker K, van Alen S, Idelevich EA, Schleimer N, Seggewiß J, Mellmann A, Kaspar U, Peters G.** 2018. Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. *Emerg Infect Dis* **24:**242–248.
109. **Ba X, Harrison EM, Edwards GF, Holden MT, Larsen AR, Petersen A, Skov RL, Peacock SJ, Parkhill J, Paterson GK, Holmes MA.** 2014. Novel mutations in penicillin-binding protein genes in clinical *Staphylococcus aureus* isolates that are methicillin resistant on susceptibility testing, but lack the *mec* gene. *J Antimicrob Chemother* **69:**594–597.
110. **Berger-Bächi B, Tschierske M.** 1998. Role of *fem* factors in methicillin resistance. *Drug Resist Updat* **1:** 325–335.
111. **Roberts MC.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev* **19:**1–24.
112. **Schwarz S, Roberts MC, Werckenthin C, Pang Y, Lange C.** 1998. Tetracycline resistance in *Staphylococcus* spp. from domestic animals. *Vet Microbiol* **63:**217–227.
113. **Khan SA, Novick RP.** 1983. Complete nucleotide sequence of pT181, a tetracycline-resistance plasmid from *Staphylococcus aureus*. *Plasmid* **10:**251–259.
114. **Schwarz S, Shen J, Wendlandt S, Feßler AT, Wang Y, Kadlec K, Wu CM.** 2014. Plasmid-mediated antimicrobial resistance in staphylococci and other firmicutes. *Microbiol Spectr* **2:** 10.1128/microbiolspec.PLAS-0020-2014.
115. **Schwarz S, Blobel H.** 1990. Isolation and restriction endonuclease analysis of a tetracycline resistance plasmid from *Staphylococcus hyicus*. *Vet Microbiol* **24:**113–122.
116. **Schwarz S, Cardoso M, Grölz-Krug S, Blobel H.** 1990. Common antibiotic resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis* from human and canine infections. *Zentralbl Bakteriol* **273:**369–377.
117. **Greene RT, Schwarz S.** 1992. Small antibiotic resistance plasmids in *Staphylococcus intermedius*. *Zentralbl Bakteriol* **276:**380–389.
118. **Schwarz S, Noble WC.** 1994. Tetracycline resistance genes in staphylococci from the skin of pigs. *J Appl Bacteriol* **76:**320–326.
119. **Hauschild T, Kehrenberg C, Schwarz S.** 2003. Tetracycline resistance in staphylococci from free-living rodents and insectivores. *J Vet Med B Infect Dis Vet Public Health* **50:**443–446.
120. **Hauschild T, Stepanović S, Dakić I, Djukić S, Ranin L, Jezek P, Schwarz S.** 2007. Tetracycline resistance and distribution of *tet* genes in members of the *Staphylococcus sciuri* group isolated from humans, animals and different environmental sources. *Int J Antimicrob Agents* **29:**356–358.
121. **Larsen J, Clasen J, Hansen JE, Paulander W, Petersen A, Larsen AR, Frees D.** 2016. Copresence of *tet*(K) and *tet*(M) in livestock-associated methicillin-resistant *Staphylococcus aureus* clonal complex 398 is associated with increased fitness during exposure to sublethal con-

centrations of tetracycline. *Antimicrob Agents Chemother* **60:**4401–4403.

122. Hauschild T, Lüthje P, Schwarz S. 2005. Staphylococcal tetracycline-MLS_B resistance plasmid pSTE2 is the product of an RS_A-mediated *in vivo* recombination. *J Antimicrob Chemother* **56:**399–402.

123. Kadlec K, Schwarz S. 2009. Identification of a novel trimethoprim resistance gene, *dfrK*, in a methicillin-resistant *Staphylococcus aureus* ST398 strain and its physical linkage to the tetracycline resistance gene *tet*(L). *Antimicrob Agents Chemother* **53:**776–778.

124. Kadlec K, Schwarz S. 2009. Novel ABC transporter gene, *vga*(C), located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* **53:**3589–3591.

125. Kadlec K, Schwarz S. 2010. Identification of a plasmid-borne resistance gene cluster comprising the resistance genes *erm*(T), *dfrK*, and *tet*(L) in a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* **54:**915–918.

126. Feßler AT, Zhao Q, Schoenfelder S, Kadlec K, Brenner Michael G, Wang Y, Ziebuhr W, Shen J, Schwarz S. 2017. Complete sequence of a plasmid from a bovine methicillin-resistant *Staphylococcus aureus* harbouring a novel *ica*-like gene cluster in addition to antimicrobial and heavy metal resistance genes. *Vet Microbiol* **200:** 95–100.

127. Schwarz S, Cardoso M, Wegener HC. 1992. Nucleotide sequence and phylogeny of the *tet*(L) tetracycline resistance determinant encoded by plasmid pSTE1 from *Staphylococcus hyicus*. *Antimicrob Agents Chemother* **36:**580–588.

128. Schwarz S, Gregory PD, Werckenthin C, Curnock S, Dyke KG. 1996. A novel plasmid from *Staphylococcus epidermidis* specifying resistance to kanamycin, neomycin and tetracycline. *J Med Microbiol* **45:**57–63.

129. Schwarz S, Wang Z. 1993. Tetracycline resistance in *Staphylococcus intermedius*. *Lett Appl Microbiol* **17:** 88–91.

130. Weiß S, Kadlec K, Feßler AT, Schwarz S. 2014. Complete sequence of a multiresistance plasmid from a methicillin-resistant *Staphylococcus epidermidis* ST5 isolated in a small animal clinic. *J Antimicrob Chemother* **69:**847–859.

131. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* **28:** 519–542.

132. Horinouchi S, Weisblum B. 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. *J Bacteriol* **150:** 815–825.

133. Brenner DG, Shaw WV. 1985. The use of synthetic oligonucleotides with universal templates for rapid DNA sequencing: results with staphylococcal replicon pC221. *EMBO J* **4:**561–568.

134. Projan SJ, Kornblum J, Moghazeh SL, Edelman I, Gennaro ML, Novick RP. 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. *Mol Gen Genet* **199:**452–464.

135. Smith MC, Thomas CD. 2004. An accessory protein is required for relaxosome formation by small staphylococcal plasmids. *J Bacteriol* **186:**3363–3373.

136. Cardoso M, Schwarz S. 1992. Chloramphenicol resistance plasmids in *Staphylococcus aureus* isolated from bovine subclinical mastitis. *Vet Microbiol* **30:**223–232.

137. Cardoso M, Schwarz S. 1992. Nucleotide sequence and structural relationships of a chloramphenicol acetyltransferase encoded by the plasmid pSCS6 from *Staphylococcus aureus*. *J Appl Bacteriol* **72:**289–293.

138. Schwarz S, Spies U, Cardoso M. 1991. Cloning and sequence analysis of a plasmid-encoded chloramphenicol acetyltransferase gene from *Staphylococcus intermedius*. *J Gen Microbiol* **137:**977–981.

139. Schwarz S, Werckenthin C, Pinter L, Kent LE, Noble WC. 1995. Chloramphenicol resistance in *Staphylococcus intermedius* from a single veterinary centre: evidence for plasmid and chromosomal location of the resistance genes. *Vet Microbiol* **43:**151–159.

140. Schwarz S, Cardoso M, Blobel H. 1989. Plasmid-mediated chloramphenicol resistance in *Staphylococcus hyicus*. *J Gen Microbiol* **135:**3329–3336.

141. Schwarz S, Noble WC. 1994. Structure and putative origin of a plasmid from *Staphylococcus hyicus* that mediates chloramphenicol and streptomycin resistance. *Lett Appl Microbiol* **18:**281–284.

142. Schwarz S, Cardoso M, Blobel H. 1990. Detection of a novel chloramphenicol resistance plasmid from "equine" *Staphylococcus sciuri*. *Zentralbl Veterinarmed B* **37:** 674–679.

143. Hauschild T, Stepanović S, Vuković D, Dakić I, Schwarz S. 2009. Occurrence of chloramphenicol resistance and corresponding resistance genes in members of the *Staphylococcus sciuri* group. *Int J Antimicrob Agents* **33:**383–384.

144. Schwarz S, Grölz-Krug S. 1991. A chloramphenicol-streptomycin-resistance plasmid from a clinical strain of *Staphylococcus sciuri* and its structural relationships to other staphylococcal resistance plasmids. *FEMS Microbiol Lett* **66:**319–322.

145. Schwarz S. 1994. Emerging chloramphenicol resistance in *Staphylococcus lentus* from mink following chloramphenicol treatment: characterisation of the resistance genes. *Vet Microbiol* **41:**51–61.

146. Schwarz S, Cardoso M. 1991. Molecular cloning, purification, and properties of a plasmid-encoded chloramphenicol acetyltransferase from *Staphylococcus haemolyticus*. *Antimicrob Agents Chemother* **35:**1277–1283.

147. Wendlandt S, Kadlec K, Schwarz S. 2015. Four novel plasmids from *Staphylococcus hyicus* and CoNS that carry a variant of the spectinomycin resistance gene *spd*. *J Antimicrob Chemother* **70:**948–949.

148. Kehrenberg C, Schwarz S. 2004. *fexA*, a novel *Staphylococcus lentus* gene encoding resistance to florfenicol and chloramphenicol. *Antimicrob Agents Chemother* **48:**615–618.

149. **Kehrenberg C, Schwarz S.** 2005. Florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn*558*. *Antimicrob Agents Chemother* **49:** 813–815.

150. **Kehrenberg C, Cuny C, Strommenger B, Schwarz S, Witte W.** 2009. Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr*. *Antimicrob Agents Chemother* **53:**779–781.

151. **Wang Y, Zhang W, Wang J, Wu C, Shen Z, Fu X, Yan Y, Zhang Q, Schwarz S, Shen J.** 2012. Distribution of the multidrug resistance gene *cfr* in *Staphylococcus* species isolates from swine farms in China. *Antimicrob Agents Chemother* **56:**1485–1490.

152. **Kehrenberg C, Schwarz S.** 2006. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob Agents Chemother* **50:**1156–1163.

153. **Kehrenberg C, Aarestrup FM, Schwarz S.** 2007. IS*21-558* insertion sequences are involved in the mobility of the multiresistance gene *cfr*. *Antimicrob Agents Chemother* **51:**483–487.

154. **Gómez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, Schwarz S.** 2013. A novel *fexA* variant from a canine *Staphylococcus pseudintermedius* isolate that does not confer florfenicol resistance. *Antimicrob Agents Chemother* **57:**5763–5766.

155. **Ramirez MS, Tolmasky ME.** 2010. Aminoglycoside modifying enzymes. *Drug Resist Updat* **13:**151–171.

156. **Ferretti JJ, Gilmore KS, Courvalin P.** 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. *J Bacteriol* **167:**631–638.

157. **Rouch DA, Byrne ME, Kong YC, Skurray RA.** 1987. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn*4001* from *Staphylococcus aureus*: expression and nucleotide sequence analysis. *J Gen Microbiol* **133:**3039–3052.

158. **Byrne ME, Rouch DA, Skurray RA.** 1989. Nucleotide sequence analysis of IS*256* from the *Staphylococcus aureus* gentamicin-tobramycin-kanamycin-resistance transposon Tn*4001*. *Gene* **81:**361–367.

159. **Lange CC, Werckenthin C, Schwarz S.** 2003. Molecular analysis of the plasmid-borne *aacA/aphD* resistance gene region of coagulase-negative staphylococci from chickens. *J Antimicrob Chemother* **51:**1397–1401.

160. **Schwarz S, Kadlec K, Strommenger B.** 2008. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004–2006 in Germany. *J Antimicrob Chemother* **61:**282–285.

161. **Wendlandt S, Li B, Ma Z, Schwarz S.** 2013. Complete sequence of the multi-resistance plasmid pV7037 from a porcine methicillin-resistant *Staphylococcus aureus*. *Vet Microbiol* **166:**650–654.

162. **Li B, Wendlandt S, Yao J, Liu Y, Zhang Q, Shi Z, Wei J, Shao D, Schwarz S, Wang S, Ma Z.** 2013. Detection and new genetic environment of the pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) in methicillin-resistant *Staphylococcus aureus* of swine origin. *J Antimicrob Chemother* **68:**1251–1255.

163. **Turutoglu H, Hasoksuz M, Ozturk D, Yildirim M, Sagnak S.** 2009. Methicillin and aminoglycoside resistance in *Staphylococcus aureus* isolates from bovine mastitis and sequence analysis of their *mecA* genes. *Vet Res Commun* **33:**945–956.

164. **Argudín MA, Vanderhaeghen W, Butaye P.** 2015. Diversity of antimicrobial resistance and virulence genes in methicillin-resistant non-*Staphylococcus aureus* staphylococci from veal calves. *Res Vet Sci* **99:**10–16.

165. **Cuny C, Kuemmerle J, Stanek C, Willey B, Strommenger B, Witte W.** 2006. Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. *Euro Surveill* **11:**44–47.

166. **Sidhu MS, Oppegaard H, Devor TP, Sørum H.** 2007. Persistence of multidrug-resistant *Staphylococcus haemolyticus* in an animal veterinary teaching hospital clinic. *Microb Drug Resist* **13:**271–280.

167. **He T, Wang Y, Schwarz S, Zhao Q, Shen J, Wu C.** 2014. Genetic environment of the multi-resistance gene *cfr* in methicillin-resistant coagulase-negative staphylococci from chickens, ducks, and pigs in China. *Int J Med Microbiol* **304:**257–261.

168. **McKenzie T, Hoshino T, Tanaka T, Sueoka N.** 1987. Correction. A revision of the nucleotide sequence and functional map of pUB110. *Plasmid* **17:**83–85.

169. **Hauschild T, Vuković D, Dakić I, Jezek P, Djukić S, Dimitrijević V, Stepanović S, Schwarz S.** 2007. Aminoglycoside resistance in members of the *Staphylococcus sciuri* group. *Microb Drug Resist* **13:**77–84.

170. **Derbise A, Dyke KG, el Solh N.** 1996. Characterization of a *Staphylococcus aureus* transposon, Tn*5405*, located within Tn*5404* and carrying the aminoglycoside resistance genes, *aphA-3* and *aadE*. *Plasmid* **35:**174–188.

171. **Boerlin P, Burnens AP, Frey J, Kuhnert P, Nicolet J.** 2001. Molecular epidemiology and genetic linkage of macrolide and aminoglycoside resistance in *Staphylococcus intermedius* of canine origin. *Vet Microbiol* **79:** 155–169.

172. **Ben Zakour NL, Bannoehr J, van den Broek AH, Thoday KL, Fitzgerald JR.** 2011. Complete genome sequence of the canine pathogen *Staphylococcus pseudintermedius*. *J Bacteriol* **193:**2363–2364.

173. **Wendlandt S, Lozano C, Kadlec K, Gómez-Sanz E, Zarazaga M, Torres C, Schwarz S.** 2013. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **68:**473–475.

174. **Wendlandt S, Li J, Ho J, Porta MA, Feßler AT, Wang Y, Kadlec K, Monecke S, Ehricht R, Boost M, Schwarz S.** 2014. Enterococcal multiresistance gene cluster in methicillin-resistant *Staphylococcus aureus* from vari-

ous origins and geographical locations. *J Antimicrob Chemother* **69:**2573–2575.

175. **Projan SJ, Moghazeh S, Novick RP.** 1988. Nucleotide sequence of pS194, a streptomycin-resistance plasmid from *Staphylococcus aureus*. *Nucleic Acids Res* **16:** 2179–2187.
176. **Schwarz S, Blobel H.** 1990. A new streptomycin-resistance plasmid from *Staphylococcus hyicus* and its structural relationship to other staphylococcal resistance plasmids. *J Med Microbiol* **32:**201–205.
177. **Stegmann R, Perreten V.** 2010. Antibiotic resistance profile of *Staphylococcus rostri*, a new species isolated from healthy pigs. *Vet Microbiol* **145:**165–171.
178. **Murphy E.** 1985. Nucleotide sequence of a spectinomycin adenyltransferase AAD(9) determinant from *Staphylococcus aureus* and its relationship to AAD(3´´) (9). *Mol Gen Genet* **200:**33–39.
179. **Feßler AT, Kadlec K, Hassel M, Hauschild T, Eidam C, Ehricht R, Monecke S, Schwarz S.** 2011. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. *Appl Environ Microbiol* **77:**7151–7157.
180. **Kehrenberg C, Ojo KK, Schwarz S.** 2004. Nucleotide sequence and organization of the multiresistance plasmid pSCFS1 from *Staphylococcus sciuri*. *J Antimicrob Chemother* **54:**936–939.
181. **Wendlandt S, Li B, Lozano C, Ma Z, Torres C, Schwarz S.** 2013. Identification of the novel spectinomycin resistance gene *spw* in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* of human and animal origin. *J Antimicrob Chemother* **68:**1679–1680.
182. **Jamrozy DM, Coldham NG, Butaye P, Fielder MD.** 2014. Identification of a novel plasmid-associated spectinomycin adenyltransferase gene *spd* in methicillin-resistant *Staphylococcus aureus* ST398 isolated from animal and human sources. *J Antimicrob Chemother* **69:**1193–1196.
183. **Wendlandt S, Feßler AT, Kadlec K, van Duijkeren E, Schwarz S.** 2014. Identification of the novel spectinomycin resistance gene *spd* in a different plasmid background among methicillin-resistant *Staphylococcus aureus* CC398 and methicillin-susceptible *S. aureus* ST433. *J Antimicrob Chemother* **69:**2000–2003.
184. **Feßler AT, Kadlec K, Schwarz S.** 2011. Novel apramycin resistance gene *apmA* in bovine and porcine methicillin-resistant *Staphylococcus aureus* ST398 isolates. *Antimicrob Agents Chemother* **55:**373–375.
185. **Kadlec K, Feßler AT, Couto N, Pomba CF, Schwarz S.** 2012. Unusual small plasmids carrying the novel resistance genes *dfrK* or *apmA* isolated from methicillin-resistant or -susceptible staphylococci. *J Antimicrob Chemother* **67:**2342–2345.
186. **Aarestrup FM, Agersø Y, Ahrens P, Jørgensen JC, Madsen M, Jensen LB.** 2000. Antimicrobial susceptibility and presence of resistance genes in staphylococci from poultry. *Vet Microbiol* **74:**353–364.
187. **Lüthje P, Schwarz S.** 2007. Molecular basis of resistance to macrolides and lincosamides among staphylococci and streptococci from various animal sources collected in the resistance monitoring program BfT-GermVet. *Int J Antimicrob Agents* **29:**528–535.
188. **Eady EA, Ross JI, Tipper JL, Walters CE, Cove JH, Noble WC.** 1993. Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. *J Antimicrob Chemother* **31:**211–217.
189. **Werckenthin C, Schwarz S.** 2000. Molecular analysis of the translational attenuator of a constitutively expressed *erm*(A) gene from *Staphylococcus intermedius*. *J Antimicrob Chemother* **46:**785–788.
190. **Nawaz MS, Khan SA, Khan AA, Khambaty FM, Cerniglia CE.** 2000. Comparative molecular analysis of erythromycin-resistance determinants in staphylococcal isolates of poultry and human origin. *Mol Cell Probes* **14:**311–319.
191. **Jaglic Z, Vlkova H, Bardon J, Michu E, Cervinkova D, Babak V.** 2012. Distribution, characterization and genetic bases of erythromycin resistance in staphylococci and enterococci originating from livestock. *Zoonoses Public Health* **59:**202–211.
192. **Werckenthin C, Schwarz S, Dyke K.** 1996. Macrolide-lincosamide-streptogramin B resistance in *Staphylococcus lentus* results from the integration of part of a transposon into a small plasmid. *Antimicrob Agents Chemother* **40:**2224–2225.
193. **Lüthje P, Schwarz S.** 2006. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. *J Antimicrob Chemother* **57:**966–969.
194. **Li L, Feng W, Zhang Z, Xue H, Zhao X.** 2015. Macrolide-lincosamide-streptogramin resistance phenotypes and genotypes of coagulase-positive *Staphylococcus aureus* and coagulase-negative staphylococcal isolates from bovine mastitis. *BMC Vet Res* **11:**168.
195. **Lodder G, Werckenthin C, Schwarz S, Dyke K.** 1997. Molecular analysis of naturally occuring *ermC*-encoding plasmids in staphylococci isolated from animals with and without previous contact with macrolide/lincosamide antibiotics. *FEMS Immunol Med Microbiol* **18:**7–15.
196. **Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, Witte W, Schwarz S.** 2006. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *J Antimicrob Chemother* **57:**461–465.
197. **Wendlandt S, Kadlec K, Feßler AT, van Duijkeren E, Schwarz S.** 2014. Two different *erm*(C)-carrying plasmids in the same methicillin-resistant *Staphylococcus aureus* CC398 isolate from a broiler farm. *Vet Microbiol* **171:**382–387.
198. **Vancraeynest D, Hermans K, Martel A, Vaneechoutte M, Devriese LA, Haesebrouck F.** 2004. Antimicrobial resistance and resistance genes in *Staphylococcus aureus* strains from rabbits. *Vet Microbiol* **101:**245–251.
199. **Schwarz S, Wegener H, Blobel H.** 1990. Plasmid-encoded resistance to macrolides and lincosamides in *Staphylococcus hyicus*. *J Appl Bacteriol* **69:**845–849.

200. **Schwarz S, Lange C, Werckenthin C.** 1998. Molecular analysis of the macrolide-lincosamide resistance gene region of a novel plasmid from *Staphylococcus hyicus*. *J Med Microbiol* **47:**63–70.

201. **Hauschild T, Lüthje P, Schwarz S.** 2006. Characterization of a novel type of MLS_B resistance plasmid from *Staphylococcus saprophyticus* carrying a constitutively expressed *erm*(C) gene. *Vet Microbiol* **115:**258–263.

202. **Hauschild T, Schwarz S.** 2010. Macrolide resistance in *Staphylococcus* spp. from free-living small mammals. *Vet Microbiol* **144:**530–531.

203. **Schwarz S, Blobel H.** 1990. Isolation of a plasmid from "canine" *Staphylococcus epidermidis* mediating constitutive resistance to macrolides and lincosamides. *Comp Immunol Microbiol Infect Dis* **13:**209–216.

204. **Lodder G, Schwarz S, Gregory P, Dyke K.** 1996. Tandem duplication in *ermC* translational attenuator of the macrolide-lincosamide-streptogramin B resistance plasmid pSES6 from *Staphylococcus equorum*. *Antimicrob Agents Chemother* **40:**215–217.

205. **Chung WO, Werckenthin C, Schwarz S, Roberts MC.** 1999. Host range of the *ermF* rRNA methylase gene in bacteria of human and animal origin. *J Antimicrob Chemother* **43:**5–14.

206. **Schwarz S, Kehrenberg C, Ojo KK.** 2002. *Staphylococcus sciuri* gene *erm*(33), encoding inducible resistance to macrolides, lincosamides, and streptogramin B antibiotics, is a product of recombination between *erm*(C) and *erm*(A). *Antimicrob Agents Chemother* **46:**3621–3623.

207. **Li D, Wu C, Wang Y, Fan R, Schwarz S, Zhang S.** 2015. Identification of multiresistance gene *cfr* in methicillin-resistant *Staphylococcus aureus* from pigs: plasmid location and integration into a staphylococcal cassette chromosome *mec* complex. *Antimicrob Agents Chemother* **59:**3641–3644.

208. **Schwendener S, Perreten V.** 2012. New MLS_B resistance gene *erm*(43) in *Staphylococcus lentus*. *Antimicrob Agents Chemother* **56:**4746–4752.

209. **Wipf JR, Schwendener S, Perreten V.** 2014. The novel macrolide-lincosamide-streptogramin B resistance gene *erm*(44) is associated with a prophage in *Staphylococcus xylosus*. *Antimicrob Agents Chemother* **58:** 6133–6138.

210. **Wendlandt S, Heß S, Li J, Feßler AT, Wang Y, Kadlec K, Gallert C, Schwarz S.** 2015. Detection of the macrolide-lincosamide-streptogramin B resistance gene *erm*(44) and a novel *erm*(44) variant in staphylococci from aquatic environments. *FEMS Microbiol Ecol* **91:** fiv090.

211. **Wipf JR, Schwendener S, Nielsen JB, Westh H, Perreten V.** 2015. The new macrolide-lincosamide-streptogramin B resistance gene *erm*(45) is located within a genomic island in *Staphylococcus fleurettii*. *Antimicrob Agents Chemother* **59:**3578–3581.

212. **Wipf JRK, Riley MC, Kania SA, Bemis DA, Andreis S, Schwendener S, Perreten V.** 2017. New macrolide-lincosamide-streptogramin B resistance gene *erm*(48) on the novel plasmid pJW2311 in *Staphylococcus xylosus*. *Antimicrob Agents Chemother* **61:**e00066–e17.

213. **Ross JI, Eady EA, Cove JH, Cunliffe WJ, Baumberg S, Wootton JC.** 1990. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Mol Microbiol* **4:** 1207–1214.

214. **Sharkey LK, Edwards TA, O'Neill AJ.** 2016. ABC-F proteins mediate antibiotic resistance through ribosomal protection. *MBio* **7:**e01975.

215. **Matsuoka M, Endou K, Kobayashi H, Inoue M, Nakajima Y.** 1998. A plasmid that encodes three genes for resistance to macrolide antibiotics in *Staphylococcus aureus*. *FEMS Microbiol Lett* **167:**221–227.

216. **Brisson-Noël A, Courvalin P.** 1986. Nucleotide sequence of gene *linA* encoding resistance to lincosamides in *Staphylococcus haemolyticus*. *Gene* **43:**247–253.

217. **Loeza-Lara PD, Soto-Huipe M, Baizabal-Aguirre VM, Ochoa-Zarzosa A, Valdez-Alarcón JJ, Cano-Camacho H, López-Meza JE.** 2004. pBMSa1, a plasmid from a dairy cow isolate of *Staphylococcus aureus*, encodes a lincomycin resistance determinant and replicates by the rolling-circle mechanism. *Plasmid* **52:**48–56.

218. **Lozano C, Aspiroz C, Sáenz Y, Ruiz-García M, Royo-García G, Gómez-Sanz E, Ruiz-Larrea F, Zarazaga M, Torres C.** 2012. Genetic environment and location of the *lnu*(A) and *lnu*(B) genes in methicillin-resistant *Staphylococcus aureus* and other staphylococci of animal and human origin. *J Antimicrob Chemother* **67:** 2804–2808.

219. **Lozano C, Aspiroz C, Rezusta A, Gómez-Sanz E, Simon C, Gómez P, Ortega C, Revillo MJ, Zarazaga M, Torres C.** 2012. Identification of novel *vga*(A)-carrying plasmids and a Tn*5406*-like transposon in meticillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* of human and animal origin. *Int J Antimicrob Agents* **40:**306–312.

220. **Lüthje P, von Köckritz-Blickwede M, Schwarz S.** 2007. Identification and characterization of nine novel types of small staphylococcal plasmids carrying the lincosamide nucleotidyltransferase gene *lnu*(A). *J Antimicrob Chemother* **59:**600–606.

221. **Zhao Q, Wendlandt S, Li H, Li J, Wu C, Shen J, Schwarz S, Wang Y.** 2014. Identification of the novel lincosamide resistance gene *lnu*(E) truncated by IS*Enfa5*-*cfr*-IS*Enfa5* insertion in *Streptococcus suis*: *de novo* synthesis and confirmation of functional activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **58:**1785–1788.

222. **Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H.** 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* **43:**2823–2830.

223. **Allignet J, El Solh N.** 1997. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. *Gene* **202:**133–138.

224. **Haroche J, Allignet J, Buchrieser C, El Solh N.** 2000. Characterization of a variant of *vga*(A) conferring resistance to streptogramin A and related compounds. *Antimicrob Agents Chemother* **44:**2271–2275.

225. **Gentry DR, McCloskey L, Gwynn MN, Rittenhouse SF, Scangarella N, Shawar R, Holmes DJ.** 2008. Genetic characterization of Vga ABC proteins conferring reduced susceptibility of *Staphylococcus aureus* to pleuromutilins. *Antimicrob Agents Chemother* **52:**4507–4509.

226. **Schwendener S, Perreten V.** 2011. New transposon Tn*6133* in methicillin-resistant *Staphylococcus aureus* ST398 contains *vga*(E), a novel streptogramin A, pleuromutilin, and lincosamide resistance gene. *Antimicrob Agents Chemother* **55:**4900–4904.

227. **Hauschild T, Feßler AT, Kadlec K, Billerbeck C, Schwarz S.** 2012. Detection of the novel *vga*(E) gene in methicillin-resistant *Staphylococcus aureus* CC398 isolates from cattle and poultry. *J Antimicrob Chemother* **67:**503–504.

228. **Kadlec K, Pomba CF, Couto N, Schwarz S.** 2010. Small plasmids carrying *vga*(A) or *vga*(C) genes mediate resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-resistant *Staphylococcus aureus* ST398 from swine. *J Antimicrob Chemother* **65:** 2692–2693.

229. **Novotna G, Janata J.** 2006. A new evolutionary variant of the streptogramin A resistance protein, $Vga(A)_{LC}$, from *Staphylococcus haemolyticus* with shifted substrate specificity towards lincosamides. *Antimicrob Agents Chemother* **50:**4070–4076.

230. **Wendlandt S, Kadlec K, Feßler AT, Schwarz S.** 2015. Identification of ABC transporter genes conferring combined pleuromutilin-lincosamide-streptogramin A resistance in bovine methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *Vet Microbiol* **177:**353–358.

231. **Li J, Li B, Wendlandt S, Schwarz S, Wang Y, Wu C, Ma Z, Shen J.** 2014. Identification of a novel *vga*(E) gene variant that confers resistance to pleuromutilins, lincosamides and streptogramin A antibiotics in staphylococci of porcine origin. *J Antimicrob Chemother* **69:** 919–923.

232. **Deng F, Wang H, Liao Y, Li J, Feßler AT, Michael GB, Schwarz S, Wang Y.** 2017. Detection and genetic environment of pleuromutilin-lincosamide-streptogramin A resistance genes in staphylococci isolated from pets. *Front Microbiol* **8:**234.

233. **Hot C, Berthet N, Chesneau O.** 2014. Characterization of *sal*(A), a novel gene responsible for lincosamide and streptogramin A resistance in *Staphylococcus sciuri*. *Antimicrob Agents Chemother* **58:**3335–3341.

234. **Schwarz S, Werckenthin C, Kehrenberg C.** 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrob Agents Chemother* **44:**2530–2533.

235. **Kehrenberg C, Schwarz S, Jacobsen L, Hansen LH, Vester B.** 2005. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. *Mol Microbiol* **57:** 1064–1073.

236. **Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B.** 2006. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* **50:**2500–2505.

237. **Witte W, Cuny C.** 2011. Emergence and spread of *cfr*-mediated multiresistance in staphylococci: an interdisciplinary challenge. *Future Microbiol* **6:**925–931.

238. **Michael GB, Freitag C, Wendlandt S, Eidam C, Feßler AT, Lopes GV, Kadlec K, Schwarz S.** 2015. Emerging issues in antimicrobial resistance of bacteria from food-producing animals. *Future Microbiol* **10:**427–443.

239. **Shen J, Wang Y, Schwarz S.** 2013. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* **68:**1697–1706.

240. **Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Feßler AT, Wu C, Yu H, Deng X, Xia X, Shen J.** 2015. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* **70:**2182–2190.

241. **Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Feßler AT, Zhang R, Wu C, Shen J.** 2016. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. *J Antimicrob Chemother* **71:**1474–1478.

242. **Fan R, Li D, Wang Y, He T, Feßler AT, Schwarz S, Wu C.** 2016. Presence of the *optrA* gene in methicillin-resistant *Staphylococcus sciuri* of porcine origin. *Antimicrob Agents Chemother* **60:**7200–7205.

243. **Fan R, Li D, Feßler AT, Wu C, Schwarz S, Wang Y.** 2017. Distribution of *optrA* and *cfr* in florfenicol-resistant *Staphylococcus sciuri* of pig origin. *Vet Microbiol* **210:**43–48.

244. **Sun C, Zhang P, Ji X, Fan R, Chen B, Wang Y, Schwarz S, Wu C.** Presence and molecular characteristics of oxazolidinone resistance in staphylococci from household animals in rural China. *J Antimicrob Chemother*. (Epub ahead of print.)

245. **Kadlec K, Schwarz S.** 2010. Identification of the novel *dfrK*-carrying transposon Tn*559* in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* **54:**3475–3477.

246. **O'Neill AJ, McLaws F, Kahlmeter G, Henriksen AS, Chopra I.** 2007. Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrob Agents Chemother* **51:**1737–1740.

247. **O'Neill AJ, Chopra I.** 2006. Molecular basis of *fusB*-mediated resistance to fusidic acid in *Staphylococcus aureus*. *Mol Microbiol* **59:**664–676.

248. **Yazdankhah SP, Asli AW, Sørum H, Oppegaard H, Sunde M.** 2006. Fusidic acid resistance, mediated by *fusB*, in bovine coagulase-negative staphylococci. *J Antimicrob Chemother* **58:**1254–1256.

249. **Loeffler A, Baines SJ, Toleman MS, Felmingham D, Milsom SK, Edwards EA, Lloyd DH.** 2008. *In vitro* activity of fusidic acid and mupirocin against coagulase-positive staphylococci from pets. *J Antimicrob Chemother* **62:**1301–1304.

250. **Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS.** 1994. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob Agents Chemother* **38:**1205–1208.

251. **Needham C, Rahman M, Dyke KG, Noble WC.** 1994. An investigation of plasmids from *Staphylococcus aureus* that mediate resistance to mupirocin and tetracycline. *Microbiology* **140:**2577–2583.

252. **Fulham KS, Lemarie SL, Hosgood G, Dick HL.** 2011. *In vitro* susceptibility testing of meticillin-resistant and meticillin-susceptible staphylococci to mupirocin and novobiocin. *Vet Dermatol* **22:**88–94.

253. **Manian FA.** 2003. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clin Infect Dis* **36:** e26–e28.

254. **Matanovic K, Pérez-Roth E, Pintarić S, Šeol Martinec B.** 2013. Molecular characterization of high-level mupirocin resistance in *Staphylococcus pseudintermedius*. *J Clin Microbiol* **51:**1005–1007.

255. **Godbeer SM, Gold RM, Lawhon SD.** 2014. Prevalence of mupirocin resistance in *Staphylococcus pseudintermedius*. *J Clin Microbiol* **52:**1250–1252.

256. **Wipf JR, Perreten V.** 2016. Methicillin-resistant *Staphylococcus aureus* isolated from dogs and cats in Switzerland. *Schweiz Arch Tierheilkd* **158:**443–450.

257. **Hauschild T, Feßler AT, Billerbeck C, Wendlandt S, Kaspar H, Mankertz J, Schwarz S, Kadlec K.** 2012. Target gene mutations among methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus* with elevated MICs of enrofloxacin obtained from diseased food-producing animals or food of animal origin. *J Antimicrob Chemother* **67:**1791–1793.

258. **Li J, Feßler AT, Jiang N, Fan R, Wang Y, Wu C, Shen J, Schwarz S.** 2016. Molecular basis of rifampicin resistance in multiresistant porcine livestock-associated MRSA. *J Antimicrob Chemother* **71:**3313–3315.

259. **Kadlec K, van Duijkeren E, Wagenaar JA, Schwarz S.** 2011. Molecular basis of rifampicin resistance in methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. *J Antimicrob Chemother* **66:**1236–1242.

260. **Kwok GM, O'Donoghue MM, Doddangoudar VC, Ho J, Boost MV.** 2013. Reduced vancomycin susceptibility in porcine ST9 MRSA isolates. *Front Microbiol* **4:**316.

261. **Moreno LZ, Dutra MC, Moreno M, Ferreira TS, Silva GF, Matajira CE, Silva AP, Moreno AM.** 2016. Vancomycin-intermediate livestock-associated methicillin-resistant *Staphylococcus aureus* ST398/t9538 from swine in Brazil. *Mem Inst Oswaldo Cruz* **111:**659–661.

262. **Rensing C, Moodley A, Cavaco LM, McDevitt SF.** 2018. Resistance to metals used in agricultural production. *Microbiol Spectr* **6**(2).

263. **Witte W, Van Dip N, Hummel R.** 1980. Resistance against mercury and cadmium by *Staphylococcus aureus* of diverse ecological origin. *Z Allg Mikrobiol* **20:**517–521. (In German.)

264. **Schwarz S, Blobel H.** 1989. Plasmids and resistance to antimicrobial agents and heavy metals in *Staphylococcus hyicus* from pigs and cattle. *Zentralbl Veterinarmed B* **36:**669–673.

265. **Aarestrup FM, Cavaco L, Hasman H.** 2010. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. *Vet Microbiol* **142:**455–457.

266. **Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T, Aarestrup FM.** 2010. Cloning and occurrence of *czrC*, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. *Antimicrob Agents Chemother* **54:**3605–3608.

267. **Cavaco LM, Hasman H, Aarestrup FM, Wagenaar JA, Graveland H, Veldman K, Mevius D, Fetsch A, Tenhagen B-A, Concepcion Porrero M, Dominguez L, Granier SA, Jouy E, Butaye P, Kaszanyitzky E, Dán A, Zmudzki J, Battisti A, Franco A, Schwarz S, Gutierrez M, Weese JS, Cui S, Pomba C, Members of MRSA-CG.** 2011. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* **150:**344–348.

268. **Li S, Skov RL, Han X, Larsen AR, Larsen J, Sørum M, Wulf M, Voss A, Hiramatsu K, Ito T.** 2011. Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* **55:**3046–3050.

269. **Argudín MA, Lauzat B, Kraushaar B, Alba P, Agerso Y, Cavaco L, Butaye P, Porrero MC, Battisti A, Tenhagen BA, Fetsch A, Guerra B.** 2016. Heavy metal and disinfectant resistance genes among livestock-associated methicillin-resistant *Staphylococcus aureus* isolates. *Vet Microbiol* **191:**88–95.

270. **Argudín MA, Butaye P.** 2016. Dissemination of metal resistance genes among animal methicillin-resistant coagulase-negative staphylococci. *Res Vet Sci* **105:**192–194.

271. **Gómez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, Schwarz S.** 2013. Novel *erm*(T)-carrying multiresistance plasmids from porcine and human isolates of methicillin-resistant *Staphylococcus aureus* ST398 that also harbor cadmium and copper resistance determinants. *Antimicrob Agents Chemother* **57:**3275–3282.

272. **Maillard J-Y.** 2018. Resistance of bacteria to biocides. *Microbiol Spectr* **6**(2).

273. **Bjorland J, Steinum T, Kvitle B, Waage S, Sunde M, Heir E.** 2005. Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. *J Clin Microbiol* **43:**4363–4368.

274. **Seier-Petersen MA, Nielsen LN, Ingmer H, Aarestrup FM, Agersø Y.** 2015. Biocide susceptibility of *Staphylococcus aureus* CC398 and CC30 isolates from pigs and identification of the biocide resistance genes, *qacG* and *qacC*. *Microb Drug Resist* **21:**527–536.

275. **Couto N, Belas A, Kadlec K, Schwarz S, Pomba C.** 2015. Clonal diversity, virulence patterns and antimicrobial and biocide susceptibility among human, animal and environmental MRSA in Portugal. *J Antimicrob Chemother* **70:**2483–2487.

276. **Slifierz MJ, Friendship RM, Weese JS.** 2015. Methicillin-resistant *Staphylococcus aureus* in commercial swine herds is associated with disinfectant and zinc usage. *Appl Environ Microbiol* **81:**2690–2695.

277. **Couto N, Belas A, Tilley P, Couto I, Gama LT, Kadlec K, Schwarz S, Pomba C.** 2013. Biocide and antimicrobial susceptibility of methicillin-resistant staphylococcal isolates from horses. *Vet Microbiol* **166:**299–303.

278. **Blodkamp S, Kadlec K, Gutsmann T, Naim HY, von Köckritz-Blickwede M, Schwarz S.** 2016. *In vitro* activity of human and animal cathelicidins against livestock-associated methicillin-resistant *Staphylococcus aureus*. *Vet Microbiol* **194:**107–111.

279. **Blodkamp S, Kadlec K, Gutsmann T, Quiblier C, Naim HY, Schwarz S, von Köckritz-Blickwede M.** 2017. Effects of SecDF on the antimicrobial functions of cathelicidins against *Staphylococcus aureus*. *Vet Microbiol* **200:**52–58.

280. **Watts JL, Sweeney MT, Lubbers BV.** 2018. Antimicrobial susceptibility testing of bacteria of veterinary origin. *Microbiol Spectr* **6**(2).

281. **Silley P, Simjee S, Schwarz S.** 2012. Surveillance and monitoring of antimicrobial resistance and antibiotic consumption in humans and animals. *Rev Sci Tech* **31:** 105–120.

282. **Swedres-Svarm.** 2016. *Consumption of Antibiotics and Occurrence of Resistance in Sweden*. Public Health Agency of Sweden and National Veterinary Institute, Solna/Uppsala, Sweden.

283. **BVL.** 2017. *BVL-Report 11.5; Berichte zu den Resistenz-monitoringstudien 2014 und 2015*. https://www.bvl.bund.de/DE/09_Untersuchungen/01_Aufgaben/03_Nationales%20Resistenz-Monitoring/untersuchungen_NatResistenzmonitoring_node.html.

284. **Guran HS, Kahya S.** 2015. Species diversity and pheno- and genotypic antibiotic resistance patterns of staphylococci isolated from retail ground meats. *J Food Sci* **80:** M1291–M1298.

285. **Truong-Bolduc QC, Dunman PM, Strahilevitz J, Projan SJ, Hooper DC.** 2005. MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. *J Bacteriol* **187:**2395–2405.

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Antimicrobial Resistance in *Streptococcus* spp.

8

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TAXONOMIC OVERVIEW OF STREPTOCOCCI

More than 60 *Streptococcus* species have been recognized so far. Some of these, such as *S. pyogenes*, *S. agalactiae*, *S. equi*, *S. canis*, and *S. iniae*, produce hemolytic factors and, when cultivated on solid media containing blood, can be classified as beta-hemolytic. However, nonhemolytic variants can also be observed (1). Isolates belonging to other species, such as *S. dysgalactiae* subsp. *dysgalactiae*, *S. pneumoniae*, *S. mutans*, *S. salivarius*, *S. sanguinis*, *S. gordonii*, *S. mitis*, and *S. oralis*, produce hydrogen peroxide that partially lyses the erythrocytes, with the subsequent oxidation of the heme group resulting in a greenish pigment in the medium that is often interpreted as alpha-hemolysis. This oxidation process is influenced by several cultivation conditions and is variably evident. For this reason, it is preferable to consider those latter-mentioned species as nonhemolytic. The truly nonhemolytic species, mainly encompassing *S. gallolyticus* (formerly *S. bovis*), were also named gamma-hemolytic. A classification of *Streptococcus* species proposed by Rebecca Lancefield in the 1930s was based on the antigenic reaction of the cell wall-associated carbohydrates and remains classically used (2). On the basis of this approach, streptococci are distributed into groups ranging from A to W, depending on the antibodies recognizing the specific carbohydrates of a definite streptococcal species. Nevertheless, the whole picture is sometimes complicated by the fact that several antibodies can react with isolates belonging to the same species. For instance, depending on the isolates, *S. dysgalactiae* subsp. *equisimilis* may be classified as belonging to the C or G group, while it may also be classified, even though less commonly, as group A or L (3); isolates from *S. phocae* may belong to either the C or G group; isolates from *S. infantarius* are sporadically considered as group D; isolates from *S. anginosus* are indifferently classified as group A, C, G, F, or N; isolates from *S. constellatus* subsp. *constellatus* belong to either group F or N; sporadic isolates belonging to *S. constellatus* subsp. *pharyngis* can be considered as group C; isolates from the *S. intermedius* species can be considered as group N; and finally, isolates belonging to *S. porcinus* are classified in either group P, U, or V.

In the following sections, the most relevant *Streptococcus* species responsible for diseases in animals and/or humans will be summarized. The relative resistances to selected antibiotics will be discussed in the sections on macrolides-lincosamides-streptogramins B tetracyclines, beta-lactam resistance, fluoroquinolone resistance, and integrative and conjugative elements (ICEs).

Group A

Streptococci have diverse ecological origins, and certain species are exclusively adapted to a unique host as exemplified by the beta-hemolytic *S. pyogenes*, which is considered as the most pathogenic type of streptococcus for humans, together with *S. pneumoniae*, and is responsible for pharyngitis, erysipelas, and other invasive diseases such as soft tissue infection, rheumatic fever, glomerulonephritis, and streptococcal toxic shock syndrome (STSS) (4, 5). The finding of *S. pyogenes* in animals has been debated. According to Copperman (6), pets could have been the source of contagious pharyngitis (6), but no expansion of these findings has been reported, supporting the hypothesis that humans are the exclusive reservoir of *S. pyogenes*.

Group B

The beta-hemolytic *S. agalactiae*, or group B streptococci, according to the Lancefield's classification, is a

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commensal of the human intestinal and urogenital tract and is infamous as a human pathogen causing severe diseases such as pneumonia, sepsis, and meningitis in newborns and pregnant women; recently, its pathogenic importance in elderly and immunocompromised patients has been re-evaluated (7). *S. agalactiae* is also an animal pathogen and has been reported from a variety of hosts such as fish with meningoencephalitis (8), camels with mastitis and joints infections (9), and horses with unspecified disease or death (10). Classically, *S. agalactiae* has been associated with mastitis in cows (11), and the zoonotic potential of *S. agalactiae* is debated. On one side, genomic comparative approaches highlight a specific host adaptation of *S. agalactiae* isolates causing infections (12); on the other side, infections of humans from consumption of fish infected by *S. agalactiae* has recently been documented (13). Also, experimental infections of fish with *S. agalactiae* isolates of human origin have resulted in fish death (14). Globally, the hygienic control measures implemented for controlling contagious bovine mastitis have contributed to a sharply decreased prevalence of *S. agalactiae* in the veterinary sector (15, 16).

Group D

Group D streptococci were divided into two diverging groups in the early 1980s: *S. feacalis* and *S. faecium*, which were renamed *Enterococcus faecalis* and *Enterococcus faecium* (17). Since then, several new species have been added to the *Enterococcus* genus (18), which will not be discussed in this review.

Formerly, *S. bovis* was included in the viridans group of streptococci, and its taxonomy has been reviewed in several studies. Overall, several previously identified *S. bovis* isolates were classified as group D according to Lancefield's reaction, as shown in Table 1. Molecular evidence has provided the basis for the classification of the former *S. bovis* isolates into five species: *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, *S. gallolyticus* subsp. *macedonicus*, *S. infantarius* subsp. *infantarius*, and *S. lutetiensis* (19). Isolates belonging to *S. gallolyticus* subsp. *gallolyticus* have been found as commensals of the gastrointestinal tract of humans and animals but also cause invasive diseases such as sepsis, endocarditis, arthritis, and meningitis in both humans and animals (Table 1). The transmission of *S. gallolyticus* subsp. *gallolyticus* between animals and humans has been reported (20), highlighting the zoonotic potential of this species. *S. gallolyticus* subsp. *pasteurianus* is an emergent infective agent in human medicine that is responsible for sepsis, bone and joint infections, and meningitis (21–23). In birds, this bacterium is responsible for similar diseases (24, 25). *S. lutetiensis* has rarely been associated with infective endocarditis and sepsis (26).

Group E

In Lancefield's group E, *S. porcinus* is typically associated with sepsis, endocarditis, pneumonia, and lymphadenitis in swine (27). Infections sustained by *S. porcinus* have also occurred in humans (28); however, *S. porcinus* isolates infecting humans seem to have a different origin when compared to *S. porcinus* isolates of nonhuman sources (29).

Groups C and G

S. dysgalactiae subsp. *dysgalactiae* belongs to Lancefield's group C and G and plays a major role in mastitis (30). On rare occasions, it has also been found in necrotic tissues of fish and in humans, as responsible for various diseases (Table 1). In the same Lancefield's groups, *S. dysgalactiae* subsp. *equisimilis* is a beta-hemolytic bacterium found associated with strangles-like diseases in horses and with arthritis and endocarditis in swine. Unfortunately, this species is also associated with invasive diseases in humans, such as STSS and sepsis (Table 1). *S. equi* subsp. *zooepidemicus* organisms react with group C and G Lancefield's antigens as well. This organism is commonly found in bovine mastitis (90, 94, 137; also see Table and Table 3) and has sporadically been found associated with mastitis in sheep (31) and is most prevalent in equine infective diseases as the causative agent of joint and respiratory tract infections (32). This species also causes severe infections in humans in association with the consumption of contaminated dairy products (33). Transmission of *S. canis* isolates belonging to Lancefield's group C and G between pets and humans seems conceivable (34). This bacterium is responsible for arthritis in cats and humans (35) and endocarditis and skin, soft tissue, and urinary tract infections in dogs and humans. Skin lesions seem to represent the entry portal for establishing infections in humans (34, 36). In contrast, *S. equi* subsp. *equi*, belonging to Lancefield's groups C and G and responsible for strangles disease in horses, is exclusively animal adapted (32).

Group R

S. suis is a major pathogen for swine and causes meningitis, endocarditis, sepsis, arthritis, and pneumonia. In humans, *S. suis* is mostly responsible for meningitis and STSS. It is a well-recognized zoonotic agent, and indeed, human exposure to swine and swine-derived food products is a risk factor for infection by *S. suis* (37, 38).

Table 1 Overview of streptococci causative of infections in humans and animals[a]

Lancefield group	Hemolysin	Species	Host	Associated disease	References
B	Beta	*S. agalactiae*	Human	Sepsis, meningitis, pneumonia, joint and urinary tract infections	7, 11, 199, 200
			Cows, camels, horses, dolphins, fish	Mastitis, joint infection, meningitis, death	
C	Alpha	*S. dysgalactiae* subsp. *dysgalactiae*	Humans	Endocarditis, joint infection, cellulitis	48, 201, 202
			Fish, cows	Tissue necrosis, mastitis	
	Beta	*S. dysgalactiae* subsp. *equisimilis*	Humans	STSS, sepsis, soft tissue infections, pneumonia, pharyngitis	33, 203–210
			Swine, seals, horses	Arthritis, endocarditis, lymphadenitis, joint infection, strangles-like disease, respiratory tract infection	
	Beta	*S. equi* subsp. *equi*	Horses	Strangles disease	32
	Beta	*S. equi* subsp. *zooepidemicus*	Humans	Nephritis, STSS	33
			Sheep, horses	Mastitis, lymphadenitis, joint and respiratory infections, endometritis	31, 32, 211
	Beta	*S. phocae*	Fish, seals	Respiratory infections, abortions, sepsis	212
D		*S. gallolyticus* spp. *gallolyticus*	Humans	Sepsis, endocarditis, arthritis, meningitis	213–216
			Koalas, birds	Intestinal colonizer, endocarditis, sepsis	
		S. gallolyticus spp. *pasteurianus*	Humans	Sepsis, bone and joint infections, meningitis	21–25
			Birds	Sepsis, meningitis	
E	Beta	*S. porcinus*	Humans	Urinary tract, placenta, and wound infections, sepsis	27, 28, 217, 218
			Swine	Endocarditis, respiratory tract infection, sepsis	
G	Beta	*S. canis*	Humans	Skin, soft-tissue and respiratory infections, sepsis	34, 35, 219, 220
			Dogs, cats	Skin, soft-tissue, and urinary tract infections, otitis, arthritis, STSS	
R	Nonhemolytic/ beta	*S. suis*	Humans	Sepsis, meningitis, endocarditis, STSS	37, 221–225
			Swine, boars, rabbits	Sepsis, meningitis, pneumonia, arthritis	
Undefined	Beta	*S. iniae*	Humans	Soft-tissue infection	48, 49
			Dolphins, fish	Abscess, streptococcosis, sepsis	226–231
		S. uberis/ *S. parauberis*	Cows, horses	Mastitis	11, 16, 30, 43

[a]Isolates belonging to *S. dysgalactiae* subsp. *equisimilis* react with antigen G with comparable prevalence of antigen C reaction, whereas reaction with antigens A and L are less common; isolates belonging to *S. phocae* react also with antigen G; isolates belonging to *S. porcinus* react also with antigens P, U, and V. Nonhemolytic variant can be recovered among isolates of *S. agalactiae* and *S. dysgalactiae* subsp. *equisimilis*.

The production of a capsule seems to have a major role in pathogenesis, and capsular types 2 and 14 are the most prevalent among *S. suis* isolates that cause disease in humans (39).

Non-Lancefield Streptococci

All other streptococci lacking the Lancefield antigens are thus considered non-Lancefield (or nontypable) streptococci. Several are frequently encountered in animals and are detailed below.

S. uberis

If hygienic measures have been effective to control the dissemination of *S. agalactiae*, the same cannot be said for *S. uberis*, which remains a major animal pathogen and a leading cause of mastitis in cattle (15, 16). This

nonserotypable organism has an environmental origin, possesses a flexible metabolism, and is almost exclusively adapted to cattle (40). Infections can occur by a variety of strains, which in some cases are able to persist and propagate among different cows within a herd (41, 42). Rarely, it has been responsible for mastitis in heifers, and even more rarely it has been isolated from shrimps (43–45). The control of *S. uberis* propagation is more challenging than that of other *Streptococcus* spp. probably because of its ability, among others, to survive on bedding material (46, 47).

S. iniae

Another nonserotypable species is *S. iniae*, which was primarily isolated from diseased dolphins and was subsequently confirmed as a major fish pathogen and as responsible for soft tissue infections in humans with zoonotic features (Table 1) (48–52).

S. pneumoniae

S. pneumoniae is a major streptococcal pathogen of humans and is responsible for serious infections such as pneumonia and meningitis; reports of infections in animals are extremely rare and concern horses with respiratory tract infections (53). A recent publication also reported *S. pneumoniae*, probably of human origin, in wild and captive chimpanzees (54). The spread of this pathogen due to the migration of infected animals to other communities or the reintroduction into wild populations of formerly captive animals might be a real danger.

Viridans streptococci

These organisms were defined as viridans because of the hemolytic features described above that produce a greenish pigmentation on blood agar, their absence of Lancefield antigens, and their resistance to the chemical compound optochin. Generally, viridans streptococci are implicated in the establishment of dental caries, arthritis, and infective endocarditis in humans (50–52).

Certain *Streptococcus* species, including *S. sobrinus* and *S. mutans*, the first representative of the mutans group; *S. salivarius*, *S. vestibularis*, and *S. infantarius* from the salivarius group; the anginosus group, including *S. anginosus*, *S. constellatus*, and *S. intermedius*; the sanguinus group, including *S. sanguinis*, *S. parasanguinis*, and *S. gordonii*; and most species of the mitis group, including *S. mitis*, *S. oralis*, *S. cristatus*, *S. infantis*, and *S. peroris*, have exclusive human adaptation; others have been found only in animal hosts, such as *S. macacae* from monkeys, *S. ferus* from wild rodents, *S. orisratti* from rats, and *S. hyointestinalis* and *S. hyovaginalis* from swine, with no associated diseases (55–59). Three species of the viridans group, namely *S. sobrinus*, *S. criceti*, and *S. ratti*, have been reported from humans and experimental rats, whereas *S. alactolyticus* has been found in swine, dogs, and humans (56, 60, 61); *S. downei* was isolated first from a monkey and more recently from human dental plaque (62, 63). Another species of the viridans group, *S. pluranimalium*, has been reported rarely. It was first reported in 1999 from bovine mastitis, and few clinical human cases have been reported since then (64, 65).

This brief introduction does not pretend to be exhaustive. The reviews by Póntigo et al. and Facklam provide a comprehensive nomenclature and classification of streptococci based on molecular and phenotypic features, respectively (1, 66).

EVOLUTION OF ANTIMICROBIAL RESISTANCE IN VETERINARY STREPTOCOCCI

Monitoring Programs

Prevalence data on antimicrobial resistance were principally obtained through dedicated studies performed at the scale of a region, a country, or a consortium of countries. However, only monitoring programs can give an evolutionary picture of antimicrobial resistance rates over time. Consequently, surveillance systems are highly valuable to follow trends and detect emergent resistant phenotypes.

Several national surveillance and monitoring programs in veterinary medicine exist in Europe (67), including the Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands program, the Swedish Veterinary Antimicrobial Resistance Monitoring program (SVARM), the Danish Integrated Antimicrobial Resistance Monitoring and Research Program, the German Resistance Monitoring in Veterinary Medicine program, and the French surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin (RESAPATH). Two similar programs cover the North American continent, the National Antimicrobial Resistance Monitoring System for Enteric Bacteria in the United States and the Canadian Integrated Program for Antimicrobial Resistance Surveillance, and one reports Japanese data (the Japanese Veterinary Antimicrobial Resistance Monitoring program). An additional industry-based pan-European monitoring program commissioned by the Executive Animal Health Study Center investigates pathogens from farm (VetPath) and companion animals (ComPath). In addition to the recurrently criticized lack

of harmonization (67, 68), a major feature of most programs is their main focus on bacteria of animal origin but of relevance for human health, such as zoonotics and commensal indicators. Accordingly, streptococci of animal origin were poorly included, and data on their resistance to antimicrobials remain limited at a global scale. Indeed, only two monitoring programs reported data on a long-term basis: the Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands program from 2002 to 2008 and RESAPATH from 2006 until today. Other programs such as the Swedish Veterinary Antimicrobial Resistance Monitoring program (in 2002), the German Resistance Monitoring in Veterinary Medicine program, and ComPath/VetPath have also documented antimicrobial resistance in streptococci, but on a sporadic basis.

Different Methodologies to Determine Antibiotic Resistance

Standard surveillance programs rely on phenotypic methods that are used in routine diagnostic laboratories. The most frequently used methods are antibiograms performed by disc diffusion and MIC performed by broth microdilution. These techniques generate qualitative or quantitative results, respectively, that are then interpreted according to official guidelines (EUCAST, CLSI, Antibiogram Committee of the French Microbiology Society (CA-SFM), etc.) so that the studied isolates can be classified as susceptible, intermediate, or resistant to the tested antibiotics. In the surveillance systems, the genotypic techniques are only optionally implemented as a second-line characterization.

This traditional approach may be disrupted by the democratization of next-generation sequencing methodologies. Recently, several publications proved the usefulness of large-scale genomic analyses for the efficient detection of resistance mechanisms and capsular types (69), for the sequence-based prediction of beta-lactam resistance using the penicillin-binding protein (PBP) transpeptidase signatures (70), and for the prediction of the antimicrobial profile and its potential evolution toward resistance over time (71). This is, of course, not an exhaustive list of publications using next-generation sequencing, especially in a field that is progressing very rapidly. There are still a couple of drawbacks to the direct implementation of next-generation sequencing in diagnostic laboratories, including the time needed to generate results (which exceeds the 48 hours traditionally needed for phenotypic testing) and the price. However, this methodology is so powerful that it will undoubtedly be used in monitoring programs, at least for long-term surveillance purposes.

Evolution of Resistance in *Streptococcus* spp.

The main *Streptococcus* species studied through monitoring programs were *S. uberis* and *S. dysgalactiae* isolated from bovine mastitis. *S. agalactiae* is also still considered a major streptococcal pathogen associated with bovine subclinical and mild to moderate clinical mastitis, but its incidence has drastically fallen in the past 20 years due to hygiene measures and guidelines for good practices. Consequently, *S. agalactiae* is now only rarely isolated from cattle mastitis, and the numbers are too small to be reliably reported. The evolution of antimicrobial resistance thus focuses on *S. uberis* and *S. dysgalactiae*. Here, we review data on resistance to the main antibiotics used in the treatment of animal infections due to *S. uberis* and *S. dysgalactiae*, i.e., penicillin G, tetracyclines, erythromycin, lincomycin, enrofloxacin, and streptomycin.

Evolution of Antimicrobial Resistance in the Netherlands

The Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands program reported antimicrobial resistance data on streptococci isolates collected from milk in the context of intramammary infections, but the monitoring of these pathogens stopped in 2008. From 2002 to 2008, the overall prevalence of resistance was quite stable for most antibiotics, with a seemingly upward trend for a few (72). Resistance to beta-lactams (represented by penicillin G and cefalotin) was only detected in rare cases of *S. uberis* and was absent in *S. dysgalactiae*. The highest rates of resistance were for tetracyclines (around 40% for *S. uberis* and 70% for *S. dysgalactiae*) and lincomycin (also around 40% for *S. uberis* and 25% for *S. dysgalactiae*). The discrepancy between the prevalence of tetracycline resistance in *S. dysgalactiae* compared to *S. uberis* is in accordance with what has been reported in other monitoring programs (see below) and numerous studies (see "Prevalence of Resistance to Macrolides among Streptococci of Bovine Origin" below). Resistance to erythromycin (around 20% for *S. uberis* and 10% for *S. dysgalactiae*) was systematically lower than for lincomycin, suggesting a significant prevalence of non-*erm*-mediated mechanisms of resistance.

RESAPATH, the Ongoing French Monitoring Program

RESAPATH is the only ongoing and long-term monitoring program for, among other topics, resistance to antimicrobials in streptococci in Europe. For *S. uberis*, from 2006 to 2015, antimicrobial resistance was tested

on 600 to 1,500 isolates, depending on the nature of the antibiotics, and the global trend was quite stable for all antibiotics (Fig. 1). The highest prevalence of resistance was observed for enrofloxacin, which may be explained by the intrinsic low-level resistance of streptococci to fluoroquinolones. Indeed, in the RESAPATH network, resistance is defined as the addition of both resistant and intermediate phenotypes, which may lead to the over-estimation of the prevalence of resistance in the case of fluoroquinolones. For erythromycin and lincomycin, the resistance rates decreased from 24% to 17% between 2006 and 2007, were stable during next 6 years until 2013, and increased again in 2014 to 2015 up to around 22% of resistant isolates. Both curves matched perfectly, indicating a cross-resistance to macrolides-lincosamides involving *erm* genes (see "Resistance to Macrolides, Lincosamides, and Streptogramin B" below). Tetracycline resistance is following a very slow upward trend (from 14% in 2006 to 21% in 2015), to be confirmed in the coming years. Finally, streptomycin is the antibiotic presenting the lowest prevalence of resistance (from 11 to 16% over the 10-year period of 2006 to 2015), albeit the highest among resistance to aminoglycosides. Indeed, rates of resistance to kanamycin and gentamicin only reached 5% and 3%, respectively, in 2015. However, these resistances are of major importance since they may result in the loss of synergy between aminoglycosides and beta-lactams, which is a frequently used combination in veterinary practice. Taken together, these data show globally high basal levels of resistance of streptococci to antimicrobials in 2006 and a slight but increasing prevalence, which will have to be monitored in the near future.

The number of *S. dysgalactiae* isolates that can be considered for global trends in prevalence is around 10 times lower than for *S. uberis* (25 compared to 235 isolates), so that the prevalence rates are subject to wider variations. However, the rates observed are different though more stable than for *S. uberis*. The antibiotic with the highest prevalence of resistance by far was tetracyclines. Up to 85% of the *S. dysgalactiae* isolates were resistant to this drug, which is in accordance with what has been reported in other studies (see "Prevalence of Resistance to Macrolides among Streptococci of Bovine Origin" below). Enrofloxacin presents fluctuating resistance rates of around 50%. Finally, erythromycin, lincomycin, and streptomycin present a stable prevalence of around 22%, 12%, and 6%, respectively. The 10% discrepancy between erythromycin and lincomycin resistance rates deserves special attention since it may signal a divergence in the resistance mechanisms involved compared to *S. uberis*.

Though originating from two countries only, these evolution rates constitute a starting point to address the issue of resistance in *Streptococcus* spp. in veterinary medicine. In line with these trends, the next sections will detail the epidemiology and mechanisms of resistance of the antibiotics mentioned above.

RESISTANCE TO MACROLIDES, LINCOSAMIDES, AND STREPTOGRAMIN B

Erythromycin was the first macrolide discovered in 1952 by McGuire as a natural product originating from *Streptomyces erythreus*. The other macrolides were derived from erythromycin by semi-synthesis, and their core unit consists of a lactone ring that can be constituted by 14, 15, and 16 carbon atoms. These molecules are bacteriostatic in staphylococci and bactericidal in streptococci, inhibiting the protein translocation by binding to the 23S or 50S ribosomal subunit at peculiar residues (i.e., the guanine 2505, the uridine 2609, and the adenines 2058, 2059, and 2062) (73). Macrolides have a broad spectrum of action, being effective against Gram-positive and Gram-negative bacteria and intracellular pathogens, and are reputed as valuable agents for their good pharmacodynamics properties, their relatively few side effects, and their good penetration in tissues. Lincosamides, including lincomycin and clindamycin, together with streptogramin B, including pristinamycin and quinupristin, are structurally unrelated to macrolides, but they have a common mechanism of action and, as a consequence, resistance to all these classes of antibiotics is crossed. In addition to the treatment of infections caused by intracellular pathogens, the usage of macrolides and lincosamides in human clinics is principally dedicated to the treatment of uncomplicated infections in patients who are allergic to beta-lactams. In veterinary medicine, macrolides and lincosamides are available as in-feed and injectable formulations and are used for the treatment of a variety of diseases ranging from respiratory tract infections to infective mastitis in food-producing animals, especially swine and cattle (74, 75). Frequently, macrolides and lincosamides are used in combination with other drugs such as aminoglycosides, ampicillin, colistin, tetracyclines, sulfonamides, and trimethoprim (76). Certain macrolides were also used as growth promoters (council regulation EC2821/98, 17 December 1998). Unfortunately, shortly after the introduction of macrolides in human therapy, resistant isolates were recovered; the emergence of resistant isolates has also occurred in animals. In the following subsections we will describe the most common mechanisms of resistance to

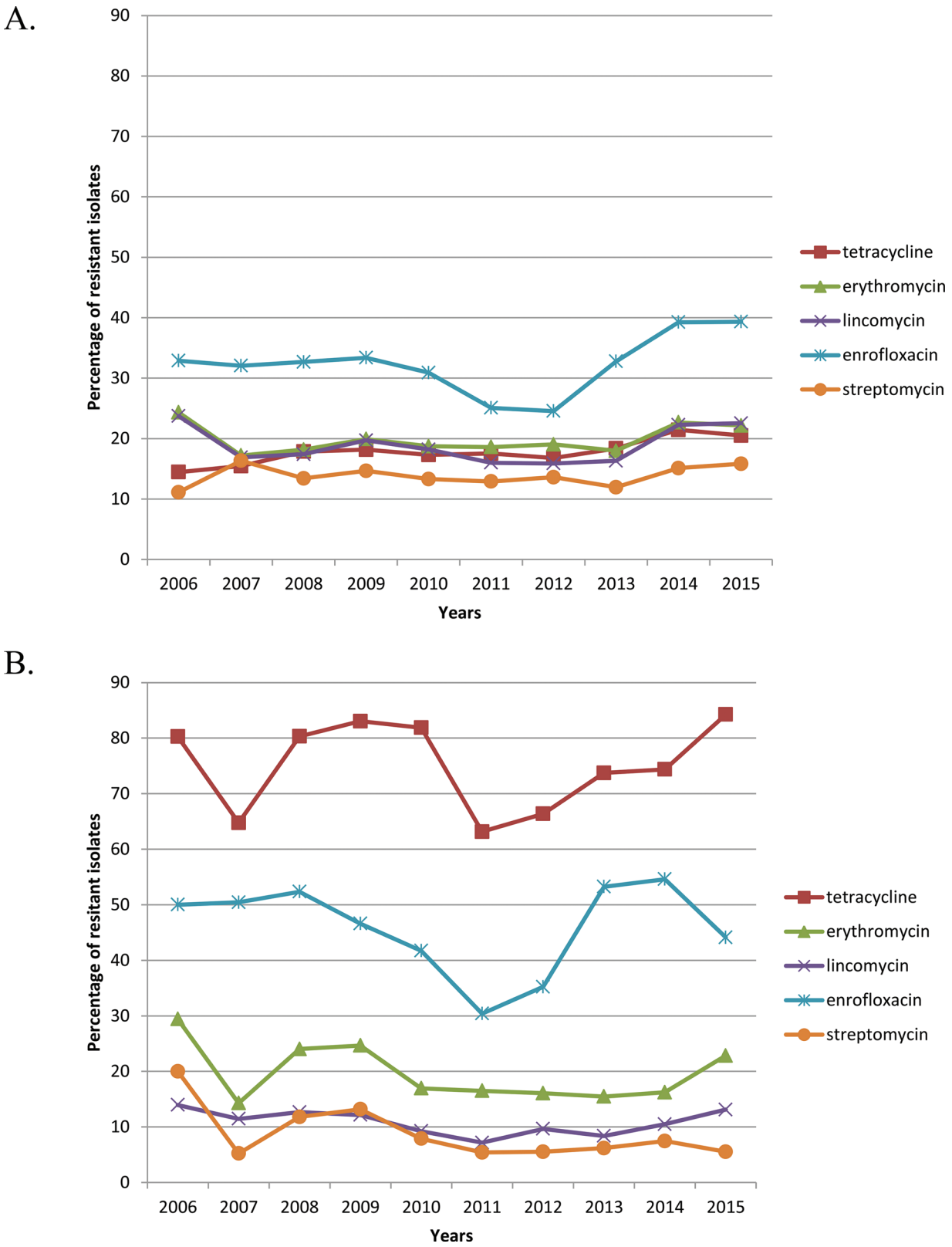


Figure 1 Ten-year evolution of resistance in France in (A) *S. uberis* and (B) *S. dysgalactiae*.

macrolides, lincosamides, and streptogramins B found in streptococci of animal origin.

Macrolides, Lincosamides, and Streptogramins B Resistance Determinants

Target modification

Ribosomal mutation in the residues crucial to the binding of macrolides results in cross-resistance to all macrolides, lincosamides, and streptogramins B conferring the so-called MLSb phenotype. Human clinical isolates with such mutations have been sporadically observed, probably because this mechanism requires mutations in all the operon copies encoding the ribosomal subunits.

Target protection

The methylation of the adenine at position 2058 is enough to confer an MLSb resistance phenotype. This reaction is mediated by the methylases encoded by the *erm* (erythromycin ribosome methylation) gene family, originated by the natural producers of macrolides. In the presence of an Erm methylase, resistance to lincosamides and streptogramin B can be either constitutive or induced by the presence of erythromycin (73). Overall, this mechanism is the most prevalent method conferring resistance to macrolides in human and veterinary clinical isolates. Currently, about 40 variants of the *erm* gene have been reported, with *erm*(B) gene being the most prevalent (77). It is often located on mobile genetic elements (MGEs) and associated with genes conferring resistance to tetracyclines (see below). These two factors have consistently contributed to the dissemination of resistance to macrolides (78).

Efflux

The second most common mechanism of resistance to macrolides in streptococci is represented by the efflux mediated by the Mef efflux pumps. The *mefA* gene was described for the first time in *S. pyogenes*, and other variants have been reported since then that are principally represented by *mefE* and *mefI* (79). These efflux systems confer resistance to 14- and 15-carbon atom macrolides only, determining an M phenotype. Transcription of *mef* genes is coupled with the expression of *msr* genes encoding for an ATP-dependent efflux system. The presence of *mef* genes seems to be necessary to confer macrolide resistance (80). Genes of the *mef* family are often located on genetic units that can transfer by transformation, such as the MEGA (macrolide efflux genetic assembly) element harboring the *mefE* gene and conjugative transposons such as the Tn*1207.3* harboring the *mefA* variant and the 5216IQ composite element harboring the *mefI* gene (81–83). With a lower prevalence, the *mreA* efflux pump has been reported as well (84). Finally, genes of the *lsa* family have been described, conferring cross-resistance to lincosamides, streptogramin A and pleuromutilins. These genes, encoding ATP-binding proteins, most likely promote the efflux of the antibiotics (192).

Drug modification

Lincosamides can be inactivated by adenylation in position 3 by a 3-lincosamide-*O*-nucleotidyltransferase encoded by the *linB* gene conferring an L phenotype (85). The *linB* gene was first found in a clinical isolate of *E. faecium*. Its origin remains unknown because no similar sequence has been found in natural lincosamide producers. The *linB* gene was reported sporadically; however, its transfer to *S. agalactiae* has occurred and has been reported from a human clinical isolate in Canada (86). After the first description, the *linB* gene was renamed *lnuB*. Later, Achard et al. unveiled the mechanism behind the lincomycin resistance in an *S. agalactiae* isolate that was surprisingly susceptible to clindamycin. It consisted of a novel nucleotidyltransferase encoded by the *lnuC* gene (87). The LnuD-adenylating clindamycin was later discovered in *S. uberis* (88).

Inactivation of macrolides can also be caused by phosphotransferases encoded by the *mph* gene families with differential affinity for the different macrolide types and lincosamides according to the variant of the *mph* gene expressed (89).

Prevalence of Resistance to Macrolides among Streptococci of Bovine Origin

Studies of the resistance to macrolides in streptococci of bovine origin have focused on isolates responsible for clinical and subclinical mastitis. During an investigation of different streptococcal species from bovine mastitis during 2002 to 2003 in Portugal, Rato et al. found a constitutive MLSb phenotype in 11/60 *S. agalactiae* isolates; among those, 10 isolates were positive for the presence of an *erm*(B) gene and one harbored an *erm*(A) gene (90). Among *S. dysgalactiae* subsp. *dysgalactiae* isolates, 4/18 demonstrated a constitutive MLSb phenotype, with the presence of *erm*(A) and *erm* (B) in 1 and 3 isolates, respectively, whereas all the MLSb-resistant *S. uberis* isolates (8/30) harbored an *erm*(B) gene. An L phenotype was demonstrated in 11 isolates, 3 belonging to *S. dysgalactiae* subsp. *dysgalactiae* and 8 to *S. uberis* species, all of them harboring an *lnuB* gene. In 2005, Duarte et al. found that 8.5% (9/38) of *S. agalactiae* isolates from Brazil were resistant

to erythromycin with a constitutive MLSb phenotype. All of the isolates harbored an *erm*(B) and an *mreA* gene; six of these coharbored *erm*(A) and were co-resistant to tetracyclines (29). Contemporaneously, Dogan et al. reported a 3.6% prevalence of erythromycin-resistant isolates among 83 *S. agalactiae* from bovines in New York. The resistance was mediated by Erm(B) in all isolates, and coresistance to tetracyclines was observed as well (91). A study from China was published in 2012 on 55 *S. agalactiae* isolates recovered from bovine mastitis during an undetermined period and reported a 23.5% rate of resistance to erythromycin. All isolates harbored the *erm*(B) gene (92). Pinto et al. analyzed 29 *S. agalactiae* isolates collected between 1980 and 2006 in Brazil. They found that 27% of the isolates were resistant to erythromycin, with *erm*(B) as the most prevalent gene, followed by the *erm*(A) variant. In this study, the presence of a *mef* allele was reported as well (93).

More recently, our investigation of 76 *S. agalactiae* isolates demonstrated an MLSb phenotype in eight isolates, including four isolates with a constitutive phenotype and four with an inducible phenotype. Six isolates harbored an *erm*(B) gene, and the two remaining ones harbored the *erm*(A) variant. In the same study, 4/32 isolates of *S. dysgalactiae* demonstrated the presence of an *erm*(B) gene; in this case also, the constitutive or inducible phenotypes were equally distributed (94). The *erm*(B) gene has also been confirmed as the most common gene conferring resistance to macrolides among *S. dysgalactiae* isolates; for instance, it was present in 4/4 isolates detected in dairy herds in the southwestern United States (95). We conducted a study to characterize the erythromycin resistance of 125 isolates of *S. uberis* collected from bovine mastitis during 2007 to 2008 in France. Overall, 111/125 isolates demonstrated an MLSb phenotype, constitutive in 42.3% of the isolates and inducible in the remaining ones. An *erm*(B) gene was present in all isolates. In this collection, 14 isolates demonstrated an L phenotype and harbored an *lnuB* gene. In one isolate, the less common *lnuD* gene was found as well (96). Contemporaneously, another study, conducted on dairy cows in Mayenne, France, confirmed MLSb resistance in 12 (12/55, 22%) *S. uberis* isolates, which all were positive for the *erm*(B) gene. An *lnuB* gene was present in four isolates with the L phenotype (97). In *S. uberis*, emergence of the *mphB* gene was documented in 2008 (98), but propagation of this mechanism has not occurred in a large scale.

Among others, the German Resistance Monitoring in Veterinary Medicine program has provided extensive data from Germany from 2007 to 2010 (Table 2). Several other studies from different parts of the world and based on the phenotypic characterization of mastitis isolates provided a comprehensive picture of the problematic link to the rise of resistance to macrolides in streptococci causing mastitis. An overview is provided in Table 2, and for exhaustive reports on temporal and geographical evolution of macrolide and lincosamide resistance in streptococci, we suggest the reports from Hendriksen et al. in 2008 and Thomas et al. in 2015 (99, 100). Overall, the lowest prevalence of macrolide and lincosamide resistance was observed in Sweden (101), and large differences in prevalence were observed among countries. However, no major variation was observed from one year to another in a single country.

Prevalence of Resistance to Macrolides among Streptococci of Porcine Origin

Macrolides, lincosamides, and streptogramins B are widely used for the treatment of infections in swine. Unfortunately, it appears that the usage of these drugs has influenced the emergence of resistance in *S. suis* (102). High rates of resistance to these drugs were observed over time, ranging from 52% (11/21 isolates) in the first observation in Norway in 1986 (103) to 94% (216/226 isolates) in Korea during 2010 to 2013 (104). The most prevalent genetic determinant is the *erm*(B) gene, whereas *mef*A/E were sporadically detected in human isolates (105). Often, such resistances occur together with tetracycline resistance (see below). *S. suis* may also act as a reservoir of lincosamide resistance genes, as exemplified by the emergence of the *lnuE* gene, previously identified in *S. suis*, in staphylococcal isolates (106). The report from Hendriksen et al. shows a certain variability of erythromycin resistance in *S. suis* among European countries during 2002 to 2004 (107). In addition, other streptococcal species were rarely found to cause diseases in swine. Within the framework of the BfT-GermVET program, Lüthje and Schwarz reported the presence of *S. dysgalactiae* subsp. *equisimilis* in diseased swine, with 21 isolates demonstrating resistance to macrolides. Among those, 13 harbored an *erm*(B) gene, one an *erm*(B) gene together with *mefA* and *msrD*, and one a *lnuB* gene (108).

In all, such an alarming prevalence of macrolide resistance in a relevant zoonotic pathogen such as *S. suis* highlights the need to prevent infections through appropriate hygienic measures.

Prevalence of Resistance to Macrolides among Streptococci from Non-Food-Producing Animals

In Brazil, six isolates of *S. dysgalactiae* subsp. *equisimilis* from horses were included in a study of antimicrobial

Table 2 Erythromycin and lincosamide resistance in streptococci in animal hosts[a]

Animal host	Country	Year	Bacterial species	No. of isolates	Genetic determinants									Percentage of resistance (%) to		Reference
					mefA	*mefE*	*msr*	*ermA*	*ermB*	*lnuB*	*lnuD*	*mph*	*mreA*	M	L	
Cattle	USA	ND	*S. dysgalactiae*	152										10	25.6	136
			S. uberis	133										9	42.9	
Cattle	France		*S. uberis*	55	0				12	4				22		97
Cattle	USA	ND	*S. dysgalactiae*	4	0	0		0	4					100		95
			S. uberis	20	0	0		0	12					60		
Cattle	China	ND	*S. agalactiae*	55	0			0	13					23.5		92
Cattle	Brazil	1980–2006	*S. agalactiae*	29	5			4	7	0				31	20.7	93
Cattle	France	1984–2008	*S. agalactiae*	76	0			2	6					10.1		94
			S. dysgalactiae	32	0			0	4					ND		
			S. uberis	101	0			0	75	5	1			ND		
Cattle	Brazil	1995–2000	*S. agalactiae*	38	0			6	9	0			9	8.5	8.5	29
Cattle	France	1995–2000	*S. agalactiae*	8										0	0	134
			S. dysgalactiae	41										16.7	11.9	
			S. uberis	50										28	36	
Cattle	Argentina	1999–2003	*S. agalactiae*	36										16.7	19.4	232
			S. dysgalactiae	8										12.5	12.5	
Cattle	USA	2000–2002	*S. agalactiae*	83	0			0	3					3.6		91
Cattle	Portugal	2002–2003	*S. agalactiae*	60	0			1	10	0				18.3	18.3	90
			S. dysgalactiae	18	0			1	3	3				22.2	38.9	
			S. uberis	30	0			0	8	8				26.7	26.7	
Cattle	Sweden	2002–2003	*S. agalactiae*	6										16.7	16.7	133
			S. dysgalactiae	152										0	0.7	
			S. uberis	113										0	0	

Cattle	Korea	2004–2008	*S. agalactiae*	5							0	60	233
			S. bovis group	24							12.5	33.3	
			S. uberis	99							34.3	41.4	
			S. oralis	30							36.7	36.7	
			S. salivarius	13							0	69.2	
			S. intermedius	7							42.8	71.4	
Cattle	Turkey	ND	*S. agalactiae*	5							0	40	169
			S. uberis	18							11	17	
Cattle	France	2007–2008	*S. uberis*	125	0		0	111	3	0	ND	ND	96
Cattle	Estonia	2007–2009	*S. agalactiae*								1.3	6.2	168
			S. dysgalactiae								6.7	7.8	
			S. uberis								8.2	6.6	
Cattle	Germany	2007–2010	*S. agalactiae*	101	2	13	0	15			16.8	ND	234
			S. dysgalactiae	100	2	1	0	10			11	ND	
			S. uberis	102	2	2	0	5			17.6	ND	
Cattle	Switzerland	2010–2012	*S. dysgalactiae*	46							2.2	2.2	238
			S. uberis	208							10.6	10.6	
Cattle	Switzerland	2011–2013	*S. dysgalactiae*	213							ND	37.4	167
			S. uberis	1,228							ND	49.7	
Swine	EU	1987–1997	*S. suis*	404							55.3		139
Swine	Denmark	1989–2002	*S. suis*	103				39			40.8		237
Swine	France	1996–2000	*S. suis*	110							78.2	78.2	235
Swine	Belgium	1999–2000	*S. suis*	87	0			62			71	71	236
Swine	Spain	1999–2001	*S. suis*	151							90.7	87.4	143
Swine	Italy	2003–2007	*S. suis*	57	0			44			81	81	140
Swine	China	2005–2007	*S. suis*	421							67.2	68.4	142
Swine	China	2005–2012	*S. suis*	96	18		0	35	0		38.5	38.5	170
Swine	China	2008–2010	*S. suis*	106	51	51		70			67.9	67.9	146
Swine	Brazil	2009–2010	*S. suis*	260							46.5	84.6	144
Swine	Korea	2010–2013	*S. suis*	227	39			218			94	95.6	104

[a]ND, not determined; M, macrolides; L, lincosamides.

resistance in humans, and prevalence data on resistance to macrolides were similar in isolates from the two sectors (109). In Germany, an *S. equi* subsp. *zooepidemicus* isolate with an M phenotype was recovered from a horse and harbored a *mefA* and an *msrD* gene. *S. canis* resistant to macrolides, mostly with an MLSb phenotype, has been reported from diseased dogs in Denmark and Germany (110). From dogs and cats, six *S. dysgalactiae* subsp. *equisimilis* macrolide-resistant isolates were found in Germany with five isolates harboring an *erm*(B) gene and one harboring a *mefA* and a *msrD* gene (108).

TETRACYCLINES

Tetracyclines, which were discovered in the late 1940s, are bacteriostatic antibiotics that block bacterial protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor A site (111). Because of their broad-spectrum activity against both Gram-positive and Gram-negative bacteria, they rapidly became one of the most widely used antibiotics (112), and consequently, the first resistant isolate was reported in 1953 in *Shigella dysenteriae* (113). Tetracycline resistance rapidly and broadly disseminated in bacteria of human, animal, and environmental origin and is now considered one of the most frequently seen resistances to antimicrobials (114). In humans, tetracyclines have largely been supplanted by beta-lactams, while they remain one of the main classes of antibiotics used in veterinary medicine (115–117). In animals, tetracyclines are also considered as growth-promoting factors when mixed with food at subtherapeutic levels, in order for food-producing animals to gain weight more quickly (111). This practice was banned in Europe at the latest on 1 January 2006 since it can promote resistance selection, as exemplified by the increase of vancomycin-resistant enterococci in animals through the use of the glycopeptide avoparcin (118). However, growth promoters are still authorized in many countries worldwide, such as in the United States, and tetracycline is again the most frequently used antibiotic class (117). The past and present excessive use of tetracyclines first of all hampers the efficacy of these molecules, but may also have unexpected side effects such as the selection of hypervirulent *S. agalactiae* clones worldwide and increasing numbers of neonate infections (119).

Tetracycline Resistance Determinants

A total of 46 *tet* or *otr* genes have been identified as tetracycline resistance determinants in 126 genera (120, 121). They are commonly divided into two main groups characterized by their mode of action: the genes coding for efflux proteins and those coding for ribosomal protection enzymes (120). In *Streptococcus* spp., *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(Q), and *tet*(T) are the most frequently reported genes (111, 113, 121). Tet(K) and Tet(L) code for membrane-associated efflux systems that share nearly 60% amino acid identity (111) and confer resistance to tetracycline but not to minocycline. The *tet*(K) gene was discovered on a pT181 plasmid in *Staphylococcus aureus* (122), whereas *tet*(L) was found associated with small non-conjugative plasmids in streptococci (123). In contrast, Tet(M), Tet(O), Tet(S), Tet(Q), Tet(T), and the more recently identified Tet(W) are enzymes that protect the ribosome from the action of tetracycline, a mechanism conferring resistance to all available antibiotics of the tetracycline family. The *tet*(M) gene was concomitantly identified with *tet*(L) on streptococcal plasmids (123). It has now been extensively detected and studied in both Gram-negative and Gram-positive species and is often found on ICEs of the Tn*916*-Tn*1545* family (124–127). Since the Tn*916*-Tn*1545* elements also encode, among others, resistance to erythromycin and kanamycin, these mobile determinants promote the emergence of multiresistant isolates, as exemplified by their frequent association with the *tet*(M) and *erm*(B) resistance genes in streptococci isolates (120). The dissemination of the remaining protecting enzymes—Tet(O) (which was first discovered in *Campylobacter coli* [128]), Tet(S) (discovered in *Listeria monocytogenes* [129]), Tet(Q) (first described in *Bacteroides* species [130]), and the closely related Tet(T) (first detected in *S. pyogenes* [131])—was less efficient than the diffusion of Tet(M), probably because of the localization of the corresponding genes, which have never been reported on conjugative transposons such as Tn*916* and Tn*1545*, mentioned above. Of note, Tet(M), Tet(O), Tet(S), and Tet(Q) are closely related since they share around 78% sequence identity (113), even though they can easily be differentiated using specific primers. Tet(W) is the latest protection enzyme detected in streptococci and was first identified in *Butyrivibrio fibrisolvens* (132).

Prevalence of Tetracycline Resistance among Streptococci of Bovine or Ovine Origin

Most studies reporting tetracycline resistance in isolates of bovine or ovine origin were performed on *S. uberis*, *S. dysgalactiae*, and *S. agalactiae* in the context of clinical or subclinical mastitis. Tetracycline is often the antibiotic presenting the highest prevalence of resistance. When considering the CLSI breakpoints which categorize as tetracycline resistant all isolates presenting an

MIC of >4 mg/liter, resistance rates in *S. uberis* ranged from 1.8 to 4% in Sweden between 2002 and 2009 up to 60% in Portugal in 2003 (90, 101, 133), with intermediate prevalence of 12.9 to 22% in France, 15% in England, 27.1% in the United States (1997 to 1999), and 44% in Italy (99, 134–136). In *S. dysgalactiae* prevalence is overall higher, ranging from 6% in Sweden to 76.6% in the Netherlands and 100% in Portugal and France (90, 94). Prevalence rate figures are less frequently available for *S. agalactiae* but also suggest a very high frequency of tetracycline resistance, with 33.4% in Sweden in 2002 and 37.5% in France in 2000 (133, 134).

Only a few studies have reported the molecular characterization of *tet* resistance genes (Table 3) in streptococci of bovine origin. When reported, the *tet* genes did not have a bacterial specificity and were often described in combination, such as *tet*(M)/*tet*(O), *tet*(M)/*tet*(K), or *tet*(O)/*tet*(K) (90, 94, 137, 138).

Most studies detailed here were performed in Europe in the 2000s. Having a better and updated view of the evolution of resistance in veterinary streptococci would require monitoring of tetracycline resistance in bacteria from animal origins at a larger scale.

Prevalence of Tetracycline Resistance among Streptococci of Porcine Origin

In line with *S. suis* being the major *Streptococcus* spp. in swine, numerous publications have reported antimicrobial resistance in this pathogen, mostly in diseased animals. Tetracycline, as already noted for streptococci of bovine or ovine origin, is often the antibiotic presenting the highest prevalence of resistance. The lowest rates were reported in the oldest European isolates studied (no resistance in Danish isolates collected in 1967 to 1981 and 7.7% in Swedish isolates collected in 1992 to 1997) and in the context of the ARBAO-II study performed in 2002 to 2004 (48% resistance in the Netherlands and 52.2% in Denmark in 2003) (102, 107). Higher rates were then reported in the United States (66.7% in 1986), Spain (68.0% in 2004), Poland (64.0% in 2004), Japan (86.9%), Italy (89.5%), and a pan-European study (75.1%) (107, 139–141). Several studies showed resistance rates greater than 90%, with 91.7%, 95.4% and 97.9% in China, Spain, and Brazil, respectively (142–144).

A few molecular studies detailed the *tet* genes responsible for the phenotypic resistance detected. Tet(O) is by far the most commonly detected enzyme in *S. suis*, while Tet(M) has also been reported (Table 3), either alone or in association with other Tet determinants, in particluar Tet(O). The distribution of these *tet* genes may also vary depending on the serotype, but further work on larger cohorts is needed to have statistically relevant data. The *tet*(W) gene was repeatedly reported in *S. suis*, initially in a human patient in Italy, but then also in pig isolates (140, 145, 146). Of note, the only occurrence of *tet*(B) in streptococci was reported in 17 *S. suis* isolates (17/111, 15%) in the United States (147).

Tetracycline Resistance among Other Streptococci

Tetracycline resistance in *S. canis* was reported from diseased cats and dogs, with a prevalence of 32.1% in France, 23.5% in Japan, and 27% in Portugal (including a few isolates from horses and humans) (34, 148, 149). In these studies, tetracycline resistance was due principally to the presence of the *tet*(M) and *tet*(O) genes, alone or in combination. A study performed in Belgium on healthy individually owned cats and groups of cats noted a higher prevalence of tetracycline resistance in a cattery (52%) compared to individual animals (22.2%), most likely due to the clonal transmission of resistant strains in the cattery (150).

BETA-LACTAM RESISTANCE

Beta-lactams are the largest family of antibiotics available in both human and veterinary medicine. All members of this family act on the bacterial cell wall by covalently blocking the PBPs and thus impairing the continuous building of this protecting structure. Currently, bacterial resistance to the last generations of beta-lactams is one of the most challenging issues in both human and animal medicine. The key threats are the worldwide emergence and dissemination of inactivating enzymes such as extended-spectrum beta-lactamases, cephalosporinases (AmpCs), and carbapenemases in Gram-negative bacteria. All these resistance determinants are carried by plasmids and thus display a high capacity to efficiently disseminate in an intra- or interspecies manner. In Gram-positive bacteria, methicillin-resistant *S. aureus*—which possesses an additional PBP2A presenting a decreased affinity to beta-lactams—remains an issue in human medicine, despite the fact that its prevalence in hospitals has been considerably reduced in the past decades with improvements of hygiene measures. In veterinary medicine, methicillin-resistant *Staphylococcus pseudintermedius* is known to cause serious treatment challenges because of its associated multiresistance. However, the success of both methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* is more due to epidemic bursts of

Table 3 Distribution of the tetracycline resistance genes in streptococci in animal hosts

Animal host	Country	Year	Bacterial species	No. of isolates	Tetracycline genes					No. of TetR isolates[b]	Percentage of resistance (%)	Reference
					Efflux		Ribosomal protection					
					tet(K)	*tet*(L)	*tet*(M)	*tet*(O)	*tet*(S)			
Cattle	USA	1990	*S. agalactiae*	39	0	0	NT[a]	7	NT	10	25.6	137
			S. dysgalactiae	21	1	1	NT	1	NT	9	42.9	
			S. uberis	11	1		NT	1	NT	2	18.2	
Cattle	France	1984–2008	*S. agalactiae*	76	NT	NT	16	13	1	30	39.5	94
			S. dysgalactiae	32	NT	NT	5	4	4	32	100.0	
			S. uberis	101	NT	NT	23	36	3	62	61.4	
Cattle	Portugal	2002–2003	*S. agalactiae*	60	34	NT	13	20	0	34	56.7	90
			S. dysgalactiae	18	0	NT	6	6	0	18	100.0	
			S. uberis	30	0	NT	2	9	8	18	60.0	
Ovine	Italy	2004–2014	*S. uberis*	51	9	NT	12	12	NT	18	35.3	138
Pig	USA	1986	*S. suis*	21	0	0	5	NT	NT	14	66.7	103
Pig	Denmark	1989–2002	*S. suis*	103	NT	0	11	6	0	25	24.3	237
Pig	Italy	2003–2007	*S. suis*	57	0	0	2	38	0	51	89.5	140
Pig	China	2005–2012	*S. suis*	62	NT	NT	53	42	NT	57	91.9	170
				34	NT	NT	24	9	NT	28	82.4	
Pig	China	2008–2010	*S. suis*	106	NT	2	16	86	1	105	99.1	146
Dog/cat	France	2010	*S. canis*	112	NT	1	31	16	5	36	32.1	148
Dog/cat	Japan	2015	*S. canis*	68	0	0	13	10	NT	16	23.5	149
Dog/cat/horse/human[c]	Portugal	2000–2010	*S. canis*	85	NT	1	11	8	1	23	27.0	34

[a]NT, not tested.
[b]Discrepancies between the number of tetracycline-resistant isolates and the genes identified may be due to either unidentified genes or to isolates presenting an association of two or three *tet* genes.
[c]Human isolates could not be individualized.

successful clones than to the mobilization of the *mecA*-carrying cassette.

Streptococci are unique among the major pathogens in the sense that they are incapable of acquiring any exogenous beta-lactam resistance genes. However, they can progressively mutate their own PBPs. Indeed, no isolate carrying a beta-lactamase (such as Gram-negative bacteria) or a new PBP (such as staphylococci) has been described yet, and a few species, including *S. pyogenes*, are even unable to develop decreased susceptibility to beta-lactams *in vitro* (151).

Beta-Lactam Resistance in Streptococci

To achieve beta-lactam resistance, streptococci sequentially modify their PBPs, specifically the class B PBP2B and PBP2X (and the class A PBP1A in the more resistant isolates). This was particularly exemplified in *S. pneumoniae*, the only *Streptococcus* spp. for which penicillin-resistance was successfully achieved and widely disseminated, where both mutated and mosaic PBPs were reported. Other less-documented genes were sporadically reported as PBP-independent penicillin-resistance mechanisms. These include the *MurMN* operon encoding enzymes that are responsible for the biosynthesis of branched muropeptide components (152), the *ciaRH* operon, a two-component signal-transducing system (153), the *adr* gene coding for a peptidoglycan *O*-acetyltransferase (154), the *stkP* gene encoding a serine/threonine kinase (155), the *pstS* gene encoding a subunit of a phosphate ABC transporter (156), and the *spr1178* gene encoding for a putative iron permease (157). Penicillin-resistant *S. pneumoniae* has widely disseminated through the success of a limited number of serotypes, selected mainly by the excessive use of antibiotics (158), but the prevalence of this resistance has considerably decreased since the early 2000s by both the reduced consumption of antibiotics and the marketing of efficient vaccines (159, 160). The presence and characterization of mutated PBPs in isolates presenting decreased susceptibilities to beta-lactam were also reported in *S. agalactiae* of human origin (69, 161, 162). Recently, a classification of *S. agalactiae* was proposed which takes into account the different mutations in the PBPs (163).

In veterinary medicine, *Streptococcus* isolates presenting full penicillin resistance have only rarely been reported, and only a few molecular studies were conducted either on laboratory strains or on field isolates presenting reduced susceptibility to beta-lactams. The presence of three groups of PBPs (PBP 1, PBP 2, and PBP 3) was demonstrated in *S. suis*, and PBP modifications were strongly suggested to be responsible for penicillin G-resistant phenotypes in both *in vitro* mutants and field isolates (164). In *S. uberis*, Haenni et al. (165) showed that both a quality control strain and field strains were capable of developing a 60-fold MIC increase after 30 cycles of exposure to penicillin G. This increase was due to the accumulation of mutations in the class B (PBP 2B and PBP 2X) and A (PBP 1A) enzymes, including the systematic presence of the two specific $E_{381}K$ and $Q_{554}E$ mutations in the PBP 2X. Interestingly, PBP analysis of seven field strains collected in Switzerland, France, and Holland and presenting MICs of 0.25 to 0.5 mg/liter also revealed the systematic presence of these two key mutations (165). However, none of the tested strains (selected either *in vitro* or by treatment on farms) could achieve full resistance, since their MICs only reached 0.25 to 2 mg/liter, which is still considered intermediately resistant.

Phenotypic Reports on Beta-Lactam Activity in Bovine Mastitis

In veterinary medicine, beta-lactam resistance has mostly been documented in bovine streptococci, namely *S. uberis*, *S. dysgalactiae*, and *S. agalactiae*. Data were gathered in cases of clinical and subclinical mastitis, a pathology for which the first-line treatment is beta-lactams. Comparison between studies can be difficult because of the heterogeneity of the methods (disc diffusion, agar diffusion, broth microdilution) and the guidelines used. If no interpretation was inferred, an isolate was considered resistant when the MIC was ≥4 mg/liter according to the CLSI breakpoints.

Most studies based on the determination of MICs report the absence of penicillin G resistance in *S. uberis* and *S. dysgalactiae*, even though isolates presenting decreased susceptibilities (0.25 to 0.5 mg/liter) were regularly reported. In France, such nonsusceptible isolates were reported in 2002, with 14.0% of the *S. uberis* showing an MIC of 0.25 mg/liter (134), and a shift toward decreased susceptibilities was suggested in 2010 based on the comparison of disk diffusion and MIC results (166). In Sweden, two studies performed successively in 2003 (133) and 2008 to 2009 (101) showed a slight shift over the years toward decreased susceptibility, with 6.0% of the *S. uberis* isolates displaying an MIC of 0.25 mg/liter and 10.0% of the *S. dysgalactiae* isolates displaying an MIC of 0.12 mg/liter in 2009, whereas only 0.9% of the *S. uberis* isolates had an MIC of 0.25 mg/liter, and all the *S. dysgalactiae* isolates were fully susceptible (MIC, <0.06 mg/liter) in 2003. In the United States, one true resistant *S. dysgalactiae* isolate (MIC, 4 mg/liter) was detected over 152 strains tested, whereas 6.8% of the *S. uberis* isolates presented

MICs of 0.5 mg/liter to penicillin, and one strain displayed an MIC of 1 mg/liter (136). The VetPath data (multicenter European data) collected between 2002 and 2006 showed no true resistance, but 29.8% of isolates presented decreased susceptibility (MICs ranging from 0.25 mg/liter to 1 mg/liter) (100). Though without any MIC values, other studies also reported data on beta-lactam resistance in veterinary streptococci, often with a very low prevalence of resistance. One pan-European study performed in 2002 to 2004 showed very low levels (0 to 3.9%) of penicillin resistance (99). A Swiss study detected susceptibilities to ampicillin of 92.3% for *S. uberis* and 94.8% for *S. dysgalactiae* from cows sampled in 2011 to 2013 (167). Kalmus et al. showed 0 to 0.4% resistance to penicillin G, ampicillin, and cefalotin in Estonia between 2007 and 2009 (168). In France, 12.9% of the *S. uberis* and 1.4% of the *S. dysgalactiae* presented resistance patterns to oxacillin, but these were not confirmed by MIC determination (135). In Turkey, 94% of the *S. uberis* isolates showed susceptibility to penicillin G (169).

These different data confirm beta-lactams as efficient antibiotics against streptococci isolated from bovine mastitis. However, this slow but clear shift of strains from full toward decreased susceptibility will have to be surveyed in the future. Beta-lactam resistance development in streptococci surely does not present the same dynamic as in Gram-negative bacteria, where plasmids play a major role. However, this should not hide rampant and silent acquisition of beta-lactam resistance in streptococci of animal origin, which may one day limit the therapeutic arsenal available for veterinarians.

Beta-Lactam Resistance Outside the Context of Cattle Mastitis

Antibiotic resistance was also monitored in *S. suis* isolated from pigs, and several studies reported the absence of resistance to any beta-lactams. Nevertheless, isolates presenting particularly high MICs to penicillin G were also recurrently reported, with unfortunately, no concomitant molecular work on the underlying mechanisms of resistance. Indeed, 4% of the Spanish isolates displayed MICs ranging from 4 to 16 mg/liter, whereas two Chinese studies reported 2.1% and 9.5% of isolates with an MIC of ≥4 mg/liter (142, 143, 170). In the Netherlands, 0.5% and 0.3% of over 1,163 isolates of *S. suis* tested were considered resistant to penicillin G and ampicillin between 2013 and 2015 (171). In Japan, Poland, and Portugal, resistance was reported in 0.9%, 8.1%, and 13.0% of the isolates, respectively (107, 141). However, despite these cases, beta-lactams can still be recommended as first-line antibiotics for the treatment of *S. suis*.

Aside from *S. suis* and streptococci isolated from bovine mastitis, reports of veterinary streptococci are quite rare. Beta-lactam resistance was described once in *S. dysgalactiae* subspecies *equisimilis* isolated from swine in Brazil and in four studies of *S. canis* isolated from pets and horses in France, Japan, Belgium, and Portugal (34, 148–150, 172), and these five studies found full susceptibility of all isolates to penicillin G.

FLUOROQUINOLONE RESISTANCE

Quinolones are not active against streptococci, because of their intrinsic resistance. However, fluoroquinolones may be an alternative to beta-lactam antibiotics to treat streptococcal infections. The main agents used in veterinary medicine are enrofloxacin, marbofloxacin, danofloxacin, and the more recent pradofloxacin.

Resistance to fluoroquinolones is generally mediated by point mutations in the quinolone resistance-determinants regions of the *gyrA* and *parC* genes (173). Furthermore, plasmidic *qnr* genes participate in the dissemination of low-level resistance, but they have never been reported in streptococci. Efflux pumps also play a role in fluoroquinolone resistance, as has been proved for the SatAB, an ABC transporter, in *S. suis* (174).

Fluoroquinolone-Resistance Phenotypes

Resistance to fluoroquinolones has rarely been reported in veterinary streptococci. Moreover, in the fluoroquinolone family, there is a wide variability of the agents tested (enrofloxacin and ciprofloxacin are the most frequently used), thus making comparisons among studies difficult.

A 1.5% prevalence of resistance to enrofloxacin in *S. uberis* and 5.5% in *S. dysgalactiae* was reported in France in 2010 (135). In the same bacterial species, MICs ranging from 0.5 to 2 mg/liter were observed in Sweden (101). These apparently elevated MICs are constitutive of streptococci, which have a basal MIC higher than that of *Enterobacteriaceae* or staphylococci. In a study performed between 1994 and 2001, the same range of MICs was reported, and no increase in the resistance rate was observed over the years (175). Except for three resistant strains (MIC, 4 mg/liter), all *S. uberis* and *S. dysgalactiae* isolates collected in a multicenter European study presented MICs to marbofloxacin ranging from 0.25 to 2 mg/liter (176). In *S. suis*, the resistance rates to enrofloxacin determined in a pan-European study performed in 2009 to 2012

and to ciprofloxacin determined in Japan in 1987 to 1996 were very similar: 0.7% and 0.3%, respectively (177). Enrofloxacin resistance was also observed in *S. canis* in France, where MICs ranged from 0.25 to 2 mg/liter (148). *Streptococcus* spp. isolated from cats and dogs were studied through the ComPath European network: no resistance was reported in dermatological samples, whereas 1.8% of the cats and 4.0% of the dogs presenting with a respiratory tract infection carried enrofloxacin-resistant streptococci (178, 179).

ROLE OF THE ICES IN THE EVOLUTION OF RESISTANCE

MGEs play a major role in the dissemination of antibiotic resistance genes. MGEs mostly comprise conjugative plasmids, transposons, phages, and ICEs (initially named conjugative transposons). ICEs are chromosomal, self-transmissible MGEs that are capable of promoting their excision, conjugation, and site-specific integration in a recipient cell (180, 181). One of the most emblematic members of the ICEs is the Tn*916*-Tn*1545* family, which carries *tet*(M) and other antibiotic resistance genes (126, 127, 182, 183).

ICEs have been widely reported in streptococci, and they were recently detected in all *Streptococcus* spp. for which at least one complete genome was available, with *S. suis* being the most "colonized" species (184, 185). In human clinical streptococcal isolates, *erm* and *tet* resistance genes were recurrently reported on ICEs, such as *erm*(B) on ICE*Sp1116* and *erm*(TR)-*tet*(O) on ICE*Sp2905* in *S. pyogenes* (186, 187), *erm*(TR) on ICE*Sag*TR7 in *S. agalactiae* (188), and *erm*(B) and *tet*(O) on ICE*SsD9* in *S. suis* (189). Interestingly, resistance genes can also be mobilized by coresident ICEs, as demonstrated by the mobilization of an *erm*(T)-carrying plasmid in *S. dysgalactiae* subsp. *equisimilis* (190). Other antibiotic resistance determinants can also be found on ICEs, as exemplified by the presence of *tet*(M) and a chloramphenicol acetyl-transferase on ICE*Sp*23FST81 from *S. agalactiae* (191), lincosamide resistance (*lsa* genes) on different ICEs in *S. agalactiae* (192), and a multidrug resistance cluster on ICE*Ssu*NC28 carried by *S. suis* (193). Moreover, ICEs originating from different streptococcal species may form hybrids that can further transfer *in vitro* to a third streptococcal species (194). This illustrates the wide distribution and the plasticity of these MGEs and thus their role in the dissemination of resistance genes. Finally, resistance determinants may be adjacent to—and not inside—an ICE. This is exemplified by the first vancomycin-resistance determinants in streptococci, the *vanG* operons, which were identified in one *S. agalactiae* and two *S. anginosus* isolates (195). These *vanG* operons were immediately followed by a large chromosomal element named ICE-r (ICE-like sequences). A plausible hypothesis is that the integration of ICE-r in the streptococcal chromosome may have favored the subsequent integration of the *vanG* element.

Except for elements from the Tn*916*-Tn*1545* family which were broadly reported in different streptococcal species originating from diverse animal hosts (9, 94, 170, 196), ICEs carrying resistance genes have rarely been reported in streptococci from animal origin. Indeed, only the mosaic *tet*(O/W/32/O) carried on ICE*Ssu32457* in *S. suis*, which could then be transferred to *S. pneumoniae*, *S. pyogenes*, and *S. agalactiae* (197), as well as the lincomycin-resistance gene *lsa* (192) were described on such MGEs. This will likely change in the near future, since new research perspectives are emerging with the democratized access to high-throughput sequencing technologies and the subsequent databases (185, 198).

CONCLUSION

In veterinary medicine streptococci are frequent pathogens not only in food-producing but also in companion animals. However, as far as public health is concerned, there are few situations in which streptococci of animal origin may cause risk for humans, and vice-versa. Among those, *S. suis* and, to a lesser extent, *S. agalactiae* are likely the most relevant examples, and are both considered zoonotic pathogens. Studies of resistance to antimicrobials in streptococci of animal origin have largely focused on tetracyclines and macrolides/lincosamides, which are widely used in the animal sector globally. Accordingly, high resistance rates to these molecules have frequently been observed, which is also in line with specific niches covering major animal diseases, such as cattle mastitis of streptococcal origin. Molecular investigations highlighted the diversity of the resistance genes of the *tet* and *erm* families, together with the pivotal role of the ICEs. Nonetheless, most data originate from Europe, and there is a need for larger prevalence and molecular studies on a global scale. Of note, despite the wide use of penicillins to treat streptococcal infections, resistance to beta-lactams does not appear to be a crucial issue in veterinary streptococci, in contrast to what has been observed with the human-specific *S. pneumoniae*. In all, as in humans, antimicrobial resistance in *Streptococcus* of animal origin may largely differ depending on the *Streptococcus* sp. and therefore should not be considered as a whole.

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References

1. **Póntigo F, Moraga M, Flores SV.** 2015. Molecular phylogeny and a taxonomic proposal for the genus *Streptococcus*. *Genet Mol Res* **14:**10905–10918.
2. **Lancefield RC, Freimer EH.** 1966. Type-specific polysaccharide antigens of group B streptococci. *J Hyg (Lond)* **64:**191–203.
3. **Tanaka D, Isobe J, Watahiki M, Nagai Y, Katsukawa C, Kawahara R, Endoh M, Okuno R, Kumagai N, Matsumoto M, Morikawa Y, Ikebe T, Watanabe H, Working Group for Group A Streptococci in Japan.** 2008. Genetic features of clinical isolates of *Streptococcus dysgalactiae* subsp. *equisimilis* possessing Lancefield's group A antigen. *J Clin Microbiol* **46:**1526–1529.
4. **Brouwer S, Barnett TC, Rivera-Hernandez T, Rohde M, Walker MJ.** 2016. *Streptococcus pyogenes* adhesion and colonization. *FEBS Lett* **590:**3739–3757.
5. **Carapetis JR, Steer AC, Mulholland EK, Weber M.** 2005. The global burden of group A streptococcal diseases. *Lancet Infect Dis* **5:**685–694.
6. **Copperman SM.** 1982. Cherchez le chien: household pets as reservoirs of persistent or recurrent streptococcal sore throats in children. *N Y State J Med* **82:**1685–1687.
7. **Farley MM.** 2001. Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis* **33:**556–561.
8. **Olivares-Fuster O, Klesius PH, Evans J, Arias CR.** 2008. Molecular typing of *Streptococcus agalactiae* isolates from fish. *J Fish Dis* **31:**277–283.
9. **Fischer A, Liljander A, Kaspar H, Muriuki C, Fuxelius HH, Bongcam-Rudloff E, de Villiers EP, Huber CA, Frey J, Daubenberger C, Bishop R, Younan M, Jores J.** 2013. Camel *Streptococcus agalactiae* populations are associated with specific disease complexes and acquired the tetracycline resistance gene *tetM* via a Tn*916*-like element. *Vet Res (Faisalabad)* **44:**86.
10. **Yildirim AO, Lämmler C, Weiss R.** 2002. Identification and characterization of *Streptococcus agalactiae* isolated from horses. *Vet Microbiol* **85:**31–35.
11. **McDonald TJ, McDonald JS.** 1976. Streptococci isolated from bovine intramammary infections. *Am J Vet Res* **37:**377–381.
12. **Brochet M, Couvé E, Zouine M, Vallaeys T, Rusniok C, Lamy MC, Buchrieser C, Trieu-Cuot P, Kunst F, Poyart C, Glaser P.** 2006. Genomic diversity and evolution within the species *Streptococcus agalactiae*. *Microbes Infect* **8:**1227–1243.
13. **Rajendram P, Mar Kyaw W, Leo YS, Ho H, Chen WK, Lin R, Pratim P, Badaruddin H, Ang B, Barkham T, Chow A.** 2016. Group B streptococcus sequence type 283 disease linked to consumption of raw fish, Singapore. *Emerg Infect Dis* **22:**1974–1977.
14. **Evans JJ, Klesius PH, Pasnik DJ, Bohnsack JF.** 2009. Human *Streptococcus agalactiae* isolate in Nile tilapia (*Oreochromis niloticus*). *Emerg Infect Dis* **15:**774–776.
15. **Bradley A.** 2002. Bovine mastitis: an evolving disease. *Vet J* **164:**116–128.
16. **Leigh JA.** 1999. *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis? *Vet J* **157:**225–238.
17. **Schleifer KH, Kilpper-Bälz R.** 1984. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Int J Syst Evol Microbiol* **34:**31–34.
18. **Köhler W.** 2007. The present state of species within the genera *Streptococcus* and *Enterococcus*. *Int J Med Microbiol* **297:**133–150.
19. **Ben-Chetrit E, Wiener-Well Y, Kashat L, Yinnon AM, Assous MV.** 2016. *Streptococcus bovis* new taxonomy: does subspecies distinction matter? *Eur J Clin Microbiol Infect Dis* **36**(2)**:**387.
20. **Dumke J, Hinse D, Vollmer T, Schulz J, Knabbe C, Dreier J.** 2015. Potential transmission pathways of *Streptococcus gallolyticus* subsp. *gallolyticus*. *PLoS One* **10:**e0126507.
21. **Gherardi G, Palmieri C, Marini E, Pompilio A, Crocetta V, Di Bonaventura G, Creti R, Facinelli B.** 2016. Identification, antimicrobial resistance and molecular characterization of the human emerging pathogen *Streptococcus gallolyticus* subsp. *pasteurianus*. *Diagn Microbiol Infect Dis* **86:**329–335.
22. **Sheng WH, Chuang YC, Teng LJ, Hsueh PR.** 2014. Bacteraemia due to *Streptococcus gallolyticus* subspecies *pasteurianus* is associated with digestive tract malignancies and resistance to macrolides and clindamycin. *J Infect* **69:**145–153.
23. **Sturt AS, Yang L, Sandhu K, Pei Z, Cassai N, Blaser MJ.** 2010. *Streptococcus gallolyticus* subspecies *pasteurianus* (biotype II/2), a newly reported cause of adult meningitis. *J Clin Microbiol* **48:**2247–2249.
24. **Barnett J, Ainsworth H, Boon JD, Twomey DF.** 2008. *Streptococcus gallolyticus* subsp. *pasteurianus* septicaemia in goslings. *Vet J* **176:**251–253.
25. **Li M, Gu C, Zhang W, Li S, Liu J, Qin C, Su J, Cheng G, Hu X.** 2013. Isolation and characterization of *Streptococcus gallolyticus* subsp. *pasteurianus* causing meningitis in ducklings. *Vet Microbiol* **162:**930–936.
26. **Marmolin ES, Hartmeyer GN, Christensen JJ, Nielsen XC, Dargis R, Skov MN, Knudsen E, Kemp M, Justesen US.** 2016. Bacteremia with the bovis group streptococci: species identification and association with infective endocarditis and with gastrointestinal disease. *Diagn Microbiol Infect Dis* **85:**239–242.
27. **Wessman GE.** 1986. Biology of the group E streptococci: a review. *Vet Microbiol* **12:**297–328.
28. **Facklam R, Elliott J, Pigott N, Franklin AR.** 1995. Identification of *Streptococcus porcinus* from human sources. *J Clin Microbiol* **33:**385–388.
29. **Duarte RS, Barros RR, Facklam RR, Teixeira LM.** 2005. Phenotypic and genotypic characteristics of *Streptococcus porcinus* isolated from human sources. *J Clin Microbiol* **43:**4592–4601.

30. **Jonsson P, Olsson SO, Olofson AS, Fälth C, Holmberg O, Funke H.** 1991. Bacteriological investigations of clinical mastitis in heifers in Sweden. *J Dairy Res* **58:** 179–185.
31. **Cuccuru C, Meloni M, Sala E, Scaccabarozzi L, Locatelli C, Moroni P, Bronzo V.** 2011. Effects of intramammary infections on somatic cell score and milk yield in Sarda sheep. *N Z Vet J* **59:**128–131.
32. **Timoney JF.** 2004. The pathogenic equine streptococci. *Vet Res* **35:**397–409.
33. **Hashikawa S, Iinuma Y, Furushita M, Ohkura T, Nada T, Torii K, Hasegawa T, Ohta M.** 2004. Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* **42:**186–192.
34. **Pinho MD, Matos SC, Pomba C, Lübke-Becker A, Wieler LH, Preziuso S, Melo-Cristino J, Ramirez M.** 2013. Multilocus sequence analysis of *Streptococcus canis* confirms the zoonotic origin of human infections and reveals genetic exchange with *Streptococcus dysgalactiae* subsp. *equisimilis*. *J Clin Microbiol* **51:**1099–1109.
35. **Iglauer F, Kunstýr I, Mörstedt R, Farouq H, Wullenweber M, Damsch S.** 1991. *Streptococcus canis* arthritis in a cat breeding colony. *J Exp Anim Sci* **34:**59–65.
36. **Lacave G, Coutard A, Troché G, Augusto S, Pons S, Zuber B, Laurent V, Amara M, Couzon B, Bédos JP, Pangon B, Grimaldi D.** 2016. Endocarditis caused by *Streptococcus canis*: an emerging zoonosis? *Infection* **44:**111–114.
37. **Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M.** 2014. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent: an update on the worldwide distribution based on serotyping and sequence typing. *Emerg Microbes Infect* **3:**e45.
38. **Segura M, Calzas C, Grenier D, Gottschalk M.** 2016. Initial steps of the pathogenesis of the infection caused by *Streptococcus suis*: fighting against nonspecific defenses. *FEBS Lett* **590:**3772–3799.
39. **Taniyama D, Sakurai M, Sakai T, Kikuchi T, Takahashi T.** 2016. Human case of bacteremia due to *Streptococcus suis* serotype *5* in Japan: the first report and literature review. *IDCases* **6:**36–38.
40. **Ward PN, Holden MT, Leigh JA, Lennard N, Bignell A, Barron A, Clark L, Quail MA, Woodward J, Barrell BG, Egan SA, Field TR, Maskell D, Kehoe M, Dowson CG, Chanter N, Whatmore AM, Bentley SD, Parkhill J.** 2009. Evidence for niche adaptation in the genome of the bovine pathogen *Streptococcus uberis*. *BMC Genomics* **10:**54–71.
41. **Phuektes P, Mansell PD, Dyson RS, Hooper ND, Dick JS, Browning GF.** 2001. Molecular epidemiology of *Streptococcus uberis* isolates from dairy cows with mastitis. *J Clin Microbiol* **39:**1460–1466.
42. **Wilesmith JW, Francis PG, Wilson CD.** 1986. Incidence of clinical mastitis in a cohort of British dairy herds. *Vet Rec* **118:**199–204.
43. **Compton CW, Heuer C, Parker K, McDougall S.** 2007. Epidemiology of mastitis in pasture-grazed peripartum dairy heifers and its effects on productivity. *J Dairy Sci* **90:**4157–4170.
44. **Hasson KW, Wyld EM, Fan Y, Lingsweiller SW, Weaver SJ, Cheng J, Varner PW.** 2009. Streptococcosis in farmed *Litopenaeus vannamei*: a new emerging bacterial disease of penaeid shrimp. *Dis Aquat Organ* **86:**93–106.
45. **Parker KI, Compton C, Anniss FM, Weir A, Heuer C, McDougall S.** 2007. Subclinical and clinical mastitis in heifers following the use of a teat sealant precalving. *J Dairy Sci* **90:**207–218.
46. **Dodd FH.** 1983. Mastitis: progress on control. *J Dairy Sci* **66:**1773–1780.
47. **Zehner MM, Farnsworth RJ, Appleman RD, Larntz K, Springer JA.** 1986. Growth of environmental mastitis pathogens in various bedding materials. *J Dairy Sci* **69:** 1932–1941.
48. **Koh TH, Sng LH, Yuen SM, Thomas CK, Tan PL, Tan SH, Wong NS.** 2009. Streptococcal cellulitis following preparation of fresh raw seafood. *Zoonoses Public Health* **56:**206–208.
49. **Weinstein MR, Litt M, Kertesz DA, Wyper P, Rose D, Coulter M, McGeer A, Facklam R, Ostach C, Willey BM, Borczyk A, Low DE.** 1997. Invasive infections due to a fish pathogen, *Streptococcus iniae*. *S. iniae* Study Group. *N Engl J Med* **337:**589–594.
50. **Nilson B, Olaison L, Rasmussen M.** 2016. Clinical presentation of infective endocarditis caused by different groups of non-beta haemolytic streptococci. *Eur J Clin Microbiol Infect Dis* **35:**215–218.
51. **Simón-Soro A, Mira A.** 2015. Solving the etiology of dental caries. *Trends Microbiol* **23:**76–82.
52. **Yombi J, Belkhir L, Jonckheere S, Wilmes D, Cornu O, Vandercam B, Rodriguez-Villalobos H.** 2012. *Streptococcus gordonii* septic arthritis: two cases and review of literature. *BMC Infect Dis* **12:**215–220.
53. **Burrell MH, Mackintosh ME, Taylor CE.** 1986. Isolation of *Streptococcus pneumoniae* from the respiratory tract of horses. *Equine Vet J* **18:**183–186.
54. **Köndgen S, Calvignac-Spencer S, Grützmacher K, Keil V, Mätz-Rensing K, Nowak K, Metzger S, Kiyang J, Becker AL, Deschner T, Wittig RM, Lankester F, Leendertz FH.** 2017. Evidence for human *Streptococcus pneumoniae* in wild and captive chimpanzees: a potential threat to wild populations. *Sci Rep* **7:**14581.
55. **Beighton D, Hayday H.** 1982. The streptococcal flora of the tongue of the monkey *Macaca fascicularis*. *Arch Oral Biol* **27:**331–335.
56. **Devriese LA, Hommez J, Pot B, Haesebrouck F.** 1994. Identification and composition of the streptococcal and enterococcal flora of tonsils, intestines and faeces of pigs. *J Appl Bacteriol* **77:**31–36.
57. **Devriese LA, Pot B, Vandamme P, Kersters K, Collins MD, Alvarez N, Haesebrouck F, Hommez J.** 1997. *Streptococcus hyovaginalis* sp. nov. and *Streptococcus thoraltensis* sp. nov., from the genital tract of sows. *Int J Syst Bacteriol* **47:**1073–1077.
58. **Freedman ML, Coykendall AL, O'Neill EM.** 1982. Physiology of "mutans-like" *Streptococcus ferus* from wild rats. *Infect Immun* **35:**476–482.
59. **Zhu H, Willcox MD, Knox KW.** 2000. A new species of oral *Streptococcus* isolated from Sprague-Dawley rats,

Streptococcus orisratti sp. nov. *Int J Syst Evol Microbiol* **50:**55–61.

60. **Rinkinen ML, Koort JM, Ouwehand AC, Westermarck E, Björkroth KJ.** 2004. *Streptococcus alactolyticus* is the dominating culturable lactic acid bacterium species in canine jejunum and feces of four fistulated dogs. *FEMS Microbiol Lett* **230:**35–39.
61. **Toepfner N, Shetty S, Kunze M, Orlowska-Volk M, Krüger M, Berner R, Hentschel R.** 2014. Fulminant neonatal sepsis due to *Streptococcus alactolyticus*: a case report and review. *APMIS* **122:**654–656.
62. **Beighton D, Russell RR, Hayday H.** 1981. The isolation of characterization of *Streptococcus mutans* serotype h from dental plaque of monkeys (*Macaca fascicularis*). *J Gen Microbiol* **124:**271–279.
63. **Yoo SY, Kim KJ, Lim SH, Kim KW, Hwang HK, Min BM, Choe SJ, Kook JK.** 2005. First isolation of *Streptococcus downei* from human dental plaques. *FEMS Microbiol Lett* **249:**323–326.
64. **Aryasinghe L, Sabbar S, Kazim Y, Awan LM, Khan HK.** 2014. *Streptococcus pluranimalium*: a novel human pathogen? *Int J Surg Case Rep* **5:**1242–1246.
65. **Devriese LA, Vandamme P, Collins MD, Alvarez N, Pot B, Hommez J, Butaye P, Haesebrouck F.** 1999. *Streptococcus pluranimalium* sp. nov., from cattle and other animals. *Int J Syst Bacteriol* **49:**1221–1226.
66. **Facklam R.** 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev* **15:**613–630.
67. **Silley P, Simjee S, Schwarz S.** 2012. Surveillance and monitoring ofantimicrobial resistance and antibiotic consumption in humans and animals. *Rev Sci Tech* **31:** 105–120.
68. **de Jong A, Thomas V, Klein U, Marion H, Moyaert H, Simjee S, Vallé M.** 2013. Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. *Int J Antimicrob Agents* **41:**403–409.
69. **Metcalf BJ, Chochua S, Gertz RE Jr, Hawkins PA, Ricaldi J, Li Z, Walker H, Tran T, Rivers J, Mathis S, Jackson D, Glennen A, Lynfield R, McGee L, Beall B, Active Bacterial Core surveillance team.** 2017. Short-read whole genome sequencing for determination of antimicrobial resistance mechanisms and capsular serotypes of current invasive *Streptococcus agalactiae* recovered in the USA. *Clin Microbiol Infect* **23:**574e577–574e514.
70. **Li Y, Metcalf BJ, Chochua S, Li Z, Gertz RE Jr, Walker H, Hawkins PA, Tran T, Whitney CG, McGee L, Beall BW.** 2016. Penicillin-binding protein transpeptidase signatures for tracking and predicting beta-lactam resistance levels in *Streptococcus pneumoniae*. *MBio* **7:**pii e00756-16.
71. **Mobegi FM, Cremers AJ, de Jonge MI, Bentley SD, van Hijum SA, Zomer A.** 2017. Deciphering the distance to antibiotic resistance for the pneumococcus using genome sequencing data. *Sci Rep* **7:**42808.
72. **MARAN.** 2009. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands in 2008. CIDC-Lelystad, Lelystad, The Netherlands.
73. **Leclercq R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* **34:**482–492.
74. **Collignon P, Powers JH, Chiller TM, Aidara-Kane A, Aarestrup FM.** 2009. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals. *Clin Infect Dis* **49:**132–141.
75. **Edmondson PW.** 1989. An economic justification of "blitz" therapy to eradicate *Streptococcus agalactiae* from a dairy herd. *Vet Rec* **125:**591–593.
76. **Pyörälä S, Baptiste KE, Catry B, van Duijkeren E, Greko C, Moreno MA, Pomba MC, Rantala M, Ružauskas M, Sanders P, Threlfall EJ, Torren-Edo J, Törneke K.** 2014. Macrolides and lincosamides in cattle and pigs: use and development of antimicrobial resistance. *Vet J* **200:**230–239.
77. **De Mouy D, Cavallo JD, Leclercq R, Fabre R, AFICORPI-BIO Network. Association de Formation Continue en Pathologie Infectieuse des Biologistes.** 2001. Antibiotic susceptibility and mechanisms of erythromycin resistance in clinical isolates of *Streptococcus agalactiae*: French multicenter study. *Antimicrob Agents Chemother* **45:**2400–2402.
78. **Puopolo KM, Klinzing DC, Lin MP, Yesucevitz DL, Cieslewicz MJ.** 2007. A composite transposon associated with erythromycin and clindamycin resistance in group B *Streptococcus*. *J Med Microbiol* **56:**947–955.
79. **Cai Y, Kong F, Gilbert GL.** 2007. Three new macrolide efflux (*mef*) gene variants in *Streptococcus agalactiae*. *J Clin Microbiol* **45:**2754–2755.
80. **Ambrose KD, Nisbet R, Stephens DS.** 2005. Macrolide efflux in *Streptococcus pneumoniae* is mediated by a dual efflux pump (*mel* and *mef*) and is erythromycin inducible. *Antimicrob Agents Chemother* **49:**4203–4209.
81. **Gay K, Stephens DS.** 2001. Structure and dissemination of a chromosomal insertion element encoding macrolide efflux in *Streptococcus pneumoniae*. *J Infect Dis* **184:** 56–65.
82. **Mingoia M, Vecchi M, Cochetti I, Tili E, Vitali LA, Manzin A, Varaldo PE, Montanari MP.** 2007. Composite structure of *Streptococcus pneumoniae* containing the erythromycin efflux resistance gene *mefI* and the chloramphenicol resistance gene *catQ*. *Antimicrob Agents Chemother* **51:**3983–3987.
83. **Santagati M, Iannelli F, Cascone C, Campanile F, Oggioni MR, Stefani S, Pozzi G.** 2003. The novel conjugative transposon *tn1207.3* carries the macrolide efflux gene *mef*(A) in *Streptococcus pyogenes*. *Microb Drug Resist* **9:**243–247.
84. **Clancy J, Dib-Hajj F, Petitpas JW, Yuan W.** 1997. Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae*. *Antimicrob Agents Chemother* **41:**2719–2723.
85. **Bozdogan B, Berrezouga L, Kuo MS, Yurek DA, Farley KA, Stockman BJ, Leclercq R.** 1999. A new resistance gene, *linB*, conferring resistance to lincosamides

by nucleotidylation in *Enterococcus faecium* HM1025. *Antimicrob Agents Chemother* **43:**925–929.

86. **de Azavedo JC, McGavin M, Duncan C, Low DE, McGeer A.** 2001. Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. *Antimicrob Agents Chemother* **45:**3504–3508.
87. **Achard A, Villers C, Pichereau V, Leclercq R.** 2005. New *lnu*(C) gene conferring resistance to lincomycin by nucleotidylation in *Streptococcus agalactiae* UCN36. *Antimicrob Agents Chemother* **49:**2716–2719.
88. **Petinaki E, Guérin-Faublée V, Pichereau V, Villers C, Achard A, Malbruny B, Leclercq R.** 2008. Lincomycin resistance gene *lnu*(D) in *Streptococcus uberis*. *Antimicrob Agents Chemother* **52:**626–630.
89. **Chesneau O, Tsvetkova K, Courvalin P.** 2007. Resistance phenotypes conferred by macrolide phosphotransferases. *FEMS Microbiol Lett* **269:**317–322.
90. **Rato MG, Bexiga R, Florindo C, Cavaco LM, Vilela CL, Santos-Sanches I.** 2013. Antimicrobial resistance and molecular epidemiology of streptococci from bovine mastitis. *Vet Microbiol* **161:**286–294.
91. **Dogan B, Schukken YH, Santisteban C, Boor KJ.** 2005. Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human hosts. *J Clin Microbiol* **43:** 5899–5906.
92. **Gao J, Yu FQ, Luo LP, He JZ, Hou RG, Zhang HQ, Li SM, Su JL, Han B.** 2012. Antibiotic resistance of *Streptococcus agalactiae* from cows with mastitis. *Vet J* **194:**423–424.
93. **Pinto TC, Costa NS, Vianna Souza AR, Silva LG, Corrêa AB, Fernandes FG, Oliveira IC, Mattos MC, Rosado AS, Benchetrit LC.** 2013. Distribution of serotypes and evaluation of antimicrobial susceptibility among human and bovine *Streptococcus agalactiae* strains isolated in Brazil between 1980 and 2006. *Braz J Infect Dis* **17:**131–136.
94. **Haenni M, Saras E, Bertin S, Leblond P, Madec JY, Payot S.** 2010. Diversity and mobility of integrative and conjugative elements in bovine isolates of *Streptococcus agalactiae*, *S. dysgalactiae* subsp. *dysgalactiae*, and *S. uberis*. *Appl Environ Microbiol* **76:**7957–7965.
95. **Loch IM, Glenn K, Zadoks RN.** 2005. Macrolide and lincosamide resistance genes of environmental streptococci from bovine milk. *Vet Microbiol* **111:**133–138.
96. **Haenni M, Saras E, Chaussière S, Treilles M, Madec JY.** 2011. *ermB*-mediated erythromycin resistance in *Streptococcus uberis* from bovine mastitis. *Vet J* **189:** 356–358.
97. **Schmitt-Van de Leemput E, Zadoks RN.** 2007. Genotypic and phenotypic detection of macrolide and lincosamide resistance in *Streptococcus uberis*. *J Dairy Sci* **90:** 5089–5096.
98. **Achard A, Guérin-Faublée V, Pichereau V, Villers C, Leclercq R.** 2008. Emergence of macrolide resistance gene *mph*(B) in *Streptococcus uberis* and cooperative effects with *rdmC*-like gene. *Antimicrob Agents Chemother* **52:**2767–2770.
99. **Hendriksen RS, Mevius DJ, Schroeter A, Teale C, Meunier D, Butaye P, Franco A, Utinane A, Amado A, Moreno M, Greko C, Stärk K, Berghold C, Myllyniemi AL, Wasyl D, Sunde M, Aarestrup FM.** 2008. Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004. *Acta Vet Scand* **50:**28–38.
100. **Thomas V, de Jong A, Moyaert H, Simjee S, El Garch F, Morrissey I, Marion H, Vallé M.** 2015. Antimicrobial susceptibility monitoring of mastitis pathogens isolated from acute cases of clinical mastitis in dairy cows across Europe: VetPath results. *Int J Antimicrob Agents* **46:**13–20.
101. **Persson Y, Nyman AK, Grönlund-Andersson U.** 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet Scand* **53:**36.
102. **Aarestrup FM, Rasmussen SR, Artursson K, Jensen NE.** 1998. Trends in the resistance to antimicrobial agents of *Streptococcus suis* isolates from Denmark and Sweden. *Vet Microbiol* **63:**71–80.
103. **Wasteson Y, Høie S, Roberts MC.** 1994. Characterization of antibiotic resistance in *Streptococcus suis*. *Vet Microbiol* **41:**41–49.
104. **Gurung M, Tamang MD, Moon DC, Kim SR, Jeong JH, Jang GC, Jung SC, Park YH, Lim SK.** 2015. Molecular basis of resistance to selected antimicrobial agents in the emerging zoonotic pathogen *Streptococcus suis*. *J Clin Microbiol* **53:**2332–2336.
105. **Palmieri C, Varaldo PE, Facinelli B.** 2011. *Streptococcus suis*, an emerging drug-resistant animal and human pathogen. *Front Microbiol* **2:**235–241.
106. **Zhao Q, Wendlandt S, Li H, Li J, Wu C, Shen J, Schwarz S, Wang Y.** 2014. Identification of the novel lincosamide resistance gene *lnu*(E) truncated by IS*Enfa5*-*cfr*-IS*Enfa5* insertion in *Streptococcus suis*: *de novo* synthesis and confirmation of functional activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **58:**1785–1788.
107. **Hendriksen RS, Mevius DJ, Schroeter A, Teale C, Jouy E, Butaye P, Franco A, Utinane A, Amado A, Moreno M, Greko C, Stärk KD, Berghold C, Myllyniemi AL, Hoszowski A, Sunde M, Aarestrup FM.** 2008. Occurrence of antimicrobial resistance among bacterial pathogens and indicator bacteria in pigs in different European countries from year 2002–2004: the ARBAO-II study. *Acta Vet Scand* **50:**19–29.
108. **Lüthje P, Schwarz S.** 2007. Molecular basis of resistance to macrolides and lincosamides among staphylococci and streptococci from various animal sources collected in the resistance monitoring program BfT-GermVet. *Int J Antimicrob Agents* **29:**528–535.
109. **Silva LG, Genteluci GL, Corrêa de Mattos M, Glatthardt T, Sá Figueiredo AM, Ferreira-Carvalho BT.** 2015. Group C *Streptococcus dysgalactiae* subsp. *equisimilis* in south-east Brazil: genetic diversity, resistance profile and the first report of human and equine isolates belonging to the same multilocus sequence typing lineage. *J Med Microbiol* **64:**551–558.

110. **Pedersen K, Pedersen K, Jensen H, Finster K, Jensen VF, Heuer OE.** 2007. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *J Antimicrob Chemother* **60:**775–781.

111. **Chopra I, Roberts M.** 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* **65:**232–260.

112. **Speer BS, Shoemaker NB, Salyers AA.** 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin Microbiol Rev* **5:**387–399.

113. **Roberts MC.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev* **19:**1–24.

114. **Kazimierczak KA, Rincon MT, Patterson AJ, Martin JC, Young P, Flint HJ, Scott KP.** 2008. A new tetracycline efflux gene, *tet*(40), is located in tandem with *tet*(O/32/O) in a human gut firmicute bacterium and in metagenomic library clones. *Antimicrob Agents Chemother* **52:**4001–4009.

115. **Moulin G, Cavalié P, Pellanne I, Chevance A, Laval A, Millemann Y, Colin P, Chauvin C, Antimicrobial Resistance ad hoc Group of the French Food Safety Agency.** 2008. A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. *J Antimicrob Chemother* **62:**617–625.

116. **Report UOH.** 2015. Joint report on human and animal antibiotic use, sales and resistance, 2013. https://www.gov.uk/government/collections/antimicrobial-resistance-amr-information-and-resources.

117. **FDA.** 2014. 2012 Summary report on antimicrobials sold or distributed for use in food-producing animals. FDA, Washington, DC. http://www.fda.gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/-UCM416983.pdf.

118. **van den Bogaard AE, Bruinsma N, Stobberingh EE.** 2000. The effect of banning avoparcin on VRE carriage in The Netherlands. *J Antimicrob Chemother* **46:**146–148.

119. **Da Cunha V, Davies MR, Douarre PE, Rosinski-Chupin I, Margarit I, Spinali S, Perkins T, Lechat P, Dmytruk N, Sauvage E, Ma L, Romi B, Tichit M, Lopez-Sanchez MJ, Descorps-Declere S, Souche E, Buchrieser C, Trieu-Cuot P, Moszer I, Clermont D, Maione D, Bouchier C, McMillan DJ, Parkhill J, Telford JL, Dougan G, Walker MJ, Holden MTG, Poyart C, Glaser P, Glaser P, DEVANI Consortium.** 2014. *Streptococcus agalactiae* clones infecting humans were selected and fixed through the extensive use of tetracycline. *Nat Commun* **5:**4544.

120. **Roberts MC.** 2005. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* **245:**195–203.

121. **Roberts MC, Schwarz S.** 2016. Tetracycline and phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans. *J Environ Qual* **45:**576–592.

122. **Khan SA, Novick RP.** 1983. Complete nucleotide sequence of pT181, a tetracycline-resistance plasmid from *Staphylococcus aureus*. *Plasmid* **10:**251–259.

123. **Burdett V, Inamine J, Rajagopalan S.** 1982. Heterogeneity of tetracycline resistance determinants in *Streptococcus*. *J Bacteriol* **149:**995–1004.

124. **Hartley DL, Jones KR, Tobian JA, LeBlanc DJ, Macrina FL.** 1984. Disseminated tetracycline resistance in oral streptococci: implication of a conjugative transposon. *Infect Immun* **45:**13–17.

125. **Courvalin P, Carlier C.** 1987. Tn*1545*: a conjugative shuttle transposon. *Mol Gen Genet* **206:**259–264.

126. **Clewell DB, Flannagan SE, Jaworski DD, Clewell DB.** 1995. Unconstrained bacterial promiscuity: the Tn*916*-Tn*1545* family of conjugative transposons. *Trends Microbiol* **3:**229–236.

127. **Rice LB.** 1998. Tn*916* family conjugative transposons and dissemination of antimicrobial resistance determinants. *Antimicrob Agents Chemother* **42:**1871–1877.

128. **Sougakoff W, Papadopoulou B, Nordmann P, Courvalin P.** 1987. Nucleotide sequence and distribution of gene *tetO* encoding tetracycline resistance in *Campylobacter coli*. *FEMS Microbiol Lett* **44:**153–159.

129. **Charpentier E, Gerbaud G, Courvalin P.** 1993. Characterization of a new class of tetracycline-resistance gene *tet*(S) in *Listeria monocytogenes* BM4210. *Gene* **131:** 27–34.

130. **Fletcher HM, Macrina FL.** 1991. Molecular survey of clindamycin and tetracycline resistance determinants in *Bacteroides* species. *Antimicrob Agents Chemother* **35:** 2415–2418.

131. **Clermont D, Chesneau O, De Cespédès G, Horaud T.** 1997. New tetracycline resistance determinants coding for ribosomal protection in streptococci and nucleotide sequence of *tet*(T) isolated from *Streptococcus pyogenes* A498. *Antimicrob Agents Chemother* **41:**112–116.

132. **Barbosa TM, Scott KP, Flint HJ.** 1999. Evidence for recent intergeneric transfer of a new tetracycline resistance gene, *tet*(W), isolated from *Butyrivibrio fibrisolvens*, and the occurrence of *tet*(O) in ruminal bacteria. *Environ Microbiol* **1:**53–64.

133. **Bengtsson B, Unnerstad HE, Ekman T, Artursson K, Nilsson-Ost M, Waller KP.** 2009. Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. *Vet Microbiol* **136:**142–149.

134. **Guérin-Faublée V, Tardy F, Bouveron C, Carret G.** 2002. Antimicrobial susceptibility of *Streptococcus* species isolated from clinical mastitis in dairy cows. *Int J Antimicrob Agents* **19:**219–226.

135. **Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY, Calavas D.** 2010. Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne Pathog Dis* **7:**479–487.

136. **Rossitto PV, Ruiz L, Kikuchi Y, Glenn K, Luiz K, Watts JL, Cullor JS.** 2002. Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies. *J Dairy Sci* **85:** 132–138.

137. **Brown MB, Roberts MC.** 1991. Tetracycline resistance determinants in streptococcal species isolated from the bovine mammary gland. *Vet Microbiol* **29:**173–180.

138. **Lollai SA, Ziccheddu M, Duprè I, Piras D.** 2016. Characterization of resistance to tetracyclines and aminoglycosides of sheep mastitis pathogens: study of the effect of gene content on resistance. *J Appl Microbiol* **121:**941–951.

139. **Wisselink HJ, Veldman KT, Van den Eede C, Salmon SA, Mevius DJ.** 2006. Quantitative susceptibility of *Streptococcus suis* strains isolated from diseased pigs in seven European countries to antimicrobial agents licensed in veterinary medicine. *Vet Microbiol* **113:**73–82.

140. **Princivalli MS, Palmieri C, Magi G, Vignaroli C, Manzin A, Camporese A, Barocci S, Magistrali C, Facinelli B.** 2009. Genetic diversity of *Streptococcus suis* clinical isolates from pigs and humans in Italy (2003–2007). *Euro Surveill* **14:**pii=19310. http://www.eurosurveillance.org/content/10.2807/ese.14.33.19310-en.

141. **Kataoka Y, Yoshida T, Sawada T.** 2000. A 10-year survey of antimicrobial susceptibility of *Streptococcus suis* isolates from swine in Japan. *J Vet Med Sci* **62:**1053–1057.

142. **Zhang C, Ning Y, Zhang Z, Song L, Qiu H, Gao H.** 2008. *In vitro* antimicrobial susceptibility of *Streptococcus suis* strains isolated from clinically healthy sows in China. *Vet Microbiol* **131:**386–392.

143. **Vela AI, Moreno MA, Cebolla JA, González S, Latre MV, Domínguez L, Fernández-Garayzábal JF.** 2005. Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. *Vet Microbiol* **105:**143–147.

144. **Soares TC, Paes AC, Megid J, Ribolla PE, Paduan KS, Gottschalk M.** 2014. Antimicrobial susceptibility of *Streptococcus suis* isolated from clinically healthy swine in Brazil. *Can J Vet Res* **78:**145–149.

145. **Manzin A, Palmieri C, Serra C, Saddi B, Princivalli MS, Loi G, Angioni G, Tiddia F, Varaldo PE, Facinelli B.** 2008. *Streptococcus suis* meningitis without history of animal contact, Italy. *Emerg Infect Dis* **14:**1946–1948.

146. **Chen L, Song Y, Wei Z, He H, Zhang A, Jin M.** 2013. Antimicrobial susceptibility, tetracycline and erythromycin resistance genes, and multilocus sequence typing of *Streptococcus suis* isolates from diseased pigs in China. *J Vet Med Sci* **75:**583–587.

147. **Chander Y, Oliveira SR, Goyal SM.** 2011. Identification of the *tet*(B) resistance gene in *Streptococcus suis*. *Vet J* **189:**359–360.

148. **Haenni M, Hourquet C, Saras E, Madec JY.** 2015. Genetic determinants of antimicrobial resistance in *Streptococcus canis* in France. *J Glob Antimicrob Resist* **3:**142–143.

149. **Tsuyuki Y, Kurita G, Murata Y, Goto M, Takahashi T.** 2017. Identification of group G streptococci isolates from companion animals in Japan and their antimicrobial resistance. *Jpn J Infect Dis* **70:**394–398.

150. **Moyaert H, De Graef EM, Haesebrouck F, Decostere A.** 2006. Acquired antimicrobial resistance in the intestinal microbiota of diverse cat populations. *Res Vet Sci* **81:**1–7.

151. **Pérez-Trallero E, Fernández-Mazarrasa C, García-Rey C, Bouza E, Aguilar L, García-de-Lomas J, Baquero F, Spanish Surveillance Group for Respiratory Pathogens.** 2001. Antimicrobial susceptibilities of 1,684 *Streptococcus pneumoniae* and 2,039 *Streptococcus pyogenes* isolates and their ecological relationships: results of a 1-year (1998–1999) multicenter surveillance study in Spain. *Antimicrob Agents Chemother* **45:**3334–3340.

152. **Filipe SR, Tomasz A.** 2000. Inhibition of the expression of penicillin resistance in *Streptococcus pneumoniae* by inactivation of cell wall muropeptide branching genes. *Proc Natl Acad Sci USA* **97:**4891–4896.

153. **Guenzi E, Gasc AM, Sicard MA, Hakenbeck R.** 1994. A two-component signal-transducing system is involved in competence and penicillin susceptibility in laboratory mutants of *Streptococcus pneumoniae*. *Mol Microbiol* **12:**505–515.

154. **Crisóstomo MI, Vollmer W, Kharat AS, Inhülsen S, Gehre F, Buckenmaier S, Tomasz A.** 2006. Attenuation of penicillin resistance in a peptidoglycan *O*-acetyl transferase mutant of *Streptococcus pneumoniae*. *Mol Microbiol* **61:**1497–1509.

155. **Dias R, Félix D, Caniça M, Trombe MC.** 2009. The highly conserved serine threonine kinase StkP of *Streptococcus pneumoniae* contributes to penicillin susceptibility independently from genes encoding penicillin-binding proteins. *BMC Microbiol* **9:**121.

156. **Soualhine H, Brochu V, Ménard F, Papadopoulou B, Weiss K, Bergeron MG, Légaré D, Drummelsmith J, Ouellette M.** 2005. A proteomic analysis of penicillin resistance in *Streptococcus pneumoniae* reveals a novel role for PstS, a subunit of the phosphate ABC transporter. *Mol Microbiol* **58:**1430–1440.

157. **Fani F, Leprohon P, Légaré D, Ouellette M.** 2011. Whole genome sequencing of penicillin-resistant *Streptococcus pneumoniae* reveals mutations in penicillin-binding proteins and in a putative iron permease. *Genome Biol* **12:**R115.

158. **Goldstein FW.** 1999. Penicillin-resistant *Streptococcus pneumoniae*: selection by both beta-lactam and non-beta-lactam antibiotics. *J Antimicrob Chemother* **44:**141–144.

159. **Anonymous.** 2008. Recent trends in antimicrobial resistance among *Streptococcus pneumoniae* and *Staphylococcus aureus* isolates: the French experience. *Euro Surveill* **13:**pii=19035. http://eurosurveillance.org/content/10.2807/ese.13.46.19035-en.

160. **Dagan R, Klugman KP.** 2008. Impact of conjugate pneumococcal vaccines on antibiotic resistance. *Lancet Infect Dis* **8:**785–795.

161. **Kimura K, Suzuki S, Wachino J, Kurokawa H, Yamane K, Shibata N, Nagano N, Kato H, Shibayama K, Arakawa Y.** 2008. First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* **52:**2890–2897.

162. **Nagano N, Nagano Y, Kimura K, Tamai K, Yanagisawa H, Arakawa Y.** 2008. Genetic heterogeneity in *pbp* genes among clinically isolated group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* **52:**4258–4267.

163. **Kimura K, Nagano N, Arakawa Y.** 2015. Classification of group B streptococci with reduced β-lactam susceptibility (GBS-RBS) based on the amino acid substitutions in PBPs. *J Antimicrob Chemother* **70:**1601–1603.

164. **Cain D, Malouin F, Dargis M, Harel J, Gottschalk M.** 1995. Alterations in penicillin-binding proteins in strains of *Streptococcus suis* possessing moderate and high levels of resistance to penicillin. *FEMS Microbiol Lett* **130:**121–127.

165. **Haenni M, Galofaro L, Ythier M, Giddey M, Majcherczyk P, Moreillon P, Madec JY.** 2010. Penicillin-binding protein gene alterations in *Streptococcus uberis* isolates presenting decreased susceptibility to penicillin. *Antimicrob Agents Chemother* **54:**1140–1145.

166. **Haenni M, Saras E, Madec JY.** 2010. Demonstration of a shift towards penicillin resistance in the *Streptococcus uberis* population. *J Med Microbiol* **59:**993–995.

167. **Rüegsegger F, Ruf J, Tschuor A, Sigrist Y, Rosskopf M, Hässig M.** 2014. Antimicrobial susceptibility of mastitis pathogens of dairy cows in Switzerland. *Schweiz Arch Tierheilkd* **156:**483–488.

168. **Kalmus P, Aasmäe B, Kärssin A, Orro T, Kask K.** 2011. Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta Vet Scand* **53:**4.

169. **Bal EB, Bayar S, Bal MA.** 2010. Antimicrobial susceptibilities of coagulase-negative staphylococci (CNS) and streptococci from bovine subclinical mastitis cases. *J Microbiol* **48:**267–274.

170. **Zhang C, Zhang Z, Song L, Fan X, Wen F, Xu S, Ning Y.** 2015. Antimicrobial resistance profile and genotypic characteristics of *Streptococcus suis* capsular type 2 isolated from clinical carrier sows and diseased pigs in China. *BioMed Res Int* **2015:**284303.

171. **van Hout J, Heuvelink A, Gonggrijp M.** 2016. Monitoring of antimicrobial susceptibility of *Streptococcus suis* in the Netherlands, 2013–2015. *Vet Microbiol* **194:** 5–10.

172. **Moreno LZ, da Costa BL, Matajira CE, Gomes VT, Mesquita RE, Silva AP, Moreno AM.** 2016. Molecular and antimicrobial susceptibility profiling of *Streptococcus dysgalactiae* isolated from swine. *Diagn Microbiol Infect Dis* **86:**178–180.

173. **Drlica K, Zhao X.** 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev* **61:** 377–392.

174. **Escudero JA, San Millan A, Gutierrez B, Hidalgo L, La Ragione RM, AbuOun M, Galimand M, Ferrándiz MJ, Domínguez L, de la Campa AG, Gonzalez-Zorn B.** 2011. Fluoroquinolone efflux in *Streptococcus suis* is mediated by SatAB and not by SmrA. *Antimicrob Agents Chemother* **55:**5850–5860.

175. **Meunier D, Acar JF, Martel JL, Kroemer S, Vallé M.** 2004. Seven years survey of susceptibility to marbofloxacin of bovine pathogenic strains from eight European countries. *Int J Antimicrob Agents* **24:**268–278.

176. **Kroemer S, Galland D, Guérin-Faublée V, Giboin H, Woehrlé-Fontaine F.** 2012. Survey of marbofloxacin susceptibility of bacteria isolated from cattle with respiratory disease and mastitis in Europe. *Vet Rec* **170:**53.

177. **El Garch F, de Jong A, Simjee S, Moyaert H, Klein U, Ludwig C, Marion H, Haag-Diergarten S, Richard-Mazet A, Thomas V, Siegwart E.** 2016. Monitoring of antimicrobial susceptibility of respiratory tract pathogens isolated from diseased cattle and pigs across Europe, 2009–2012: VetPath results. *Vet Microbiol* **194:**11–22.

178. **Morrissey I, Moyaert H, de Jong A, El Garch F, Klein U, Ludwig C, Thiry J, Youala M.** 2016. Antimicrobial susceptibility monitoring of bacterial pathogens isolated from respiratory tract infections in dogs and cats across Europe: ComPath results. *Vet Microbiol* **191:**44–51.

179. **Ludwig C, de Jong A, Moyaert H, El Garch F, Janes R, Klein U, Morrissey I, Thiry J, Youala M.** 2016. Antimicrobial susceptibility monitoring of dermatological bacterial pathogens isolated from diseased dogs and cats across Europe (ComPath results). *J Appl Microbiol* **121:** 1254–1267.

180. **Burrus V, Pavlovic G, Decaris B, Guédon G.** 2002. Conjugative transposons: the tip of the iceberg. *Mol Microbiol* **46:**601–610.

181. **Wozniak RA, Waldor MK.** 2010. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat Rev Microbiol* **8:** 552–563.

182. **Santoro F, Vianna ME, Roberts AP.** 2014. Variation on a theme; an overview of the Tn*916*/Tn*1545* family of mobile genetic elements in the oral and nasopharyngeal streptococci. *Front Microbiol* **5:**535–545.

183. **Roberts AP, Mullany P.** 2011. Tn*916*-like genetic elements: a diverse group of modular mobile elements conferring antibiotic resistance. *FEMS Microbiol Rev* **35:** 856–871.

184. **Ambroset C, Coluzzi C, Guédon G, Devignes MD, Loux V, Lacroix T, Payot S, Leblond-Bourget N.** 2016. New insights into the classification and integration specificity of *Streptococcus* integrative conjugative elements through extensive genome exploration. *Front Microbiol* **6:**1483–1504.

185. **Huang J, Ma J, Shang K, Hu X, Liang Y, Li D, Wu Z, Dai L, Chen L, Wang L.** 2016. Evolution and diversity of the antimicrobial resistance associated mobilome in *Streptococcus suis:* a probable mobile genetic elements reservoir for other streptococci. *Front Cell Infect Microbiol* **6:**118.

186. **Brenciani A, Tiberi E, Morici E, Oryasin E, Giovanetti E, Varaldo PE.** 2012. ICE*Sp1116*, the genetic element responsible for *erm*(B)-mediated, inducible resistance to erythromycin in *Streptococcus pyogenes*. *Antimicrob Agents Chemother* **56:**6425–6429.

187. **Giovanetti E, Brenciani A, Tiberi E, Bacciaglia A, Varaldo PE.** 2012. ICE*Sp2905*, the *erm*(TR)-*tet*(O) element of *Streptococcus pyogenes*, is formed by two independent integrative and conjugative elements. *Antimicrob Agents Chemother* **56:**591–594.

188. **Mingoia M, Morici E, Marini E, Brenciani A, Giovanetti E, Varaldo PE.** 2016. Macrolide resistance gene *erm*(TR) and *erm*(TR)-carrying genetic elements in *Streptococcus agalactiae*: characterization of ICE*Sag*TR7, a new composite element containing IME*Sp*2907. *J Antimicrob Chemother* **71:**593–600.

189. **Huang K, Song Y, Zhang Q, Zhang A, Jin M.** 2016. Characterisation of a novel integrative and conjugative element ICESsD9 carrying *erm*(B) and *tet*(O) resistance determinants in *Streptococcus suis*, and the distribution of ICESsD9-like elements in clinical isolates. *J Glob Antimicrob Resist* **7:**13–18.

190. **Palmieri C, Magi G, Creti R, Baldassarri L, Imperi M, Gherardi G, Facinelli B.** 2013. Interspecies mobilization of an *erm*T-carrying plasmid of *Streptococcus dysgalactiae* subsp. *equisimilis* by a coresident ICE of the ICE*Sa*2603 family. *J Antimicrob Chemother* **68:**23–26.

191. **Croucher NJ, Walker D, Romero P, Lennard N, Paterson GK, Bason NC, Mitchell AM, Quail MA, Andrew PW, Parkhill J, Bentley SD, Mitchell TJ.** 2009. Role of conjugative elements in the evolution of the multidrug-resistant pandemic clone *Streptococcus pneumoniae*^{Spain23F} ST81. *J Bacteriol* **191:**1480–1489.

192. **Douarre PE, Sauvage E, Poyart C, Glaser P.** 2015. Host specificity in the diversity and transfer of *lsa* resistance genes in group B *Streptococcus*. *J Antimicrob Chemother* **70:**3205–3213.

193. **Huang K, Zhang Q, Song Y, Zhang Z, Zhang A, Xiao J, Jin M.** 2016. Characterization of spectinomycin resistance in *Streptococcus suis* leads to two novel insights into drug resistance formation and dissemination mechanism. *Antimicrob Agents Chemother* **60:**6390–6392.

194. **Marini E, Palmieri C, Magi G, Facinelli B.** 2015. Recombination between *Streptococcus suis* ICESsu32457 and *Streptococcus agalactiae* ICESa2603 yields a hybrid ICE transferable to *Streptococcus pyogenes*. *Vet Microbiol* **178:**99–104.

195. **Srinivasan V, Metcalf BJ, Knipe KM, Ouattara M, McGee L, Shewmaker PL, Glennen A, Nichols M, Harris C, Brimmage M, Ostrowsky B, Park CJ, Schrag SJ, Frace MA, Sammons SA, Beall B.** 2014. *vanG* element insertions within a conserved chromosomal site conferring vancomycin resistance to *Streptococcus agalactiae* and *Streptococcus anginosus*. *MBio* **5:**e01386-14.

196. **Meng F, Kanai K, Yoshikoshi K.** 2009. Structural characterization of Tn*916*-like element in *Streptococcus parauberis* serotype II strains isolated from diseased Japanese flounder. *Lett Appl Microbiol* **48:**770–776.

197. **Palmieri C, Magi G, Mingoia M, Bagnarelli P, Ripa S, Varaldo PE, Facinelli B.** 2012. Characterization of a *Streptococcus suis tet*(O/W/32/O)-carrying element transferable to major streptococcal pathogens. *Antimicrob Agents Chemother* **56:**4697–4702.

198. **Richards VP, Zadoks RN, Pavinski Bitar PD, Lefébure T, Lang P, Werner B, Tikofsky L, Moroni P, Stanhope MJ.** 2012. Genome characterization and population genetic structure of the zoonotic pathogen, *Streptococcus canis*. *BMC Microbiol* **12:**293–309.

199. **Bekele T, Molla B.** 2001. Mastitis in lactating camels (*Camelus dromedarius*) in Afar Region, north-eastern Ethiopia. *Berl Munch Tierarztl Wochenschr* **114:**169–172.

200. **Evans JJ, Pasnik DJ, Klesius PH, Al-Ablani S.** 2006. First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild bottlenose dolphin (*Tursiops truncatus*). *J Wildl Dis* **42:**561–569.

201. **Jordal S, Glambek M, Oppegaard O, Kittang BR.** 2015. New tricks from an old cow: infective endocarditis caused by *Streptococcus dysgalactiae* subsp. *dysgalactiae*. *J Clin Microbiol* **53:**731–734.

202. **Nomoto R, Munasinghe LI, Jin DH, Shimahara Y, Yasuda H, Nakamura A, Misawa N, Itami T, Yoshida T.** 2004. Lancefield group C *Streptococcus dysgalactiae* infection responsible for fish mortalities in Japan. *J Fish Dis* **27:**679–686.

203. **Hilmarsdóttir I, Valsdóttir F.** 2007. Molecular typing of beta-hemolytic streptococci from two patients with lower-limb cellulitis: identical isolates from toe web and blood specimens. *J Clin Microbiol* **45:**3131–3132.

204. **Kawata K, Minakami T, Mori Y, Katsumi M, Kataoka Y, Ezawa A, Kikuchi N, Takahashi T.** 2003. rDNA sequence analyses of *Streptococcus dysgalactiae* subsp. *equisimilis* isolates from pigs. *Int J Syst Evol Microbiol* **53:**1941–1946.

205. **Lopardo HA, Vidal P, Sparo M, Jeric P, Centron D, Facklam RR, Paganini H, Pagniez NG, Lovgren M, Beall B.** 2005. Six-month multicenter study on invasive infections due to *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* in Argentina. *J Clin Microbiol* **43:**802–807.

206. **Nei T, Akutsu K, Shima A, Tsuboi I, Suzuki H, Yamamoto T, Tanaka K, Shinoyama A, Kojima Y, Washio Y, Okawa S, Sonobe K, Norose Y, Saito R.** 2012. A case of streptococcal toxic shock syndrome due to group G streptococci identified as *Streptococcus dysgalactiae* subsp. *equisimilis*. *J Infect Chemother* **18:**919–924.

207. **Savini V, Catavitello C, Talia M, Manna A, Pompetti F, Di Bonaventura G, Di Giuseppe N, Febbo F, Balbinot A, Di Zacomo S, Esattore F, D'Antonio D.** 2008. Beta-lactam failure in treatment of two group G *Streptococcus dysgalactiae* subsp. *equisimilis* pharyngitis patients. *J Clin Microbiol* **46:**814–816.

208. **Siljander T, Karppelin M, Vähäkuopus S, Syrjänen J, Toropainen M, Kere J, Vuento R, Jussila T, Vuopio-Varkila J.** 2008. Acute bacterial, nonnecrotizing cellulitis in Finland: microbiological findings. *Clin Infect Dis* **46:**855–861.

209. **Wajima T, Morozumi M, Hanada S, Sunaoshi K, Chiba N, Iwata S, Ubukata K.** 2016. Molecular characterization of invasive *Streptococcus dysgalactiae* subsp. *equisimilis*, Japan. *Emerg Infect Dis* **22:**247–254.

210. **Zoric M, Nilsson E, Lundeheim N, Wallgren P.** 2009. Incidence of lameness and abrasions in piglets in identical farrowing pens with four different types of floor. *Acta Vet Scand* **51:**23–32.

211. **Casagrande Proietti P, Bietta A, Coppola G, Felicetti M, Cook RF, Coletti M, Marenzoni ML, Passamonti F.** 2011. Isolation and characterization of β-haemolytic-streptococci from endometritis in mares. *Vet Microbiol* **152:**126–130.

212. **Imai D, Jang S, Miller M, Conrad PA.** 2009. Characterization of beta-hemolytic streptococci isolated from southern sea otters (*Enhydra lutris nereis*) stranded along the California coast. *Vet Microbiol* **136:**378–381.

213. **García-País MJ, Rabuñal R, Armesto V, López-Reboiro M, García-Garrote F, Coira A, Pita J, Rodríguez-**

Macías AI, López-Álvarez MJ, Alonso MP, Corredoira J. 2016. *Streptococcus bovis* septic arthritis and osteomyelitis: a report of 21 cases and a literature review. *Semin Arthritis Rheum* **45:**738–746.

214. **Osawa R, Sly LI.** 1991. Phenotypic characterization of CO2-requiring strains of *Streptococcus bovis* from koalas. *Appl Environ Microbiol* **57:**3037–3039.
215. **Sekizaki T, Nishiya H, Nakajima S, Nishizono M, Kawano M, Okura M, Takamatsu D, Nishino H, Ishiji T, Osawa R.** 2008. Endocarditis in chickens caused by subclinical infection of *Streptococcus gallolyticus* subsp. *gallolyticus*. *Avian Dis* **52:**183–186.
216. **van Samkar A, Brouwer MC, Pannekoek Y, van der Ende A, van de Beek D.** 2015. *Streptococcus gallolyticus* meningitis in adults: report of five cases and review of the literature. *Clin Microbiol Infect* **21:**1077–1083.
217. **Katsumi M, Kataoka Y, Takahashi T, Kikuchi N, Hiramune T.** 1997. Bacterial isolation from slaughtered pigs associated with endocarditis, especially the isolation of *Streptococcus suis*. *J Vet Med Sci* **59:**75–78.
218. **O'Sullivan T, Friendship R, Blackwell T, Pearl D, McEwen B, Carman S, Slavić D, Dewey C.** 2011. Microbiological identification and analysis of swine tonsils collected from carcasses at slaughter. *Can J Vet Res* **75:**106–111.
219. **Miller CW, Prescott JF, Mathews KA, Betschel SD, Yager JA, Guru V, DeWinter L, Low DE.** 1996. Streptococcal toxic shock syndrome in dogs. *J Am Vet Med Assoc* **209:**1421–1426.
220. **Reissmann S, Friedrichs C, Rajkumari R, Itzek A, Fulde M, Rodloff AC, Brahmadathan KN, Chhatwal GS, Nitsche-Schmitz DP.** 2010. Contribution of *Streptococcus anginosus* to infections caused by groups C and G streptococci, southern India. *Emerg Infect Dis* **16:**656–663.
221. **Hatrongjit R, Kerdsin A, Gottschalk M, Takeuchi D, Hamada S, Oishi K, Akeda Y.** 2015. First human case report of sepsis due to infection with *Streptococcus suis* serotype 31 in Thailand. *BMC Infect Dis* **15:**392–399.
222. **Mancini F, Adamo F, Creti R, Monaco M, Alfarone G, Pantosti A, Ciervo A.** 2016. A fatal case of streptococcal toxic shock syndrome caused by *Streptococcus suis* carrying *tet* (40) and *tet* (O/W/32/O), Italy. *J Infect Chemother* **22:**774–776.
223. **Sánchez del Rey V, Fernández-Garayzábal JF, Briones V, Iriso A, Domínguez L, Gottschalk M, Vela AI.** 2013. Genetic analysis of *Streptococcus suis* isolates from wild rabbits. *Vet Microbiol* **165:**483–486.
224. **Sánchez del Rey V, Fernández-Garayzábal JF, Domínguez L, Gottschalk M, Vela AI.** 2016. Screening of virulence-associated genes as a molecular typing method for characterization of *Streptococcus suis* isolates recovered from wild boars and pigs. *Vet J* **209:**108–112.
225. **Staats JJ, Feder I, Okwumabua O, Chengappa MM.** 1997. *Streptococcus suis*: past and present. *Vet Res Commun* **21:**381–407.
226. **Anshary H, Kurniawan RA, Sriwulan S, Ramli R, Baxa DV.** 2014. Isolation and molecular identification of the etiological agents of streptococcosis in Nile tilapia (*Oreochromis niloticus*) cultured in net cages in Lake Sentani, Papua, Indonesia. *Springerplus* **3:**627–638.
227. **Bonar CJ, Wagner RA.** 2003. A third report of "golf ball disease" in an Amazon River dolphin (*Inia geoffrensis*) associated with *Streptococcus iniae*. *J Zoo Wildl Med* **34:**296–301.
228. **Chou L, Griffin MJ, Fraites T, Ware C, Ferguson H, Keirstead N, Brake J, Wiles J, Hawke JP, Kearney MT, Getchell RG, Gaunt P, Soto E.** 2014. Phenotypic and genotypic heterogeneity among *Streptococcus iniae* isolates recovered from cultured and wild fish in North America, Central America and the Caribbean islands. *J Aquat Anim Health* **26:**263–271.
229. **El Aamri F, Padilla D, Acosta F, Caballero M, Roo J, Bravo J, Vivas J, Real F.** 2010. First report of *Streptococcus iniae* in red porgy (*Pagrus pagrus*, L.). *J Fish Dis* **33:**901–905.
230. **Figueiredo HC, Netto LN, Leal CA, Pereira UP, Mian GF.** 2012. *Streptococcus iniae* outbreaks in Brazilian Nile tilapia (*Oreochromis niloticus* L:) farms. *Braz J Microbiol* **43:**576–580.
231. **Keirstead ND, Brake JW, Griffin MJ, Halliday-Simmonds I, Thrall MA, Soto E.** 2014. Fatal septicemia caused by the zoonotic bacterium *Streptococcus iniae* during an outbreak in Caribbean reef fish. *Vet Pathol* **51:**1035–1041.
232. **Denamiel G, Llorente P, Carabella M, Rebuelto M, Gentilini E.** 2005. Anti-microbial susceptibility of *Streptococcus* spp. isolated from bovine mastitis in Argentina. *J Vet Med B Infect Dis Vet Public Health* **52:**125–128.
233. **Nam HM, Lim SK, Kang HM, Kim JM, Moon JS, Jang KC, Joo YS, Kang MI, Jung SC.** 2009. Antimicrobial resistance of streptococci isolated from mastitic bovine milk samples in Korea. *J Vet Diagn Invest* **21:**698–701.
234. **Entorf M, Feßler AT, Kaspar H, Kadlec K, Peters T, Schwarz S.** 2016. Comparative erythromycin and tylosin susceptibility testing of streptococci from bovine mastitis. *Vet Microbiol* **194:**36–42.
235. **Marie J, Morvan H, Berthelot-Hérault F, Sanders P, Kempf I, Gautier-Bouchardon AV, Jouy E, Kobisch M.** 2002. Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. *J Antimicrob Chemother* **50:**201–209.
236. **Martel A, Baele M, Devriese LA, Goossens H, Wisselink HJ, Decostere A, Haesebrouck F.** 2001. Prevalence and mechanism of resistance against macrolides and lincosamides in *Streptococcus suis* isolates. *Vet Microbiol* **83:**287–297.
237. **Tian Y, Aarestrup FM, Lu CP.** 2004. Characterization of *Streptococcus suis* serotype 7 isolates from diseased pigs in Denmark. *Vet Microbiol* **103:**55–62.
238. **Overesch G, Stephan R, Perreten V.** 2013. Antimicrobial susceptibility of gram-positive udder pathogens from bovine mastitis milk in Switzerland. *Schweiz Arch Tierheilkd* **155:**339–350.

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Antimicrobial Resistance in *Enterococcus* spp. of animal origin

9

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INTRODUCTION

Enterococcus species are natural inhabitants of the intestinal tract in humans and animals, and due to their ubiquity in human and animal feces and their persistence in the environment, enterococci are considered indicators of fecal contamination in water (1). Moreover, enterococci serve as important key indicator bacteria for several human and veterinary resistance surveillance systems.

During the evisceration process at slaughterhouses, fecal enterococci can contaminate food products of animal origin. Some studies reported that over 90% of food samples of animal origin are contaminated with enterococci at the slaughterhouse, mostly with *Enterococcus faecalis*, followed by *Enterococcus faecium* (1, 2). In addition, enterococci are opportunistic pathogens which have become one of the main causes of nosocomial and community-acquired human infections, including septicemia, endocarditis, and urinary tract infections, among others (3).

The genus *Enterococcus* presently contains over 50 species, and *E. faecalis* and *E. faecium* are the predominant isolated species, accounting for more than 80% of isolates. In addition, these two species are considered the third- and fourth-most prevalent nosocomial pathogens worldwide (4). Other *Enterococcus* species, such as *E. hirae*, *E. avium*, *E. durans*, *E. gallinarum*, *E. casseliflavus*, and *E. raffinosus*, are rare causes of human clinical infections and are thought to be more opportunistic in nature than *E. faecium* and *E. faecalis* (5–10). *E. faecalis* and *E. faecium* are also the most representative enterococcal species detected in the human intestine, whereas other species, such as *E. durans* and *E. avium*, are occasionally detected (11). The most commonly encountered enterococcal species in the guts of animals are *E. faecalis*, *E. faecium*, *E. hirae*, and *E. durans*; other species are also detected sporadically or in particular age groups (such as *E. cecorum* in older poultry) (11, 12). Several members of the genus *Enterococcus* can cause bovine mastitis, endocarditis, septicemia and amyloid encephalopathy with sudden death in chickens (13), and diarrhea in dogs, cats, pigs, and rats (12). In the past decade, *E. cecorum* has emerged as an important poultry pathogen, associated with arthritis and osteomyelitis (14–15).

The intrinsic resistance of these bacteria to several antimicrobial agents has compromised the choice of therapeutic options to treat enterococcal infections. Those intrinsic resistances confer resistance to semisynthetic penicillins (low-level resistance), aminoglycosides (low-level resistance), vancomycin (*E. gallinarum*, *E. casseliflavus*, and *E. flavescens*), and polymyxins and streptogramins (*E. faecalis*) (11). Moreover, enterococci frequently acquire antimicrobial resistance genes through plasmids and/or transposons. The antibiotic resistances in *Enterococcus* species have been reviewed previously (3, 16–18), with focuses on specific agents (such as vancomycin [19–22] or aminoglycosides [23]) or sources (livestock/food [24–26]). The zoonotic transmission potential of antimicrobial-resistant enterococci has also been reviewed (27). In this review, we update the available knowledge on the prevalence and molecular mechanisms of antimicrobial resistance in enterococcal isolates from a wide range of animals (livestock, pets, and wildlife) and animal-derived food, with

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particular emphasis on beta-lactams, vancomycin, and linezolid. Furthermore, we outline the major clonal lineages and plasmids responsible for antimicrobial resistance in *Enterococcus* from farm and companion animals.

DIVERSITY OF ENTEROCOCCAL SPECIES IN THE ANIMAL INTESTINAL TRACT

Enterococci are ubiquitous bacteria in the gastrointestinal tract of humans and a wide range of animals (mammals, reptiles, birds, and some invertebrates). In addition, they are commonly found in vegetables, water, soil, and food derived from animals (including fermented and dairy products) (11). Enterococci are classified as lactic acid bacteria and are highly adaptable to different environmental conditions. They survive over a wide range of temperature (10 to 45°C), and pH (4.8 to 9.6) and are able to grow at high salt concentrations (up 6.5% NaCl). Most of them can hydrolyze esculin in the presence of 40% bile salts, a characteristic used for phenotypic identification processes (11). These and other properties explain the utilization of enterococci in diverse roles; for instance, they have been used as probiotics, starter cultures, biopreservatives, and indicators of fecal contamination of water and sanitary quality of food (28–30).

Genomic analysis revealed that members of the genus *Enterococcus* have a low G+C content, ranging from 34.29 to 44.75% (31). For a long time, *Enterococcus* species were considered streptococci of Lancefield group D. In 1984, application of nucleic hybridization and 16S rRNA sequencing led to a reclassification of *Streptococcus faecium* and *Streptococcus faecalis* in the genus *Enterococcus* (32). Currently, this genus includes around 50 species (33). Many of them were discovered in this century, mostly recovered from nonhuman sources, such as plants (*E. plantarum*, *E. ureilyticus*), water (*E. quebecensis*, *E. rivorum*, *E. ureasiticus*), animals (*E. canis*, *E. phoeniculicola*, *E. devriesei*), and food products (*E. thailandicus*, *E. italicus*) (34–42).

A recent genomic study which compared the concatenated nucleotide sequences of the core genes of 37 enterococci belonging to a variety of species divided these strains into 6 branches: (i) the *E. faecium* branch (containing *E. faecium*, *E. mundtii*, *E. durans*, *E. hirae*, *E. ratti*, *E. villorum*, *E. thailandicus*, *E. phoeniculicola*), (ii) the *E. faecalis* branch (*E. faecalis*, *E. termitis*, *E. quebecensis*, *E. moraviensis*, *E. caccae*, *E. haemoperoxidus*, *E. silesiacus*), (iii) the *E. dispar* branch (*E. dispar*, *E. canintestini*, *E. asini*), (iv) the *E. casseliflavus* branch (*E. casseliflavus*, *E. gallinarum*, *E. aquimarinus*, *E. saccharolyticus*, *E. italicus*, *E. sulfureus*, *E. cecorum*, *E. columbae*), (v) the *E. pallens* branch (*E. pallens*, *E. hermanniensis*, *E. devriesei*, *E. gilvus*, *E. malodoratus*, *E. avium*, *E. raffinosus*), and (vi) the *E. canis* branch, which contained only one strain (31). Results showed that most strains from human and other mammals were clustered into the *E. faecium*, *E. faecalis*, *E. dispar*, and *E. pallens* branches, whereas the majority of the bird isolates belonged to the *E. casseliflavus* branch.

In 1963, Mundt and colleagues carried out a survey of the occurrence of enterococci among animals living in the wild environment (43). They obtained enterococci from the feces of 71% of the studied mammals, 86% of the reptiles, and 32% of the birds. In addition, patterns of food and animal species dependence were observed. In general, enterococci were only isolated sporadically in samples recovered from herbivorous mammals. However, they were abundant in rodents, bats, and larger animals with omnivorous or carnivorous diets (43), but as demonstrated in several other reports, the differences in the proportions of enterococci in each niche, as well as the species distributions, varied not only according to the diet, but also according to seasonal changes, individual characteristics (gender, age), and geographic location (11, 44).

In general, *E. faecium*, *E. faecalis*, *E. hirae*, and *E. durans* are the most prevalent enterococcal species in the gastrointestinal tract of humans and other mammals (11). *E. cecorum* is also a relevant member of the normal enterococcal microbiota in the gut of farm and pet animals (cattle, pigs, dogs, cats) and birds (poultry and pigeons) (45–47). However, in chickens, a significant age-dependent increase in gut colonization has been reported for this species. *E. cecorum* has been found to be a dominant part of the enterococcal gastrointestinal microbiota in mature chickens (48). Some other species, such as *E. gallinarum* and *E. avium*, which were first described in chickens, have not been frequently detected among enterococcal gut populations in poultry (49, 50).

In cattle and swine, the proportions of the enterococcal species vary across studies. *E. faecium*, *E. durans*, *E. hirae*, and *E. faecalis* were unanimously found in different surveys (46, 50–52). In some works, *E. faecalis* was the predominant enterococcal species in the gut of bovines and swines (46, 53). In others, *E. hirae* and *E. faecium* were described as the more abundant bacteria in both livestock species (44, 51, 52). As observed, variations between geographical regions might explain these differences in the composition of the enterococcal populations (44). *E. casseliflavus*, *E. gallinarum*, *E. avium*, and *E. cecorum* have also been

reported as part of the bovine and swine microbiota, but they were present in lower proportions (46, 50, 51). Additionally, some minoritary species, such as *E. villorum* and *E. thailandicus*, have been sporadically detected in feces from cattle and pigs (52, 54, 55).

The enterococcal microbiota of the intestinal tract of dogs and cats showed a predominance of *E. faecalis* and *E. faecium*, followed by *E. hirae* (56–59). *E. avium* has been commonly isolated in canines and also, although in smaller proportions, in feline feces (56, 57). Other species, such as *E. durans*, *E. gallinarum*, *E. casseliflavus*, *E. cecorum*, and *E. raffinosus*, have been occasionally reported (56, 58, 59). In addition, some newly characterized species were isolated from anal swabs and chronic otitis externa (*E. canis*) and fecal samples (*E. canintestini*) of dogs (34, 60).

Enterococci are also normal residents of the gut of a wide range of free-living animals. In pigeons, the predominant species is *E. columbae* and, to a lesser extent, *E. cecorum*. However, *E. faecium* and *E. faecalis* are rare in these birds (61). Another study reported a high prevalence of enterococci among three species of coraciiform birds (74%), with a dominance of *E. faecalis*, followed by *E. casseliflavus* (62). In Portugal, *E. faecium* was the most frequently encountered species in buzzard fecal samples (63), and *E. faecium*, *E. durans*, and *E. gallinarum* were found in the feces of a variety of wild birds (64). The enterococcal gut microbiota has also been analyzed in wild marine species. *E. faecium* was identified as the most abundant species in echinoderms collected from Azorean waters. Minor species, such as *E. hirae*, *E. faecalis*, and *E. gallinarum*, were also detected (65). In a recent study in southern Brazil, different wild marine animals were analyzed using real-time quantitative PCR to identify and quantify enterococci in feces. These bacteria were found in all the studied animal species, with a dominance of *E. faecalis* and *E. mundtii* in most of the marine mammals; *E. faecalis* in green turtles, Magellanic penguins, and albatross; and *E. hirae* and *E. gallinarum* in white-backed stilts (66). Enterococci are also a relevant part of the facultative anaerobic microbiota of the gastrointestinal tract of large wild mammals (wolf, wildboar, deer, etc.) and rodents (67–69).

Administration of antibiotics in both human and animal medicine may shift the gut microbial community, allowing drug-resistant strains (e.g., vancomycin-resistant enterococci) to proliferate dramatically. Because many enterococcal infections are caused by normal inhabitants of the gastrointestinal tract that become opportunistic pathogens, the selection of antibiotic-resistant strains raises the risk of developing difficult-to-treat infections. The following sections give an overview of the mechanisms and prevalence of antimicrobial resistance in enterococci in the animal setting.

ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI OF ANIMALS AND FOODS OF ANIMAL ORIGIN

Beta-Lactam Resistance

Enterococci are intrinsically resistant to cephalosporins and present a natural reduced susceptibility to penicillins, due to the expression of low-affinity penicillin binding proteins (PBPs) that bind weakly to beta-lactam antibiotics. For this reason, the MICs for penicillins are higher in enterococci than in streptococci or other Gram-positive organisms, which do not produce chromosomally encoded low-affinity PBPs (17). *E. faecalis* isolates normally exhibit lower MIC values for penicillins than *E. faecium* (18).

All enterococci have at least five PBPs, and six putative PBP genes have been detected by genomic analysis in *E. faecalis* and *E. faecium* (class A: *ponA*, *pbpF*, *pbpZ*; class B: *pbp5*, *pbpA*, *pbpB*) (18). The expression of the species-specific chromosomally located *pbp5* gene, which encodes PBP5, with low affinity binding for penicillins and cephalosporins, is associated with intrinsic resistance to beta-lactams. In *E. faecium*, the *pbp5* gene is included within an operon, together with two other genes that are also implicated in cell wall synthesis (*psr* and *ftsW*) (18).

Acquired (enhanced) resistance for penicillins (penicillin or ampicillin) has been frequently detected among clinical *E. faecium* isolates, being rare in *E. faecalis*. High-level ampicillin resistance in *E. faecium* (MIC, ≥128 μg/ml) has been associated with increased production of PBP5 (requiring a higher concentration of the agent to saturate the active site) or with specific amino acid changes in its sequence, which make the low-affinity PBP5 even less susceptible to inhibition by penicillins (70, 71). The amino acid substitutions near the Ser-Thr-Phe-Lys, Ser-Asp-Ala, and Lys-Thr-Gly motifs, which are part of the active-site cavity, seem to be the most significant ones (16).

Combinations of specific amino acid changes in the C-terminal transpeptidase domain of PBP5 (especially the substitution Met-485-Ala/Thr, but also the changes Ala-499-Ile/Thr, Glu-629-Val, and Pro-667-Ser), and the insertion of serine or aspartic acid after position 466, have been associated with ampicillin resistance in *E. faecium* isolates (72–76). It has been found that single substitutions at positions 485, 499, 629, and

466-insertion have only slight influence on ampicillin MIC, but when combined, the effect increases. Mutations in genes encoding other species-specific proteins that participate in cell wall synthesis may also slightly increase the MIC value (76).

Two distinct allelic forms have been identified when the whole sequence of the *pbp5* gene is considered, which differ in 5% of the sequence, yielding two types of PBP5 (PBP5-S and PBP5-R) with changes in 21 amino acid residues. PBP5-S is usually detected in community-associated ampicillin-susceptible *E. faecium* isolates (MIC of usually ≤2 μg/ml), and PBP5-R is usually detected in hospital-associated ampicillin-resistant isolates (MIC of usually ≥16 μg/ml) (77, 78). A hybrid-like type of PBP5 (PBP5-S/R), with a sequence between the other two types, has been observed in some isolates, with a MIC for ampicillin of around 4 μg/ml (77, 78).

Considering the population structure of *E. faecium*, two main lineages have been postulated in humans: (i) subclade A1, hospital-associated, enriched in mobile genetic elements, usually implicated in human infections, and in most cases, ampicillin-resistant (MIC, ≥16 μg/ml) with the consensus allele *pbp5*-R, and (ii) clade B: community-associated, detected in isolates from healthy humans (not implicated in infections), generally ampicillin-susceptible (MIC, ≤2 μg/ml), and harboring the consensus allele *pbp5*-S. The subclade A2 includes *E. faecium* isolates mostly from animal settings, exhibits a wide range of ampicillin MIC values (0.5 to 128 μg/ml), and generally carries the hybrid-like *pbp5* allele (*pbp5*-S/R) (72, 78, 79). In addition to amino acid sequence alteration in PBP5, elevated levels of this protein are also observed in highly ampicillin-resistant isolates of clade A (subclade A1 and part of A2), but not in the ampicillin-susceptible isolates of subclade A2 and clade B, suggesting a differential regulation process in each clade. The upstream region of *pbp5* seems to have a role in the level of expression of the gene (72).

In *E. faecalis*, acquired ampicillin resistance is unusual but is generally mediated by mutations in *pbp*4 (27, 80). Selected strains of *E. faecalis* produce a plasmid-mediated beta-lactamase that is similar to the enzyme produced by *Staphylococcus aureus* (17, 81), encoded by the *bla*Z gene, although some polymorphisms in this gene have also been detected in some isolates. This beta-lactamase is expressed in a constitutive way in *E. faecalis*, in contrast to the inducible production in *S. aureus*. The enzyme is produced in low amounts in *E. faecalis*, and for this reason, the strain can appear as ampicillin susceptible when the MIC is tested *in vitro*. In any case, this mechanism of resistance is very infrequently seen in *E. faecalis*. Very unusual beta-lactamase-producing *E. faecium* strains have also been reported (82). Chromosomal beta-lactamase-encoding genes conferring ampicillin resistance have also been detected in *E. faecium* isolates (83).

The *in vitro* transferability of *pbp5* in *E. faecium* isolates (84), which suggests a mechanism by which high-level ampicillin resistance conferred by mutated *pbp5* alleles could be disseminated among clinical isolates, has been reported. Moreover, Novais *et al.* (85) demonstrated *in vitro* ampicillin-resistance transference by conjugation in 28% of the *E. faecium* isolates from a pig farm environment, although the genetic basis of this transference was not determined. Codiversification of the *E. faecium* core genome and *pbp5* has been recently analyzed, showing evidence of *pbp5* horizontal transfer (86).

Various studies have evaluated the prevalence of penicillin or ampicillin resistance in enterococci from food-producing animals, pets, or wild animals, as well as in those from food of animal origin. For *E. faecium*, the prevalence of resistance is variable depending on the country and the type of animal. Reflecting this, no resistant *E. faecium* isolates were detected in a surveillance study performed in a cattle population at slaughter in Australia (87), but a rate of 30% resistance was detected in isolates of poultry in Portugal (88). For pets, the following ampicillin resistance rates were reported among *E. faecium* isolates: 63% and 37% in dogs and cats, respectively, in the United States and 3% in pets in Portugal (58, 88). Moreover, ampicillin-resistant *E. faecium* isolates were detected in 23% of the dogs screened in a cross-sectional study in the United Kingdom and in 76% of the dogs analyzed in a longitudinal study in Denmark (89). Most of these resistant isolates belonged to the hospital-adapted clonal complex CC17. Frequencies of ampicillin resistance in the range of 4.5 to 7.7% have been detected in *E. faecium* isolates recovered from wild animals (wild boar, Iberian wolf, and gilt-head seabream) (74, 90, 91), but no resistant isolates were detected in Iberian lynx (92).

A surveillance study was performed analyzing the prevalence of antimicrobial resistance in 21,077 *Enterococcus* isolates obtained from retail meat samples in the United States between 2002 and 2014, through the National Antimicrobial Resistance Monitoring System (NARMS) (2). A low frequency of ampicillin resistance was detected among *E. faecium* isolates from ground beef and pork chops (4% and 2.7%, respectively), but higher percentages were detected in retail chicken (26%), and even higher in ground turkey (62.6%). Bortolaia *et al.* (25) reviewed ampicillin

resistance data reported in European countries (Denmark, Sweden, The Netherlands, Slovenia) and the United States for *E. faecium* isolates recovered from poultry meat, comparing them to human isolates in the same countries (93–95). Human isolates showed very high rates of ampicillin resistance in all countries (>80%), but resistance in food isolates was significantly lower than in those of humans. Of note is the detection of 10% ampicillin resistance in *E. faecium* of (imported) broiler meat in Denmark and >50% resistance in isolates of turkey meat in the United States. Almost no ampicillin-resistant *E. faecalis* isolates (with very few exceptions) have been reported in animals or food of animal origin.

Glycopeptide Resistance

Mechanism of resistance

Vancomycin and teicoplanin are important members of the glycopeptide family and are used for the treatment of severe human infections. Avoparcin, another member of this family, has been extensively used in the past as a growth-promoter in food-producing animals in many countries.

The mechanism of action of glycopeptides is the inhibition of the synthesis of the bacterial cell wall, by the link to the D-Ala-D-Ala terminus of the pentapeptide precursor of the peptidoglycan, preventing cross-linking of the peptidoglycan chain and inhibiting cell wall synthesis. The main mechanism of glycopeptide resistance in enterococci implicates the alteration of the peptidoglycan synthesis pathway. In this sense, the terminus D-Ala-D-Ala of the pentapeptide to which vancomycin binds is modified to D-Ala-D-Lac (causing high-level vancomycin resistance; MIC, >64 μg/ml) or to D-Ala-D-Ser (low-level vancomycin resistance; MIC, 4 to 32 μg/ml). These modified cell-wall precursors bind glycopeptides with reduced affinity (about 1,000-fold and 7-fold for D-Lac and D-Ser substitutions, respectively) (18, 22).

The first vancomycin-resistant enterococci (VRE) with an acquired mechanism of resistance were detected three decades ago in clinical *E. faecium* isolates in France and the United Kingdom (96, 97). Since then, VRE have been extensively described in hospitals worldwide; they have been seen especially frequently in the United States since the 1990, mostly in patients in intensive care units, and at lower levels in Europe since the 2000s (21). According to surveillance data from the European Centre for Disease Prevention and Control (EARS-Net), the European Union/European Economic Area population-weighted mean percentage of vancomycin resistance in *E. faecium* was 11.8% in 2016, and national percentages ranged from 0 to 46.3%; the prevalence of vancomycin resistance for *E. faecalis* was lower (98).

Vancomycin resistance is mediated by *van* operons, which encode the modified peptidoglycan precursors. To date, eight *van* operons have been identified in enterococci mediating acquired vancomycin resistance (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*), and one additional operon in intrinsic vancomycin resistance (*vanC*) (18, 19, 99–102). Three variants of the gene *vanC* have been described (*vanC1*, *vanC2*, and *vanC3*), intrinsic to *E. gallinarum*, *E. casseliflavus*, and *E. flavescens*, respectively. Moreover, different subtypes have been identified for *vanB* (*vanB1*, *vanB2*, and *vanB3*), *vanD* (*vanD1* to *vanD5*) and *vanG* (*vanG1*, *vanG2*) (100, 103, 104). An additional variant, *vanF*, has also been described, but until now only in the environmental microorganism *Paenibacillus popilliae* (105).

vanA and *vanB* are the most frequent genotypes among VRE with acquired resistance mechanisms of humans and animals, mostly among *E. faecalis* and *E. faecium*. The genotypes *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN* are very unusual in VRE isolates, and *E. faecalis* (*vanE/G/L*) and *E. faecium* (*vanD/M/N*) are the most common carriers (22).

The *vanA* operon is associated with the transposon Tn*1546* and includes seven open reading frames transcribed under two different promoters (106). Regulation is mediated by a *vanS-vanR* (sensor-kinase-response regulator) two-component system, transcribed with a common promoter (107). The remaining genes are transcribed from a second promoter (22). The proteins encoded by *vanH* (dehydrogenase that converts pyruvate into lactate) and *vanA* (ligase that forms a D-Ala-D-Lac dipeptide) modify the synthesis of peptidoglycan precursors; moreover, the proteins encoded by both *vanX* (dipeptidase that cleaves D-Ala-D-Ala) and *vanY* (D,D-carboxipeptidase), interrupt the formation of the D-Ala-D-Ala end of the pentapeptide, and the *vanZ* gene is related to teicoplanin resistance (22, 108). Different insertion elements (ISs) can be included in the *vanA* operon, rendering different variants (109).

The *vanB* operon has been associated with different transposons (Tn*1547*, Tn*1549*, and Tn*5382*). Tn*1549* is widely prevalent among *vanB*-type enterococci, usually located in the chromosome and less frequently on plasmids (22). The structure of the *vanB* operon is similar to that of *vanA*, with two promoters and seven open reading frames, but with important differences, mostly in the two-component signaling regulatory

system (encoded by $vanR_B$ and $vanS_B$) and in the absence of a homolog of *vanZ* (substituted by *vanW*, of unknown function); consequently, *vanB* enterococci show vancomycin resistance (high or low level) but teicoplanin susceptibility (22, 108). The structure of the different *van* operons and their mechanisms of action have been extensively reviewed in previous studies (17–19, 21, 22, 108, 110).

Origin of vancomycin resistance

Partially preassembled glycopeptide resistance-associated gene clusters present in environmental organisms are suggested as the source of the vancomycin resistance genes in VRE (105, 111). The environmental organism *P. popilliae*, carrier of a *vanF* variant with high similarity at the amino acid level to *vanA*, has been suggested as the potential origin of vancomycin resistance in enterococci. To a lesser extent, this role could also be attributed to glycopeptide-producing organisms (e.g., the vancomycin-producing organism *Amycolatopsis orientalis*), which require these genes to inhibit the action of produced glycopeptides (111). Nevertheless, the genes in these organisms are probably not the direct source of the enterococcal vancomycin resistance genes since they are similar but not identical; in this sense, transference could have occurred from a common ancestral bacterium or via one or more bacterial intermediaries. In addition, considering the differences in G+C content, as well as the sequence homology among different organisms, it is possible that the genes of the *van* cluster could have more than one origin (111).

Historical aspects related to glycopeptide resistance

During the 1990s, VRE with the *vanA* genotype emerged in food-producing animals, healthy humans, food products, and environmental samples throughout Europe and other countries; this emergence was linked to the use of the glycopeptide avoparcin since the mid-1970s, in subtherapeutic concentrations, as an animal growth promoter (22, 26, 112, 113). This hypothesis was tested in poultry flocks and pig herds receiving or not receiving avoparcin, confirming the significant role of avoparcin in VRE selection in the animals (112, 113). This association was also corroborated in an animal model with young chickens receiving avoparcin supplementation (114). Avoparcin was banned as a growth promoter in the European Union in 1997, and a clear decrease in VRE fecal carriage in food-producing animals and healthy humans was observed (115), as well as in food-derived products. Nevertheless, VRE persisted in the animal setting many years after the avoparcin ban (116, 117). A similar situation happened in Taiwan after the ban of avoparcin in 2000 that resulted in a clear decrease of VRE prevalence in chickens, although it still persisted in this animal population (118). In dogs, high rates of fecal VRE carriage were reported before the avoparcin ban in the European Union (119), although no VRE was detected in dogs in Spain a decade after the ban (120). The frequency of human infection by VRE in the European Union was low during the period of high prevalence in animals, but an increase in the frequency of VRE-related human infections was evidenced since 1999 (22).

The situation in the United States and Canada was completely different than that in the European Union. Avoparcin use has never been approved in animal production in those countries, and VRE was not reported in animals until the end of the 2000s (20, 76, 121, 122). Nevertheless, in North America, VRE was a frequent cause of human infections, especially in patients in intensive care units, which was attributed to the high use of vancomycin in humans (22, 123). The differences in VRE prevalence in humans and animals in the European Union and the United States before and after the avoparcin ban in the European Union introduce some doubts about the possible routes of transmission of VRE determinants between animals and humans (22, 124).

Different theories have been postulated to explain the persistence of VRE in food-producing animals after the avoparcin ban in the European Union and in other countries, such as coselection by the use of other antimicrobials (e.g., erythromycin and tetracycline). It has been shown that *vanA* and *erm*(B) genes (the latter implicated in erythromycin resistance) are frequently located in the same transferable plasmids (113). Moreover, the gene *tcrB*, implicated in copper resistance, has been detected in pig *E. faecium* isolates in the same plasmid as *vanA* and *erm*(B) (125). However, the presence of plasmid addition systems in the same plasmid that carries the *vanA* gene could force bacteria to retain the resistance (125).

VRE in food-producing animals and food of animal origin

Tables 1 and 2 summarize the papers that have been published related to the prevalence and mechanisms of vancomycin resistance in enterococci isolated from food-producing animals and food of animal origin, respectively, as well as the genetic lineages of the isolates (when available). The data are organized by animal species (poultry, pigs, and cattle, among others) and by the year the isolates were recovered. Many of the studies

were performed in European countries, but studies in the American, African and Asian countries, as well as Australia and New Zealand are also included.

Most of the surveys of food-producing animals reported *E. faecium* as the major species of the genus *Enterococcus* exhibiting acquired resistance to vancomycin, in most cases with the *vanA* genotype. However, *vanA*-containing *E. faecalis* isolates, and to a lesser extent *E. durans* and *E. hirae* isolates, have also been frequently detected in food-producing animals (Table 1) (27, 85, 87, 114, 121, 122, 125–165). Other enterococcal species have occasionally been reported as *vanA* carriers, such as *E. mundtii* in poultry in Hungary (130) and *E. casseliflavus* in cattle in France (158) and in horses and swine in Italy (159). Available data indicate that *vanA* was, by far, the main gene responsible for acquired VRE in food-producing animals worldwide, regardless of the species. Nevertheless, *vanB* (and especially the *vanB2* variant) was occasionally detected. The first detection of *vanB2* in animals was in a vancomycin-resistant *E. hirae* isolate recovered from a pig in Spain in 2008 (145); later, *vanB*-positive *E. faecium* and *E. faecalis* isolates were detected in poultry in Czech Republic (132) and in *Enterococcus* species in pigs in South Africa (147). Moreover, *vanC1* was detected as an acquired gene in isolates of *E. faecium*, *E. faecalis*, and *E. mundtii* in poultry in Australia (140). In most of the studies, VRE were detected when a selective protocol with media supplemented with vancomycin was used (Table 1). Resistance frequencies varied depending on the type of animals tested (poultry, 0 to 77%; pigs, 0 to 25.3%; cattle, 0 to 0.5%), the year the study was performed, the country, and the protocol used for VRE recovery (see Table 1). *vanA*-containing enterococci have also been detected in ostriches and mullet fish in Portugal (prevalence of resistance of 7.4% and 3.9%, respectively) (164). In eight of the reviewed papers in which VRE were detected in food-producing animals, the multilocus sequence typing (MLST) data were provided for *vanA*-positive *E. faecium* (most isolates) or *E. faecalis* isolates. A wide variety of sequence types were identified among the *E. faecium* isolates from poultry and pigs (>30 sequence types) (27, 85, 121, 122, 127, 129, 144, 156). Also, the lineage sequence type 6 (ST6) (CC2) was identified in *E. faecalis* of pig origin (85).

The *E. faecium* species carrier of the *vanA* gene was the most frequent VRE detected in food of animal origin. Nevertheless, *vanA*-containing *E. faecalis*, *E. durans*, and *E. hirae* isolates were also frequently detected in these types of samples (Table 2) (2, 118, 128, 133, 162, 166–194). VRE with the *vanB* gene was found in *E. faecium* isolates from veal and chicken in Spain (ST17-*vanB2*) (188) and in different types of food in Greece (*vanB2/3*) and Spain (*vanB*) (181, 190). The identification of the unusual *vanN* gene in five *E. faecium* isolates from chicken meat in Japan is interesting, showing a low level of vancomycin resistance (MIC, 12 μg/ml) (177). Also notable is the unusual detection of *vanA*-containing *E. cecorum* isolates in chicken samples in Japan (168), *vanA*-positive *E. gallinarum* in fish in Egypt (193), and *vanC1*-positive *E. faecalis* isolates from sheep milk samples in Spain (192). The frequencies of detection of VRE with acquired resistance in food samples were variable (Table 1). In chicken and pork food samples analyzed from 1996 to 1999, the prevalence was in the range of 4.2 to 34% (Table 2), with a few exceptions (1.3%) (167). Very high frequencies were detected in different types of food in Korea (44%) (133), but no VRE were found in the studies performed in the United States (2, 171, 185). In some cases, isolates showing a phenotype usually associated with the *vanB* genotype (high-level resistance to vancomycin, susceptibility to teicoplanin) were detected in *Enterococcus* strains harboring the *vanA* gene (118, 168, 173).

VRE in companion animals

Table 3 shows the detection of VRE with acquired mechanisms of resistance in companion animals. *vanA*-containing *E. faecium* is a unique type of VRE with acquired resistance reported in dogs and cats (136, 145, 195–202). These isolates, recovered from fecal samples from 1996 to 2003, were found in the United States, Spain, and Portugal, with variable frequencies of detection (ranging from 2.8 to 22.7%) (136, 145, 195, 196). No VRE were detected in studies performed in the following years (Table 3), not even in sick dogs (197, 200). Vancomycin-resistant *E. faecium* and *E. durans* isolates were detected in fecal samples of equids obtained in 2007 to 2008 (prevalence 4.4%) in a study performed in Portugal (202).

VRE in free-living animals

Table 3 also shows the detection of VRE with acquired mechanisms of resistance in free-living animals, including different species of mammals and birds (136, 165, 203–226). Many studies have been performed with this type of animal, including in various countries in Europe, the Americas (United States, Canada, and Brazil), and Africa (Tunisia and Tanzania). The most frequently detected mechanism of resistance was *vanA*, mainly among *E. faecium* isolates, followed by *E. faecalis* (*E. durans* and *E. hirae* were infrequently detected).

Table 1 Summary of reports about detection of VRE with acquired mechanisms of resistance in healthy food-producing animals

Animal species	Year of recovery of tested isolates	Country	% Prevalence[a]	Species ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Poultry	1996	Spain	11/15	*E. hirae*/*E. faecium* (*vanA*)	+	114
Poultry	1997	Netherlands	29 (poultry and farmers)	*E. faecium*/*E. durans*/*E. hirae* (*vanA*) *E. faecalis* (*vanA*)	+	126
Poultry	1997	Norway	0.8–4.6	*E. faecium* ST26/ST146/ST195/ST242/ ST248/ST9/ST241/ST244/ ST245 (*vanA*)	+	127
Poultry	1997–1998	Spain	16	*E. durans* (*vanA*)	+	128
Poultry	1998–1999	Norway	34	*E. faecium* (*vanA*)	+	125
Poultry	2000–2007	Sweden	<1 (2000), 40 (2005), 30 (2006–2007)	*E. faecium* ST13/ST370/ST310 (*vanA*)	+	129
Poultry	2001–2004	Hungary	8.6 (2001), 23 (2002), 0 (2003–2007)	*E. faecium*/*E. durans*/ *E. mundtii* (*vanA*)	+	130
Poultry	2002–2003	New Zealand	5.8	*E. faecium*/*E. faecalis*/*E. durans* (*vanA*)	+	131
Poultry	2002–2004	Czech Republic	2.1	*E. faecium* (*vanA*), *E. faecium*/ *E. faecalis* (*vanB*)	–	132
Poultry	2003	Korea	1.4	*E. faecium* (*vanA*)	+	133
Poultry	2003–2004	Canada	0		–	134
Poultry	2003–2004	Canada	0		–	135
Poultry	2004	Portugal	9.2	*E. faecium*/*E. durans*/*E. hirae* (*vanA*)	+	136
Poultry	2005	Hungary	1.1	*E. faecium*/*E. faecalis* (*vanA*)	+	137
Poultry	2005–2008	Greece	14.4	*E. faecium* (*vanA*)	+	138
Poultry	2009	Germany	17.6	*Enterococcus* spp. (*vanA*)	+	139
Poultry	2000	Australia	8.6	*E. faecium*/*E. mundtii*/ *E. faecalis* (*vanC1*)	+	140
	2008–2009		0	-	+	
Poultry	2010	Denmark	47	*E. faecium* ST10/ST12/ST22/ST26/ ST38/ST157/ST417/ST520/ST587/ ST784/ST785/ST839/ST840/ST841/ ST842 (*vanA*)	+	27
Poultry	2010–2011	Germany	0		–	141
Poultry	2013–2014	Poland	0.11	NS[c]	–	142
Poultry	NS	Italy	8.7	NS	–	143
Poultry	NS	Sweden	55–70 (few samples)	*E. faecium*- ST310- (*vanA*)	+	144
Pigs	1998	Spain	6.1	*E. faecium* (*van*A), *E. hirae* (*vanB2*)	+	145
Pigs	1998–1999	Spain	8.0	*E. faecium* (NS), *E. hirae* (NS), *E. durans* (NS)	+	146
Pigs and farm facilities	2006–2007	Portugal	5	*E. faecium* ST132/ST185 (CC5) (*van*A), *E. faecium* ST443 (*vanA*), *E. faecalis* ST6 (CC2) (*vanA*)	+	85
Pigs	2009	USA	10.9	*E. faecium* ST5/ST6/ST185 (CC5) (*vanA*)	+	121
Pigs	2009–2010	USA	8.2	*E. faecium* ST5/ST6/ST185 (CC5) (*vanA*)	+	122
Pigs	2014	South Africa	NS	*Enterococcus* spp. (*vanB*)	–	147
Pigs	NS	Australia	0		NS	148
Poultry and pigs	1998–1999	Costa Rica	14.8 (poultry), 10.9 (pigs)	*E. faecium*/*E. faecalis*/*E. durans*/ *E. hirae* (*vanA*)	+	149

(Continued)

Table 1 *(Continued)*

Animal species	Year of recovery of tested isolates	Country	% Prevalence[a]	Species ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Poultry and pigs	1998–2000	European countries	8.2	*E. faecium*/*E. faecalis*/*E. hirae* (*vanA*)	+	150
Poultry and pigs	2002	UK and Wales	24 (poultry), 5 (pigs)	*E. faecium* (*vanA*)	+	151
Poultry and pigs	2005	France	1.6 (poultry), 6.2 (pigs)	*E. faecium*/*E. faecalis*/*Enterococcus* spp. (*vanA*)	+	152
Poultry and pigs	2009	China	0		–	153
Poultry and cattle	2014	Nigeria	0		–	154
Poultry and cattle	NS	Ethiopia	30–54	NS	–	155
Poultry, pigs, cattle	2008–2013	China	0.2 (pigs), 0 (poultry), 0 (cattle)	*E. faecium* ST6 (CC5) (*vanA*)	+	156
Poultry, pigs, cattle	NS[a]	Austria	47.8 (poultry), 0.5 (cattle), 0 (pigs)	*E. faecium*/*E. durans* (*vanA*)	+	157
Cattle	2003–2004 (healthy cattle)–2006 (sick cattle)	France	0.1 (healthy cattle), 0.4 (sick cattle)	*E. faecium*/*E. faecalis*/*E. casseliflavus* (*vanA*)	+	158
Cattle	NS[a]	Australia	0		–	87
Equines and swine	2005	Italy	6.7 (equine), 16.1 (swine),	*E. faecium*/*E. faecalis*/*E. casseliflavus* (*vanA*)	+	159
Sheep, pigs, cattle	2008–2009	Portugal	25.3 (pigs), 2.7 (sheep), 0 (cattle)	*E. faecium*/*E. hirae* (*vanA*)	+	160
Farm animals	1998–2003	USA	0		+	161
Farm animals	2000–2001	Korea	0.67	*E. faecium* (*vanA*)	+	162
Farm animals	2001	Korea	16.7 (chicken), 1.9 (pigs), 0 (cattle)	*E. faecium* (*vanA*)	+	163
Ostriches	2009–2010	Portugal	7.4	*E. durans* (*vanA*)	+	164
Mullet fish	2006–2007	Portugal	3.9	*E. faecium* (*vanA*)	+	165

[a]Some characteristics (year of isolation, or type of samples) are included in parenthesis.
[b]ST (CC), sequence type (clonal complex), if data are available.
[c]NS, not specified.

Occasionally, enterococci were found to be *vanB* carriers: two small mammals (*Rattus rattus*) harbored *vanB2*-containing *E. faecalis* ST6 isolates in Spain (204), and *E. faecium vanB* was detected in wild game meat, also in Spain (226). The frequencies of detection of *vanA*-containing enterococci in wild animals ranged from 0 to 13.5%, with the highest values detected in red foxes, seagulls, and buzzards in Portugal (9 to 13.5%) (216, 220, 222). Interestingly, *vanA*-containing *E. faecium* isolates detected were ascribed to different sequence types included in the high-risk clonal complex CC17 (ST18, ST262, ST273, ST280, ST313, ST362, ST412, ST448, and ST555). These isolates were detected in corvids in the United States and in mullet fish, gilt-head seabream, seagulls, buzzards, partridges, red foxes, and Iberian wolves in Portugal (Table 3).

Resistance to Linezolid

The widespread occurrence of VRE in many countries makes it necessary to look for other therapeutic options, and linezolid is an important one. This oxazolidinone, introduced in 2000 in the United States and in 2001 in the United Kingdom, is an important agent for the treatment not only of VRE, but also of other Gram-positive bacteria, such as methicillin-resistant *S. aureus*.

Table 2 Summary of reports about detection of VRE with acquired mechanisms of resistance in food samples of animal origin

Origin of the food sample	Year of recovery of tested isolates	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Chicken	1995–1996	Japan	3.0	*E. faecium*/*E. faecalis* (*vanA*)	–	166
Chicken	1997–1998	Spain	27.2	*E. faecium*/*E. faecalis*/*E. durans*/*E. hirae* (*vanA*)	+	128
Chicken	1997–1998	Belgium	1.3	*E. faecium* (NS[c])	–	167
Chicken	2000–2001	Korea	60 (15 samples)	*E. faecium* (*vanA*)	+	162
Chicken	2000–2003	Taiwan	13.7 (2000), 3.7 (2003) (*E. faecalis*), 3.4 (2000)–0 (2003) (*E. faecium*)	*E. faecium*/*E. faecalis* (*vanA*)	+	118
Chicken	2009	Japan	4.5 (22 samples)	*E. cecorum* (*vanA*)	+	168
Chicken	2009	Colombia	3.7	NS[c]	–	169
Chicken	2009–2010	Turkey	0	–	–	170
Chicken	NS	USA	0	–	–	171
Chicken	NS	UK	18.5	NS	+	172
Food and poultry	1999–2001	Portugal	34.0	*E. faecium*/*E. faecalis*/*Enterococcus* spp. (*vanA*)	+	173
Chicken and pork	1996–1997	Germany	12.3	*E. faecium*/*E. durans*/*E. faecalis*/*E. hirae* (*vanA*)	+	174
Chicken and pork	1997–1998	Italy	15.9 (1997), 8.1 (1998) (poultry product) 4.2 (1997), 6.9 (1998) (pork products)	*E. faecium*/*E. faecalis*/*E. durans*/*E. hirae* (*vanA*)	+	175
Chicken and pork	1999	France	10.2	*E. faecium*/*E. durans* (*vanA*)	+	176
Chicken and pork	2011	Japan	0.6 (chicken meat)	*E. faecium* (*vanN*)	+	177
Various types of food	2000–2002	Germany	0		–	178
Various types of food	2010	Argentina	7.5	*E. faecium* (*vanA*)	–	179
Various types of food	2010	Iran	NS	*E. faecium* ST669 (*vanA*)	+	180
Various types of food	2010–2012	Greece	21.9	*E. faecium* (*van*A), *E. faecium* (*vanA* + *van*B2/3), *E. faecium* (*vanB2/3*)	+	181
Various types of food	2012	Italy	3.53	NS	–	182
Various types of food	NS	Spain	5.4	NS	–	183
Various meats	1997–1998	Italy	14.6 (1997), 8.0 (1998)	*E. faecium*/*E. faecalis*/*E. durans*/*E. hirae* (*vanA*)	+	184
Various meats	2001–2002	USA	0		–	185
Various meats	2003	Korea	44	*E. faecium* (*vanA*)	+	133
Various meats and feces	2004–2006	Japan	0		+	186
Various meats	2007–2008	Canada	0		–	187
Various meats	2007–2009	Spain	3.9	*E. faecium* ST78 (CC17)/ST425 (*vanA*), *E. durans*/*E. hirae* (*van*A), *E. faecium* ST17 (CC17) (*vanB2*)	+	188
Various meats	NS	Canada	0		–	189
Various meats	NS	Spain	19.4	*E. faecium*/*E. durans*/*E. hirae* (*vanA*), *E. faecium* (*vanB*)	+	190
Retail meats	2002–2014	USA	0		–	2
Cheese	2005	France	0		+	191
Milk (sheep)	NS	Spain	1.7	*E. faecalis* ST168 (*vanC1*)	–	192
Shellfish	NS	UK	2.7	NS	–	172
Fish (Tilapia)	NS	Egypt	37.5 (8 samples)	*E. faecalis*/*E. gallinarum* (*vanA*)	–	193
Frozen food	NS	Thailand	9.7	NS	–	194

[a]Some characteristics (year of isolation or type of samples) are included in parenthesis.
[b]ST (CC), Sequence type (clonal complex), if data are available.
[c]NS, not specified.

Linezolid resistance is still unusual among enterococci but has emerged in recent years in human and animal isolates (227). Mutations in the central loop of domain V of the 23S rDNA is the most common mechanism of resistance in enterococci, the amino acid change G2576T being the predominant one, although other changes have also been described (G2505A, U2500A, G2447U, C2534U, and G2603U) (18). *E. faecalis* and *E. faecium* possess four and six 23S rDNA alleles per genome, respectively, and depending on the number of mutated versus wild-type alleles per genome, these correlate with the level of resistance of the isolates (227). In some cases, this mechanism appears during the course of treatment with oxazolidinones, and nosocomial transmission of linezolid-resistant enterococci has been reported (228). Linezolid-resistant *E. faecalis* and *E. gallinarum* isolates of swine origin were detected in China (MIC, 8 to 16 μg/ml), and the nucleic acid change G2576T was identified in the 23S rDNA of these isolates (229). Mutations in the ribosomal proteins L3, L4, and L22 can confer decreased susceptibility to linezolid in enterococci and staphylococci (230).

In recent years, there has been concern about the emergence of transferable resistance to linezolid, associated with the acquisition of the *cfr* gene or with the recently described *optr*A gene. The *cfr* gene has been detected in enterococci of both human and animal origin (231) and encodes an rRNA methyltransferase that modifies the adenine residue at position 2503 in domain V of the 23S rRNA; it confers resistance to oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A (the phenotype named $PhLOPS_A$) (18). Among oxazolidinones, linezolid is mostly affected by *cfr*; tedizolid, a new compound of this family, showed increased activity in *cfr*-positive enterococci, so these isolates are susceptible to this agent. Table 4 summarizes the data published until now in relation to linezolid resistance mechanisms in enterococci of animal and food origin, as well as in enterococci of environmental origin (229, 232–241).

The *cfr* gene was identified for the first time in enterococci in 2011, specifically in an *E. faecalis* isolate recovered on a dairy farm in China (232). Since then, *cfr* has been detected in human clinical *E. faecalis* isolates (242), as well as in swine *E. casseliflavus*, *E. gallinarum*, and *E. faecalis* isolates in China and Brazil (233–235) and in a cattle *E. faecalis* isolate in China (234). A second variant of the *cfr* gene, named *cfr*(B), has been described in *E. faecium* isolates of human origin. This new plasmid-located variant is more similar to a *cfr*-like gene of *Clostridium difficile* than to the *cfr* genes of staphylococci or other enterococcal species (243, 244), and it has so far not been detected in enterococci of animal origin.

The novel *optr*A gene confers transferable resistance to oxazolidinones (both linezolid and telizolid) and phenicoles (chloramphenicol and florfenicol) and has been detected in *E. faecalis* and *E. faecium* isolates of both human and animal origin (236). This gene encodes an ABC transporter and has been detected more frequently in *E. faecalis* than in *E. faecium* isolates and more frequently in isolates from food-producing animals (pigs and chickens) than in those of human origin (236). The *optr*A gene has been detected both in chromosomal and in plasmidic locations in animal and human *E. faecalis* and *E. faecium* isolates. As shown in Table 4, *optr*A-positive enterococci have been detected in food-producing animals (poultry, pigs, and occasionally, cattle) in Asiatic countries, mostly in *E. faecalis* and *E. faecium* belonging to many different sequence types, and sporadically in *E. gallinarum*. The prevalence of *optr*A-positive enterococci represents 10% and 5.7% of total *E. faecalis* and *E. faecium* isolates, respectively, obtained from fecal samples of poultry and pigs in a study performed in China (236). In a recent study carried out in Korea, 11,659 *E. faecalis* and *E. faecium* isolates obtained from fecal and carcass samples of healthy cattle, pigs, and chickens from farms and slaughter houses from 2003 to 2014 were tested for linezolid resistance, detecting a rate of resistance of 0.33%, mainly attributed to *optr*A carriage (238). The *optr*A gene has also been detected in sporadic isolates of *E. faecalis* and *E. faecium* ($n = 3$) obtained in meat samples in Denmark (imported poultry and veal), which represented <0.1% of total enterococci recovered from these samples (239). In Colombia, *optr*A has been detected in three *E. faecalis* isolates from poultry meat, coharboring the *fexA*, *tet*(L), and *Isa*(A) resistance genes (240). Both *cfr* and *optr*A have been detected in VRE isolates of human origin (245), but not in animal isolates so far.

The *optr*A gene has also been detected in two *E. faecalis* isolates of the lineage ST86 recovered from urban wastewater in Tunisia, accounting for 1% of all chloramphenicol-resistant enterococci tested (241); *optr*A was located within a transferable mosaic plasmid, which also contained the *fexA* and *erm*(A) genes.

At least 12 and 5 polymorphic variants of the *optr*A gene have been detected among human and animal enterococci, respectively (237, 246–248). The wild OptrA type ($OptrA_{E349}$) and the variants Tyr176Asp + Lys3Glu-Gly393Asp or Thr481Pro or Thr112Lys or Gly393Asp have been found in animal isolates (237,

Table 3 Summary of reports about detection of VRE with acquired mechanisms of resistance in companion and free-living animals

Animal species	Isolation year	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Companion animals						
Dogs (sick)	1996–1998	USA	2.8	*E. faecium* (*vanA*)	–	195
Dogs/cats	1998	Spain	22.7 (22 samples)	*E. faecium* (*vanA*)	+	145
Dogs	1998–2003	Spain	12.6	*E. faecium* (*vanA*)	+	196
Dogs/cats	2003	Portugal	2.6 (dogs) 0 (cats)	*E. faecium* (*vanA*)	+	136
Dogs (sick)	2008–2009	USA	0		–	197
Dogs	2009	Spain	0		+	120
Dogs/cats (antibiotic-treated)	2011–2012	Japan	0		–	198
Dogs/cats	2014	Egypt	0		–	199
Dogs (sick)	NS[c]	Portugal	0		–	200
Dogs/cats	NS	Turkey	3.8 (dogs) 0 (cats)	NS[c]	–	201
Equids	2007–2008	Portugal	4.4	*E. faecium*/*E. durans* (*vanA*)	+	202
Free-living animals						
Wild mammals	1997–2000	England	1.2 (badgers) 4.6 (woodmice)	*E. faecium* (*vanA*)	–	203
Wild animals	2004	Portugal	0		+	136
Wild animals	2008–2013	Spain	2.0	*E. faecium*-ST915 (*vanA*), *E. faecalis* ST6 (CC2) (*vanB2*)	+	204
Wild animals	2012	Poland	14.9	NS	–	205
Wild animals	2014–2015	Spain	0.3	*E. faecium* ST993 (*vanA*)	+	206
Wild birds	2006–2010	Portugal	2.7	*E. faecium*/*E. durans* (*vanA*)	+	207
Wild birds	2009–2010	Portugal	1.3	*E. faecium*/*E. durans*/*E. hirae* (*vanA*)	+	208
Wild birds	2010	Portugal	0.7	*E. faecalis* (*vanA*)		209
Wild birds	2012	Tunisia	3.6	*E. faecium* (*vanA*)	+	210
Corvids (American crows)	2012	USA	2.5	*E. faecium* ST18 (CC17)/ST555 (CC17)/ ST749/ST750/ST751/ST752–(*vanA*), *E. faecalis* ST179 (CC16)/ST16 (CC16)/ ST6 (CC2) (*vanA*)	+	211
Corvids (American crows)	2012–2015	USA	2.7	*E. faecium*-ST362/ST412 (CC17)-(*vanA*)	+	212
Corvids	2012–2013	Canada	0.7	*E. faecium* ST448 (*vanA*)	+	213
Corvids	2013	Slovakia	1.3	*E. faecium* ST917/ST6 (CC5) (*vanA*)	+	214
Wild boars	2005–2006	Portugal	3	*E. faecium* (*vanA*)	+	215
Mullets fish	2006–2007	Portugal	3.8	*E. faecium* ST273 (CC17)/ST280 (CC17) (*vanA*)	+	165
Seagulls	2007	Portugal	10.5	*E. faecium*-ST5 (CC17)-(*vanA*), *E. durans*-(*vanA*)	+	216
Gilt-head seabream	2007	Portugal	5.9	*E. faecium* ST273 (CC17)/ST313 (CC17)/ST76 (*vanA*), *E. faecalis* ST6 (CC2) (*vanA*), *E. durans* (*vanA*)	+	217
Wild rabbits	2007–2008	Portugal	3.9	*E. faecium*-(*vanA*)	+	218
Pigeons	2007–2008	Brazil	0		–	219
Buzzards	2007–2009	Portugal	9	*E. faecium* ST273 (CC17)/ST5 (*vanA*), *E. durans* (*vanA*)	+	220
Partridges	2015–2016	Portugal	2	*E. faecium*-ST18(CC17)/ST448(CC17)/ ST139(CC5)-(*vanA*)	+	221

(Continued)

Table 3 *(Continued)*

Animal species	Isolation year	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Red foxes	2008–2009	Portugal	13.5	*E. faecium* ST262 (CC17)/ST273 (CC17) (*vanA*), *E. durans* (*vanA*)	+	222
Iberian wolf, Iberian lynx	2008–2010	Portugal	0.5 (Iberian wolf) 0 (Iberian lynx)	*E. faecium* ST18 (CC17)/ST573 (*vanA*)	+	223
Buffalo, wildebeest, zebra	2010–2011	Tanzania	2.8 (buffalo) 20 (zebra) 2.5 (wildebeest)	NS	+	224
Camels	NS	Spain	0		–	225
Wild game meat	NS	Spain	30.9	*E. faecium*/*E. faecalis*/*E. durans* (*vanA*), *E. faecium* (*vanB*)	–	226

[a]Some characteristics (year of isolation, type of samples) are included in parenthesis.
[b]ST (CC), sequence type (clonal complex), if data are available.
[c]NS, not specified.

246). Functional *cfr* and *optrA* genes have been identified in both enterococci and *S. aureus*. In most of the animal isolates, *optr*A is located close to other genes, as is the case of *fexA* (implicated in phenicol resistance) and a novel *erm*(A)-like gene. This *erm*(A)-like gene encodes an rRNA methylase, which shows 85.2% amino acid identity to the Erm(A) protein of transposon Tn*554* of *S. aureus* (237).

Most of the *cfr*-positive enterococci of food-producing animals (>90%) showed a MIC for linezolid of ≥8 μg/ml, but two *E. faecalis* isolates presented a MIC of 4 μg/ml. *optr*A-positive isolates of food-producing animal and food origin showed a linezolid MIC in the range of 2 to >8 μg/ml, presenting 19% of the isolates' MICs in the range of 2 to 4 μg/ml (categorized as susceptible according to EUCAST breakpoints and susceptible-intermediate according to CLSI) (Table 4). It is interesting to note that *cfr*- and *optr*A-positive enterococci could appear as linezolid-susceptible, probably leading to an underestimation of their actual incidence.

Oxazolidinones are not used in food-producing animals. Nevertheless, the emergent detection in these animals of linezolid-resistant enterococci carrying the *optr*A gene in transferable plasmids, linked to resistance genes for antibiotics commonly used in animals (phenicols, tetracyclines, lincosamides, and aminoglycosides), suggests its role in the coselection of multiresistant bacteria, which poses a risk for public health.

To summarize, transferable linezolid resistance genes, mostly *optr*A, have been detected in enterococci of food-producing animals and food of animal origin in various European, South American, and Asian countries, but so far not in Africa. These mechanisms of resistance have not been detected so far, to our knowledge, in pets or in wild animals.

Resistance to Aminoglycosides

Enterococci are intrinsically resistant to clinically achievable concentrations of aminoglycosides due to their low cell wall permeability. In addition, some species, such as *E. faecium* [*aac*(6′)-*Ii*], *E. durans* [*aac*(6′)-*Id*], and *E. hirae* [*aac*(6′)-*Ih*], intrinsically express a chromosomal-encoded acetyltransferase that confers resistance to tobramycin, kanamycin, and amikacin (249). The chromosomally encoded methyltransferase EfmM has been exceptionally described in an *E. faecium* isolate (250) codifying resistance to kanamycin and tobramycin. Acquired resistances to aminoglycosides are detected in strains from both animals and humans and usually confer a high level of resistance to gentamicin, kanamycin, and streptomycin.

High-level resistance to gentamicin in enterococcal isolates of animal origin was first described in 1998 in Denmark (251) and in 2001 in the United States (252). The acquired genetic mechanisms identified in animal isolates are identical to those described in human isolates. The most frequent ones are the bifunctional enzyme encoded by *aac(6′)-Ie-aph(2″)-Ia* (conferring resistance to gentamicin, kanamycin, amikacin, netilmicin, and tobramycin) and *aph(3)-III*a (conferring resistance to kanamycin and amikacin) (23, 253). High-level gentamicin resistance can also be due to the expression of the unusual *aph(2″)-Ic*, *aph(2″)-Id*, *aph(2″)-Ie*, and *aph(2″)-Ib* genes (17, 23); *aph(2‴)-Ic* seems to be more frequent in enterococci of animal origin, and some farm animals could be a reservoir of this gene (252). High-level resistance to streptomycin is

Table 4 Mechanisms implicated in linezolid resistance in enterococci of animals, food of animal origin, and the environment

Origin	Species	Sample	Year of isolation	Country	Number of linezolid resistant isolates (%)	Linezolid MIC (μg/ml)	Mechanism of linezolid resistance (genetic location, number of isolates)[a]	ST (number of isolates)[b]	Characteristics (number of isolates)	Reference
Food-producing animals										
Cattle	*E. faecalis*	Feces	2009	China	1	4	*cfr* (P)		*fexB*-linked	232
Pigs	*E. faecalis*	Feces	2009	China	1 (0.3% of *E. faecalis*)	4	*cfr* (P)	ST21	*fexA* (in a different plasmid)	233
Pigs	*E. faecalis*	Rectal swabs	2013	Brazil	5 (2% of *E. faecalis*)	8–>8	*cfr* (5) Mutation in ribosomal L3 protein (V149I) (1)	ST591 (2); ST29, ST590, ST592 (1 each)		234
Pigs	*E. gallinarum* (*n* = 1), *E. casseliflavus* (*n* = 24)	Rectal swabs	2012	China	25 (31.5% of florfenicol-resistant enterococci)	8–16	*cfr* (25) (C,1; P, 8)		*fexA*-linked (5), *fexA-fexB*-linked (1)	235
Poultry and pigs	*E. faecalis*	Feces	2012–2013	China	20 (10% of *E. faecalis* of animal origin)	2[c]–8	*optrA* (C,8; P, 9; P/C, 3)	ST21 (2); ST27, ST59, ST74, ST93, ST116, ST256, ST330, ST403, ST475, ST476, ST480, ST593, ST618, ST619, ST620, ST621, ST622, ST623 (1 each)	*fexA*-linked (14), *fexB*-linked (2), *fexA-fexB*-linked (1) *optrA* amino acid changes: Y176D, T481P (2); K3E, Y176D, G393D (1); T112K, Y176D (2); Y176D, G393D (1)	236, 237
Poultry and pigs	*E. faecium*	Feces	2005, 2009, 2012, 2014	China	5 (5.7% of *E. faecium* of animal origin)	4[d]–8	*optrA* (C, 1; P/C, 4)	ST957 (2); ST32, ST184, ST29 (1 each)	*fexB*-linked (4)	236, 237

Poultry and pigs	*E. faecalis*	Feces and carcass	2008, 2012, 2014	Korea	12 (0.2% of *E. faecalis*)	8–>16	*optrA* (12)	ST21 (4); ST49 (2); ST16, ST32, ST256, ST403, ST728, ST729 (1 each)	*fexA*-linked (11)	238
Poultry and cattle	*E. faecium*	Feces and carcass	2008, 2010, 2012–2014	Korea	27 (0.7% of *E. faecium*)	8–>16	*optrA* (23) Mutation in ribosomal L4 protein (N130K) (7)	ST195 (6); ST32 (2); ST157 (2); ST1171 (2); ST1168 (2); ND (4); ST8, ST120, ST121, ST236, ST241, ST1166, ST1167, ST1169, ST1170 (1 each)	*fexA*-linked (19)	238
Pigs	*E. faecalis* (*n* = 5), *E. gallinarum* (*n* = 1)			China	6 (17.14% of enterococci)	8–16	23S rRNA mutation: G2576T	ST29, ST146, ST220, ST283, ST535		229
Meat										
Cattle	*E. faecalis*	Danish veal	2015	Denmark	1 (<0.1% of *E. faecalis*)	8	*optrA* (P)	ST22	*fexA*-linked	239
Poultry	*E. faecium*	Imported turkey and broiler meat	2012, 2013	Denmark	2 (<0.1% of *E. faecium*)	8	*optrA* (P, 1)	ST22, ST873		239
Poultry	*E. faecalis*	Meat	2010–2011	Colombia	3 (0.5% of enterococci)	8	*optrA* (P, 3)	ST59 (2), ST489	*fexA*-linked	240
Environment										
Wastewater	*E. faecalis*	Urban wastewater treatment plant	2014	Tunisia	2 (1% of chloramphenicol-resistant enterococci)	4	*optrA* (P)	ST86 (2)	*fexA*-linked (2), *optrA* amino acid changes:M1L (2), K3E (1) and I622M (1)	241

[a]P, plasmid; C, chromosome.
[b]ND, not determined.
[c]Five *optr*A-positive *E. faecalis* isolates showed a linezolid MIC of 2 μg/ml, and five isolates showed an MIC of 4 μg/ml.
[d]Two *optr*A-positive *E. faecium* isolates showed a linezolid MIC of 4 mcg/ml.

commonly caused by punctual ribosomal mutations, although acquisition of some modifying enzymes has been also described [*ant(3´´)-Ia* and *ant(6´)-Ia*]. Table 5 summarizes papers (from 2013 to 2017) that analyzed the rates of antimicrobial resistance (high level to gentamicin and others such as tetracycline, erythromycin, and ciprofloxacin) in enterococcal isolates from animals (65, 15, 87, 90, 92, 135, 141, 143, 147, 153, 154, 198, 205, 209, 254–276).

Resistance to Tetracycline

This family of antimicrobials integrates several antibacterial active compounds (277), although tetracycline, chlortetracycline, oxytetracycline, and doxycycline are the most used in veterinary. Despite Roberts' extensive 1996 review about tetracycline resistance mechanisms (278), a more recent update was published in 2005 (279). Almost 60 tetracycline resistance genes have been described, although the most frequent ones in *Enterococcus* are those implicated in ribosomal protection [*tet*(M), *tet*(O), *tet*(S)], efflux, or enzymatic inactivation [*tet*(K), *tet*(L)]. In *Enterococcus*, as occurs in other Gram-positive microorganisms, the ribosomal protection protein mechanism encoded by the *tet*(M) gene is the most frequent, independent of the origin of the strains. The transferability of the tetracycline resistance determinants in the absence of plasmids has been described (280); the Tn*916*/Tn*1545* conjugative transposon family carrying the *tet*(M) gene is responsible, usually in combination with *erm*(B).

Resistance to Macrolides/ Lincosamines/Streptogramins

Numerous chemically diverse compounds are integrated into the macrolide family, with erythromycin being the most representative. Resistance to this antibiotic was immediately reported after its introduction in human clinical use in 1952; moreover, enterococci are intrinsically resistant to clindamycin and lincomycin. Tylosin, spiramycin, and virginiamycin were widely used in pigs and other animals before the European Union limited their use. After the ban, erythromycin resistance in *Enterococcus* strains from animals decreased spectacularly (281), demonstrating the link between consumption of the antibiotic and the increase in resistance rates, even in different environments.

Chromosomal intrinsic resistance to macrolides by *msr*(A) and to lincosamides by *linB* in *E. faecium* has been described (282, 283). Acquired resistance to macrolides can be codified by various genetic determinants (up to 92 have been described) (284), although the most common worldwide is *erm*(B), usually carried by Tn*917*, which is widespread in human and animal isolates. Other relevant genes in the genus *Enterococcus* are the efflux genes *mef*(A), conferring resistance to macrolides, *vgb*(A), conferring resistance to virginiamycin, *lnu*(B), conferring resistance to lincosamide, and *vat*(D) and *vat*(E), conferring resistance to streptogramins.

Resistance to Quinolones

Fluoroquinolones have reduced antimicrobial activity against enterococci, with levofloxacin and moxifloxacin being the most active compounds. Acquired resistance is the consequence of mutations in the *gyrA* and *parC* genes (285, 286, 287) or the acquisition of the *qnr* genes (287). Efflux pumps such as EmeA for *E. faecalis* (288) and NorA-like for *E. faecium* (289) have also been described, although their frequency is low. Resistance to ciprofloxacin is a conserved feature among the high-risk *E. faecium* CC17 clone linked to nosocomial outbreaks (290) and among almost all isolates with resistance to glycopeptides. Fluoroquinolones have never been used as growth promoters, although their use for veterinary therapy is common.

MOLECULAR EPIDEMIOLOGY AND POPULATION STRUCTURE OF ENTEROCOCCI IN FARM AND COMPANION ANIMALS

Epidemiological studies of farm and companion animals were originally driven by the interest in establishing a relationship between antibiotic-resistant isolates from human and nonhuman hosts. At present, the resistance phenotypes of clinical relevance that may be linked to animals mainly comprise resistance to ampicillin, gentamicin, quinupristin-dalfopristin, vancomycin, and linezolid.

Molecular typing of enterococci strains has been performed by different methods, including pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), MLST, cgMLST, Bayesian analysis of population structure (BAPS) and whole-genome sequencing (WGS) (revised in 291).

The emergence of VRE in European food-producing animals and food of animal origin in the early 1990s (128, 291, 292–296), as well as in the feces of healthy volunteers and food handlers (297–299), led to surveillance studies in the community setting that suggested a relationship between the extensive use of animal growth promoters in veterinary medicine (e.g., avoparcin and tylosin), the colonization pressure in animals, and the subsequent transmission to human hosts throughout the food chain (300, 301).

The first report of VRE in nonhuman hosts occurred in 1993 in the United Kingdom and documented the similarity between isolates of different origins (300). This study was followed by others, which confirmed the similarity of VRE strains from humans and farm animals exposed to avoparcin in different European countries (26, 292, 302–305). The potential selection of antibiotic-resistant enterococci by antibiotics led to the ban of avoparcin as an animal growth promoter in Sweden in 1986, Denmark and Switzerland in 1995, and in the rest of the European countries 2 years later (Commission Directive 97/6/EC). By 1999, other antibiotics (such as bacitracin, virginiamycin, and tylosin) were also banned as growth promoters for healthy animals in Europe, and this was followed in 2006 by a ban on all antibiotics as growth promoters. In this way, Europe led the first intervention against VRE at a global level. In contrast to western countries, the use of antimicrobials in livestock and poultry, as well as the standard policies on antimicrobial use, varies significantly among Asian countries (reviewed in 306). In Korea, avoparcin was used in the management of poultry and swine from 1983 to 1997 but was banned thereafter to reduce exposure of humans to VRE (133). After several years of avoparcin discontinuance in Korea, the prevalence of VRE in Korean livestock was investigated, and some studies reported that the VRE incidence rate in chicken samples was higher than that in pig samples (163, 307).

The ban led to a significant reduction of VRE colonization in animals, foods, and fecal samples of community-based people in different countries. However, VRE was recovered in feces of animals and humans years later, reflecting the important effects of previous livestock practices in the population structure of enterococci in animals.

Most information came from the species *E. faecium* and *E. faecalis*, the predominant species in the gastrointestinal tract of mammals, along with *E. hirae*, *E. durans*, and *E. cecorum* (11, 45, 46).

E. faecium

PFGE remained the "gold standard" for molecular typing of *E. faecium* until the recent introduction of WGS-based epidemiology (291, 308). By using PFGE, clonal dissemination of *E. faecium* strains with clinically relevant phenotypes (ampicillin, gentamicin, quinupristin-dalfopristin, and vancomycin) has been extensively documented between animals from the same or different farms and has also been suggested between animals and humans (309, 310). The data vary greatly among geographic areas and are normally associated with the use of antibiotics.

Ecological differentiation of *E. faecium* has been documented in epidemiological studies using AFLP, MLST, and/or BAPS (311–314). AFLP analysis originally revealed different subpopulations (or ecotypes) corresponding to hospitalized patients, community-based people, and farm animals, including veal calves, poultry, and swine (311, 315). Later, MLST results using eBURST confirmed the split of *E. faecium* into host-specific subgroups, one from hospitalized patients (originally termed clonal complex 17 [CC17]) and others from domesticated animals (291, 316). More recently, BAPS analysis allowed the partitioning of 519 sequence types of 1,720 *E. faecium* isolates into 13 nonoverlapping groups. Again, BAPS groups were significantly associated with isolates from hospitalized patients (BAPS 3-3) and farm animals (BAPS 2-1 and 2-4) (313). More recently, single-nucleotide polymorphism-based phylogenetic analysis of WGS data split *E. faecium* into isolates causing infections (clade A1), isolates from healthy humans (clade B), and isolates from healthy humans and animals (clade A2) (79). Clade A1 mostly comprises isolates from hospitalized humans associated with lineages 17 (including ST16 and ST17), 18 (ST18), and 78 (ST78 and ST192), although isolates from animals have been extensively reported (89, 304, 313). The ST78 isolates show putative evolutionary hallmarks with respect to pets (dogs and cats) and poultry isolates and diversified mainly through recombination and acquisition or loss of mobile genetic elements, which eventually led to adaptation to different ecological niches. Thus, ecological distinction is not absolute, and the main zoonotic risk linked to *E. faecium* isolates is represented by transfer of mobile genetic elements harboring antimicrobial resistance genes.

Poultry

E. faecium isolates resistant to macrolides, quinupristin-dalfoprisitin, or other streptogramins were extensively reported in poultry farms, revealing high heterogeneity of PFGE types and sequence types, although some similar patterns were eventually detected on farms in Europe, the United States, and Asia (317–319). Clonal dissemination of VRE of the *E. faecium* species (VREfm) in poultry farms exposed to antibiotics before and after the avoparcin ban (109, 302) were documented in European and Asian countries, with sequence types belonging to CC9 or CC96 being predominant in Europe and Malaysia, respectively (320). A dramatic increase of VREfm in Sweden from 2000 to 2009 was due to the clonal expansion of the clone ST310, despite the absence of selection by antibiotics in this country, where the use of antibiotics as animal growth promoters has

Table 5 Summary of articles on the antimicrobial resistance in *Enterococcus* isolated from animals from 2013 to 2017

Animal	Country	Enterococcal specie	Percentage of resistance to[a]				Reference
			TET	ERY	HLR-GEN	CIP	
Food-producing and farm animals							
Camels	Tunisia	*Enterococcus* spp.	4	11	0	ND	254
Chickens and pigs	China	*Enterococcus* spp.	92.5	72.8	30	ND	153
Aquaculture	Spain	*E. faecalis*	0	5	ND	62	255
		E. faecium	5	37	ND	26	
Rainbow trout	Spain	*Enterococcus* spp.	27.1	41	7	0	256
Marine aquaculture	Italy	*Enterococcus* spp.	66	25	0	ND	257
Tibetan pigs	Tibet	*E. faecalis*	93.5	93.5	0	0	258
		E. faecium	44.4	20	0	0	
Farm animals	Argentina	*E. faecalis*	100	50	0	ND	259
Chickens	Southeast Asian countries	*E. faecalis*	69.2	70.9	11.1	17.9	260
		E. faecium	92.2	79.4	13.9	82.8	
Poultry	Italy	*Enterococcus* spp.	65.2	87.8	60	70.4	143
Beef and sheep	Tunisia	*E. faecalis*	15	30	2.5	22.5	261
Pigs	South Africa	*E. faecalis*	ND	100	ND	45	147
		E. faecium	ND	98.3	ND	94.1	
Poultry	Germany	*E. faecalis*	82	70	98	14	141
		E. faecium	67	89	89	72	
Poultry	Nigeria	*Enterococcus* spp.	81.6	100	20	ND	262
Chicken and turkey	Canada	*Enterococcus* spp.	94	64–74	8–15	2–8	135
Fish and seafood	Switzerland	*E. faecalis*	16	ND	4	0	263
Infection in poultry	Poland	*E. cecorum*	29	51	1	ND	15
Poultry and cattle	Nigeria	*Enterococcus* spp.	61	61	32.7	9.7	154
Mastitis in cows	China	*Enterococcus* spp.	6.7	ND	50	25	264
Cattle	Australia	*E. faecalis*	7.3	10.4	0	ND	87
		E. faecium	11.7	8.3	0	ND	
Pigs	Nigeria	*E. faecalis*	60	68.7	85	0.5	265
		E. faecium	20	31.5	40	7.4	
Donkey	Spain	*E. faecium*	68	44	0	60	266
Pets and companion animals							
Healthy dogs and cats	Japan	*Enterococcus* spp.	ND	40	44	25	267
Hospitalized dogs	Korea	*E. faecalis*	64.3	50	7.1	0	268
		E. faecium	66.7	44.4	33.3	55.6	
Antibiotic-treated dogs and cats	Japan	*E. faecalis*	58	52	38	30	198
		E. faecium	15	28	21	8	
Various animals	Poland	*E. faecalis*	36	48	ND	38	205
		E. faecium	7	60	ND	46	
Healthy cats and dogs	Italy	*Enterococcus* spp.	97.4	81.7	40.8	7.8	269
Wild animals							
Iberian lynx	Spain	*Enterococcus* spp.	33	30	4	7	92
Iberian wolf	Portugal	*Enterococcus* spp.	55	22	1	15	90
Echinoderms	Portugal	*E. faecium*	31.7	33	0.8	30.8	65
Red foxes	Portugal	*E. faecalis*	81	36	18	ND	270
		E. faecium	96	56	12	ND	
Wild birds	Portugal	*E. faecalis*	32.3	15.3	0	15.3	209
		E. faecium	45	15	0	32.5	
Eurasian otter	Portugal	*E. faecalis*	32	ND	52	ND	271
		E. faecium	78	ND	11	ND	
House flies	USA	*E. faecalis*	60	18	5	0	272

(Continued)

Table 5 *(Continued)*

Animal	Country	Enterococcal specie	Percentage of resistance to[a]				Reference
			TET	ERY	HLR-GEN	CIP	
Wild birds	Tunisia	*E. faecalis*	33	33	50	66	261
		E. faecium	16	53	11	38	
Wild fur seals	Brazil	*E. faecalis*	0	25	0	10	273
Wild marine animals	Brazil	*Enterococcus* spp.	14.5	32.2	0	20.9	274
Seafood	Tunisia	*E. faecalis*	42.9	38.1	14.3	52.4	275
		E. faecium	18.2	27.3	0	63.6	
House flies	Thailand	*Enterococcus* spp.	75	42.5	ND	ND	276

[a]TET, tetracycline; ERY, erythromycin; HLR-GEN, high-level resistance to gentamicin; CIP, ciprofloxacin.

been forbidden since 1986 (129). A Danish study showed a high rate of VREfm in Danish farms after the 15-year ban of avoparcin, with different sequence types and the presence of an ST842 clone in 36 flocks analyzed corresponding to eight farms broadly distributed across the country (85). Recently, clonally unrelated *E. faecium* isolates resistant to linezolid emerged on farms in China (236, 237). Common PFGE profiles or sequence types between humans and broilers have also been documented (321–323), but the human health risk associated with the presence of *E. faecium* in poultry meat is under debate (25).

Swine

VREfm has been extensively reported in pig farms in European countries before and after the avoparcin ban (113, 324, 325). Clonal spread of VREfm was documented in Denmark, Norway, Finland (113), Switzerland (326), Portugal (304), and Spain (327), with predominance of sequence types belonging to the CC5 lineage (ST5, ST6, ST185). The persistence of VREfm in pig farms after the avoparcin ban was associated later with the use of tylosin, which facilitated the coselection of strains resistant to both glycopeptides and macrolides due to the presence of both *vanA* and *erm*(B) genes in the same plasmid (113). VREfm was also detected in county fairs in Michigan from 2008 to 2010, which represents the first and only report of VREfm in livestock in the United States to date (121, 122). In Asia, the occurrence varies among countries and is sporadic in China (156). In all these studies, CC5 strains were predominantly identified. A particular ST6 (CC5) clone was identified on farms in different European Union countries and the United States, as well as in healthy volunteers and hospitalized patients, all carrying a Tn*1546* in *orf1* and a G-T point mutation in position 8234 at *vanX* (304, 328). In addition to tylosin, copper is frequently added to pig and cattle feeds, so colocation of heavy metal resistance determinants has also been demonstrated in Europe and the United States (329, 330). Copper resistance is often associated with resistance to macrolides [*erm*(B)], tetracyclines [*tet*(M)], and glycopeptides (*vanA*). Although clonal dissemination has been reported (330), a great diversity has been observed on farms (331). Major human clones CC9 and CC22 (previously classified as CC17) have also been documented in some studies (85, 332, 333).

Companion animals

A few studies have analyzed the fecal carriage of ampicillin-resistant *E. faecium* (AREfm) and VREfm in companion animals. High rates of AREfm were observed among fecal samples of dogs collected in the United Kingdom and Denmark in 2006 and 2008 (23% and 76%, respectively) (89, 334). Most of these isolates belonged to the major human clonal lineage originally called CC17, which suggested a possible transmission between hosts. Later, de Regt *et al*. demonstrated some unique metabolic features in these CC17 canine isolates that could have facilitated niche adaptation (335). A recent large Dutch country-wide population-based study reported a higher prevalence of fecal carriers of AREfm in dogs and cats than in a healthy human population (25.6%, 5.1%, and 1.5%, respectively). This study concluded that isolates from pets were genetically distinct from those of humans based on the lack of cooccurrence and the cgMLST results (336). Prior antibiotic use and eating raw meat were considered a risk factor for acquiring AREfm in all the available studies (197, 336). Clinical isolates from dogs and cats treated with amoxicillin belong to high clonal complex risks and were similar to those from humans (197, 337).

E. faecalis

A plethora of molecular methods have been used to type this species, including PFGE, AFLP, and MLST. In

contrast to *E. faecium*, *E. faecalis* isolated from different sources/hosts cannot be grouped using MLST or AFLP. Studies using MLST data revealed the presence of many sequence types in different hosts, including farm animals, companion animals, and hospitalized patients (338, 339). Moreover, some sequence types are associated with a higher prevalence of antibiotic resistance, represented by ST2, ST8, ST9, ST16, ST40, and ST87 (303, 339, 340), all of them being overrepresented in humans. To date, ST16 has been recovered from humans and farm animals and is considered a zoonotic lineage (25), involved in the spread of resistance to all antibiotics used in animals, including bacitracin, phenicols, oxazolidinones (341). Clonal outbreaks of *E. faecalis* ST82, a common cause of amyloid arthropathy in poultry, have been reported on farms in Denmark, the United States, France, and Germany (342).

Although the detection of more-prevalent *E. faecalis* sequence types in distant geographical locations and different hosts suggests frequent horizontal gene transfer between different host populations (69, 211, 241, 339, 340, 343), some studies using comparative genomics discarded the idea of global transmission (344).

The incongruence in the topologies of the seven MLST gene trees revealed that this species is highly recombinogenic (291, 343). Subsequent analysis of the *E. faecalis* population structure based on MLST data using BAPS also yielded incongruent results and confirmed the lack of host-specific groups or ecotypes (313, 314). This issue was also demonstrated by studies that characterized the phylogenetic diversity of *E. faecalis* using whole genomes (phylogenomics and cgMLST) of clinical, human commensal, and animal isolates and that observed a lack of distinct clustering of isolates according to the source (291, 345).

Additional WGS studies are necessary to characterize and describe the role of animals in the evolution, genetic diversity, and population structure of *E. faecalis*.

PLASMIDS IN ENTEROCOCCI FROM FOODBORNE AND COMPANION ANIMALS

Horizontal gene transfer plays a relevant role in the dissemination of antibiotic resistance in nonhuman hosts, and plasmids play a central role in this dissemination. Classically, plasmid categorization is based on the presence and diversity of their replication machinery (346), which were established by replication-initiator protein (*rep*) schemes (347, 348) identified in Gram-positive species to date. In Fig. 1 we show the plasmid content (percentage and diversity of *rep* sequences) of the 67 *E. faecium* and 47 *E. faecalis* genomes of animal origin obtained from the WGS database of the NCBI. The enterococcal genomes from public databases were classified according to their origin (Table 6), information obtained from the Pathosystems Resource Integration Center (PATRIC) database (349). The *rep* genes obtained by the PlasmidFinder bioinformatics tool (350) belong to plasmid families with theta (RepaA_N, Inc18, Rep3_small tetha) or rolling-circle replication mechanisms (Fig. 1).

Plasmids conferring resistance in enterococci to vancomycin, macrolides, tetracycline, aminoglycosides, and heavy metals (copper, cadmium, bacitracin zinc) have been detected on farms that were exposed to antimicrobials used as growth promoters (avoparcin, virginiamycin, tylosin, or bacitracin zinc), therapeutically (tetracyclines, gentamicin, penicillins), or as dietary supplements (e.g., copper). Antibiotic-resistant plasmids have also been recovered from areas where selection was not apparent. Some emblematic examples are transferable *vanA* in commercial animal husbandry on farms in Michigan, where avoparcin has never been licensed for use in growth promotion (121, 122), and persistent *vanA*-Inc18 plasmids in Norwegian broiler flocks after the ban of some antibiotics. These studies suggest alternative routes of selection, introduction, and spread of *vanA*-type vancomycin resistance, plasmid fitness, and other phenomena (351).

Plasmids Conferring Resistance to Glycopeptides

Tn*1546* (*vanA*), the predominant mechanism of glycopeptide resistance in enterococci, has been successfully disseminated among poultry and swine through plasmids of the Inc18 and RepA_N families, respectively (352, 353). In poultry, an 18- to 25-kb fragment that includes the 10.85 kb of Tn*1546* (*vanA*), is conserved in Inc18 plasmids detected in Norwegian broiler flocks for more than 1 decade (from 1999 to years after the avoparcin ban) and in pIP186, the first Inc18 (*vanA*) plasmid described, in 1986, in an *E. faecium* clinical isolate (354, 355). The persistence of *vanA* plasmids on Norwegian poultry farms is attributed to the toxin-antitoxin system ω-ε-ζ originally described in pRE25, a plasmid of *E. faecalis* that carries resistance to different antibiotic families and is prevalent in animals and foods (127, 354). Analyses of the Tn*1546* insertion sites and plasmid backbones suggest spread of the *vanA* transposon across clonal lines in the broiler industry (125, 354–356). Bortolaia and Guardabassi recently associated the persistence of glycopeptide resistance in Danish poultry flocks after 15 years of the avoparcin ban with a nontransferable 54-kb plasmid in isolates

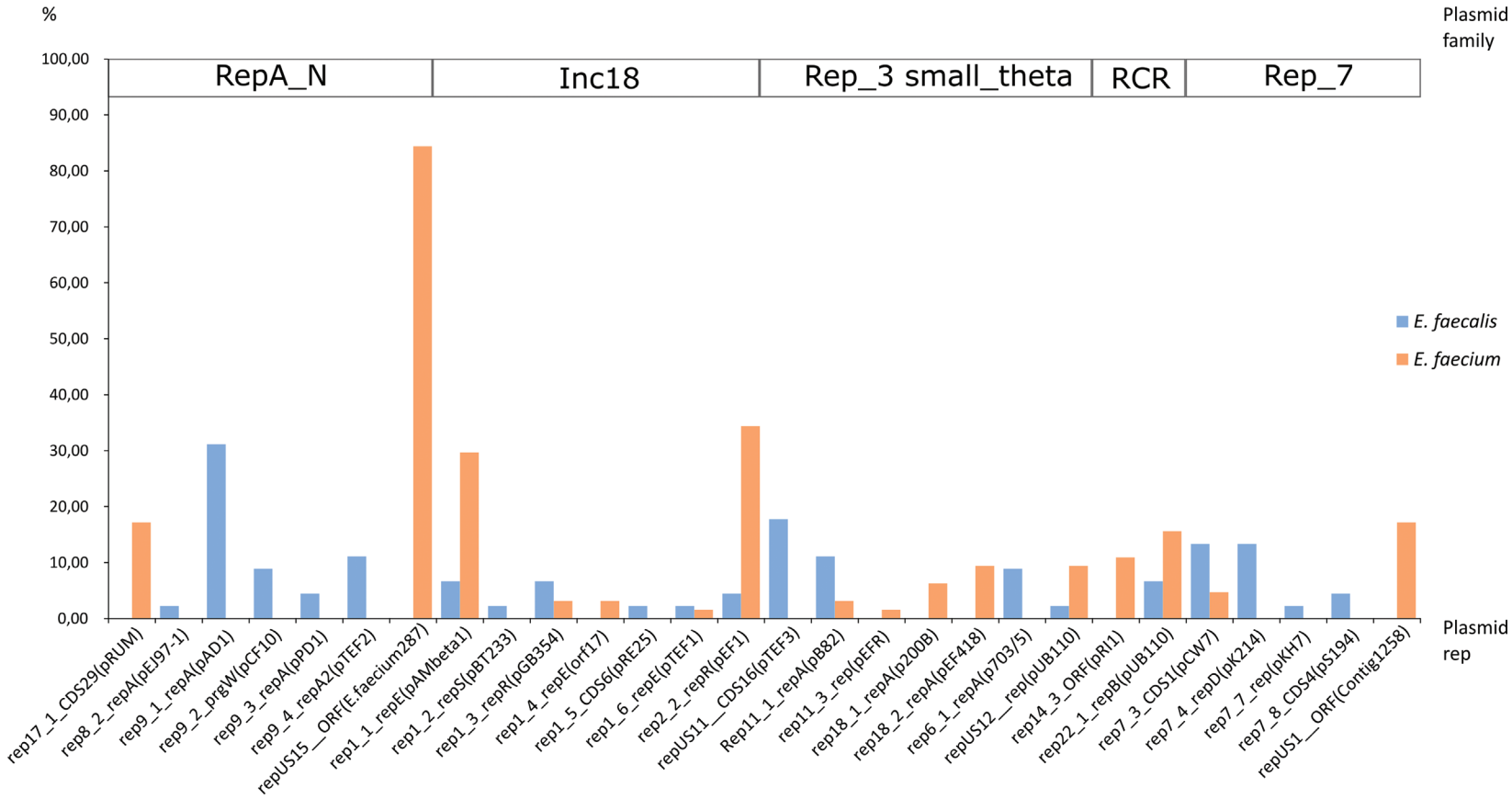


Figure 1 Plasmid gene content of 67 *E. faecium* and 47 *E. faecalis* genomes of animal origin from the NCBI whole-genome database. Plasmid data were obtained by the PlasmidFinder bioinformatics tool. The genomes from the database were classified by source, extracting the isolate information from the Pathosystems Resource Integration Center (PATRIC) database (344). Reps, replicases.

Table 6 *Enterococcus* isolates with animal source from the Genbank database included in the plasmid *rep* genes *in silico* screening

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
Enterococcus faecalis	PF3		0	Adélie penguin (*Pygoscelis adeliae*)	2010	Antarctica	GCA_000505585.1
	DBH18		1	Mallard duck (*Anas platyrhynchos*)	2005	Spain	GCF_001563075.1
	ATCC 27959	40	0	Cattle	1975	United States	GCA_000395265.1
	ATCC 35038	59	2	Chicken (*Gallus gallus*)	1980		GCA_000392955.1
	ATCC 29212		0	Chicken (*Gallus gallus*)			GCA_900117325.1
	ATCC 29212		2	Chicken (*Gallus gallus*)			GCA_900117335.1
	F1	72	2	Cow	1900		GCA_000393095.1
	ATCC 6055	113	2	Cow	1937		GCA_000393375.1
	ARO1/DG	108	3	Dog (*Canis lupus familiaris*)			GCA_000157275.1
	UCD-PD3		1	*Felis catus*	2015	United States	GCF_001692955.1
	Fly1	101	0	Fruit fly (*Drosophila*)		United States	GCA_000157415.1
	Fly 2	102	1	Fruit fly (*Drosophila*)	2005		GCA_000393215.1
	P9-1		2	Magellanic penguin (*Spheniscus magellanicus*)	2013	Brazil	GCF_001400065.1
	P8-1		3	Magellanic penguin, (*Spheniscus magellanicus*)	2013	Brazil	GCF_001400055.1
	KB1		1	*Mus musculus*	2011	Germany	GCA_002221625.2
	KB1		0	*Mus musculus*	2015	Switzerland	GCF_001689055.1
	7330257-1	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390805.1
	7330259-5	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390825.1
	7330948-5	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390845.1
	7330112-3	16	6	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390765.1
	7430416-3	16	0	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390905.1
	19116	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390685.1
	7430275-3	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390865.1
	7430821-4	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390925.1
	D6	16	0	Pig (*Sus scrofa*)		Denmark	GCA_000157455.1
	7430315-3	35	3	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390885.1
	D1	40	0	Pig (*Sus scrofa*)			GCA_000393575.1
	7330082-2	40	0	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390745.1
	D32	40	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000281195.1
	D3	47	2	Pig (*Sus scrofa*)			GCA_000392915.1
	7330245-2	47	2	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390785.1
	L9		0	Pig (*Sus scrofa*)	2013	Brazil	GCF_001878735.1
	L12		0	Pig (*Sus scrofa*)	2013	Brazil	GCF_001886675.1
	IBUN9046YE		5	Pig (*Sus scrofa*)	2014	Colombia	GCA_002250145.1
	19		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788165.1
	17		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788185.1
	1		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788175.1
	32		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788255.1
	12		3	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788155.1
	18		3	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788235.1
	Enfs51		2	Pig (*Sus scrofa*)	2012	Malaysia	GCF_001806515.1
	P.En250		3	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009545.1
	P. En090		3	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009565.1
	Enfs85		5	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009465.1
	XJ05		1	Sheep (*Ovis aries*)	2002	China	GCF_001263775.1
	OG1X	92	1	Tunicate (*Lissoclinum patella*)	2006	Solomon Islands	GCA_000320305.1
	2924	116	1	Turkey meat	2005	Denmark	GCA_000390725.1

(Continued)

Table 6 *(Continued)*

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
Enterococcus faecium	E1573	21	1	Bison (*Bison* sp.)	1994	Belgium	GCA_000321765.1
	825		4	Cattle	2004	United States	GCA_002360185.1
	E0045	9	3	Chicken (*Gallus gallus*)	1992	United Kingdom	GCA_000321465.1
	E2134	12	6	Chicken (*Gallus gallus*)	2004	Netherlands	GCA_000322185.1
	VAN 342	22	6	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395785.1
	VAN 332	38	4	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395745.1
	VAN 345	38	5	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395805.1
	E1575	158	4	Chicken (*Gallus gallus*)	1995	Belgium	GCA_000321805.1
	F9730129-1	245	4	Chicken (*Gallus gallus*)	1997	Denmark	GCA_000395965.1
	VAN 335	417	4	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395765.1
	VAN 219	784	3	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395705.1
	VAN 222	784	3	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395725.1
	7527		3	Chicken (*Gallus gallus*)	2003	United States	GCA_002360135.1
	6605		4	Chicken (*Gallus gallus*)	2003	United States	GCA_002360225.1
	5209		6	Chicken (*Gallus gallus*)	2003	United States	GCA_002360255.1
	E1574	27	3	Dog (*Canis lupus familiaris*)	1995	Belgium	GCA_000321785.1
	E4389	78	5	Dog (*Canis lupus familiaris*)		Denmark	GCA_000322425.1
	E4453	192	5	Dog (*Canis lupus familiaris*)			GCA_000239095.2
	E4452	266	5	Dog (*Canis lupus familiaris*)	2008	Netherlands	GCA_000239115.2
	GL3		0	Dromedary	2013	Algeria	GCF_001277795.1
	ICIS 96		5	*Equus caballus*	2014	Russia	GCA_002265255.1
	LS170308		2	*Moschus berezovskii*	2017	China	GCA_002831505.1
	E1622	104	0	Mouse	1959	Netherlands	GCA_000321945.1
	E1576	159	3	Ostrich (*Struthio camelus*)	2001	South Africa	GCA_000321825.1
	E0688	5	4	Pig (*Sus scrofa*)		Spain	GCA_000321605.1
	HF50104	5	2	Pig (*Sus scrofa*)	2008	United States	GCA_000396685.1
	HF50215	5	4	Pig (*Sus scrofa*)	2008	United States	GCA_000396785.1
	A17 Sv1	6	1	Pig (*Sus scrofa*)	1995	Denmark	GCA_000392125.1
	E8sv3	6	1	Pig (*Sus scrofa*)	1995	Denmark	GCA_000392145.1
	9730219-1	6	1	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391945.1
	9730357-1	6	1	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391965.1
	9731352-4	6	2	Pig (*Sus scrofa*)	1997	Denmark	GCA_000392005.1
	9830091-5	6	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392025.1
	9830512-2	6	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392045.1
	9931110-4	6	3	Pig (*Sus scrofa*)	1999	Denmark	GCA_000392105.1
	7330446-2	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391825.1
	7330519-3	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391845.1
	7330884-2	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391885.1
	7430166-3	6	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000391905.1
	S658-3	6	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000395425.1
	HF50204	6	1	Pig (*Sus scrofa*)	2008	United States	GCA_000396765.1
	HF50105	6	3	Pig (*Sus scrofa*)	2008	United States	GCA_000396705.1
	HF50106	6	3	Pig (*Sus scrofa*)	2008	United States	GCA_000396725.1
	9731349-1	82	4	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391985.1
	9930238-2	133	1	Pig (*Sus scrofa*)	1999	Denmark	GCA_000392085.1
	7330381-1	133	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391805.1
	E0679	150	2	Pig (*Sus scrofa*)		Belgium	GCA_000321565.1
	E0680	151	3	Pig (*Sus scrofa*)		Germany	GCA_000321585.1
	E1578	160	1	Pig (*Sus scrofa*)	2001	Germany	GCA_000321845.1
	9830565-4	185	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392065.1

(Continued)

Table 6 *(Continued)*

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
	7330614-1	185	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391865.1
	HF50203	185	1	Pig (*Sus scrofa*)	2008	United States	GCA_000396745.1
	CICYT-205	437	3	Pig (*Sus scrofa*)		Spain	GCF_001622975.1
	7230532-1		1	Pig (*Sus scrofa*)	2000	Denmark	GCA_000391785.1
	2006-70-121		0	Pig (*Sus scrofa*)	2006	Denmark	GCA_000391765.1
	1970-07-08		0	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767345.1
	70-40-11		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804405.1
	70-61-3		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767355.1
	1970-08-02		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804415.1
	70-36-8		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767365.1
	70-61-7		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804385.1
	E2071	27	1	Poultry	2001	Denmark	GCA_000322165.1
	FDAARGOS_397		2	Tonsil crypt			GCA_002554355.1
	E0269	9	3	Turkey (*Meleagris gallopavo*)	1996	Netherlands	GCA_000321525.1
	E0164	26	3	Turkey (*Meleagris gallopavo*)	1996	Netherlands	GCA_000321505.1
	M3K31		3	Vulture	2010	Spain	GCF_001039515.1
	58M		1	Wooly mammoth (*Mammuthus primigenius*)	2014	Russia	GCF_001280775.1

that only confer resistance to glycopeptides (27). It is notable that broiler flocks raised in Denmark come from parent birds imported from Sweden, and the high occurrence of VREfm was also observed in Swedish broiler flocks until 2011 (129).

In swine, large plasmids belonging to the RepA_N family (150 to 190 kb, rep_{pLG1}), which carry a truncated variant of Tn*1546* and *tcrB* (coding for resistance to copper), have been detected in a pandemic CC5 *E. faecium* clone that has been circulating in swine farms in Spain, Portugal, Denmark, Switzerland, and the United States for decades and in other *E. faecium* lineages of pigs and humans, which suggests transmission (304). These plasmids used to carry the *erm*(B) gene (macrolide resistance) and, eventually, *trcB* (copper resistance) (see below).

Also, sporadic reports have documented the occurrence of strains carrying other *vanB* or *vanN* operons on plasmids in poultry meat (178, 188), farmed game meat and wild game meat (226). Finally, vancomycin-susceptible *E. faecalis* strains carrying *vanC1* on transferable elements (plasmids, transposons, and integrons) have also been reported in cloacal swabs of broilers (357) and feces of diseased pigs from different farms (358). Transmission of species-specific *vanC1* and *vanC2/C3* genes could be currently underestimated given the high prevalence of *E. gallinarum* and *E. casseliflavus*, respectively, in food-producing animals (159, 359–360) and the scarcity of studies that screen *vanC* genes in other species.

Plasmids Conferring Resistance to Macrolides, Streptogramins, and Lincosamides

These plasmids have been extensively recovered in enterococci from poultry and pig farms where macrolides (spiramycin and tylosin) and streptogramins (virginiamycin) were used as growth promoters and pleuromutilins (tiamulin and valnemulin) were used to treat infections. Lincomycin, alone or in combination with spectinomycin, has been widely used to control respiratory and gastrointestinal bacterial pathogens in cattle, swine, poultry, dogs, and cats, and pirlimycin has been only used to treat bovine mastitis cases. Clindamycin is a common therapeutic option for topical infections in dogs and cats.

Macrolides

The most widespread gene that confers resistance to macrolides in enterococci is *erm*(B), which is located in different transposons and plasmids in species of the *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Clostridium* genera (346, 361). pRE25, a multidrug-resistant plasmid originally recovered from an *E. faecalis* isolate from a sausage sample, is the paradigm of the Inc18 family and has greatly contributed to the spread of *erm*(B) among animals and humans (346, 353, 362). This plasmid encodes resistance to 12 antimicrobials from 5 structural classes (macrolides, lincosamides, streptothricin, chloramphenicol, aminoglycosides) due to the presence of *erm*(B) (macrolide-lincosamide-

streptogramin B), cat_{pIP501} (chloramphenicol), and Tn*5405*, which comprises the genes *aadE-sat4-aphA3* (aminoglycoside-streptothricin) (363, 364). The genes carried by pRE25 are present in several animal pathogens, namely, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *S. aureus*, *Bacillus subtilis*, *Campylobacter coli*, *Clostridium perfringens*, and *C. difficile*. The *erm*(B) gene has also been found in small plasmids in poultry samples (365) and in large plasmids in food samples in addition to other genes such as *msr*(C) and *lnu*(B), *tet*(L), and *tet*(W) (366). Its presence in chromosomes is also frequent.

The gene *erm*(A), associated with Tn*554* and commonly found in staphylococci from swine, has also been found in streptococci and sporadic isolates of *E. faecalis* and *E. faecium* from pigs, suggesting transfer events (282, 367). More recently, a novel *erm*(A)-like gene that confers high-level resistance to erythromycin (MIC, >128 μg/ml) has been detected in Inc18 plasmids with genes encoding resistance to phenicols and oxazolidinones (see below). This gene differs from the widespread *erm*(A) gene on Tn*554* and the *erm*(A) gene formerly called *ermTR*, predominant in staphylococci and streptococci, respectively (82 to 85% homology at the amino acid level). This *erm*(A) enterococcal variant has a 116-bp deletion in the translational attenuator (237).

Streptogramins

Genes conferring resistance to streptogramins (acetyltransferases encoded by *satG/vatE* and *satA/vatA* genes and ABC transporters encoded by *vgb/vgb*B) and macrolides [23S rRNA methylases encoded by *erm*(B), *erm*(A), *erm*(C) genes] are observed in a diversity of plasmids and clonal backgrounds. In addition, *vat* genes are often cotranscribed and cotransferred along with *vga*, *vgaB*, *vgb*, *vgbB*, or *erm*(B) genes through transposable elements, some of them previously observed in staphylococci (364, 368–373). Transferability of *vat* genes and streptogramin resistance in *E. faecium* strains through contaminated pork and chicken meat, raw manure, and surface/ground water has been extensively documented (374, 375).

Lincosamides

Resistance to this antibiotic family can be due to the presence of genes coding for ABC transporters or modifying enzymes, most of them located on plasmids and/or transposable elements. These elements have been extensively documented in staphylococci and to a lesser extent in streptococci, *Clostridium*, and other species of Gram-positive bacteria in animals.

ABC transporters that confer resistance to pleuromutilins, lincosamides, and streptogramin A antibiotics (PLS_A) include the genes *vga* and *vga*(A)v, *vga*(C), *vga*(E), *vga*(E)v, *eat*(A)v, *sal*(A), *lsa*(A), *lsa*(C), and *lsa*(E). They frequently appear within clusters in plasmids or transferable chromosomal regions previously reported in *S. aureus* (230). A 8,705-bp region flanked by IS*Efa8* and IS*1216*, and comprising genes coding for one or more antibiotics, namely *lnu*(B) (lincosamide), *lsa*(E) (PLS_A), *spw* (spectinomycin), *aadE* (streptomycin), and *erm*(B) (macrolide-lincosamide-streptogramin B), is common for plasmids of *S. aureus* (pV7037) and *E. faecium* (pY13, pXD4, pXD5) strains recovered from pigs (230, 376, 377). The pY13 plasmid also contains a copy of the genes *lnu*(B) (lincosamide) and *aphA3* (kanamycin/neomycin) and a second copy of *erm*(B), highlighting the redundancy of determinants in settings under high selective pressure.

Two genes coding for nucleotidyl transferases (*lnu*), which only confer resistance to lincosamides, have been described in *Enterococcus* from swine recovered on Chinese farms (229, 378). *lnu*(G) is part of a 4,738-bp functionally active transposon designated Tn*6260*, which was first detected in an *E. faecalis* isolate of swine origin; this element is similar to others of the Tn*554* family, which includes different antibiotic resistance genes (378). *lnu*(B) has been detected in porcine *E. faecium* isolates, and it has been found in a nonconjugative plasmid linked to the *erm*(B), *lsa*(E), *spw*, *aadE*, and *aphA3* genes, which account for resistance to macrolides, lincosamides, streptogramins, pleuromutilins, streptomycin, spectinomycin, and kanamycin/neomycin (229).

Plasmids Conferring Resistance to Phenicols and Oxazolidinones

Genes coding for resistance to nonfluorinated phenicols (*cat*), nonfluorinated and fluorinated phenicols (*fexA*, *fexB*), and to both phenicols and oxazolidinones (*cfr*, *optrA*) have been detected in enterococcal species from animals, foods, and humans.

The production of chloramphenicol acetyltransferase (CAT) enzymes seems to be the main mechanism of resistance to chloramphenicol, although the number of studies addressing the diversity and the genetic context of *cat* genes in *Enterococcus* is still scarce. The predominant *cat* variants are *cat*(A-7), associated with pRE25-like plasmids of the Inc18 family, which are widely disseminated in food and farm animals, predominantly poultry (241), and *cat*(A-8), also known as cat_{pC223}, associated with pC223 plasmids originally detected in *S. aureus* that are now predominant in *E. faecalis* from

swine. This gene eventually appears in tandem with *tet* (M) and *tet*(L) genes within the transposon Tn*6245*, and relics of this transposon have been observed in plasmids that also carry *fexA* and *optrA* (237). Although isolates positive for the *cat*(A-9) gene have been recently identified in *E. faecalis* from swine, their genetic context has not been characterized (A. Freitas, personal communication).

The florfenicol exporter gene *fexB* was initially detected in nonconjugative plasmids of *E. faecium*, *E. faecalis*, and *E. hirae* isolates collected on Chinese swine farms heavily exposed to florfenicol (379). These plasmids share common regions with the backbone of Inc18 plasmid derivatives (e.g., pVEF4), widely disseminated in Norwegian poultry farms (355). The *fexB* gene is bracketed by IS*1216* and would have been acquired by widespread pRE25-like plasmids, as occurred for other antimicrobial resistance genes flanked by this IS. The *fexB* gene has also been identified in enterococci from other farm animals (bovine) and aquaculture, although the plasmids have not been characterized (241, 380). A different epidemiological landscape occurs for the *fexA* gene, which is located on plasmids (241) and chromosomes (236) of enterococcal animal isolates, often in tandem with the *optrA* gene (237, 241) or the *cfr* gene (235). The *fexA* gene is inserted in the emblematic Tn*554* of staphylococci, although in enterococci traces of this transposon might be absent as a consequence of different events of horizontal gene transfer (237).

Enterococcal plasmids carrying *optrA* have been detected in poultry, swine, and humans. Despite differences in size (30 to 80 kb) and the backbone, all share similar regions upstream and downstream of the *optrA* gene (236, 237, 241). The presence of a novel *erm*(A)-like gene that confers a high level of resistance to erythromycin is notable (237). The genetic context of *optrA* is flanked by copies of IS*1216* in the same or opposite direction, which determine the mobility.

Conjugative and nonconjugative plasmids carrying the *cfr* gene flanked by different ISs (IS*1216*, IS*Enfa4*, IS*Enfa5*, IS*256*) have been described in animal isolates of various Gram-positive species, including enterococci. The nonconjugative pEF-01 (32.2 kb) plasmid represents the first description of a *cfr*-plasmid in this bacterial genus and was identified in a fecal *E. faecalis* isolate of bovine origin collected in 2009 on a Chinese farm (232). This plasmid has three Rep proteins of the Inc18 and Rep3 plasmid families and 9-kb and 6-kb regions which exhibit high similarity with the backbone of *vanA* Inc18 plasmids (pVEF1-2-3), which have been widely isolated on poultry farms (232). Moreover, the *cfr* gene was flanked by IS*1216*, which would facilitate recombination processes, and the plasmid also contains the *fexA* gene, which provides resistance to phenicols. Conjugative plasmids carrying the *cfr* gene bracketed by IS*Enfa4* copies were isolated from *E. thailandicus* and *E. faecalis* from Chinese swine farms. These are closely related to another emblematic Inc18 plasmid, pAMb1, and contained *erm*(B) and *erm*(A) genes, conferring the MLS_B phenotype and the ω-ε-ζ toxin-antitoxin module, which may promote the persistence of plasmids by encoding a system that kills or prevents the growth of plasmid-free cells (55). This genetic context has also been detected in streptococci and staphylococci and points to independent acquisition events for the *cfr* gene. The *cfr* gene bracketed by two copies of IS*Enfa5* has been documented in *E. gallinarum* and *E. casseliflavus* of swine origin (235).

Plasmids Conferring Resistance to Bacitracin

Bacitracin has been used as an animal growth promoter in China, and recent reports documented *E. faecalis* isolates with high-level resistance to this antibiotic (MIC, ≥256 μg/ml), due to the presence of the *bcrABDR* cluster, which is composed by the *bcrABD operon* and its regulatory gene, *bcrR*. The cluster bracketed by either two, one, or no IS*Enfa1* copies is located on transferable plasmids (341) or chromosomes. The structure IS*Enfa1-bcrABDR*-IS*Enfa1* may be circulating and may have been transferred to other species by IS-mediated recombination. A multiresistant 79-kb pheromone-responsive plasmid carrying this IS*Enfa1-bcrABDR*-IS*Enfa1* platform as well as *optrA*, *fexA*, Tn*6425* (cat_{pC223}-*tetM-tetL*), Tn*5405* (*aph-sat-str*), and genes for resistance to copper and cadmium seems to be disseminated in Chinese farms (341), frequently associated with *E. faecalis* ST16. This *bcrABDR* cluster is also common in *E. cecorum,* a chicken commensal species (341).

Plasmids Conferring Resistance to Copper

Transferable resistance to copper (*tcrB*) in enterococci has been detected in piglets, calves, poultry, and humans in Europe, Asia, Australia, and North America (148, 331, 368, 381–383). Plasmids carrying *tcrB* are identified in intensively copper-supplemented livestock species, but plasmids with additional linkage with erythromycin [*erm*(B)] and/or vancomycin resistance (*vanA*) genes have only been observed in heavily copper-exposed swine (often with different copper compounds) in European countries where avoparcin was used as growth enhancer in the 1990s (148, 329, 381, 383, 384). The plasmids were detected in several enterococcal species (*E. faecium*, *E. faecalis*, *E. gallinarum*,

E. casseliflavus, *E. mundtii*, *E. hirae*), and conjugation has been experimentally demonstrated from *E. faecalis* to *E. faecium* (381). Copper fed to feedlot cattle at a growth-promotion concentration (10 × basal requirement) was associated with increased frequencies of *tcrB*-positive, macrolide-resistant *erm*(B) and, eventually, tetracycline-resistant *tet*(M) enterococci; however, copper susceptibilities were not increased in piglets in which the effect of in-feed tylosin or chlortetracycline was evaluated (382, 385). Cotransmission of *tcrB* and *erm*(B) genes between *E. hirae* from a sediment-derived livestock isolate and *E. faecalis* has been experimentally demonstrated (386). A recent analysis of WGS of *E. faecalis* from copper-supplemented Danish pigs also documented the presence of a chromosomal cluster of genes involved in susceptibility to copper, including the *tcrYAZB* operon, in three of six isolates analyzed, all containing plasmids (387). A detailed characterization of this chromosomal region was not provided, although other authors, who also identified redundancy of copper genes in chromosomes, demonstrated its cotransferability with ampicillin resistance (331).

CONCLUDING REMARKS

This review summarizes the current knowledge concerning the epidemiology and population structure of antibiotic-resistant *Enterococcus* species from food-producing, wild, and companion animals. Members of this genus are normal components of the intestinal microbiota of animals, and some species may also be etiological agents of a wide variety of infections with *E. faecalis* ST16 (considered a zoonotic pathogen) or ST82 (an etiological agent of the amyloid encephalopathy in chickens).

Enterococcus species are frequent contaminants on foods (especially poultry meat), although the risk of transmission from animals to humans through the food chain is based on indirect evidence, and thus, the bacterial load necessary to colonize the human gut remains greatly unknown. The food and animal industries seem to have contributed to the spread of certain pathogenic lineages (*E. faecalis* ST82 and ST16 lineages) and multidrug-resistant strains. Other species adapted to animals seem to act as important reservoirs of adaptive traits (*E. cecorum*). However, transmission of antimicrobial resistance by horizontal gene transfer events represents the main risk of foods contaminated by enterococci. Genes encoding resistance to vancomycin, macrolides, phenicols, and linezolid have been extensively documented in animals, frequently in response to heavy selection by antimicrobials (antibiotics and heavy metals) used in prophylaxis or as growth promoters. Although the same genes and plasmids may be present in humans and animals, particular plasmid variants are often documented on farms, suggesting certain host specificity and transmission at a local level. Deep analysis of antimicrobial-resistant genes reveals a wide diversity of alleles [e.g., *erm*(A), *optrA, cfr*] and also the frequent presence of ISs (e.g., IS*1216*) that highlight the risk of frequent and independent acquisition and selection events of antimicrobial resistance on farms. More studies are necessary to establish the risks of the emergence and transmission of antibiotic-resistant enterococci from animals to humans.

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References

1. **Boehm AB, Sassoubre LM.** 2014. Enterococci as indicators of environmental fecal contamination. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), *Enterococci: from Commensals to Leading Causes of Drug Resistant Infection*. Eye and Ear Infirmary, Boston, MA.
2. **Tyson GH, Nyirabahizi E, Crarey E, Kabera C, Lam C, Rice-Trujillo C, McDermott PF, Tate H.** 2017. Prevalence and antimicrobial resistance of enterococci isolated from retail meats in the United States, 2002–2014. *Appl Environ Microbiol* **84:**e01902–e01917.
3. **Arias CA, Murray BE.** 2012. The rise of the *Enterococcus:* beyond vancomycin resistance. *Nat Rev Microbiol* **10:**266–278.
4. **European Centre for Disease Prevention and Control (ECDC).** 2011. Annual epidemiological report on communicable diseases in Europe. *Euro Surveill* **16:**20012.
5. **Iaria C, Stassi G, Costa GB, Di Leo R, Toscano A, Cascio A.** 2005. Enterococcal meningitis caused by *Enterococcus casseliflavus*. First case report. *BMC Infect Dis* **5:**3.
6. **Canalejo E, Ballesteros R, Cabezudo J, García-Arata MI, Moreno J.** 2008. Bacteraemic spondylodiscitis caused by *Enterococcus hirae*. *Eur J Clin Microbiol Infect Dis* **27:**613–615.

7. **Mastroianni A.** 2009. *Enterococcus raffinosus* endocarditis. First case and literature review. *Infez Med* **17:** 14–20.
8. **Antonello VS, Zenkner FM, França J, Santos BR.** 2010. *Enterococcus gallinarum* meningitis in an immunocompetent host: a case report. *Rev Inst Med Trop São Paulo* **52:**111–112.
9. **Escribano JA, Solivera J, Vidal E, Rivin E, Lozano J.** 2013. Otogenic cerebellar abscess by *Enterococcus avium*, a very rare infectious agent. *J Neurol Surg A Cent Eur Neurosurg* **74**(Suppl 1)**:**e155–e158.
10. **Kenzaka T, Takamura N, Kumabe A, Takeda K.** 2013. A case of subacute infective endocarditis and blood access infection caused by *Enterococcus durans*. *BMC Infect Dis* **13:**594.
11. **Lebreton F, Willems RJL, Gilmore MS.** 2014. *Enterococcus* diversity, origins in nature, and gut colonization. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), *Enterococci: from Commensals to Leading Causes of Drug Resistant Infection*. Eye and Ear Infirmary, Boston, MA.
12. **Aarestrup FM, Butaye P, Witte W.** 2002. Nonhuman reservoirs of enterococci, p 1281–1289. *In* Gilmore MS, Clewell DB, Courvalin P, Dunny GM, Murray BE, Rice LB (ed), *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. ASM Press, Washington, DC.
13. **Aarestrup FM (ed).** 2006. *Antimicrobial Resistance in Bacteria of Animal Origin*. ASM Press, Washington, DC.
14. **Stalker MJ, Brash ML, Weisz A, Ouckama RM, Slavic D.** 2010. Arthritis and osteomyelitis associated with *Enterococcus cecorum* infection in broiler and broiler breeder chickens in Ontario, Canada. *J Vet Diagn Invest* **22:**643–645.
15. **Dolka B, Chrobak-Chmiel D, Makrai L, Szeleszczuk P.** 2016. Phenotypic and genotypic characterization of *Enterococcus cecorum* strains associated with infections in poultry. *BMC Vet Res* **12:**129.
16. **Kak V, Chow JM.** 2002. Acquired antibiotic resistances in enterococci, p 355–383. *In* Gilmore MS, Clewell B, Courvalin P, Dunny GM, Murray BM, Rice LB (ed), *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. ASM Press, Washington, DC.
17. **Hollenbeck BL, Rice LB.** 2012. Intrinsic and acquired resistance mechanisms in *enterococcus*. *Virulence* **3:** 421–433.
18. **Miller WR, Munita JM, Arias CA.** 2014. Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti Infect Ther* **12:**1221–1236.
19. **Courvalin P.** 2006. Vancomycin resistance in Gram-positive cocci. *Clin Infect Dis* **42**(Suppl 1)**:**S25–S34.
20. **Nilsson O.** 2012. Vancomycin resistant enterococci in farm animals: occurrence and importance. *Infect Ecol Epidemiol* **2:**16959.
21. **Cattoir V, Leclercq R.** 2013. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? *J Antimicrob Chemother* **68:**731–742.
22. **Ahmed MO, Baptiste KE.** 2017. Vancomycin-resistant enterococci: a review of antimicrobial resistant mechanisms and perspectives of human and animal health. *Microb Drug Resist*. Epub ahead of print.
23. **Chow JW.** 2000. Aminoglycoside resistance in enterococci. *Clin Infect Dis* **31:**586–589.
24. **Hammerum AM, Lester CH, Heuer OE.** 2010. Antimicrobial-resistant enterococci in animals and meat: a human health hazard? *Foodborne Pathog Dis* **7:** 1137–1146.
25. **Bortolaia V, Espinosa-Gongora C, Guardabassi L.** 2016. Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. *Clin Microbiol Infect* **22:**130–140.
26. **Hammerum AM.** 2012. Enterococci of animal origin and their significance for public health. *Clin Microbiol Infect* **18:**619–625.
27. **Bortolaia V, Guardabassi L.** 2015. Zoonotic transmission of antimicrobial resistant enterococci: a threat to public health or an overemphasized risk? p 407–431. *In* Sing A (ed), *Zoonoses: Infections Affecting Humans and Animals*. Focus on Public Health Aspects. Springer, New York, NY.
28. **Stiles ME, Holzapfel WH.** 1997. Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol* **36:**1–29.
29. **Lund B, Adamsson I, Edlund C.** 2002. Gastrointestinal transit survival of an *Enterococcus faecium* probiotic strain administered with or without vancomycin. *Int J Food Microbiol* **77:**109–115.
30. **Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L.** 2006. The role and application of enterococci in food and health. *Int J Food Microbiol* **106:**1–24.
31. **Zhong Z, Zhang W, Song Y, Liu W, Xu H, Xi X, Menghe B, Zhang H, Sun Z.** 2017. Comparative genomic analysis of the genus *Enterococcus*. *Microbiol Res* **196:**95–105.
32. **Schleifer KH, Kilpper-Balz R.** 1984. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Int J Syst Bacteriol* **34:**31–34.
33. **Parte AC.** 2014. LPSN: list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* **42**(D1)**:** D613–D616.
34. **De Graef EM, Devriese LA, Vancanneyt M, Baele M, Collins MD, Lefebvre K, Swings J, Haesebrouck F.** 2003. Description of *Enterococcus canis* sp. nov. from dogs and reclassification of *Enterococcus porcinus* Teixeira *et al*. 2001 as a junior synonym of *Enterococcus villorum* Vancanneyt *et al*. 2001. *Int J Syst Evol Microbiol* **53:**1069–1074.
35. **Law-Brown J, Meyers PR.** 2003. *Enterococcus phoeniculicola* sp. nov., a novel member of the enterococci isolated from the uropygial gland of the red-billed woodhoopoe, *Phoeniculus purpureus*. *Int J Syst Evol Microbiol* **53:**683–685.
36. **Fortina MG, Ricci G, Mora D, Manachini PL.** 2004. Molecular analysis of artisanal Italian cheeses reveals *Enterococcus italicus* sp. nov. *Int J Syst Evol Microbiol* **54:**1717–1721.
37. **Švec P, Vancanneyt M, Koort J, Naser SM, Hoste B, Vihavainen E, Vandamme P, Swings J, Björkroth J.**

2005. *Enterococcus devriesei* sp. nov., associated with animal sources. *Int J Syst Evol Microbiol* **55:**2479–2484.

38. **Tanasupawat S, Sukontasing S, Lee JS.** 2008. *Enterococcus thailandicus* sp. nov., isolated from fermented sausage ('mum') in Thailand. *Int J Syst Evol Microbiol* **58:**1630–1634.
39. **Švec P, Vandamme P, Bryndová H, Holochová P, Kosina M, Maslanová I, Sedlácek I.** 2012. *Enterococcus plantarum* sp. nov., isolated from plants. *Int J Syst Evol Microbiol* **62:**1499–1505.
40. **Niemi RM, Ollinkangas T, Paulin L, Švec P, Vandamme P, Karkman A, Kosina M, Lindström K.** 2012. *Enterococcus rivorum* sp. nov., from water of pristine brooks. *Int J Syst Evol Microbiol* **62:**2169–2173.
41. **Sistek V, Maheux AF, Boissinot M, Bernard KA, Cantin P, Cleenwerck I, De Vos P, Bergeron MG.** 2012. *Enterococcus ureasiticus* sp. nov. and *Enterococcus quebecensis* sp. nov., isolated from water. *Int J Syst Evol Microbiol* **62:**1314–1320.
42. **Sedláček I, Holochová P, Mašlaňová I, Kosina M, Spröer C, Bryndová H, Vandamme P, Rudolf I, Hubálek Z, Švec P.** 2013. *Enterococcus ureilyticus* sp. nov. and *Enterococcus rotai* sp. nov., two urease-producing enterococci from the environment. *Int J Syst Evol Microbiol* **63:**502–510.
43. **Mundt JO.** 1963. Occurrence of enterococci in animals in a wild environment. *Appl Microbiol* **11:**136–140.
44. **Kühn I, Iversen A, Burman LG, Olsson-Liljequist B, Franklin A, Finn M, Aarestrup F, Seyfarth AM, Blanch AR, Vilanova X, Taylor H, Caplin J, Moreno MA, Dominguez L, Herrero IA, Möllby R.** 2003. Comparison of enterococcal populations in animals, humans, and the environment: a European study. *Int J Food Microbiol* **88:**133–145.
45. **Devriese LA, Ceyssens K, Haesebrouck F.** 1991a. Characteristics of *Enterococcus cecorum* strains from the intestines of different animal species. *Lett Appl Microbiol* **12:**137–139.
46. **Devriese LA, Laurier L, De Herdt P, Haesebrouck F.** 1992. Enterococcal and streptococcal species isolated from faeces of calves, young cattle and dairy cows. *J Appl Bacteriol* **72:**29–31.
47. **Scupham AJ, Patton TG, Bent E, Bayles DO.** 2008. Comparison of the cecal microbiota of domestic and wild turkeys. *Microb Ecol* **56:**322–331.
48. **Gong J, Forster RJ, Yu H, Chambers JR, Wheatcroft R, Sabour PM, Chen S.** 2002. Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. *FEMS Microbiol Ecol* **41:**171–179.
49. **Devriese LA, Hommez J, Wijfels R, Haesebrouck F.** 1991. Composition of the enterococcal and streptococcal intestinal flora of poultry. *J Appl Bacteriol* **71:**46–50.
50. **Kojima A, Morioka A, Kijima M, Ishihara K, Asai T, Fujisawa T, Tamura Y, Takahashi T.** 2010. Classification and antimicrobial susceptibilities of *Enterococcus* species isolated from apparently healthy food-producing animals in Japan. *Zoonoses Public Health* **57:**137–141.
51. **Iweriebor BC, Obi LC, Okoh AI.** 2016. Macrolide, glycopeptide resistance and virulence genes in *Enterococcus* species isolates from dairy cattle. *J Med Microbiol* **65:**641–648.
52. **Beukers AG, Zaheer R, Cook SR, Stanford K, Chaves AV, Ward MP, McAllister TA.** 2015. Effect of in-feed administration and withdrawal of tylosin phosphate on antibiotic resistance in enterococci isolated from feedlot steers. *Front Microbiol* **6:**483.
53. **Hwang IY, Ku HO, Lim SK, Park CK, Jung GS, Jung SC, Nam HM.** 2009. Species distribution and resistance patterns to growth-promoting antimicrobials of enterococci isolated from pigs and chickens in Korea. *J Vet Diagn Invest* **21:**858–862.
54. **Vancanneyt M, Snauwaert C, Cleenwerck I, Baele M, Descheemaeker P, Goossens H, Pot B, Vandamme P, Swings J, Haesebrouck F, Devriese LA.** 2001. *Enterococcus villorum* sp. nov., an enteroadherent bacterium associated with diarrhoea in piglets. *Int J Syst Evol Microbiol* **51:**393–400.
55. **Liu Y, Wang Y, Schwarz S, Li Y, Shen Z, Zhang Q, Wu C, Shen J.** 2013. Transferable multiresistance plasmids carrying *cfr* in *Enterococcus* spp. from swine and farm environment. *Antimicrob Agents Chemother* **57:**42–48.
56. **Devriese LA, Cruz Colque JI, De Herdt P, Haesebrouck F.** 1992. Identification and composition of the tonsillar and anal enterococcal and streptococcal flora of dogs and cats. *J Appl Bacteriol* **73:**421–425.
57. **Rodrigues J, Poeta P, Martins A, Costa D.** 2002. The importance of pets as reservoirs of resistant *Enterococcus* strains, with special reference to vancomycin. *J Vet Med B Infect Dis Vet Public Health* **49:**278–280.
58. **Jackson CR, Fedorka-Cray PJ, Davis JA, Barrett JB, Frye JG.** 2009. Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. *J Appl Microbiol* **107:**1269–1278.
59. **Ben Said L, Dziri R, Sassi N, Lozano C, Ben Slama K, Ouzari I, Torres C, Klibi N.** 2017. Species distribution, antibiotic resistance and virulence traits in canine and feline enterococci in Tunisia. *Acta Vet Hung* **65:**173–184.
60. **Naser SM, Vancanneyt M, De Graef E, Devriese LA, Snauwaert C, Lefebvre K, Hoste B, Svec P, Decostere A, Haesebrouck F, Swings J.** 2005. *Enterococcus canintestini* sp. nov., from faecal samples of healthy dogs. *Int J Syst Evol Microbiol* **55:**2177–2182.
61. **Baele M, Devriese LA, Butaye P, Haesebrouck F.** 2002. Composition of enterococcal and streptococcal flora from pigeon intestines. *J Appl Microbiol* **92:**348–351.
62. **Splichalova P, Svec P, Ghosh A, Zurek L, Oravcova V, Radimersky T, Bohus M, Literak I.** 2015. Prevalence, diversity and characterization of enterococci from three coraciiform birds. *Antonie van Leeuwenhoek* **107:**1281–1289.
63. **Radhouani H, Poeta P, Gonçalves A, Pacheco R, Sargo R, Igrejas G.** 2012. Wild birds as biological indicators of environmental pollution: antimicrobial resistance patterns of *Escherichia coli* and enterococci isolated

from common buzzards (*Buteo buteo*). *J Med Microbiol* **61:**837–843.

64. **Silva N, Igrejas G, Rodrigues P, Rodrigues T, Gonçalves A, Felgar AC, Pacheco R, Gonçalves D, Cunha R, Poeta P.** 2011. Molecular characterization of vancomycin-resistant enterococci and extended-spectrum β-lactamase-containing *Escherichia coli* isolates in wild birds from the Azores Archipelago. *Avian Pathol* **40:**473–479.
65. **Marinho C, Silva N, Pombo S, Santos T, Monteiro R, Gonçalves A, Micael J, Rodrigues P, Costa AC, Igrejas G, Poeta P.** 2013. Echinoderms from Azores islands: an unexpected source of antibiotic resistant *Enterococcus* spp. and *Escherichia coli* isolates. *Mar Pollut Bull* **69:** 122–127.
66. **Medeiros AW, Blaese Amorim D, Tavares M, de Moura TM, Franco AC, d'Azevedo PA, Frazzon J, Frazzon AP.** 2017. *Enterococcus* species diversity in fecal samples of wild marine species as determined by real-time PCR. *Can J Microbiol* **63:**129–136.
67. **Farnleitner AH, Ryzinska-Paier G, Reischer GH, Burtscher MM, Knetsch S, Kirschner AK, Dirnböck T, Kuschnig G, Mach RL, Sommer R.** 2010. *Escherichia coli* and enterococci are sensitive and reliable indicators for human, livestock and wildlife faecal pollution in alpine mountainous water resources. *J Appl Microbiol* **109:**1599–1608.
68. **Radhouani H, Igrejas G, Carvalho C, Pinto L, Gonçalves A, Lopez M, Sargo R, Cardoso L, Martinho A, Rego V, Rodrigues R, Torres C, Poeta P.** 2011. Clonal lineages, antibiotic resistance and virulence factors in vancomycin-resistant enterococci isolated from fecal samples of red foxes (*Vulpes vulpes*). *J Wildl Dis* **47:** 769–773.
69. **Lozano C, González-Barrio D, García JT, Ceballos S, Olea PP, Ruiz-Fons F, Torres C.** 2015. Detection of vancomycin-resistant *Enterococcus faecalis* ST6-*vanB2* and *E. faecium* ST915-*vanA* in faecal samples of wild *Rattus rattus* in Spain. *Vet Microbiol* **177:**168–174.
70. **Fontana R, Aldegheri M, Ligozzi M, Lopez H, Sucari A, Satta G.** 1994. Overproduction of a low-affinity penicillin-binding protein and high-level ampicillin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **38:** 1980–1983.
71. **Ligozzi M, Pittaluga F, Fontana R.** 1996. Modification of penicillin-binding protein 5 associated with high-level ampicillin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **40:**354–357.
72. **Montealegre MC, Roh JH, Rae M, Davlieva MG, Singh KV, Shamoo Y, Murray BE.** 2016. Differential penicillin-binding protein 5 (PBP5) levels in the *Enterococcus faecium* clades with different levels of ampicillin resistance. *Antimicrob Agents Chemother* **61:**e02034-16.
73. **Jureen R, Top J, Mohn SC, Harthug S, Langeland N, Willems RJ.** 2003. Molecular characterization of ampicillin-resistant *Enterococcus faecium* isolates from hospitalized patients in Norway. *J Clin Microbiol* **41:** 2330–2336.
74. **Poeta P, Costa D, Igrejas G, Sáenz Y, Zarazaga M, Rodrigues J, Torres C.** 2007. Polymorphisms of the *pbp5* gene and correlation with ampicillin resistance in *Enterococcus faecium* isolates of animal origin. *J Med Microbiol* **56:**236–240.
75. **Klibi N, Sáenz Y, Zarazaga M, Ben Slama K, Masmoudi A, Ruiz-Larrea F, Boudabous A, Torres C.** 2008. Polymorphism in *pbp5* gene detected in clinical *Enterococcus faecium* strains with different ampicillin MICs from a Tunisian hospital. *J Chemother* **20:**436–440.
76. **Kristich CJ, Rice LB, Arias CA.** 2014. Enterococcal infection treatment and antibiotic resistance. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), *Enterococci: from Commensals to Leading Causes of Drug Resistant Infection*. Eye and Ear Infirmary, Boston: MA.
77. **Galloway-Peña JR, Rice LB, Murray BE.** 2011. Analysis of PBP5 of early U.S. isolates of *Enterococcus faecium*: sequence variation alone does not explain increasing ampicillin resistance over time. *Antimicrob Agents Chemother* **55:**3272–3277.
78. **Pietta E, Montealegre MC, Roh JH, Cocconcelli PS, Murray BE.** 2014. *Enterococcus faecium* PBP5-S/R, the missing link between PBP5-S and PBP5-R. *Antimicrob Agents Chemother* **58:**6978–6981.
79. **Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, Zeng Q, Wortman J, Birren B, Willems RJ, Earl AM, Gilmore MS.** 2013. Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. *MBio* **4:**e00534-13.
80. **Ono S, Muratani T, Matsumoto T.** 2005. Mechanisms of resistance to imipenem and ampicillin in *Enterococcus faecalis*. *Antimicrob Agents Chemother* **49:**2954–2958.
81. **Murray BE, Lopardo HA, Rubeglio EA, Frosolono M, Singh KV.** 1992. Intrahospital spread of a single gentamicin-resistant, beta-lactamase-producing strain of *Enterococcus faecalis* in Argentina. *Antimicrob Agents Chemother* **36:**230–232.
82. **Coudron PE, Markowitz SM, Wong ES.** 1992. Isolation of a beta-lactamase-producing, aminoglycoside-resistant strain of *Enterococcus faecium*. *Antimicrob Agents Chemother* **36:**1125–1126.
83. **Sarti M, Campanile F, Sabia C, Santagati M, Gargiulo R, Stefani S.** 2012. Polyclonal diffusion of beta-lactamase-producing *Enterococcus faecium*. *J Clin Microbiol* **50:**169–172.
84. **Rice LB, Carias LL, Rudin S, Lakticová V, Wood A, Hutton-Thomas R.** 2005. *Enterococcus faecium* low-affinity *pbp5* is a transferable determinant. *Antimicrob Agents Chemother* **49:**5007–5012.
85. **Novais C, Freitas AR, Silveira E, Antunes P, Silva R, Coque TM, Peixe L.** 2013. Spread of multidrug-resistant *Enterococcus* to animals and humans: an underestimated role for the pig farm environment. *J Antimicrob Chemother* **68:**2746–2754.
86. **Novais C, Tedim AP, Lanza VF, Freitas AR, Silveira E, Escada R, Roberts AP, Al-Haroni M, Baquero F, Peixe L, Coque TM.** 2016. Co-diversification of *Enterococcus faecium* core genomes and PBP5: evidences of *pbp5* horizontal transfer. *Front Microbiol* **7:**1581.
87. **Barlow RS, McMillan KE, Duffy LL, Fegan N, Jordan D, Mellor GE.** 2017. Antimicrobial resistance status

of *Enterococcus* from Australian cattle populations at slaughter. *PLoS One* **12:**e0177728.

88. **Poeta P, Costa D, Rodrigues J, Torres C.** 2006. Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. *Int J Antimicrob Agents* **27:**131–137.
89. **Damborg P, Top J, Hendrickx AP, Dawson S, Willems RJ, Guardabassi L.** 2009. Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. *Appl Environ Microbiol* **75:**2360–2365.
90. **Gonçalves A, Igrejas G, Radhouani H, Correia S, Pacheco R, Santos T, Monteiro R, Guerra A, Petrucci-Fonseca F, Brito F, Torres C, Poeta P.** 2013. Antimicrobial resistance in faecal enterococci and *Escherichia coli* isolates recovered from Iberian wolf. *Lett Appl Microbiol* **56:**268–274.
91. **Barros J, Igrejas G, Andrade M, Radhouani H, López M, Torres C, Poeta P.** 2011. Gilthead seabream (*Sparus aurata*) carrying antibiotic resistant enterococci. A potential bioindicator of marine contamination? *Mar Pollut Bull* **62:**1245–1248.
92. **Gonçalves A, Igrejas G, Radhouani H, Santos T, Monteiro R, Pacheco R, Alcaide E, Zorrilla I, Serra R, Torres C, Poeta P.** 2013. Detection of antibiotic resistant enterococci and *Escherichia coli* in free range Iberian Lynx (*Lynx pardinus*). *Sci Total Environ* **456-457:** 115–119.
93. **DANMAP.** 2014. *Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*. www.danmap.org.
94. **Swedres-Svarm.** 2015. *Consumption of antibiotics and occurrence of antibiotic resistance in Sweden*. Solna/Uppsala, Sweden.
95. **NethMap-MARAN.** 2015. *Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2014*. Nigmegen. SWAB. https://www.swab.nl/nethmap
96. **Uttley AH, George RC, Naidoo J, Woodford N, Johnson AP, Collins CH, Morrison D, Gilfillan AJ, Fitch LE, Heptonstall J.** 1989. High-level vancomycin-resistant enterococci causing hospital infections. *Epidemiol Infect* **103:**173–181.
97. **Leclercq R, Derlot E, Duval J, Courvalin P.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* **319:** 157–161.
98. **European Centre for Disease Prevention and Control.** 2017. *Antimicrobial resistance surveillance in Europe 2016. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)*. ECDC, Stockholm, Sweden.
99. **Lebreton F, Depardieu F, Bourdon N, Fines-Guyon M, Berger P, Camiade S, Leclercq R, Courvalin P, Cattoir V.** 2011. D-Ala-D-Ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **55:**4606–4612.
100. **Boyd DA, Du T, Hizon R, Kaplen B, Murphy T, Tyler S, Brown S, Jamieson F, Weiss K, Mulvey MR.** 2006. VanG-type vancomycin-resistant *Enterococcus faecalis* strains isolated in Canada. *Antimicrob Agents Chemother* **50:**2217–2221.
101. **Boyd DA, Willey BM, Fawcett D, Gillani N, Mulvey MR.** 2008. Molecular characterization of *Enterococcus faecalis* N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, vanL. *Antimicrob Agents Chemother* **52:**2667–2672.
102. **Xu X, Lin D, Yan G, Ye X, Wu S, Guo Y, Zhu D, Hu F, Zhang Y, Wang F, Jacoby GA, Wang M.** 2010. *vanM*, a new glycopeptide resistance gene cluster found in *Enterococcus faecium*. *Antimicrob Agents Chemother* **54:**4643–4647.
103. **Depardieu F, Foucault ML, Bell J, Dubouix A, Guibert M, Lavigne JP, Levast M, Courvalin P.** 2009. New combinations of mutations in VanD-type vancomycin-resistant *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus avium* strains. *Antimicrob Agents Chemother* **53:**1952–1963.
104. **López M, Rezusta A, Seral C, Aspiroz C, Marne C, Aldea MJ, Ferrer I, Revillo MJ, Castillo FJ, Torres C.** 2012. Detection and characterization of a ST6 clone of *vanB2-Enterococcus faecalis* from three different hospitals in Spain. *Eur J Clin Microbiol Infect Dis* **31:** 257–260.
105. **Patel R, Piper K, Cockerill FR III, Steckelberg JM, Yousten AA.** 2000. The biopesticide *Paenibacillus popilliae* has a vancomycin resistance gene cluster homologous to the enterococcal VanA vancomycin resistance gene cluster. *Antimicrob Agents Chemother* **44:** 705–709.
106. **Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A.** 2010. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. *Clin Microbiol Infect* **16:**541–554.
107. **Arthur M, Quintiliani R Jr.** 2001. Regulation of VanA- and VanB-type glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* **45:**375–381.
108. **Arthur M, Courvalin P.** 1993. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* **37:**1563–1571.
109. **López M, Sáenz Y, Alvarez-Martínez MJ, Marco F, Robredo B, Rojo-Bezares B, Ruiz-Larrea F, Zarazaga M, Torres C.** 2010. Tn*1546* structures and multilocus sequence typing of *vanA*-containing enterococci of animal, human and food origin. *J Antimicrob Chemother* **65:**1570–1575.
110. **Werner G, Strommenger B, Witte W.** 2008. Acquired vancomycin resistance in clinically relevant pathogens. *Future Microbiol* **3:**547–562.
111. **Patel R.** 2003. Clinical impact of vancomycin-resistant enterococci. *J Antimicrob Chemother* **51**(Suppl 3)**:** iii13–iii21.
112. **Bager F, Madsen M, Christensen J, Aarestrup FM.** 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* **31:**95–112.

113. **Aarestrup FM, Kruse H, Tast E, Hammerum AM, Jensen LB.** 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. *Microb Drug Resist* **6:**63–70.

114. **Robredo B, Singh KV, Baquero F, Murray BE, Torres C.** 1999. From *vanA Enterococcus hirae* to *vanA Enterococcus faecium*: a study of feed supplementation with avoparcin and tylosin in young chickens. *Antimicrob Agents Chemother* **43:**1137–1143.

115. **Klare I, Badstübner D, Konstabel C, Böhme G, Claus H, Witte W.** 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microb Drug Resist* **5:**45–52.

116. **Borgen K, Sørum M, Wasteson Y, Kruse H.** 2001. VanA-type vancomycin-resistant enterococci (VRE) remain prevalent in poultry carcasses 3 years after avoparcin was banned. *Int J Food Microbiol* **64:**89–94.

117. **Bortolaia V, Mander M, Jensen LB, Olsen JE, Guardabassi L.** 2015. Persistence of vancomycin resistance in multiple clones of *Enterococcus faecium* isolated from Danish broilers 15 years after the ban of avoparcin. *Antimicrob Agents Chemother* **59:**2926–2929.

118. **Lauderdale TL, Shiau YR, Wang HY, Lai JF, Huang IW, Chen PC, Chen HY, Lai SS, Liu YF, Ho M.** 2007. Effect of banning vancomycin analogue avoparcin on vancomycin-resistant enterococci in chicken farms in Taiwan. *Environ Microbiol* **9:**819–823.

119. **van Belkun A, van den Braak N, Thomassen R, Verbrugh H, Endtz H.** 1996. Vancomycin-resistant enterococci in cats and dogs. *Lancet* **348:**1038–1039.

120. **López M, Tenorio C, Torres C.** 2013. Study of vancomycin resistance in faecal enterococci from healthy humans and dogs in Spain a decade after the avoparcin ban in Europe. *Zoonoses Public Health* **60:**160–167.

121. **Donabedian SM, Perri MB, Abdujamilova N, Gordoncillo MJ, Naqvi A, Reyes KC, Zervos MJ, Bartlett P.** 2010. Characterization of vancomycin-resistant *Enterococcus faecium* isolated from swine in three Michigan counties. *J Clin Microbiol* **48:**4156–4160.

122. **Gordoncillo MJ, Donabedian S, Bartlett PC, Perri M, Zervos M, Kirkwood R, Febvay C.** 2013. Isolation and molecular characterization of vancomycin-resistant *Enterococcus faecium* from swine in Michigan, USA. *Zoonoses Public Health* **60:**319–326.

123. **Centers for Disease Control and Prevention (CDC).** 1993. Nosocomial enterococci resistant to vancomycin: United States, 1989–1993. *MMWR Morb Mortal Wkly Rep* **42:**597–599.

124. **Goossens H.** 1998. Spread of vancomycin-resistant enterococci: differences between the United States and Europe. *Infect Control Hosp Epidemiol* **19:**546–551.

125. **Johnsen PJ, Østerhus JI, Sletvold H, Sørum M, Kruse H, Nielsen K, Simonsen GS, Sundsfjord A.** 2005. Persistence of animal and human glycopeptide-resistant enterococci on two Norwegian poultry farms formerly exposed to avoparcin is associated with a widespread plasmid-mediated *vanA* element within a polyclonal *enterococcus faecium* population. *Appl Environ Microbiol* **71:**159–168.

126. **van den Bogaard AE, Willems R, London N, Top J, Stobberingh EE.** 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *J Antimicrob Chemother* **49:**497–505.

127. **Sørum M, Johnsen PJ, Aasnes B, Rosvoll T, Kruse H, Sundsfjord A, Simonsen GS.** 2006. Prevalence, persistence, and molecular characterization of glycopeptide-resistant enterococci in Norwegian poultry and poultry farmers 3 to 8 years after the ban on avoparcin. *Appl Environ Microbiol* **72:**516–521.

128. **Robredo B, Singh KV, Baquero F, Murray BE, Torres C.** 2000. Vancomycin-resistant enterococci isolated from animals and food. *Int J Food Microbiol* **54:**197–204.

129. **Nilsson O, Greko C, Top J, Franklin A, Bengtsson B.** 2009. Spread without known selective pressure of a vancomycin-resistant clone of *Enterococcus faecium* among broilers. *J Antimicrob Chemother* **63:**868–872.

130. **Ghidán A, Kaszanyitzky EJ, Dobay O, Nagy K, Amyes SG, Rozgonyi F.** 2008a. Distribution and genetic relatedness of vancomycin-resistant enterococci (VRE) isolated from healthy slaughtered chickens in Hungary from 2001 to 2004. *Acta Vet Hung* **56:**13–25.

131. **Manson JM, Smith JM, Cook GM.** 2004. Persistence of vancomycin-resistant enterococci in New Zealand broilers after discontinuation of avoparcin use. *Appl Environ Microbiol* **70:**5764–5768.

132. **Kolar M, Pantucek R, Bardon J, Cekanova L, Kesselova M, Sauer P, Vagnerova I, Koukalová D.** 2005. Occurrence of vancomycin-resistant enterococci in humans and animals in the Czech Republic between 2002 and 2004. *J Med Microbiol* **54:**965–967.

133. **Jung WK, Lim JY, Kwon NH, Kim JM, Hong SK, Koo HC, Kim SH, Park YH.** 2007. Vancomycin-resistant enterococci from animal sources in Korea. *Int J Food Microbiol* **113:**102–107.

134. **Tremblay CL, Letellier A, Quessy S, Boulianne M, Daignault D, Archambault M.** 2011. Multiple-antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* from cecal contents in broiler chicken and turkey flocks slaughtered in Canada and plasmid colocalization of *tetO* and *ermB* genes. *J Food Prot* **74:** 1639–1648.

135. **Boulianne M, Arsenault J, Daignault D, Archambault M, Letellier A, Dutil L.** 2016. Drug use and antimicrobial resistance among *Escherichia coli* and *Enterococcus* spp. isolates from chicken and turkey flocks slaughtered in Quebec, Canada. *Can J Vet Res* **80:**49–59.

136. **Poeta P, Costa D, Rodrigues J, Torres C.** 2005. Study of faecal colonization by *vanA*-containing *Enterococcus* strains in healthy humans, pets, poultry and wild animals in Portugal. *J Antimicrob Chemother* **55:**278–280.

137. **Ghidán A, Dobay O, Kaszanyitzky EJ, Samu P, Amyes SG, Nagy K, Rozgonyi F.** 2008. Vancomycin resistant enterococci (VRE) still persist in slaughtered poultry in hungary 8 years after the ban on avoparcin. *Acta Microbiol Immunol Hung* **55:**409–417.

138. **Tzavaras I, Siarkou VI, Zdragas A, Kotzamanidis C, Vafeas G, Bourtzi-Hatzopoulou E, Pournaras S, Sofianou D.** 2012. Diversity of *vanA*-type vancomycin-resistant *Enterococcus faecium* isolated from broilers, poultry slaughterers and hospitalized humans in Greece. *J Antimicrob Chemother* **67:**1811–1818.

139. **Sting R, Richter A, Popp C, Hafez HM.** 2013. Occurrence of vancomycin-resistant enterococci in turkey flocks. *Poult Sci* **92:**346–351.

140. **Obeng AS, Rickard H, Ndi O, Sexton M, Barton M.** 2013. Comparison of antimicrobial resistance patterns in enterococci from intensive and free range chickens in Australia. *Avian Pathol* **42:**45–54.

141. **Maasjost J, Mühldorfer K, Hafez HM, Hafez HM, Cortez de Jäckel S.** 2015. Antimicrobial susceptibility patterns of *Enterococcus faecalis* an *Enterococcus faecium* isolated from poultry flocks in Germany. *Avian Dis* **59:**143–148.

142. **Stępień-Pyśniak D, Marek A, Banach T, Adaszek Ł, Pyzik E, Wilczyński J, Winiarczyk S.** 2016. Prevalence and antibiotic resistance of *Enterococcus* strains isolated from poultry. *Acta Vet Hung* **64:**148–163.

143. **Bertelloni F, Salvadori C, Moni A, Cerri D, Mani P, Ebani VV.** 2015. Antimicrobial resistance in *Enterococcus* spp. isolated from laying hens of backyard poultry floks. *Ann Agric Environ Med* **22:**665–669.

144. **Nilsson O, Greko C, Bengtsson B.** 2009. Environmental contamination by vancomycin resistant enterococci (VRE) in Swedish broiler production. *Acta Vet Scand* **51:**49.

145. **Torres C, Tenorio C, Portillo A, García M, Martínez C, Del Campo R, Ruiz-Larrea F, Zarazaga M.** 2003. Intestinal colonization by *vanA*- or *vanB2*-containing enterococcal isolates of healthy animals in Spain. *Microb Drug Resist* **9**(Suppl 1)**:**S47–S52.

146. **Herrero IA, Teshager T, Garde J, Moreno MA, Domínguez L.** 2000. Prevalence of vancomycin-resistant *Enterococcus faecium* (VREF) in pig faeces from slaughterhouses in Spain. *Prev Vet Med* **47:**255–262.

147. **Iweriebor BC, Obi LC, Okoh AI.** 2015. Virulence and antimicrobial resistance factors of *Enterococcus* spp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa. *BMC Microbiol* **15:**136.

148. **Fard RM, Heuzenroeder MW, Barton MD.** 2011. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. *Vet Microbiol* **148:**276–282.

149. **Bustamante W, Alpízar A, Hernández S, Pacheco A, Vargas N, Herrera ML, Vargas A, Caballero M, García F.** 2003. Predominance of *vanA* genotype among vancomycin-resistant *Enterococcus* isolates from poultry and swine in Costa Rica. *Appl Environ Microbiol* **69:**7414–7419.

150. **Kühn I, Iversen A, Finn M, Greko C, Burman LG, Blanch AR, Vilanova X, Manero A, Taylor H, Caplin J, Domínguez L, Herrero IA, Moreno MA, Möllby R.** 2005. Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. *Appl Environ Microbiol* **71:**5383–5390.

151. **García-Migura L, Pleydell E, Barnes S, Davies RH, Liebana E.** 2005. Characterization of vancomycin-resistant *Enterococcusfaecium* isolates from broiler poultry and pig farms in England and Wales. *J Clin Microbiol* **43:**3283–3289.

152. **Kempf I, Hellard G, Perrin-Guyomard A, Gicquel-Bruneau M, Sanders P, Leclercq R.** 2008. Prevalence of high-level vancomycin-resistant enterococci in French broilers and pigs. *Int J Antimicrob Agents* **32:**463–464.

153. **Liu Y, Liu K, Lai J, Wu C, Shen J, Wang Y.** 2013. Prevalence and antimicrobial resistance of *Enterococcus* species of food animal origin from Beijing and Shandong Province, China. *J Appl Microbiol* **114:**555–563.

154. **Ngbede EO, Raji MA, Kwanashie CN, Kwaga JKP.** 2017. Antimicrobial resistance and virulence profile of enterococci isolated from poultry and cattle sources in Nigeria. *Trop Anim Health Prod* **49:**451–458.

155. **Bekele B, Ashenafi M.** 2010. Distribution of drug resistance among enterococci and *Salmonella* from poultry and cattle in Ethiopia. *Trop Anim Health Prod* **42:** 857–864.

156. **Ho PL, Lai E, Chan PY, Lo WU, Chow KH.** 2013. Rare occurrence of vancomycin-resistant *Enterococcus faecium* among livestock animals in China. *J Antimicrob Chemother* **68:**2948–2949.

157. **Eisner A, Feierl G, Gorkiewicz G, Dieber F, Kessler HH, Marth E, Köfer J.** 2005. High prevalence of VanA-type vancomycin-resistant enterococci in Austrian poultry. *Appl Environ Microbiol* **71:**6407–6409.

158. **Haenni M, Saras E, Châtre P, Meunier D, Martin S, Lepage G, Ménard MF, Lebreton P, Rambaud T, Madec JY.** 2009. *vanA* in *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus casseliflavus* detected in French cattle. *Foodborne Pathog Dis* **6:** 1107–1111.

159. **de Niederhäusern S, Sabia C, Messi P, Guerrieri E, Manicardi G, Bondi M.** 2007. VanA-type vancomycin-resistant enterococci in equine and swine rectal swabs and in human clinical samples. *Curr Microbiol* **55:**240–246.

160. **Ramos S, Igrejas G, Rodrigues J, Capelo-Martínez JL, Poeta P.** 2012. Genetic characterisation of antibiotic resistance and virulence factors in vanA-containing enterococci from cattle, sheep and pigs subsequent to the discontinuation of the use of avoparcin. *Vet J* **193:** 301–303.

161. **Hershberger E, Oprea SF, Donabedian SM, Perri M, Bozigar P, Bartlett P, Zervos MJ.** 2005. Epidemiology of antimicrobial resistance in enterococci of animal origin. *J Antimicrob Chemother* **55:**127–130.

162. **Song JY, Hwang IS, Eom JS, Cheong HJ, Bae WK, Park YH, Kim WJ.** 2005. Prevalence and molecular epidemiology of vancomycin-resistant enterococci (VRE) strains isolated from animals and humans in Korea. *Korean J Intern Med (Korean Assoc Intern Med)* **20:**55–62.

163. **Lim SK, Kim TS, Lee HS, Nam HM, Joo YS, Koh HB.** 2006. Persistence of *vanA*-type *Enterococcus faecium* in Korean livestock after ban on avoparcin. *Microb Drug Resist* **12:**136–139.

164. **Gonçalves A, Poeta P, Silva N, Araújo C, López M, Ruiz E, Uliyakina I, Direitinho J, Igrejas G, Torres C.** 2010. Characterization of vancomycin-resistant enterococci isolated from fecal samples of ostriches by molecular methods. *Foodborne Pathog Dis* **7:**1133–1136.

165. **Araújo C, Torres C, Gonçalves A, Carneiro C, López M, Radhouani H, Pardal M, Igrejas G, Poeta P.** 2011. Genetic detection and multilocus sequence typing of vanA-containing *Enterococcus* strains from mullets fish (*Liza ramada*). *Microb Drug Resist* **17:**357–361.

166. **Yoshimura H, Ishimaru M, Endoh YS, Suginaka M, Yamatani S.** 1998. Isolation of glycopeptide-resistant enterococci from chicken in Japan. *Antimicrob Agents Chemother* **42:**3333.

167. **Butaye P, Van Damme K, Devriese LA, Van Damme L, Bael M, Lauwers S, Haesebrouck F.** 2000. *In vitro* susceptibility of *Enterococcus faecium* isolated from food to growth-promoting and therapeutic antibiotics. *Int J Food Microbiol* **54:**181–187.

168. **Harada T, Kawahara R, Kanki M, Taguchi M, Kumeda Y.** 2012. Isolation and characterization of *vanA* genotype vancomycin-resistant *Enterococcus cecorum* from retail poultry in Japan. *Int J Food Microbiol* **153:** 372–377.

169. **Donado-Godoy P, Byrne BA, León M, Castellanos R, Vanegas C, Coral A, Arevalo A, Clavijo V, Vargas M, Romero Zuñiga JJ, Tafur M, Pérez-Gutierrez E, Smith WA.** 2015. Prevalence, resistance patterns, and risk factors for antimicrobial resistance in bacteria from retail chicken meat in Colombia. *J Food Prot* **78:**751–759.

170. **Kasimoglu-Dogru A, Gencay YE, Ayaz ND.** 2010. Prevalence and antibiotic resistance profiles of *Enterococcus* species in chicken at slaughter level; absence of *vanA* and *vanB* genes in *E. faecalis* and *E. faecium*. *Res Vet Sci* **89:**153–158.

171. **Harwood VJ, Brownell M, Perusek W, Whitlock JE.** 2001. Vancomycin-resistant *Enterococcus* spp. isolated from wastewater and chicken feces in the United States. *Appl Environ Microbiol* **67:**4930–4933.

172. **Wilson IG, McAfee GG.** 2002. Vancomycin-resistant enterococci in shellfish, unchlorinated waters, and chicken. *Int J Food Microbiol* **79:**143–151.

173. **Novais C, Coque TM, Costa MJ, Sousa JC, Baquero F, Peixe LV.** 2005. High occurrence and persistence of antibiotic-resistant enterococci in poultry food samples in Portugal. *J Antimicrob Chemother* **56:**1139–1143.

174. **Lemcke R, Bülte M.** 2000. Occurrence of the vancomycin-resistant genes *vanA*, *vanB*, *vanCl*, *vanC2* and *vanC3* in *Enterococcus* strains isolated from poultry and pork. *Int J Food Microbiol* **60:**185–194.

175. **Del Grosso M, Caprioli A, Chinzari P, Fontana MC, Pezzotti G, Manfrin A, Giannatale ED, Goffredo E, Pantosti A.** 2000. Detection and characterization of vancomycin-resistant enterococci in farm animals and raw meat products in Italy. *Microb Drug Resist* **6:** 313–318.

176. **Gambarotto K, Ploy MC, Dupron F, Giangiobbe M, Denis F.** 2001. Occurrence of vancomycin-resistant enterococci in pork and poultry products from a cattle-rearing area of France. *J Clin Microbiol* **39:**2354–2355.

177. **Nomura T, Tanimoto K, Shibayama K, Arakawa Y, Fujimoto S, Ike Y, Tomita H.** 2012. Identification of VanN-type vancomycin resistance in an *Enterococcus faecium* isolate from chicken meat in Japan. *Antimicrob Agents Chemother* **56:**6389–6392.

178. **Peters J, Mac K, Wichmann-Schauer H, Klein G, Ellerbroek L.** 2003. Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. *Int J Food Microbiol* **88:** 311–314.

179. **Delpech G, Pourcel G, Schell C, De Luca M, Basualdo J, Bernstein J, Grenovero S, Sparo M.** 2012. Antimicrobial resistance profiles of *Enterococcus faecalis* and *Enterococcus faecium* isolated from artisanal food of animal origin in Argentina. *Foodborne Pathog Dis* **9:** 939–944.

180. **Talebi M, Sadeghi J, Rahimi F, Pourshafie MR.** 2015. Isolation and biochemical fingerprinting of vancomycin-resistant *Enterococcus faecium* from meat, chicken and cheese. *Jundishapur J Microbiol* **8:**e15815.

181. **Gousia P, Economou V, Bozidis P, Papadopoulou C.** 2015. Vancomycin-resistance phenotypes, vancomycin-resistance genes, and resistance to antibiotics of enterococci isolated from food of animal origin. *Foodborne Pathog Dis* **12:**214–220.

182. **Pesavento G, Calonico C, Ducci B, Magnanini A, Lo Nostro A.** 2014. Prevalence and antibiotic resistance of *Enterococcus* spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat. *Food Microbiol* **41:**1–7.

183. **Sánchez Valenzuela A, Lavilla Lerma L, Benomar N, Gálvez A, Pérez Pulido R, Abriouel H.** 2013. Phenotypic and molecular antibiotic resistance profile of *Enterococcus faecalis* and *Enterococcus faecium* isolated from different traditional fermented foods. *Foodborne Pathog Dis* **10:**143–149.

184. **Pantosti A, Del Grosso M, Tagliabue S, Macrì A, Caprioli A.** 1999. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. *Lancet* **354:**741–742.

185. **Hayes JR, English LL, Carter PJ, Proescholdt T, Lee KY, Wagner DD, White DG.** 2003. Prevalence and antimicrobial resistance of *Enterococcus* species isolated from retail meats. *Appl Environ Microbiol* **69:**7153–7160.

186. **Hiroi M, Kawamori F, Harada T, Sano Y, Miwa N, Sugiyama K, Hara-Kudo Y, Masuda T.** 2012. Antibiotic resistance in bacterial pathogens from retail raw meats and food-producing animals in Japan. *J Food Prot* **75:** 1774–1782.

187. **Aslam M, Diarra MS, Checkley S, Bohaychuk V, Masson L.** 2012. Characterization of antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from retail meats in Alberta, Canada. *Int J Food Microbiol* **156:**222–230.

188. **López M, Sáenz Y, Rojo-Bezares B, Martínez S, del Campo R, Ruiz-Larrea F, Zarazaga M, Torres C.** 2009. Detection of *vanA* and *vanB2*-containing enterococci

from food samples in Spain, including *Enterococcus faecium* strains of CC17 and the new singleton ST425. *Int J Food Microbiol* **133:**172–178.

189. **Jahan M, Krause DO, Holley RA.** 2013. Antimicrobial resistance of *Enterococcus* species from meat and fermented meat products isolated by a PCR-based rapid screening method. *Int J Food Microbiol* **163:** 89–95.
190. **Guerrero-Ramos E, Molina-González D, Blanco-Morán S, Igrejas G, Poeta P, Alonso-Calleja C, Capita R.** 2016a. Prevalence, antimicrobial resistance, and genotypic characterization of vancomycin-resistant enterococci in meat preparations. *J Food Prot* **79:**748–756.
191. **Jamet E, Akary E, Poisson MA, Chamba JF, Bertrand X, Serror P.** 2012. Prevalence and characterization of antibiotic resistant *Enterococcus faecalis* in French cheeses. *Food Microbiol* **31:**191–198.
192. **de Garnica ML, Valdezate S, Gonzalo C, Saez-Nieto JA.** 2013. Presence of the *vanC1* gene in a vancomycin-resistant *Enterococcus faecalis* strain isolated from ewe bulk tank milk. *J Med Microbiol* **62:**494–495.
193. **Osman KM, Ali MN, Radwan I, ElHofy F, Abed AH, Orabi A, Fawzy NM.** 2016. Dispersion of the vancomycin resistance genes *vanA* and *vanC* of *Enterococcus* isolated from Nile Tilapia on retail sale: a public health hazard. *Front Microbiol* **7:**1354.
194. **Tansuphasiri U, Khaminthakul D, Pandii W.** 2006. Antibiotic resistance of enterococci isolated from frozen foods and environmental water. *Southeast Asian J Trop Med Public Health* **37:**162–170.
195. **Simjee S, White DG, McDermott PF, Wagner DD, Zervos MJ, Donabedian SM, English LL, Hayes JR, Walker RD.** 2002. Characterization of Tn*1546* in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. *J Clin Microbiol* **40:**4659–4665.
196. **Herrero IA, Fernández-Garayzábal JF, Moreno MA, Domínguez L.** 2004. Dogs should be included in surveillance programs for vancomycin-resistant enterococci. *J Clin Microbiol* **42:**1384–1385.
197. **Ghosh A, Dowd SE, Zurek L.** 2011. Dogs leaving the ICU carry a very large multi-drug resistant enterococcal population with capacity for biofilm formation and horizontal gene transfer. *PLoS One* **6:**e22451.
198. **Kataoka Y, Umino Y, Ochi H, Harada K, Sawada T.** 2014. Antimicrobial susceptibility of enterococcal species isolated from antibiotic-treated dogs and cats. *J Vet Med Sci* **76:**1399–1402.
199. **Abdel-Moein KA, El-Hariri MD, Wasfy MO, Samir A.** 2017. Occurrence of ampicillin-resistant *Enterococcus faecium* carrying *esp* gene in pet animals: an upcoming threat for pet lovers. *J Glob Antimicrob Resist* **9:**115–117.
200. **Oliveira M, Tavares M, Gomes D, Touret T, São Braz B, Tavares L, Semedo-Lemsaddek T.** 2016. Virulence traits and antibiotic resistance among enterococci isolated from dogs with periodontal disease. *Comp Immunol Microbiol Infect Dis* **46:**27–31.
201. **Gulhan T, Boynukara B, Ciftci A, Sogut MU, Findik A.** 2015. Characterization of *Enterococcus faecalis* isolates originating from different sources for their virulence factors and genes, antibiotic resistance patterns, genotypes and biofilm production. *Majallah-i Tahqiqat-i Dampizishki-i Iran* **16:**261–266.
202. **Moura I, Radhouani H, Torres C, Poeta P, Igrejas G.** 2010. Detection and genetic characterisation of *vanA*-containing *Enterococcus* strains in healthy Lusitano horses. *Equine Vet J* **42:**181–183.
203. **Mallon DJ, Corkill JE, Hazel SM, Wilson JS, French NP, Bennett M, Hart CA.** 2002. Excretion of vancomycin-resistant enterococci by wild mammals. *Emerg Infect Dis* **8:**636–638.
204. **Lozano C, González-Barrio D, García JT, Ceballos S, Olea PP, Ruiz-Fons F, Torres C.** 2015. Detection of vancomycin-resistant *Enterococcus faecalis* ST6-vanB2 and *E. faecium* ST915-vanA in faecal samples of wild *Rattus rattus* in Spain. *Vet Microbiol* **177:**168–174.
205. **Nowakiewicz A, Ziółkowska G, Zięba P, Kostruba A.** 2014. Undomesticated animals as a reservoir of multidrug-resistant *Enterococcus* in eastern Poland. *J Wildl Dis* **50:**645–650.
206. **Lozano C, González-Barrio D, Camacho MC, Lima-Barbero JF, de la Puente J, Höfle U, Torres C.** 2016. Characterization of fecal vancomycin-resistant enterococci with acquired and intrinsic resistance mechanisms in wild animals, Spain. *Microb Ecol* **72:**813–820.
207. **Silva N, Igrejas G, Rodrigues P, Rodrigues T, Gonçalves A, Felgar AC, Pacheco R, Gonçalves D, Cunha R, Poeta P.** 2011. Molecular characterization of vancomycin-resistant enterococci and extended-spectrum β-lactamase-containing *Escherichia coli* isolates in wild birds from the Azores Archipelago. *Avian Pathol* **40:**473–479.
208. **Silva N, Igrejas G, Felgar A, Gonçalves A, Pacheco R, Poeta P.** 2012. Molecular characterization of *vanA*-containing *Enterococcus* from migratory birds: song thrush (*Turdus philomelos*). *Braz J Microbiol* **43:**1026–1029.
209. **Santos T, Silva N, Igrejas G, Rodrigues P, Micael J, Rodrigues T, Resendes R, Gonçalves A, Marinho C, Gonçalves D, Cunha R, Poeta P.** 2013. Dissemination of antibiotic resistant *Enterococcus* spp. and *Escherichia coli* from wild birds of Azores Archipelago. *Anaerobe* **24:**25–31.
210. **Klibi N, Ben Amor I, Rahmouni M, Dziri R, Douja G, Ben Said L, Lozano C, Boudabous A, Ben Slama K, Mansouri R, Torres C.** 2015. Diversity of species and antibiotic resistance among fecal enterococci from wild birds in Tunisia. Detection of *vanA*-containing *Enterococcus faecium* isolates. *Eur J Wildl Res* **61:**319–323.
211. **Oravcova V, Zurek L, Townsend A, Clark AB, Ellis JC, Cizek A, Literak I.** 2014. American crows as carriers of vancomycin-resistant enterococci with *vanA* gene. *Environ Microbiol* **16:**939–949.
212. **Roberts MC, No DB, Marzluff JM, Delap JH, Turner R.** 2016. Vancomycin resistant *Enterococcus* spp. from crows and their environment in metropolitan Washington State, USA: is there a correlation between

VRE positive crows and the environment? *Vet Microbiol* **194:**48–54.

213. **Oravcova V, Janecko N, Ansorge A, Masarikova M, Literak I.** 2014. First record of vancomycin-resistant *Enterococcus faecium* in Canadian wildlife. *Environ Microbiol Rep* **6:**210–211.
214. **Oravcova V, Hadelova D, Literak I.** 2016. Vancomycin-resistant *Enterococcus faecium* with *vanA* gene isolated for the first time from wildlife in Slovakia. *Vet Microbiol* **194:**43–47.
215. **Poeta P, Costa D, Igrejas G, Rojo-Bezares B, Sáenz Y, Zarazaga M, Ruiz-Larrea F, Rodrigues J, Torres C.** 2007. Characterization of *vanA*-containing *Enterococcus faecium* isolates carrying Tn*5397*-like and Tn*916*/Tn*1545*-like transposons in wild boars (*Sus Scrofa*). *Microb Drug Resist* **13:**151–156.
216. **Radhouani H, Poeta P, Pinto L, Miranda J, Coelho C, Carvalho C, Rodrigues J, López M, Torres C, Vitorino R, Domingues P, Igrejas G.** 2010. Proteomic characterization of *vanA*-containing *Enterococcus* recovered from seagulls at the Berlengas Natural Reserve, W Portugal. *Proteome Sci* **8:**48.
217. **Barros J, Andrade M, Radhouani H, López M, Igrejas G, Poeta P, Torres C.** 2012. Detection of *vanA*-containing *Enterococcus* species in faecal microbiota of gilthead seabream (*Sparus aurata*). *Microbes Environ* **27:**509–511.
218. **Figueiredo N, Radhouani H, Gonçalves A, Rodrigues J, Carvalho C, Igrejas G, Poeta P.** 2009. Genetic characterization of vancomycin-resistant enterococci isolates from wild rabbits. *J Basic Microbiol* **49:**491–494.
219. **da Silva VL, Caçador NC, da Silva CS, Fontes CO, Garcia GD, Nicoli JR, Diniz CG.** 2012. Occurrence of multidrug-resistant and toxic-metal tolerant enterococci in fresh feces from urban pigeons in Brazil. *Microbes Environ* **27:**179–185.
220. **Radhouani H, Pinto L, Coelho C, Sargo R, Araújo C, López M, Torres C, Igrejas G, Poeta P.** 2010. MLST and a genetic study of antibiotic resistance and virulence factors in *vanA*-containing *Enterococcus* from buzzards (*Buteo buteo*). *Lett Appl Microbiol* **50:**537–541.
221. **Silva V, Igrejas G, Carvalho I, Peixoto F, Cardoso L, Pereira JE, Del Campo R, Poeta P.** 2018. Genetic characterization of *vanA-Enterococcus faecium* isolates from wild red-legged partridges in Portugal. *Microb Drug Resist* **24:**89–94.
222. **Radhouani H, Igrejas G, Carvalho C, Pinto L, Gonçalves A, Lopez M, Sargo R, Cardoso L, Martinho A, Rego V, Rodrigues R, Torres C, Poeta P.** 2011. Clonal lineages, antibiotic resistance and virulence factors in vancomycin-resistant enterococci isolated from fecal samples of red foxes (*Vulpes vulpes*). *J Wildl Dis* **47:**769–773.
223. **Gonçalves A, Igrejas G, Radhouani H, López M, Guerra A, Petrucci-Fonseca F, Alcaide E, Zorrilla I, Serra R, Torres C, Poeta P.** 2011. Detection of vancomycin-resistant enterococci from faecal samples of Iberian wolf and Iberian lynx, including *Enterococcus faecium* strains of CC17 and the new singleton ST573. *Sci Total Environ* **410-411:**266–268.
224. **Katakweba AA, Møller KS, Muumba J, Muhairwa AP, Damborg P, Rosenkrantz JT, Minga UM, Mtambo MM, Olsen JE.** 2015. Antimicrobial resistance in faecal samples from buffalo, wildebeest and zebra grazing together with and without cattle in Tanzania. *J Appl Microbiol* **118:**966–975.
225. **Tejedor Junco MT, González-Martin M, Rodríguez González NF, Gutierrez C.** 2015. Identification, antimicrobial susceptibility, and virulence factors of *Enterococcus* spp. strains isolated from Camels in Canary Islands, Spain. *Vet Ital* **51:**179–183.
226. **Guerrero-Ramos E, Cordero J, Molina-González D, Poeta P, Igrejas G, Alonso-Calleja C, Capita R.** 2016. Antimicrobial resistance and virulence genes in enterococci from wild game meat in Spain. *Food Microbiol* **53** (Pt B)**:**156–164.
227. **Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, Mischnik A, Werner G.** 2015. Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. *J Glob Antimicrob Resist* **3:**128–131.
228. **Herrero IA, Issa NC, Patel R.** 2002. Nosocomial spread of linezolid-resistant, vancomycin-resistant *Enterococcus faecium*. *N Engl J Med* **346:**867–869.
229. **Si H, Zhang WJ, Chu S, Wang XM, Dai L, Hua X, Dong Z, Schwarz S, Liu S.** 2015. Novel plasmid-borne multidrug resistance gene cluster including *lsa*(E) from a linezolid-resistant *Enterococcus faecium* isolate of swine origin. *Antimicrob Agents Chemother* **59:**7113–7116.
230. **Mendes RE, Deshpande LM, Jones RN.** 2014. Linezolid update: stable *in vitro* activity following more than a decade of clinical use and summary of associated resistance mechanisms. *Drug Resist Updat* **17:**1–12.
231. **Shen J, Wang Y, Schwarz S.** 2013. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* **68:**1697–1706.
232. **Liu Y, Wang Y, Wu C, Shen Z, Schwarz S, Du XD, Dai L, Zhang W, Zhang Q, Shen J.** 2012. First report of the multidrug resistance gene *cfr* in *Enterococcus faecalis* of animal origin. *Antimicrob Agents Chemother* **56:**1650–1654.
233. **Liu Y, Wang Y, Schwarz S, Wang S, Chen L, Wu C, Shen J.** 2014. Investigation of a multiresistance gene *cfr* that fails to mediate resistance to phenicols and oxazolidinones in *Enterococcus faecalis*. *J Antimicrob Chemother* **69:**892–898.
234. **Filsner PHLN, de Almeida LM, Moreno M, Moreno LZ, Matajira CEC, Silva KC, Pires C, Cerdeira LT, Sacramento AG, Mamizuka EM, Lincopan N, Moreno AM.** 2017. Identification of the *cfr* methyltransferase gene in *Enterococcus faecalis* isolated from swine: first report in Brazil. *J Glob Antimicrob Resist* **8:**192–193.
235. **Liu Y, Wang Y, Dai L, Wu C, Shen J.** 2014. First report of multiresistance gene *cfr* in *Enterococcus* species *casseliflavus* and *gallinarum* of swine origin. *Vet Microbiol* **170:**352–357.
236. **Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y,**

Feßler AT, Wu C, Yu H, Deng X, Xia X, Shen J. 2015. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* **70:**2182–2190.

237. He T, Shen Y, Schwarz S, Cai J, Lv Y, Li J, Feßler AT, Zhang R, Wu C, Shen J, Wang Y. 2016. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. *J Antimicrob Chemother* **71:**1466–1473.

238. Tamang MD, Moon DC, Kim SR, Kang HY, Lee K, Nam HM, Jang GC, Lee HS, Jung SC, Lim SK. 2017. Detection of novel oxazolidinone and phenicol resistance gene *optrA* in enterococcal isolates from food animals and animal carcasses. *Vet Microbiol* **201:**252–256.

239. Cavaco LM, Korsgaard H, Kaas RS, Seyfarth AM, Leekitcharoenphon P, Hendriksen RS. 2017. First detection of linezolid resistance due to the *optrA* gene in enterococci isolated from food products in Denmark. *J Glob Antimicrob Resist* **9:**128–129.

240. Cavaco LM, Bernal JF, Zankari E, Léon M, Hendriksen RS, Perez-Gutierrez E, Aarestrup FM, Donado-Godoy P. 2017. Detection of linezolid resistance due to the *optrA* gene in *Enterococcus faecalis* from poultry meat from the American continent (Colombia). *J Antimicrob Chemother* **72:**678–683.

241. Freitas AR, Elghaieb H, León-Sampedro R, Abbassi MS, Novais C, Coque TM, Hassen A, Peixe L. 2017. Detection of *optrA* in the African continent (Tunisia) within a mosaic *Enterococcus faecalis* plasmid from urban wastewaters. *J Antimicrob Chemother* **72:**3245–3251.

242. Diaz L, Kiratisin P, Mendes RE, Panesso D, Singh KV, Arias CA. 2012. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother* **56:**3917–3922.

243. Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, Farrell DJ, Mendes RE. 2015. Detection of a new *cfr*-like gene, *cfr*(B), in *Enterococcus faecium* recovered from human specimens in the United States: report from the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother* **59:**6256–6261.

244. Bender JK, Fleige C, Klare I, Fiedler S, Mischnik A, Mutters NT, Dingle KE, Werner G. 2016. Detection of a *cfr*(B) variant in German *Enterococcus faecium* clinical isolates and the impact on linezolid resistance in *Enterococcus* spp. *PLoS One* **11:**e0167042.

245. Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, Earls MR, Boyle B, O'Connell B, Brennan GI, Shore AC. 2017. Novel multiresistance *cfr* plasmids in linezolid-resistant methicillin-resistant *Staphylococcus epidermidis* and vancomycin-resistant *Enterococcus faecium* (VRE) from a hospital outbreak: co-location of *cfr* and *optrA* in VRE. *J Antimicrob Chemother* **72:**3252–3257.

246. Morroni G, Brenciani A, Simoni S, Vignaroli C, Mingoia M, Giovanetti E. 2017. Nationwide surveillance of novel oxazolidinone resistance gene *optrA* in *Enterococcus* isolates in China from 20004 to 2014. *Front Microbiol* **8:**1631.

247. Cai J, Wang Y, Schwarz S, Lv H, Li Y, Liao K, Yu S, Zhao K, Gu D, Wang X, Zhang R, Shen J. 2015. Enterococcal isolates carrying the novel oxazolidinone resistance gene *optrA* from hospitals in Zhejiang, Guangdong, and Henan, China, 2010–2014. *Clin Microbiol Infect* **21:**1095.e1–1095.e4.

248. Cui L, Wang Y, Lv Y, Wang S, Song Y, Li Y, Liu J, Xue F, Yang W, Zhang J. 2016. Nationwide surveillance of novel oxazolidinone resistance gene *optrA* in *Enterococcus* isolates in China from 2004 to 2014. *Antimicrob Agents Chemother* **60:**7490–7493.

249. Del Campo R, Galán JC, Tenorio C, Ruiz-Garbajosa P, Zarazaga M, Torres C, Baquero F. 2005. New aac (6′)-I genes in *Enterococcus hirae* and *Enterococcus durans*: effect on beta-lactam/aminoglycoside synergy. *J Antimicrob Chemother* **55:**1053–1055.

250. Galimand M, Schmitt E, Panvert M, Desmolaize B, Douthwaite S, Mechulam Y, Courvalin P. 2011. Intrinsic resistance to aminoglycosides in *Enterococcus faecium* is conferred by the 16S rRNA m5C1404-specific methyltransferase EfmM. *RNA* **17:**251–262.

251. Bager F, Emborg HD (ed). 1998. *DANMAP 1997. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*. National Food Institute, Technical University of Denmark, Søborg, Denmark.

252. Donabedian SM, Thal LA, Hershberger E, Perri MB, Chow JW, Bartlett P, Jones R, Joyce K, Rossiter S, Gay K, Johnson J, Mackinson C, Debess E, Madden J, Angulo F, Zervos MJ. 2003. Molecular characterization of gentamicin-resistant enterococci in the United States: evidence of spread from animals to humans through food. *J Clin Microbiol* **41:**1109–1113.

253. Werner G, Coque TM, Franz CM, Grohmann E, Hegstad K, Jensen L, van Schaik W, Weaver K. 2013. Antibiotic resistant enterococci: tales of a drug resistance gene trafficker. *Int J Med Microbiol* **303:**360–379.

254. Klibi N, Ben Lagha A, Ben Slama K, Boudabous A, Torres C. 2013. Faecal enterococci from camels in Tunisia: species, antibiotic resistance and virulent genes. *Vet Rec* **172:**213.

255. Muñoz-Atienza E, Gómez-Sala B, Araújo C, Campanero C, del Campo R, Hernández PE, Herranz C, Cintas LM. 2013. Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiol* **13:**15.

256. Araújo C, Muñoz-Atienza E, Hernández PE, Herranz C, Cintas LM, Igrejas G, Poeta P. 2015. Evaluation of *Enterococcus* spp. from rainbow trout (*Oncorhynchus mykiss*, Walbaum), feed, and rearing environment against fish pathogens. *Foodborne Pathog Dis* **12:**311–322.

257. Di Cesare A, Pasquaroli S, Vignaroli C, Paroncini P, Luna GM, Manso E, Biavasco F. 2014. The marine environment as a reservoir of enterococci carrying resistance and virulence genes strongly associated with clinical strains. *Environ Microbiol Rep* **6:**184–190.

258. **Li P, Wu D, Liu K, Suolang S, He T, Liu X, Wu C, Wang Y, Lin D.** 2014. Investigation of antimicrobial resistance in *Escherichia coli* and enterococci isolated from Tibetan pigs. *PLoS One* **9:**e95623.

259. **Pantozzi FL, Ibar MP, Nievas VF, Vigo GB, Moredo FA, Giacoboni GI.** 2014. Wild-type minimal inhibitory concentration distributions in bacteria of animal origin in Argentina. *Rev Argent Microbiol* **46:**34–40.

260. **Usui M, Ozawa S, Onozato H, Kuge R, Obata Y, Uemae T, Ngoc PT, Heriyanto A, Chalemchaikit T, Makita K, Muramatsu Y, Tamura Y.** 2014. Antimicrobial susceptibility of indicator bacteria isolated from chickens in Southeast Asian countries (Vietnam, Indonesia and Thailand). *J Vet Med Sci* **76:**685–692.

261. **Klibi N, Aouini R, Borgo F, Ben Said L, Ferrario C, Dziri R, Boudabous A, Torres C, Ben Slama K.** 2015. Antibiotic resistance and virulence of faecal enterococci isolated from food-producing animals in Tunisia. *Ann Microbiol* **65:**695–702.

262. **Ayeni FA, Odumosu BT, Oluseyi AE, Ruppitsch W.** 2016. Identification and prevalence of tetracycline resistance in enterococci isolated from poultry in Ilishan, Ogun State, Nigeria. *J Pharm Bioallied Sci* **8:**69–73.

263. **Boss R, Overesch G, Baumgartner A.** 2016. Antimicrobial resistance of *Escherichia coli*, enterococci, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from raw fish and seafood imported into Switzerland. *J Food Prot* **79:**1240–1246.

264. **Wu X, Hou S, Zhang Q, Ma Y, Zhang Y, Kan W, Zhao X.** 2016. Prevalence of virulence and resistance to antibiotics in pathogenic enterococci isolated from mastitic cows. *J Vet Med Sci* **78:**1663–1668.

265. **Beshiru A, Igbinosa IH, Omeje FI, Ogofure AG, Eyong MM, Igbinosa EO.** 2017. Multi-antibiotic resistant and putative virulence gene signatures in *Enterococcus* species isolated from pig farms environment. *Microb Pathog* **104:**90–96.

266. **Carvalho I, Campo RD, Sousa M, Silva N, Carrola J, Marinho C, Santos T, Carvalho S, Nóvoa M, Quaresma M, Pereira JE, Cobo M, Igrejas G, Poeta P.** 2017. Antimicrobial-resistant *Escherichia coli* and *Enterococcus* spp. isolated from Miranda donkey (*Equus asinus*): an old problem from a new source with a different approach. *J Med Microbiol* **66:**191–202.

267. **Kataoka Y, Ito C, Kawashima A, Ishii M, Yamashiro S, Harada K, Ochi H, Sawada T.** 2013. Identification and antimicrobial susceptibility of enterococci isolated from dogs and cats subjected to differing antibiotic pressures. *J Vet Med Sci* **75:**749–753.

268. **Chung YS, Kwon KH, Shin S, Kim JH, Park YH, Yoon JW.** 2014. Characterization of veterinary hospital-associated isolates of *Enterococcus* species in Korea. *J Microbiol Biotechnol* **24:**386–393.

269. **Iseppi R, Messi P, Anacarso I, Bondi M, Sabia C, Condò C, de Niederhausern S.** 2015. Antimicrobial resistance and virulence traits in *Enterococcus* strains isolated from dogs and cats. *New Microbiol* **38:**369–378.

270. **Radhouani H, Igrejas G, Gonçalves A, Pacheco R, Monteiro R, Sargo R, Brito F, Torres C, Poeta P.** 2013. Antimicrobial resistance and virulence genes in *Escherichia coli* and enterococci from red foxes (*Vulpes vulpes*). *Anaerobe* **23:**82–86.

271. **Semedo-Lemsaddek T, Nóbrega CS, Ribeiro T, Pedroso NM, Sales-Luís T, Lemsaddek A, Tenreiro R, Tavares L, Vilela CL, Oliveira M.** 2013. Virulence traits and antibiotic resistance among enterococci isolated from Eurasian otter (*Lutra lutra*). *Vet Microbiol* **163:**378–382.

272. **Doud CW, Scott HM, Zurek L.** 2014. Role of house flies in the ecology of *Enterococcus faecalis* from wastewater treatment facilities. *Microb Ecol* **67:**380–391.

273. **Santestevan NA, de Angelis Zvoboda D, Prichula J, Pereira RI, Wachholz GR, Cardoso LA, de Moura TM, Medeiros AW, de Amorin DB, Tavares M, d'Azevedo PA, Franco AC, Frazzon J, Frazzon AP.** 2015. Antimicrobial resistance and virulence factor gene profiles of *Enterococcus* spp. isolates from wild *Arctocephalus australis* (South American fur seal) and *Arctocephalus tropicalis* (Subantarctic fur seal). *World J Microbiol Biotechnol* **31:**1935–1946.

274. **Prichula J, Pereira RI, Wachholz GR, Cardoso LA, Tolfo NC, Santestevan NA, Medeiros AW, Tavares M, Frazzon J, d'Azevedo PA, Frazzon AP.** 2016. Resistance to antimicrobial agents among enterococci isolated from fecal samples of wild marine species in the southern coast of Brazil. *Mar Pollut Bull* **105:**51–57.

275. **Ben Said L, Hamdaoui M, Klibi A, Ben Slama K, Torres C, Klibi N.** 2017. Diversity of species and antibiotic resistance in enterococci isolated from seafood in Tunisia. *Ann Microbiol* **67:**135–141.

276. **Chaiwong T, Srivoramas T, Panya M, Wanram S, Panomket P.** 2014. Antibiotic resistance patterns of *Enterococcus* spp. isolated from *Musca domestica* and *Chrysomya megacephala* in ubon Ratchathani. *J Med Assoc Thai* **97**(Suppl 4)**:**S1–S6.

277. **Chopra I, Roberts M.** 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* **65:**232–260.

278. **Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H.** 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* **43:**2823–2830.

279. **Roberts MC.** 2005. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* **245:**195–203.

280. **Franke AE, Clewell DB.** 1980. Evidence for conjugal transfer of a *Streptococcus faecalis* tranposon (Tn*916*) from a chromosomal site in the absence of plasmid DNA. *Cold Spring Harb Symp Quant Biol* **46:**7780.

281. **Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F.** 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* **45:**2054–2059.

282. **Portillo A, Ruiz-Larrea F, Zarazaga M, Alonso A, Martinez JL, Torres C.** 2000. Macrolide resistance genes in *Enterococcus* spp. *Antimicrob Agents Chemother* **44:**967–971.

283. **Bozdogan B, Berrezouga L, Kuo MS, Yurek DA, Farley KA, Stockman BJ, Leclercq R.** 1999. A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. *Antimicrob Agents Chemother* **43:**925–929.

284. **Roberts MC.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev* **19:**1–24.

285. **Hooper DC.** 2002. Fluoroquinolone resistance among Gram-positive cocci. *Lancet Infect Dis* **2:**530–538.

286. **López M, Tenorio C, Del Campo R, Zarazaga M, Torres C.** 2011. Characterization of the mechanisms of fluoroquinolone resistance in vancomycin-resistant enterococci of different origins. *J Chemother* **23:**87–91.

287. **Arsène S, Leclercq R.** 2007. Role of a *qnr*-like gene in the intrinsic resistance of *Enterococcus faecalis* to fluoroquinolones. *Antimicrob Agents Chemother* **51:**3254–3258.

288. **Jonas BM, Murray BE, Weinstock GM.** 2001. Characterization of *emeA*, a *NorA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. *Antimicrob Agents Chemother* **45:**3574–3579.

289. **Oyamada Y, Ito H, Fujimoto K, Asada R, Niga T, Okamoto R, Inoue M, Yamagishi J.** 2006. Combination of known and unknown mechanisms confers high-level resistance to fluoroquinolones in *Enterococcus faecium*. *J Med Microbiol* **55:**729–736.

290. **Werner G, Fleige C, Ewert B, Laverde-Gomez JA, Klare I, Witte W.** 2010. High-level ciprofloxacin resistance among hospital-adapted *Enterococcus faecium* (CC17). *Int J Antimicrob Agents* **35:**119–125.

291. **Guzman Prieto AM, van Schaik W, Rogers MR, Coque TM, Baquero F, Corander J, Willems RJ.** 2016. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? *Front Microbiol* **7:**788.

292. **van den Bogaard AE, Jensen LB, Stobberingh EE.** 1997. Vancomycin-resistant enterococci in turkeys and farmers. *N Engl J Med* **337:**1558–1559.

293. **Klein G, Pack A, Reuter G.** 1998. Antibiotic resistance patterns of enterococci and occurrence of vancomycin-resistant enterococci in raw minced beef and pork in Germany. *Appl Environ Microbiol* **64:**1825–1830.

294. **Butaye P, Devriese LA, Goossens H, Ieven M, Haesebrouck F.** 1999. Enterococci with acquired vancomycin resistance in pigs and chickens of different age groups. *Antimicrob Agents Chemother* **43:**365–366.

295. **Klare I, Heier H, Claus H, Böhme G, Marin S, Seltmann G, Hakenbeck R, Antanassova V, Witte W.** 1995. *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. *Microb Drug Resist* **1:**265–272.

296. **Wegener HC, Madsen M, Nielsen N, Aarestrup FM.** 1997. Isolation of vancomycin resistant *Enterococcus faecium* from food. *Int J Food Microbiol* **35:**57–66.

297. **Balzereit-Scheuerlein F, Stephan R.** 2001. Prevalence of colonisation and resistance patterns of vancomycin-resistant enterococci in healthy, non-hospitalised persons in Switzerland. *Swiss Med Wkly* **131:**280–282.

298. **Van der Auwera P, Pensart N, Korten V, Murray BE, Leclercq R.** 1996. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. *J Infect Dis* **173:**1129–1136.

299. **del Campo R, Ruiz-Garbajosa P, Sánchez-Moreno MP, Baquero F, Torres C, Cantón R, Coque TM.** 2003. Antimicrobial resistance in recent fecal enterococci from healthy volunteers and food handlers in Spain: genes and phenotypes. *Microb Drug Resist* **9:**47–60.

300. **Bates J, Jordens Z, Selkon JB.** 1993. Evidence for an animal origin of vancomycin-resistant enterococci. *Lancet* **342:**490–491.

301. **Nannini E, Murray BE.** 2006. Vancomycin resistant enterococci, p 155–188. *In* Fong IW, Drlica K (ed), *Reemergence of Established Pathogens in the 21st Century*. Kluwer Academic, New York, NY.

302. **Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L.** 2009. Dispersion of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. *Appl Environ Microbiol* **75:**4904–4908.

303. **Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L.** 2009. Clonal expansion within clonal complex 2 and spread of vancomycin-resistant plasmids among different genetic lineages of *Enterococcus faecalis* from Portugal. *J Antimicrob Chemother* **63:**1104–1111.

304. **Freitas AR, Coque TM, Novais C, Hammerum AM, Lester CH, Zervos MJ, Donabedian S, Jensen LB, Francia MV, Baquero F, Peixe L.** 2011. Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn*1546* on indistinguishable plasmids. *J Clin Microbiol* **49:**925–931.

305. **Robredo B, Singh KV, Torres C, Murray BE.** 2000. Streptogramin resistance and shared pulsed-field gel electrophoresis patterns in *vanA*-containing *Enterococcus faecium* and *Enterococcus hirae* isolated from humans and animals in Spain. *Microb Drug Resist* **6:**305–311.

306. **Daniel DS, Lee SM, Dykes GA, Rahman S.** 2015. Public health risks of multiple-drug-resistant *Enterococcus* spp. in Southeast Asia. *Appl Environ Microbiol* **81:**6090–6097.

307. **Seo KS, Lim JY, Yoo HS, Bae WK, Park YH.** 2005. Comparison of vancomycin-resistant enterococci isolates from human, poultry and pigs in Korea. *Vet Microbiol* **106:**225–233.

308. **Howden BP, Holt KE, Lam MM, Seemann T, Ballard S, Coombs GW, Tong SY, Grayson ML, Johnson PD, Stinear TP.** 2013. Genomic insights to control the emergence of vancomycin-resistant enterococci. *MBio* **4:**e00412-13.

309. **Hammerum AM, Lester CH, Neimann J, Porsbo LJ, Olsen KE, Jensen LB, Emborg HD, Wegener HC, Frimodt-Moller N.** 2004. A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is

possibly related to pig isolates. *J Antimicrob Chemother* **53:**547–549.

310. **van den Bogaard AE, Stobberingh EE.** 2000. Epidemiology of resistanceto antibiotics. Links between animals and humans. *Int J Antimicrob Agents* **14:**327–335.
311. **Willems RJ, Top J, van Den Braak N, van Belkum A, Endtz H, Mevius D, Stobberingh E, van Den Bogaard A, van Embden JD.** 2000. Host specificity of vancomycin-resistant *Enterococcus faecium*. *J Infect Dis* **182:**816–823.
312. **Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, Van Embden JD, Willems RJ.** 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. *J Clin Microbiol* **40:**1963–1971.
313. **Willems RJ, Top J, van Schaik W, Leavis H, Bonten M, Sirén J, Hanage WP, Corander J.** 2012. Restricted gene flow among hospital subpopulations of *Enterococcus faecium*. *MBio* **3:**e00151-12.
314. **Tedim AP, Ruiz-Garbajosa P, Corander J, Rodríguez CM, Cantón R, Willems RJ, Baquero F, Coque TM.** 2015. Population biology of intestinal enterococcus isolates from hospitalized and nonhospitalized individuals in different age groups. *Appl Environ Microbiol* **81:** 1820–1831.
315. **Bruinsma N, Willems RJ, van den Bogaard AE, van Santen-Verheuvel M, London N, Driessen C, Stobberingh EE.** 2002. Different levels of genetic homogeneity in vancomycin-resistant and -susceptible *Enterococcus faecium* isolates from different human and animal sources analyzed by amplified-fragment length polymorphism. *Antimicrob Agents Chemother* **46:**2779–2783.
316. **Willems RJ, Top J, van Santen M, Robinson DA, Coque TM, Baquero F, Grundmann H, Bonten MJ.** 2005. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* **11:**821–828.
317. **Hwang IY, Ku HO, Lim SK, Lee KJ, Park CK, Jung GS, Jung SC, Park YH, Nam HM.** 2010. Distribution of streptogramin resistance genes and genetic relatedness among quinupristin/dalfopristin-resistant *Enterococcus faecium* recovered from pigs and chickens in Korea. *Res Vet Sci* **89:**1–4.
318. **Donabedian SM, Perri MB, Vager D, Hershberger E, Malani P, Simjee S, Chow J, Vergis EN, Muder RR, Gay K, Angulo FJ, Bartlett P, Zervos MJ.** 2006. Quinupristin-dalfopristin resistance in *Enterococcus faecium* isolates from humans, farm animals, and grocery store meat in the United States. *J Clin Microbiol* **44:**3361–3365.
319. **De Graef EM, Decostere A, De Leener E, Goossens H, Baele M, Haesebrouck F.** 2007. Prevalence and mechanism of resistance against macrolides, lincosamides, and streptogramins among *Enterococcus faecium* isolates from food-producing animals and hospital patients in Belgium. *Microb Drug Resist* **13:**135–141.
320. **Cha JO, Jung YH, Lee HR, Yoo JI, Lee YS.** 2012. Comparison of genetic epidemiology of vancomycin-resistant *Enterococcus faecium* isolates from humans and poultry. *J Med Microbiol* **61:**1121–1128.
321. **Stobberingh E, van den Bogaard A, London N, Driessen C, Top J, Willems R.** 1999. Enterococci with glycopeptide resistance in turkeys, turkey farmers, turkey slaughterers, and (sub)urban residents in the south of The Netherlands: evidence for transmission of vancomycin resistance from animals to humans? *Antimicrob Agents Chemother* **43:**2215–2221.
322. **Simonsen GS, Haaheim H, Dahl KH, Kruse H, Løvseth A, Olsvik O, Sundsfjord A.** 1998. Transmission of VanA-type vancomycin-resistant enterococci and *vanA* resistance elements between chicken and humans at avoparcin-exposed farms. *Microb Drug Resist* **4:**313–318.
323. **De Leener E, Martel A, De Graef EM, Top J, Butaye P, Haesebrouck F, Willems R, Decostere A.** 2005. Molecular analysis of human, porcine, and poultry *Enterococcus faecium* isolates and their *erm*(B) genes. *Appl Environ Microbiol* **71:**2766–2770.
324. **Aarestrup FM, Carstensen B.** 1998. Effect of tylosin used as a growth promoter on the occurrence of macrolide-resistant enterococci and staphylococci in pigs. *Microb Drug Resist* **4:**307–312.
325. **Bager F, Madsen M, Christensen J, Aarestrup FM.** 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* **31:**95–112.
326. **Boerlin P, Wissing A, Aarestrup FM, Frey J, Nicolet J.** 2001. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in enterococci from pigs. *J Clin Microbiol* **39:**4193–4195.
327. **Novais C, Coque TM, Boerlin P, Herrero I, Moreno MA, Dominguez L, Peixe L.** 2005. Vancomycin-resistant *Enterococcus faecium* clone in swine, Europe. *Emerg Infect Dis* **11:**1985–1987.
328. **Novais C, Freitas AR, Sousa JC, Baquero F, Coque TM, Peixe LV.** 2008. Diversity of Tn*1546* and its role in the dissemination of vancomycin-resistant enterococci in Portugal. *Antimicrob Agents Chemother* **52:** 1001–1008.
329. **Hasman H, Aarestrup FM.** 2002. *tcrB*, a gene conferring transferable copper resistance in *Enterococcus faecium*: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. *Antimicrob Agents Chemother* **46:**1410–1416.
330. **Amachawadi RG, Shelton NW, Shi X, Vinasco J, Dritz SS, Tokach MD, Nelssen JL, Scott HM, Nagaraja TG.** 2011. Selection of fecal enterococci exhibiting *tcrB*-mediated copper resistance in pigs fed diets supplemented with copper. *Appl Environ Microbiol* **77:**5597–5603.
331. **Silveira E, Freitas AR, Antunes P, Barros M, Campos J, Coque TM, Peixe L, Novais C.** 2014. Co-transfer of resistance to high concentrations of copper and first-line antibiotics among *Enterococcus* from different origins (humans, animals, the environment and foods) and clonal lineages. *J Antimicrob Chemother* **69:**899–906.
332. **Freitas AR, Tedim AP, Novais C, Ruiz-Garbajosa P, Werner G, Laverde-Gomez JA, Cantón R, Peixe L, Baquero F, Coque TM.** 2010. Global spread of the *hyl* (Efm) colonization-virulence gene in megaplasmids of the *Enterococcus faecium* CC17 polyclonal subcluster. *Antimicrob Agents Chemother* **54:**2660–2665.

333. Getachew Y, Hassan L, Zakaria Z, Abdul Aziz S. 2013. Genetic variability of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans, chickens, and pigs in Malaysia. *Appl Environ Microbiol* **79:**4528–4533.

334. Damborg P, Sørensen AH, Guardabassi L. 2008. Monitoring of antimicrobial resistance in healthy dogs: first report of canine ampicillin-resistant *Enterococcus faecium* clonal complex 17. *Vet Microbiol* **132:**190–196.

335. de Regt MJ, van Schaik W, van Luit-Asbroek M, Dekker HA, van Duijkeren E, Koning CJ, Bonten MJ, Willems RJ. 2012. Hospital and community ampicillin-resistant *Enterococcus faecium* are evolutionarily closely linked but have diversified through niche adaptation. *PLoS One* **7:**e30319.

336. van den Bunt G, Top J, Hordijk J, de Greeff SC, Mughini-Gras L, Corander J, van Pelt W, Bonten MJM, Fluit AC, Willems RJL. Intestinal carriage of ampicillin- and vancomycin-resistant *Enterococcus faecium* in humans, dogs and cats in the Netherlands. *J Antimicrob Chemother*. Epub ahead of print.

337. Marques C, Belas A, Franco A, Aboim C, Gama LT, Pomba C. 2018. Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. *J Antimicrob Chemother* **73:** 377–384.

338. Ruiz-Garbajosa P, Bonten MJM, Robinson DA, Top J, Nallapareddy SR, Torres C, Coque TM, Cantón R, Baquero F, Murray BE, del Campo R, Willems RJL. 2006. Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. *J Clin Microbiol* **44:**2220–2228.

339. McBride SM, Fischetti VA, Leblanc DJ, Moellering RC Jr, Gilmore MS. 2007. Genetic diversity among *Enterococcus faecalis*. *PLoS One* **2:**e582.

340. Kuch A, Willems RJL, Werner G, Coque TM, Hammerum AM, Sundsfjord A, Klare I, Ruiz-Garbajosa P, Simonsen GS, van Luit-Asbroek M, Hryniewicz W, Sadowy E. 2012. Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe. *J Antimicrob Chemother* **67:**551–558.

341. Chen MY, Lira F, Liang HQ, Wu RT, Duan JH, Liao XP, Martínez JL, Liu YH, Sun J. 2016. Multilevel selection of *bcr*ABDR-mediated bacitracin resistance in *Enterococcus faecalis* from chicken farms. *Sci Rep* **6:**34895.

342. Petersen A, Christensen H, Philipp H-C, Bisgaard M. 2009. Clonality of *Enterococcus faecalis* associated with amyloid arthropathy in chickens evaluated by multilocus sequence typing (MLST). *Vet Microbiol* **134:**392–395.

343. Ruiz-Garbajosa P, Cantón R, Pintado V, Coque TM, Willems R, Baquero F, del Campo R. 2006. Genetic and phenotypic differences among *Enterococcus faecalis* clones from intestinal colonisation and invasive disease. *Clin Microbiol Infect* **12:**1193–1198.

344. Raven KE, Reuter S, Gouliouris T, Reynolds R, Russell JE, Brown NM, Török ME, Parkhill J, Peacock SJ. 2016. Genome-based characterization of hospital-adapted *Enterococcus faecalis* lineages. *Nat Microbiol* **1:**15033.

345. Palmer KL, Godfrey P, Griggs A, Kos VN, Zucker J, Desjardins C, Cerqueira G, Gevers D, Walker S, Wortman J, Feldgarden M, Haas B, Birren B, Gilmore MS. 2012. Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. *MBio* **3:**e00318-11.

346. Clewell DB, Weaver KE, Dunny GM, Coque TM, Francia MV, Hayes F. 2014. Extrachromosomal and mobile elements in enterococci: transmission, maintenance, and epidemiology. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), *Enterococci: from Commensals to Leading Causes of Drug Resistant Infection*. Eye and Ear Infirmary, Boston, MA.

347. Jensen LB, Garcia-Migura L, Valenzuela AJS, Løhr M, Hasman H, Aarestrup FM. 2010. A classification system for plasmids from enterococci and other Gram-positive bacteria. *J Microbiol Methods* **80:**25–43.

348. Wardal E, Gawryszewska I, Hryniewicz W, Sadowy E. 2013. Abundance and diversity of plasmid-associated genes among clinical isolates of *Enterococcus faecalis*. *Plasmid* **70:**329–342.

349. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* **42**(D1)**:**D581–D591.

350. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* **58:**3895–3903.

351. Johnsen PJ, Townsend JP, Bøhn T, Simonsen GS, Sundsfjord A, Nielsen KM. 2011. Retrospective evidence for a biological cost of vancomycin resistance determinants in the absence of glycopeptide selective pressures. *J Antimicrob Chemother* **66:**608–610.

352. Freitas AR, Tedim AP, Francia MV, Jensen LB, Novais C, Peixe L, Sánchez-Valenzuela A, Sundsfjord A, Hegstad K, Werner G, Sadowy E, Hammerum AM, Garcia-Migura L, Willems RJ, Baquero F, Coque TM. 2016. Multilevel population genetic analysis of *vanA* and *vanB Enterococcus faecium* causing nosocomial outbreaks in 27 countries (1986–2012). *J Antimicrob Chemother* **71:** 3351–3366.

353. Lanza VF, Tedim AP, Martínez JL, Baquero F, Coque TM. 2015. The plasmidome of *Firmicutes*: impact on the emergence and the spread of resistance to antimicrobials. *Microbiol Spectr* **3:**PLAS-0039-2014.

354. Sletvold H, Johnsen PJ, Hamre I, Simonsen GS, Sundsfjord A, Nielsen KM. 2008. Complete sequence of *Enterococcus faecium* pVEF3 and the detection of an

omega-epsilon-zeta toxin-antitoxin module and an ABC transporter. *Plasmid* **60:**75–85.

355. **Sletvold H, Johnsen PJ, Wikmark OG, Simonsen GS, Sundsfjord A, Nielsen KM.** 2010. Tn*1546* is part of a larger plasmid-encoded genetic unit horizontally disseminated among clonal *Enterococcus faecium* lineages. *J Antimicrob Chemother* **65:**1894–1906.
356. **Garcia-Migura L, Hasman H, Svendsen C, Jensen LB.** 2008. Relevance of hot spots in the evolution and transmission of Tn*1546* in glycopeptide-resistant *Enterococcus faecium* (GREF) from broiler origin. *J Antimicrob Chemother* **62:**681–687.
357. **Moura TM, Cassenego AP, Campos FS, Ribeiro AM, Franco AC, d'Azevedo PA, Frazzon J, Frazzon AP.** 2013. Detection of *vanC1* gene transcription in vancomycin-susceptible *Enterococcus faecalis*. *Mem Inst Oswaldo Cruz* **108:**453–456.
358. **Schwaiger K, Bauer J, Hörmansdorfer S, Mölle G, Preikschat P, Kämpf P, Bauer-Unkauf I, Bischoff M, Hölzel C.** 2012. Presence of the resistance genes *vanC1* and *pbp5* in phenotypically vancomycin and ampicillin susceptible *Enterococcus faecalis*. *Microb Drug Resist* **18:**434–439.
359. **Batista Xavier D, Moreno Bernal FE, Titze-de-Almeida R.** 2006. Absence of *VanA*- and *Van*B-containing enterococci in poultry raised on nonintensive production farms in Brazil. *Appl Environ Microbiol* **72:**3072–3073.
360. **Mammina C, Di Noto AM, Costa A, Nastasi A.** 2005. *VanB-VanC1 Enterococcus gallinarum*, Italy. *Emerg Infect Dis* **11:**1491–1492.
361. **Mikalsen T, Pedersen T, Willems R, Coque TM, Werner G, Sadowy E, van Schaik W, Jensen LB, Sundsfjord A, Hegstad K.** 2015. Investigating the mobilome in clinically important lineages of *Enterococcus faecium* and *Enterococcus faecalis*. *BMC Genomics* **16:**282.
362. **Teuber M, Schwarz F, Perreten V.** 2003. Molecular structure and evolution of the conjugative multiresistance plasmid pRE25 of *Enterococcus faecalis* isolated from a raw-fermented sausage. *Int J Food Microbiol* **88:**325–329.
363. **Schwarz FV, Perreten V, Teuber M.** 2001. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from *Enterococcus faecalis* RE25. *Plasmid* **46:**170–187.
364. **Werner G, Hildebrandt B, Witte W.** 2003. Linkage of *erm*(B) and *aadE-sat4-aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. *Microb Drug Resist* **9**(Suppl 1)**:**S9–S16.
365. **Khan AA, Nawaz MS, Khan SA, Steele R.** 2002. Detection and characterization of erythromycin-resistant methylase genes in Gram-positive bacteria isolated from poultry litter. *Appl Microbiol Biotechnol* **59:**377–381.
366. **Thumu SC, Halami PM.** 2014. Phenotypic expression, molecular characterization and transferability of erythromycin resistance genes in *Enterococcus* spp. isolated from naturally fermented food. *J Appl Microbiol* **116:**689–699.
367. **Schwaiger K, Bauer J.** 2008. Detection of the erythromycin rRNA methylase gene *erm*(A) in *Enterococcus faecalis*. *Antimicrob Agents Chemother* **52:**2994–2995.
368. **Werner G, Hildebrandt B, Klare I, Witte W.** 2000. Linkage of determinants for streptogramin A, macrolide-lincosamide-streptogramin B, and chloramphenicol resistance on a conjugative plasmid in *Enterococcus faecium* and dissemination of this cluster among streptogramin-resistant enterococci. *Int J Med Microbiol* **290:**543–548.
369. **Werner G, Klare I, Heier H, Hinz KH, Böhme G, Wendt M, Witte W.** 2000. Quinupristin/dalfopristin-resistant enterococci of the *satA* (*vatD*) and *satG* (*vatE*) genotypes from different ecological origins in Germany. *Microb Drug Resist* **6:**37–47.
370. **Hammerum AM, Flannagan SE, Clewell DB, Jensen LB.** 2001. Indication of transposition of a mobile DNA element containing the *vat*(D) and *erm*(B) genes in *Enterococcus faecium*. *Antimicrob Agents Chemother* **45:**3223–3225.
371. **Jackson CR, Fedorka-Cray PJ, Barrett JB, Hiott LM, Woodley TA.** 2007. Prevalence of streptogramin resistance in enterococci from animals: identification of *vatD* from animal sources in the USA. *Int J Antimicrob Agents* **30:**60–66.
372. **Jensen LB, Hammerum AM, Aarestrup FM.** 2000. Linkage of *vat*(E) and *erm*(B) in streptogamin-resistant *Enterococcus faecium* isolates from Europe. *Antimicrob Agents Chemother* **44:**2231–2232.
373. **Jensen LB, Hammerum AM, Bager F, Aarestrup FM.** 2002. Streptogramin resistance among *Enterococcus faecium* isolated from production animals in Denmark in 1997. *Microb Drug Resist* **8:**369–374.
374. **Sørensen TL, Blom M, Monnet DL, Frimodt-Møller N, Poulsen RL, Espersen F.** 2001. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. *N Engl J Med* **345:**1161–1166.
375. **Smith DL, Johnson JA, Harris AD, Furuno JP, Perencevich EN, Morris JG Jr.** 2003. Assessing risks for a pre-emergent pathogen: virginiamycin use and the emergence of streptogramin resistance in *Enterococcus faecium*. *Lancet Infect Dis* **3:**241–249.
376. **Li XS, Dong WC, Wang XM, Hu GZ, Wang YB, Cai BY, Wu CM, Wang Y, Du XD.** 2014. Presence and genetic environment of pleuromutilin-lincosamide-streptogramin A resistance gene lsa(E) in enterococci of human and swine origin. *J Antimicrob Chemother* **69:**1424–1426.
377. **Wang XM, Li XS, Wang YB, Wei FS, Zhang SM, Shang YH, Du XD.** 2015. Characterization of a multidrug resistance plasmid from *Enterococcus faecium* that harbours a mobilized bcrABDR locus. *J Antimicrob Chemother* **70:**609–611.
378. **Zhu XQ, Wang XM, Li H, Shang YH, Pan YS, Wu CM, Wang Y, Du XD, Shen JZ.** 2017. Novel *lnu*(G) gene conferring resistance to lincomycin by nucleotidylation, located on Tn*6260* from *Enterococcus faecalis* E531. *J Antimicrob Chemother* **72:**993–997.
379. **Liu H, Wang Y, Wu C, Schwarz S, Shen Z, Jeon B, Ding S, Zhang Q, Shen J.** 2012. A novel phenicol exporter gene, *fexB*, found in enterococci of animal origin. *J Antimicrob Chemother* **67:**322–325.

380. **Novais C, Campos J, Freitas AR, Barros M, Silveira E, Coque TM, Antunes P, Peixe L.** 2018. Water supply and feed as sources of antimicrobial-resistant *Enterococcus* spp. in aquacultures of rainbow trout (*Oncorhyncus mykiss*), Portugal. *Sci Total Environ* **625:** 1102–1112.

381. **Aarestrup FM, Hasman H, Jensen LB, Moreno M, Herrero IA, Domínguez L, Finn M, Franklin A.** 2002. Antimicrobial resistance among enterococci from pigs in three European countries. *Appl Environ Microbiol* **68:**4127–4129.

382. **Amachawadi RG, Scott HM, Alvarado CA, Mainini TR, Vinasco J, Drouillard JS, Nagaraja TG.** 2013. Occurrence of the transferable copper resistance gene *tcrB* among fecal enterococci of U.S. feedlot cattle fed copper-supplemented diets. *Appl Environ Microbiol* **79:** 4369–4375.

383. **Kim J, Lee S, Choi S.** 2012. Copper resistance and its relationship to erythromycin resistance in *Enterococcus* isolates from bovine milk samples in Korea. *J Microbiol* **50:**540–543.

384. **Hasman H, Kempf I, Chidaine B, Cariolet R, Ersbøll AK, Houe H, Bruun Hansen HC, Aarestrup FM.** 2006. Copper resistance in *Enterococcus faecium*, mediated by the *tcrB* gene, is selected by supplementation of pig feed with copper sulfate. *Appl Environ Microbiol* **72:**5784–5789.

385. **Amachawadi RG, Scott HM, Vinasco J, Tokach MD, Dritz SS, Nelssen JL, Nagaraja TG.** 2015. Effects of in-feed copper, chlortetracycline, and tylosin on the prevalence of transferable copper resistance gene, *tcrB*, among fecal enterococci of weaned piglets. *Foodborne Pathog Dis* **12:**670–678.

386. **Pasquaroli S, Di Cesare A, Vignaroli C, Conti G, Citterio B, Biavasco F.** 2014. Erythromycin- and copper-resistant *Enterococcus hirae* from marine sediment and co-transfer of *erm*(B) and *tcr*B to human *Enterococcus faecalis*. *Diagn Microbiol Infect Dis* **80:**26–28.

387. **Zhang S, Wang D, Wang Y, Hasman H, Aarestrup FM, Alwathnani HA, Zhu YG, Rensing C.** 2015. Genome sequences of copper resistant and sensitive *Enterococcus faecalis* strains isolated from copper-fed pigs in Denmark. *Stand Genomic Sci* **10:**35.

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Antimicrobial Resistance in *Rhodococcus equi*

10

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INTRODUCTION

Rhodococcus equi, a Gram-positive facultative intracellular pathogen, is one of the most important causes of disease in foals between 3 weeks and 5 months of age. *R. equi* has also emerged as a common opportunistic pathogen in immunocompromised people, especially those infected with the human immunodeficiency virus (1–3). Infection in both foals and people is most commonly characterized by life-threatening pyogranulomatous pneumonia, but extrapulmonary infections are also common (3, 4). In foals, extrapulmonary disorders might occur concurrent with or independent of pneumonia, and some foals have multiple extrapulmonary disorders concurrently (5, 6). Because ultrasonographic screening for early detection has become routine practice at many farms endemic for pneumonia caused by *R. equi*, the most frequently recognized form of *R. equi* infection on those farms is a subclinical form in which foals develop sonographic evidence of peripheral pulmonary consolidation or abscessation without manifesting clinical signs (7, 8).

R. equi is commonly cultured from the submaxillary lymph nodes of pigs with granulomatous lymphadenitis. However, the causative role of *R. equi* in these lesions is unclear, because it can be isolated from 3 to 5% of apparently healthy pigs, and experimental infection studies have failed to reproduce granulomatous lymphadenitis (9–11). *R. equi* is also occasionally isolated from abscesses or granulomas in the lymph nodes of cattle (12). It has been cultured from rare cases of pulmonary or extrapulmonary infections in cattle, goats, camelids, dogs, and cats (13–17).

Since the 1980s the standard treatment recommendation for foals infected with *R. equi* has been the combination of a macrolide (erythromycin initially and, more recently, clarithromycin or azithromycin) and rifampin with, until recently, very few documented instances of resistance to these drugs. This text reviews the available data regarding antimicrobial resistance in *R. equi*, with emphasis on the molecular mechanisms of the recent emergence of resistance to macrolides and rifampin in equine isolates of *R. equi*.

IN VITRO ACTIVITY

In vitro Susceptibility Testing of *R. equi*

Antimicrobial susceptibility testing can be done using a variety of methods, with broth dilution, disk diffusion, and concentration gradient test (Etest) being commonly used by veterinary diagnostic laboratories. The decision regarding which method to use is based on cost, ease of use, and flexibility to meet the needs of the laboratory. The methods all assess inhibition of growth rather than killing of the bacterium as the endpoint. *In vitro* susceptibility tests must be performed using standardized procedures to provide valid and reproducible results. Although numerous studies have investigated the *in vitro* activity of a variety of antimicrobial drugs against *R. equi* over the years, different methods, media, and incubation conditions have been used. A protocol for antimicrobial susceptibility testing of *R. equi* by broth microdilution was recently adopted by the Veterinary Antimicrobial Susceptibility Testing subcommittee of the Clinical and Laboratory Standards Institute (CLSI) and will be included in the upcoming CLSI document (18). The approved protocol recommends a standard inoculum of 1×10^5 CFU/ml, an incubation temperature of 35 ± 2°C, the use of cation-adjusted Mueller-Hinton broth supplemented with 2% (vol/vol) lysed horse blood, and reading of the results after 24 h (19). This protocol was recently evaluated in 18 laboratories.

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All 18 participating laboratories were able to perform broth microdilution according to guidelines established by the CLSI, with over 98% of all MIC determinations for the *Escherichia coli* quality control strain being within the acceptable range (18). There was more interlaboratory variability for *R. equi*, with 72 to 100% of all MICs being within the acceptable range (modal MIC ± 1 dilution), with only one laboratory being below 80% (18).

The results generated by *in vitro* susceptibility tests, whether they are determined by disk diffusion, concentration gradient, or dilution methodologies, are usually presented to the clinician by designating the pathogen as susceptible, intermediate, or resistant. Despite adopting a standardized protocol for antimicrobial susceptibility testing, the CLSI has not established interpretive criteria for the classification of isolates of *R. equi* as susceptible, intermediate, or resistant. Therefore, interpretive criteria reported by diagnostic laboratories are extrapolated from criteria established for other bacterial agents and, for most drugs, based on therapeutic concentrations achievable in people or other animal species. The results of broth macrodilution, disk diffusion, and Etest for *in vitro* susceptibility testing of macrolide-susceptible and macrolide-resistant isolates of *R. equi* were compared recently. Categorical agreement between methods ranged between 85.1 and 100% depending on the drug tested (20). Overall, the agreement between Etest and disk diffusion was better than the agreement between broth macrodilution and the agar-based methods (20). Etest tended to overestimate MICs relative to broth macrodilution for clarithromycin and gentamicin and underestimate MICs for erythromycin and doxycycline (20).

MIC and MBC of Drugs Used Against *R. equi*

Drugs with the highest *in vitro* activity against *R. equi* based on MIC are clarithromycin, rifampin, imipenem, telithromycin, erythromycin, gentamicin, apramycin, vancomycin, azithromycin, gamithromycin, doxycycline, enrofloxacin, and linezolid (Table 1). Trimethoprim-sulfonamide combinations are also active *in vitro* against the majority of isolates. Not all macrolides are equally active, with tildipirosin, tilmicosin, tulathromycin, spiramycin, and tylosin being poorly active against *R. equi in vitro*. Most drugs including macrolides and rifampin exert only bacteriostatic activity against *R. equi*, with only amikacin, gentamicin, enrofloxacin, and vancomycin being bactericidal (21). Combinations including a macrolide (erythromycin, clarithromycin, or azithromycin) and either rifampin or doxycycline, and the combination doxycycline-rifampin are highly synergistic against *R. equi in vitro* (21–23). In contrast, combinations containing amikacin and erythromycin, clarithromycin, azithromycin, or rifampin and the combination gentamicin-rifampin are antagonistic *in vitro* (21–23). However, the clinical significance of these *in vitro* findings has not been established.

Mutant Prevention Concentration (MPC)

Given the recent emergence of resistance to macrolides and rifampin among isolates of *R. equi* (see below), the relative propensity of currently available antimicrobial agents to selectively enrich for resistant mutant subpopulations among *R. equi* isolates has been studied. A common way to compare drugs for selective enrichment of resistant mutants is based on measurement of the MPC, which is defined as the drug concentration that prevents selective enrichment of first-step resistant mutants within a large susceptible bacterial population. The range of concentrations between the MIC and the MPC is known as the mutant selection window, which represents the danger zone for emergence of resistant mutants (24). Minimizing the length of time that the drug concentrations remain in the mutant selection window may reduce the likelihood of development of resistance during therapy. Of 10 antimicrobial agents studied (erythromycin, clarithromycin, azithromycin, rifampin, amikacin, gentamicin, enrofloxacin, vancomycin, imipenem, and doxycycline), rifampin had the highest MPC, indicating that rifampin monotherapy is likely to select for resistance (25). However, combining rifampin with erythromycin, clarithromycin, or azithromycin resulted in a profound and significant decrease in MPC (25). The MPC was well above clinically achievable plasma concentrations for most antimicrobial drugs studied (25).

Activity of Drugs Against Intracellular *R. equi*

The ability of *R. equi* to survive and replicate in macrophages is the basis of its pathogenicity, and strains unable to replicate intracellularly are avirulent for foals (26). Many drugs active against *R. equi in vitro* have been hypothesized to be ineffective *in vivo* because of poor cellular uptake and resulting low intracellular concentrations. Studies with facultative intracellular bacterial pathogens have shown that evaluation of the bactericidal activity of antimicrobial agents against intracellular bacteria is more closely associated with *in vivo* efficacy than with traditional *in vitro* susceptibility testing. In one study, clarithromycin was more active than azithromycin, erythromycin, and gamithromycin against intracellular *R. equi* (27). More recently, equine monocyte-derived macrophages were infected

Table 1 MIC data for *R. equi* isolates susceptible to macrolides and rifampin[a]

Antimicrobial classes/agents	*n*	MIC (g/ml)		
		MIC_{90}[b]	MIC_{50}[c]	Range
Macrolides/azalides/ketolides				
Azithromycin	264	1	1	0.06–4
Clarithromycin	264	0.06	0.06	0.008–0.5
Erythromycin	263	0.5	0.5	0.06–1
Gamithromycin	30	1	1	0.5–1
Spiramycin	200	32	32	2–64
Telithromycin	25	0.25	0.25	0.25–0.5
Tildipirosin	40	32	16	8–32
Tilmicosin	278	64	32	0.5–>64
Tulathromycin	278	>64	64	8–>64
Tylosin	200	32	32	1–64
Rifamycins				
Rifampin	194	0.12	0.06	0.008–0.25
β-lactams				
Ampicillin	264	8	4	0.06–8
Amoxicillin-clavulanic acid[d]	264	8	4	0.06–16
Penicillin G	200	4	4	0.03–8
Cefazolin	64	16	≤2	≤2–>16
Cefoperazone	200	32	16	2–64
Cefotaxime	200	8	8	0.12–16
Ceftazidime	64	>32	>32	≤0.25–>32
Ceftiofur	278	16	8	0.25–16
Cefquinome	200	4	2	0.12–8
Imipenem	200	0.12	0.12	0.015–0.25
Aminoglycosides				
Amikacin	64	4	≤2	≤2–8
Apramycin	200	0.5	0.25	0.12–8
Gentamicin	264	0.5	0.5	0.12–1
Quinolones				
Enrofloxacin	264	1	1	0.25–4
Nalidixic acid	200	128	128	16–256
Tetracycline				
Tetracycline	264	8	4	0.5–16
Doxycycline	278	1	1	0.25–2
Other classes				
Chloramphenicol	342	16	8	≤4–32
Clindamycin	264	8	2	0.8–8
Florfenicol	200	16	16	4–32
Linezolid	78	1	1	0.5–2
Quinupristin/dalfopristin	200	18	8	0.5–16
Trimethoprim/sulfamethoxazole[e]	342	1	0.5	0.06–>4
Vancomycin	278	0.5	0.5	0.12–1

[a] Adapted from references 27, 43, 49, 57.
[b] MIC that inhibits at least 90% of the isolates tested.
[c] MIC that inhibits at least 50% of the isolates tested.
[d] Expressed as MIC of amoxicillin.
[e] Expressed as MIC of trimethoprim.

with virulent *R. equi* and exposed to erythromycin, clarithromycin, azithromycin, rifampin, ceftiofur, gentamicin, enrofloxacin, vancomycin, imipenem, or doxycycline at concentrations achievable in plasma at clinically recommended dosages in foals. Enrofloxacin, gentamicin, and vancomycin were significantly more active than other drugs against intracellular *R. equi*, whereas doxycycline was the least active drug (21).

EMERGENCE OF RESISTANCE TO MACROLIDES AND RIFAMPIN IN ISOLATES FROM HORSES

The combination of a macrolide such as erythromycin, clarithromycin, or azithromycin with rifampicin has been the mainstay of therapy in foals infected with *R. equi* since the early 1980s (28–30), with only one report of macrolide resistance in foals before 1999 (31). During the same period, reports of rifampin resistance were rare and typically associated with the use of the drug in monotherapy (31–33). Over the past 15 years, however, the incidence of macrolide-resistant *R. equi* from foals has increased considerably, at least in the United States. The overall prevalence of macrolide and rifampin-resistant isolates in Texas and Florida between 1997 and 2008 was 4%, with most resistant isolates being identified after 2001 (34). In the same study, the odds of death were approximately 7 times higher in foals infected with resistant isolates (34). More recently, it has been documented that mass antimicrobial treatment of subclinically affected foals has selected for antimicrobial resistance over time, with isolates of *R. equi* resistant to all macrolides and rifampin now being cultured from the environment and from up to 40% of pneumonic foals at one farm (35). While there was considerable chromosomal heterogeneity among susceptible *R. equi* isolates, macrolide- and rifampin-resistant isolates from the farm were all closely related and formed two distinct genotypic clusters (35). To date, isolates of *R. equi* resistant to macrolides and rifampin have been identified from at least five U.S. states. However, the true prevalence of macrolide- and/or rifampin-resistant *R. equi* in the United States and elsewhere is unknown. Reports of macrolide resistance in veterinary isolates of *R. equi* outside the United States have been extremely rare. There is one report of isolation of a macrolide- and rifampin-resistant strain from a foal in China (36). Resistance to macrolides or rifampin has been reported in 3 to 4% of isolates cultured from people (37).

MOLECULAR BASIS OF DRUG RESISTANCE

Macrolides, Lincosamides, and Streptogramin B

Three main mechanisms account for acquired macrolide resistance in bacteria: rRNA methylation, active efflux, and enzymatic inactivation (38). rRNA methylation and active efflux are the mechanisms responsible for the majority of resistant isolates. Most macrolide-resistance genes are associated with mobile elements and thus have the capacity to spread among strains, species, and bacterial ecosystems. rRNA methylation, encoded by erythromycin-resistant methylase (*erm*) genes, results in cross-resistance to the macrolides, lincosamides, and streptogramin B. Efflux of macrolide antimicrobial agents is mediated by members of the ATP binding cassette family of proteins or by major facilitator superfamily transporters. These proteins pump antimicrobial agents out of the cell or cellular membrane, thereby allowing the bacterial ribosomes to function again. The third and less common mechanism of resistance is due to enzymatic inactivation of macrolides by bacteria (39). A very small proportion of macrolide-resistant bacteria do not carry any of the known acquired macrolide-resistance genes described above. These isolates typically have mutations in the V domain of the 23S rRNA genes and/or the genes coding the ribosomal proteins L4 and L22 (40).

Macrolide resistance in macrolide-resistant isolates of *R. equi* cultured in the United States is caused by *erm*(46), an erythromycin-resistant methylase gene that has been identified only in *R. equi* to date (41). The *erm*(46) gene encodes a predicted methyltransferase that targets the 50S subunit of the bacterial ribosome and is most similar (68 to 69% nucleotide sequence identity) to the mycobacterial rRNA methyltransferases encoded by *erm*(38), *erm*(39), and *erm*(40). The *erm*(46) gene is flanked upstream by an open reading frame encoding a putative AAA-family P-loop ATPase/nucleotide kinase domain, and downstream by an open reading frame encoding a putative integrase/Tra5-like transposase with closely related homologs in other *Actinobacteria*. Erm(46) and its mycobacterial counterparts form a distinct branch within an Erm subclade populated by enzymes from various *Actinobacteria* genera including *Streptomyces*, *Corynebacterium*, and *Micrococcus* (41), suggesting that the *R. equi erm*(46) gene has an actinobacterial origin and was likely horizontally acquired via a transposable mobile element.

There was complete agreement between the macrolide resistance phenotype and detection of the *erm*(46) gene, with 100% of resistant (n = 62) and susceptible (n = 62) isolates testing positive and negative, respectively (41). Expression of *erm*(46) in the macrolide-susceptible strain 103^{+} conferred high-level (>256 g/ml) resistance to all macrolides tested (azithromycin, clarithromycin, erythromycin, gamithromycin, tildipirosin), lincosamides, and streptogramin B (41). Expression of the gene conferred a lower level of resistance (8 to 16 g/ml) to ketolides and to the combination of quinupristin and dalfopristin. Expression of the gene did not confer resistance to aminoglycosides, tetracyclines, glycopep-

tides, β-lactams, fluoroquinolones, and rifampin. Mating experiments confirmed horizontal transfer of *erm*(46) from resistant to susceptible strains of *R. equi*, with transfer frequencies ranging from 3×10^{-3} to 1×10^{-2} (41). To the authors' knowledge, the presence of *erm* (46) in *R. equi* isolates resistant to macrolides cultured outside the United States has not been examined. Macrolide resistance in an isolate of *R. equi* cultured from a foal in China was associated with an A to G mutation at position 2063 in domain V of the 23S rRNA gene (36). It is unknown if the isolate was also carrying *erm*(46), given that the report was published before the discovery of the gene.

The vast majority of macrolide-resistant *R. equi* isolates identified so far are also resistant to rifampin. There are no known mechanisms of cross-resistance between macrolides and rifampin. Rifampin resistance in *R. equi* has been shown to be the result of mutations in the *rpoB* gene (see below). Selection pressure caused by the combined use of rifampin with a macrolide for the treatment and prevention of *R. equi* infection at endemic horse breeding farms might have coselected for the acquisition of *erm*(46) alongside an *rpoB* mutation in specific strains.

Rifampin

Rifampin resistance in several bacterial genera typically results from the substitution of a limited number of highly conserved amino acids in the RNA polymerase β subunit encoded by the *rpoB* gene. This gene is divided into three regions: clusters I, II, and III. In *Mycobacterium* spp., the vast majority of substitutions conferring rifampin resistance are found in cluster I within an 81-bp resistance-determining region corresponding to codons 507 to 533 (*E. coli* numbering) of the *rpoB* gene. Most substitutions associated with rifampin resistance detected to date in *R. equi* are single substitutions within the same resistance-determining region, with mutations at codons 526 or 531 being the most commonly reported (Table 2) (32, 42, 43). The level of antimicrobial resistance has been reported to be dependent on both the location and the nature of the base substitution. However, different isolates with the same substitution have been found occasionally to have considerably different MICs (43). Rare isolates of *R. equi* resistant to rifampin do not have mutations in the 81-bp resistance-determining region (43). It is possible that such isolates have mutations in other regions of the *rpoB* gene, as reported in a small percentage of rifampin-resistant strains of *Mycobacterium tuberculosis*. Alternatively, resistance in these isolates of *R. equi* might be caused by one or more mechanism(s). Several rifampin-inactivating enzymes have been identified in environmental and pathogenic Gram-positive bacteria such as *Bacillus* spp., *Nocardia* spp., *Mycobacterium smegmatis*, and *Listeria monocytogenes* (44–46). A gene encoding a monooxigenase-like protein was identified in one strain of *R. equi* and shown to confer low-level resistance to rifampin by inactivating the drug (47). The role played by rifampin-inactivating enzymes in rifampin resistance in foals in a clinical setting is unknown.

Fluoroquinolones

Fluoroquinolones are rarely used to treat infections caused by *R. equi* in foals because of the risk of arthropathy (48). *R. equi* is highly resistant to first-generation quinolones such as nalidixic acid. However, resistance to higher-generation fluoroquinolones in clinical use is

Table 2 Mutations in the *rpoB* gene associated with rifampin resistance in *R. equi*[a]

Origin[b]	Country	Rifampin MIC (g/ml)	Substitution	
			Codon[c]	Amino acid exchange
Human	Thailand	8	509	Ser → Pro
Horse	United States	64	513	Gln → Leu
Horse	Germany, France	2–4	516	Asp → Val
Horse	France, United States	128–256	526	His → Asp
Horse	France	8	526	His → Asn
Human, *in vitro*	Thailand, NA[d]	≥256	526	His → Tyr
In vitro	NA	≥256	526	His → Arg
Horse	France, United States	8–128	531	Ser → Leu
Horse	United States	≥256	531	Ser → Phe
Human	Thailand	64	531	Ser → Trp

[a] Adapted from references 32, 42, 43.
[b] Species of origin or *in vitro* mutations generated in the laboratory.
[c] *E. coli* numbering.
[d] NA: not applicable.

rare in equine isolates of *R. equi*, with less than 5% of isolates being classified as resistant to enrofloxacin or ciprofloxacin using CLSI breakpoints for other bacterial agents (MIC ≥ 4 g/ml as resistant), and with most isolates classified as being of intermediate susceptibility (MIC 1 to 2 g/ml) to enrofloxacin and susceptible to ciprofloxacin (43, 49, 50). Resistance to ciprofloxacin appears to be more common in isolates cultured from people, with approximately 85% of isolates classified as susceptible (37, 51). Bacterial resistance to fluoroquinolones can be the result of chromosomal mutations coding for modifications in target subunits of bacterial topoisomerases II and IV, by active efflux, or by alterations in the expression of outer membrane proteins.

The mechanisms of fluoroquinolone resistance have not been studied in clinical isolates of *R. equi*. However, *in vitro* selection for resistance to ciprofloxacin in *R. equi* is typically associated with one of seven identified single amino acid substitutions in the quinolone resistance-determining region of DNA gyrase subunit A (52, 53). Mutants with amino acid substitutions at Ser-83 of GyrA were particularly resistant (MIC > 64 g/ml) (53). In the same study, a single amino acid in the quinolone resistance-determining region of *gyrB* was identified and associated with a lower level of resistance (MIC=4g/ml) (53). Some ciprofloxacin-resistant mutants did not have substitutions in the quinolone resistance-determining region of *gyrA* or *gyrB*, indicating that other fluoroquinolone resistance mechanisms such as efflux pumps or alteration in membrane permeability might also occur. The MPC for enrofloxacin and ciprofloxacin ranged between 32 and 64 g/ml, which is well above concentrations achievable *in vivo*, indicating that monotherapy with fluoroquinolones might result in emergence of resistant mutants (25, 52).

Other Antimicrobial Agents

Resistance to aminoglycosides in clinical use such as gentamicin and amikacin and to glycopeptides such as vancomycin is extremely rare (20, 37, 43, 49). In one study, an inducible glycopeptide-resistance operon (*vanO*) was described in a single isolate of *R. equi* from soil (54). The *vanO* operon had unique gene organization compared to the *vanA* operon in enterococci and displayed structural similarities to putative operon-like clusters detected in actinomycetes (54). The *vanO* operon is located on the chromosome, and attempts at transferring vancomycin resistance by conjugation or transformation were not successful (54). The molecular mechanisms of antimicrobial resistance to other antimicrobial agents in *R. equi* have not been studied. The genome of the *R. equi* reference strain 103S contains an array of putative antimicrobial-resistance determinants, including a variety of antibiotic-inactivation enzymes, β-lactamases, and multidrug efflux systems (55). However, the functionality of these putative determinants has not been confirmed. Isolates of *R. equi* have been reported to be resistant to a variety of antimicrobial agents and drug classes. These findings must be interpreted in the context of the lack of CLSI-approved breakpoints for *R. equi*. Therefore, breakpoints for the classification of isolates of *R. equi* as susceptible, intermediate, or resistant are CLSI breakpoints for other species, and bacterial agents used have not been consistent between studies.

R. equi isolates are typically resistant or of intermediate susceptibility to most β-lactam antimicrobial agents, with the exception of the carbapenems such as imipenem or meropenem (Table 1). Resistance to carbapenems is rare, and low-level imipenem resistance has been associated with an altered penicillin-binding protein pattern (56). Most isolates of *R. equi* are of intermediate susceptibility to tetracyclines and chloramphenicol, and resistance is not uncommon. However, resistance to doxycycline is extremely rare (<1% of isolates) (20, 43, 49). Most isolates of *R. equi* are susceptible to combinations of trimethoprim-sulfonamide, but resistance is not uncommon.

CONCLUSIONS

Our understanding of the molecular mechanisms of macrolide and rifampin resistance in *R. equi* has improved considerably in the past few years. However, further work is needed to assess the overall prevalence of macrolide and rifampin resistance in isolates of *R. equi*. Resistance to other antimicrobial agents such as tetracyclines, chloramphenicol, trimethoprim-sulfa, and fluoroquinolones is not uncommon, and more work is needed to characterize the molecular mechanisms of resistance to these drugs.

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References

1. **Arlotti M, Zoboli G, Moscatelli GL, Magnani G, Maserati R, Borghi V, Andreoni M, Libanore M, Bonazzi L, Piscina A, Ciammarughi R.** 1996. *Rhodococcus equi* infection in HIV-positive subjects: a retrospective analysis of 24 cases. *Scand J Infect Dis* **28:**463–467.

2. **Donisi A, Suardi MG, Casari S, Longo M, Cadeo GP, Carosi G.** 1996. *Rhodococcus equi* infection in HIV-infected patients. *AIDS* **10:**359–362.
3. **Yamshchikov AV, Schuetz A, Lyon GM.** 2010. *Rhodococcus equi* infection. *Lancet Infect Dis* **10:**350–359.
4. **Giguère S, Cohen ND, Chaffin MK, Hines SA, Hondalus MK, Prescott JF, Slovis NM.** 2011. *Rhodococcus equi*: clinical manifestations, virulence, and immunity. *J Vet Intern Med* **25:**1221–1230.
5. **Reuss SM, Chaffin MK, Cohen ND.** 2009. Extrapulmonary disorders associated with *Rhodococcus equi* infection in foals: 150 cases (1987–2007). *J Am Vet Med Assoc* **235:**855–863.
6. **Zink MC, Yager JA, Smart NL.** 1986. *Corynebacterium equi* infections in horses, 1958–1984: a review of 131 cases. *Can Vet J* **27:**213–217.
7. **Slovis NM, McCracken JL, Mundy G.** 2005. How to use thoracic ultrasound to screen foals for *Rhodococcus equi* at affected farms. *Proc Am Assoc Equine Pract* **51:**274–278.
8. **Venner M, Kerth R, Klug E.** 2007. Evaluation of tulathromycin in the treatment of pulmonary abscesses in foals. *Vet J* **174:**418–421.
9. **Komijn RE, Wisselink HJ, Rijsman VM, Stockhofe-Zurwieden N, Bakker D, van Zijderveld FG, Eger T, Wagenaar JA, Putirulan FF, Urlings BA.** 2007. Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for *Mycobacterium avium* subsp. *avium* and positive for *Rhodococcus equi*. *Vet Microbiol* **120:**352–357.
10. **Madarame H, Yaegashi R, Fukunaga N, Matsukuma M, Mutoh K, Morisawa N, Sasaki Y, Tsubaki S, Hasegawa Y, Takai S.** 1998. Pathogenicity of *Rhodococcus equi* strains possessing virulence-associated 15- to 17-kDa and 20-kDa antigens: experimental and natural cases in pigs. *J Comp Pathol* **119:**397–405.
11. **Takai S, Fukunaga N, Ochiai S, Sakai T, Sasaki Y, Tsubaki S.** 1996. Isolation of virulent and intermediately virulent *Rhodococcus equi* from soil and sand on parks and yards in Japan. *J Vet Med Sci* **58:**669–672.
12. **Flynn O, Quigley F, Costello E, O'Grady D, Gogarty A, Mc Guirk J, Takai S.** 2001. Virulence-associated protein characterisation of *Rhodococcus equi* isolated from bovine lymph nodes. *Vet Microbiol* **78:**221–228.
13. **Hong CB, Donahue JM.** 1995. *Rhodococcus equi*-associated necrotizing lymphadenitis in a llama. *J Comp Pathol* **113:**85–88.
14. **Kinne J, Madarame H, Takai S, Jose S, Wernery U.** 2011. Disseminated *Rhodococcus equi* infection in dromedary camels (*Camelus dromedarius*). *Vet Microbiol* **149:**269–272.
15. **Tkachuk-Saad O, Lusis P, Welsh RD, Prescott JF.** 1998. *Rhodococcus equi* infections in goats. *Vet Rec* **143:**311–312.
16. **Bryan LK, Clark SD, Diaz-Delgado J, Lawhon SD, Edwards JF.** 2016. *Rhodococcus equi* infections in dogs. *Vet Pathol*.
17. **Takai S, Martens RJ, Julian A, Garcia Ribeiro M, Rodrigues de Farias M, Sasaki Y, Inuzuka K, Kakuda T, Tsubaki S, Prescott JF.** 2003. Virulence of *Rhodococcus equi* isolated from cats and dogs. *J Clin Microbiol* **41:**4468–4470.
18. **Riesenberg A, Kaspar H, Feßler AT, Werckenthin C, Schwarz S.** 2016. Susceptibility testing of *Rhodococcus equi*: an interlaboratory test. *Vet Microbiol* **194:**30–35.
19. **Riesenberg A, Feßler AT, Frömke C, Kadlec K, Klarmann D, Kreienbrock L, Werckenthin C, Schwarz S.** 2013. Harmonization of antimicrobial susceptibility testing by broth microdilution for *Rhodococcus equi* of animal origin. *J Antimicrob Chemother* **68:**2173–2175.
20. **Berghaus LJ, Giguère S, Guldbech K, Warner E, Ugorji U, Berghaus RD.** 2015. Comparison of Etest, disk diffusion, and broth macrodilution for *in vitro* susceptibility testing of *Rhodococcus equi*. *J Clin Microbiol* **53:**314–318.
21. **Giguère S, Lee EA, Guldbech KM, Berghaus LJ.** 2012. *In vitro* synergy, pharmacodynamics, and postantibiotic effect of 11 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol* **160:**207–213.
22. **Nordmann P, Ronco E.** 1992. *In-vitro* antimicrobial susceptibility of *Rhodococcus equi*. *J Antimicrob Chemother* **29:**383–393.
23. **Prescott JF, Nicholson VM.** 1984. The effects of combinations of selected antibiotics on the growth of *Corynebacterium equi*. *J Vet Pharmacol Ther* **7:**61–64.
24. **Blondeau JM.** 2009. New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. *Vet Dermatol* **20:**383–396.
25. **Berghaus LJ, Giguère S, Guldbech K.** 2013. Mutant prevention concentration and mutant selection window for 10 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol* **166:**670–675.
26. **Giguère S, Hondalus MK, Yager JA, Darrah P, Mosser DM, Prescott JF.** 1999. Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein A in intracellular survival and virulence of *Rhodococcus equi*. *Infect Immun* **67:**3548–3557.
27. **Berghaus LJ, Giguère S, Sturgill TL, Bade D, Malinski TJ, Huang R.** 2012. Plasma pharmacokinetics, pulmonary distribution, and *in vitro* activity of gamithromycin in foals. *J Vet Pharmacol Ther* **35:**59–66.
28. **Giguère S, Jacks S, Roberts GD, Hernandez J, Long MT, Ellis C.** 2004. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med* **18:**568–573.
29. **Giguère S, Cohen ND, Chaffin MK, Slovis NM, Hondalus MK, Hines SA, Prescott JF.** 2011. Diagnosis, treatment, control, and prevention of infections caused by *Rhodococcus equi* in foals. *J Vet Intern Med* **25:**1209–1220.
30. **Hillidge CJ.** 1987. Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. *Vet Microbiol* **14:**337–342.
31. **Kenney DG, Robbins SC, Prescott JF, Kaushik A, Baird JD.** 1994. Development of reactive arthritis and resistance to erythromycin and rifampin in a foal during

treatment for *Rhodococcus equi* pneumonia. *Equine Vet J* **26:**246–248.

32. **Fines M, Pronost S, Maillard K, Taouji S, Leclercq R.** 2001. Characterization of mutations in the *rpoB* gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. *J Clin Microbiol* **39:**2784–2787.
33. **Takai S, Takeda K, Nakano Y, Karasawa T, Furugoori J, Sasaki Y, Tsubaki S, Higuchi T, Anzai T, Wada R, Kamada M.** 1997. Emergence of rifampin-resistant *Rhodococcus equi* in an infected foal. *J Clin Microbiol* **35:**1904–1908.
34. **Giguère S, Lee E, Williams E, Cohen ND, Chaffin MK, Halbert N, Martens RJ, Franklin RP, Clark CC, Slovis NM.** 2010. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R equi*. *J Am Vet Med Assoc* **237:**74–81.
35. **Burton AJ, Giguère S, Sturgill TL, Berghaus LJ, Slovis NM, Whitman JL, Levering C, Kuskie KR, Cohen ND.** 2013. Macrolide- and rifampin-resistant *Rhodococcus equi* on a horse breeding farm, Kentucky, USA. *Emerg Infect Dis* **19:**282–285.
36. **Liu H, Wang Y, Yan J, Wang C, He H, Forbes BA.** 2014. Appearance of multidrug-resistant virulent *Rhodococcus equi* clinical isolates obtained in China. *J Clin Microbiol* **52:**703.
37. **McNeil MM, Brown JM.** 1992. Distribution and antimicrobial susceptibility of *Rhodococcus equi* from clinical specimens. *Eur J Epidemiol* **8:**437–443.
38. **Roberts MC.** 2004. Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. *Mol Biotechnol* **28:**47–62.
39. **Roberts MC.** 2008. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. *FEMS Microbiol Lett* **282:**147–159.
40. **Franceschi F, Kanyo Z, Sherer EC, Sutcliffe J.** 2004. Macrolide resistance from the ribosome perspective. *Curr Drug Targets Infect Disord* **4:**177–191.
41. **Anastasi E, Giguère S, Berghaus LJ, Hondalus MK, Willingham-Lane JM, MacArthur I, Cohen ND, Roberts MC, Vazquez-Boland JA.** 2015. Novel transferable *erm* (46) determinant responsible for emerging macrolide resistance in *Rhodococcus equi*. *J Antimicrob Chemother* **70:**3184–3190.
42. **Asoh N, Watanabe H, Fines-Guyon M, Watanabe K, Oishi K, Kositsakulchai W, Sanchai T, Kunsuikmengrai K, Kahintapong S, Khantawa B, Tharavichitkul P, Sirisanthana T, Nagatake T.** 2003. Emergence of rifampin-resistant *Rhodococcus equi* with several types of mutations in the rpoB gene among AIDS patients in northern Thailand. *J Clin Microbiol* **41:**2337–2340.
43. **Riesenberg A, Feßler AT, Erol E, Prenger-Berninghoff E, Stamm I, Böse R, Heusinger A, Klarmann D, Werckenthin C, Schwarz S.** 2014. MICs of 32 antimicrobial agents for *Rhodococcus equi* isolates of animal origin. *J Antimicrob Chemother* **69:**1045–1049.
44. **Quan S, Venter H, Dabbs ER.** 1997. Ribosylative inactivation of rifampin by *Mycobacterium smegmatis* is a principal contributor to its low susceptibility to this antibiotic. *Antimicrob Agents Chemother* **41:**2456–2460.
45. **Spanogiannopoulos P, Waglechner N, Koteva K, Wright GD.** 2014. A rifamycin inactivating phosphotransferase family shared by environmental and pathogenic bacteria. *Proc Natl Acad Sci USA* **111:**7102–7107.
46. **Hoshino Y, Fujii S, Shinonaga H, Arai K, Saito F, Fukai T, Satoh H, Miyazaki Y, Ishikawa J.** 2010. Monooxygenation of rifampicin catalyzed by the *rox* gene product of *Nocardia farcinica*: structure elucidation, gene identification and role in drug resistance. *J Antibiot (Tokyo)* **63:**23–28.
47. **Andersen SJ, Quan S, Gowan B, Dabbs ER.** 1997. Monooxygenase-like sequence of a *Rhodococcus equi* gene conferring increased resistance to rifampin by inactivating this antibiotic. *Antimicrob Agents Chemother* **41:**218–221.
48. **Vivrette S, Bostian A, Bermingham E, Papich MG.** 2001. Quinolone induced arthropathy in neonatal foals. *Proc. Am. Assoc. Equine Pract* **47:**376–377.
49. **Jacks SS, Giguère S, Nguyen A.** 2003. *In vitro* susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother* **47:**1742–1745.
50. **Giguère S.** 2006. Antimicrobial drug use in horses, p 449–462. *In* Giguère S, Prescott JF, Baggot JD, Walker RD, Dowling PM (ed), *Antimicrobial Therapy in Veterinary Medicine*, 4th ed. Blackwell Publishing, Ames IA.
51. **Gundelly P, Suzuki Y, Ribes JA, Thornton A.** 2016. Differences in *Rhodococcus equi* infections based on immune status and antibiotic susceptibility of clinical isolates in a case series of 12 patients and cases in the literature. *BioMed Res Int* **2016:**2737295.
52. **Niwa H, Hobo S, Anzai T.** 2006. A nucleotide mutation associated with fluoroquinolone resistance observed in *gyrA* of *in vitro* obtained *Rhodococcus equi* mutants. *Vet Microbiol* **115:**264–268.
53. **Niwa H, Lasker BA.** 2010. Mutant selection window and characterization of allelic diversity for ciprofloxacin-resistant mutants of *Rhodococcus equi*. *Antimicrob Agents Chemother* **54:**3520–3523.
54. **Gudeta DD, Moodley A, Bortolaia V, Guardabassi L.** 2014. *vanO*, a new glycopeptide resistance operon in environmental *Rhodococcus equi* isolates. *Antimicrob Agents Chemother* **58:**1768–1770.
55. **Letek M, González P, Macarthur I, Rodríguez H, Freeman TC, Valero-Rello A, Blanco M, Buckley T, Cherevach I, Fahey R, Hapeshi A, Holdstock J, Leadon D, Navas J, Ocampo A, Quail MA, Sanders M, Scortti MM, Prescott JF, Fogarty U, Meijer WG, Parkhill J, Bentley SD, Vázquez-Boland JA.** 2010. The genome of a pathogenic *Rhodococcus*: cooptive virulence underpinned by key gene acquisitions. *PLoS Genet* **6:**e1001145.
56. **Nordmann P, Nicolas MH, Gutmann L.** 1993. Penicillin-binding proteins of *Rhodococcus equi*: potential role in resistance to imipenem. *Antimicrob Agents Chemother* **37:**1406–1409.
57. **Carlson KL, Kuskie KR, Chaffin KM, Libal MC, Giguère S, Lawhon SD, Cohen ND.** 2010. Antimicrobial activity of tulathromycin and 14 other antimicrobials against virulent *Rhodococcus equi in vitro*. *Vet Ther* **11:**E1–E9.

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Antimicrobial Resistance in *Listeria* Species

11

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Currently, multidrug resistance is not a common feature encountered in *Listeria* species. However, as has been observed with other pathogens of importance to humans, *Listeria* species have the ability to rapidly develop resistance to any antimicrobial agent, a feature that represents an emerging and increasing threat to human and animal health. Isolates of *Listeria* have been reported with varying degrees of resistance to commonly used antibiotics, and the first multidrug-resistant *Listeria* isolate was identified in France in 1988. These isolates developed resistance through a number of well-known mechanisms, including target gene mutations, such as within genes encoding efflux pumps, together with the acquisition of mobile genetic elements. This article will focus, in particular, on describing the mechanisms that confer resistance in *Listeria* species to antibiotics, biocides, and heavy metals.

INTRODUCTION TO THE GENUS *LISTERIA* AND SPECIES

Listeria species are small rod-shaped bacteria, typically 0.5 μm in diameter and 1 to 2 μm in length. They are Gram-positive bacteria, facultative anaerobes, and nonsporulating. They are catalase-positive, oxidase-negative, and motile at low temperatures. They grow at temperatures in the range of 4 to 45°C and pH values of 4.7 to 9.6, and have a low GC content. The genus *Listeria* comprises 17 recognized species: *L. monocytogenes*, *L. ivanovii*, *L. grayi*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. marthii*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae* (Fig. 1) (1). *Listeria* species can be classified into *Listeria sensu stricto* (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, and *L. marthii*) and *Listeria sensu lato* (*L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae*) (1).

Phylogenetic studies indicate that the most closely related genus is *Brochothrix*, and there is a relatedness to other Gram-positive bacteria with low GC content, such as *Bacillus*, *Clostridium*, *Enterococcus*, *Staphylococcus*, and *Streptococcus* (2, 3). Orsi and Wiedmann (1) proposed a reclassification of the *Listeria* genus into four genera, *Listeria*, *Mesolisteria*, *Paenilisteria*, and *Murraya* (Fig. 1). The species that belong to the *Mesolisteria* genus are unable to grow at temperatures below 7°C. *Listeria sensu stricto* species and *L. grayi* possess motility genes that enable them to move, in contrast to the rest of the *Listeria* species.

Listeria species can be classified into serotypes based on the serological reactions of listerial somatic antigen (O-antigen) and flagellar antigen (H-antigen) with specific antisera. *L. monocytogenes* can be differentiated into at least 13 serotypes, and these are divided into 4 lineages, I to IV (Table 1) (4). The majority of *L. monocytogenes* isolates cluster into lineages I and II, described by Piffaretti et al. (5). Lineage IV is the most recently identified, and currently few isolates belong to this lineage (6, 7).

L. monocytogenes is an important facultative human foodborne pathogen, and it is the third leading cause

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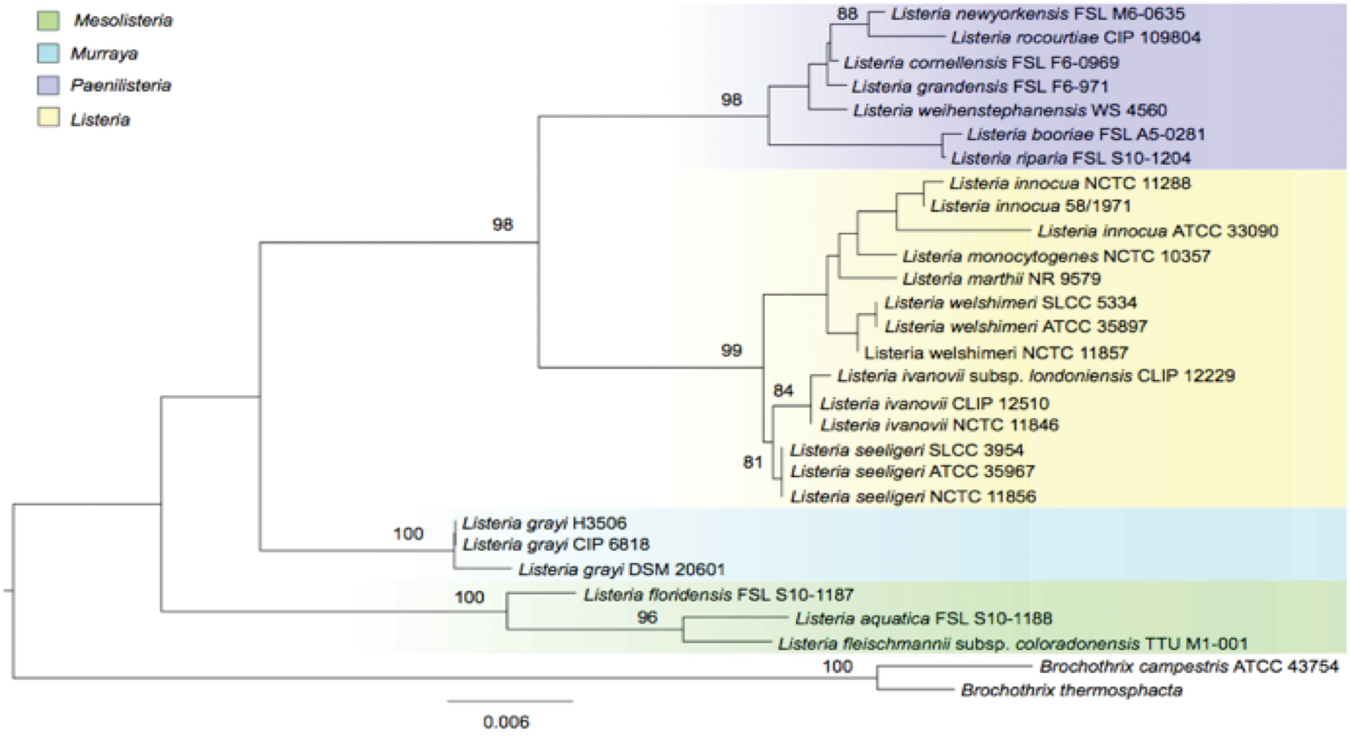


Figure 1 *Listeria* species maximum likelihood phylogenetic tree based on concatenated nucleotide sequences of the 16S rRNA genes from all *Listeria* species. Values on branches represent bootstrap values based on 500 bootstrap replicates; bootstrap values >80% are not displayed. *Listeria* species are color coded according the new genera classification proposed by Orsi et al. (1).

of deaths due to foodborne bacteria the United States (8). *Listeria* outbreaks are distributed globally, causing a significant economic impact on the food industry and public health. This bacterium was first isolated in rabbits and guinea pigs with pronounced monocytosis (9). *Listeria* species are ubiquitous and widespread in the environment. The pathogenicity of *Listeria* species is associated with intercellular replication, and its reservoir extends from the environment to humans and animals, providing an ecological niche (10).

LISTERIA SPECIES IN DOMESTIC ANIMALS AND THE FARM ENVIRONMENT

Listeria species have been isolated from a wide range of domestic and wild animals including mammals, birds, fish, and crustaceans (11, 12). The clinical disease is usually observed in domestic ruminants and humans, though occasionally also in poultry, pigs, rabbits, and other species. Clinical listeriosis in birds is rare, but birds tend to be asymptomatic carriers, because they can ingest this bacterium through contaminated food,

Table 1 *Listeria monocytogenes* lineages and serotype distribution

Lineage	Serotypes	Distribution	Genetic characteristics
I	1/2b, 3b, 4b	Commonly isolated from various sources; overrepresented among human isolates	Lowest diversity among the lineages; lowest levels of recombination among the lineages
II	1/2a, 1/2c, 3a, 3c	Commonly isolated from various sources; overrepresented among food and food-related as well as natural environments	Most diverse, highest recombination levels
III	4a, 4b, 4c	Most isolates obtained from ruminants	Very diverse; recombination levels between those for lineage I and lineage II
IV	4a, 4b, 4c	Most isolates obtained from ruminants	Few isolates analyzed to date

water, bedding, or soil (13). Younger cohorts of bird populations seem to be more susceptible (14). Like rabbits, guinea pigs are also susceptible, and *L. monocytogenes* has also been isolated in canine cutaneous infection (15). There have been reports of symptoms such as miscarriages and septicemia associated with *L. monocytogenes* equine infections (16). The prevalence of *Listeria* in wildlife species ranges from 1 to 60%, although species were mainly captive or domestic (17–19). Less than 1% prevalence was recorded in wild birds and mammals in their natural habitats in Japan (20), while *Listeria* species were recorded in 5% of sampled red fox, beech marten, and raccoon populations in Poland (21). Contact with contaminated silage is believed to be an important transmission vector for livestock infection (22).

Listeria can be classified as a saprophytic bacterium, because its reservoirs are the soil and the intestinal tracts of asymptomatic animals, with soil and fecal contamination resulting in the presence of these bacteria on plants and fodder, particularly silage, along with walls, floors, and drains in the agricultural environment (23, 24).

Evidence indicates that farm systems with cattle species have a higher prevalence of *L. monocytogenes*, including the species that cause human listeriosis, compared to other animals (25). In the addition, it appears that cattle play an important part in the amplification and spread of *L. monocytogenes* in agricultural environments (Fig. 2), while there is evidence of a difference in epidemiology and transmission between ruminant species (25, 26). *L. monocytogenes* can be isolated from cattle farms more frequently during the spring, a feature that may be related to variables including housing with silage feeding during the winter and the application of slurry (27). This would indicate that farmyard environments are reservoirs. The latter feature is not surprising since healthy bovine species with fecal carriage of *L. monocytogenes* as high as 46.3% have been reported (28, 29).

However, the disease ecology caused by *Listeria* species is not fully understood, and fundamental questions regarding the interaction between the hosts, the pathogen, and environment remain to be answered. Specifically, the determinants and mechanisms of transmission from the principal reservoirs, i.e., ruminants, within agricultural ecosystems merit further investigations (30).

OVERVIEW OF THE PATHOGENESIS OF LISTERIOSIS

The genus *Listeria* contains two pathogenic species, *L. monocytogenes* and *L. ivanovii*. However, on rare occasions infections caused by *L. innocua* and *L. seeligeri* have occurred (31, 32). Listeriosis in humans and many vertebrate species, including birds, is caused by *L. monocytogenes*, with *L. ivanovii* infections being specific to ruminants (33). Most infections caused by

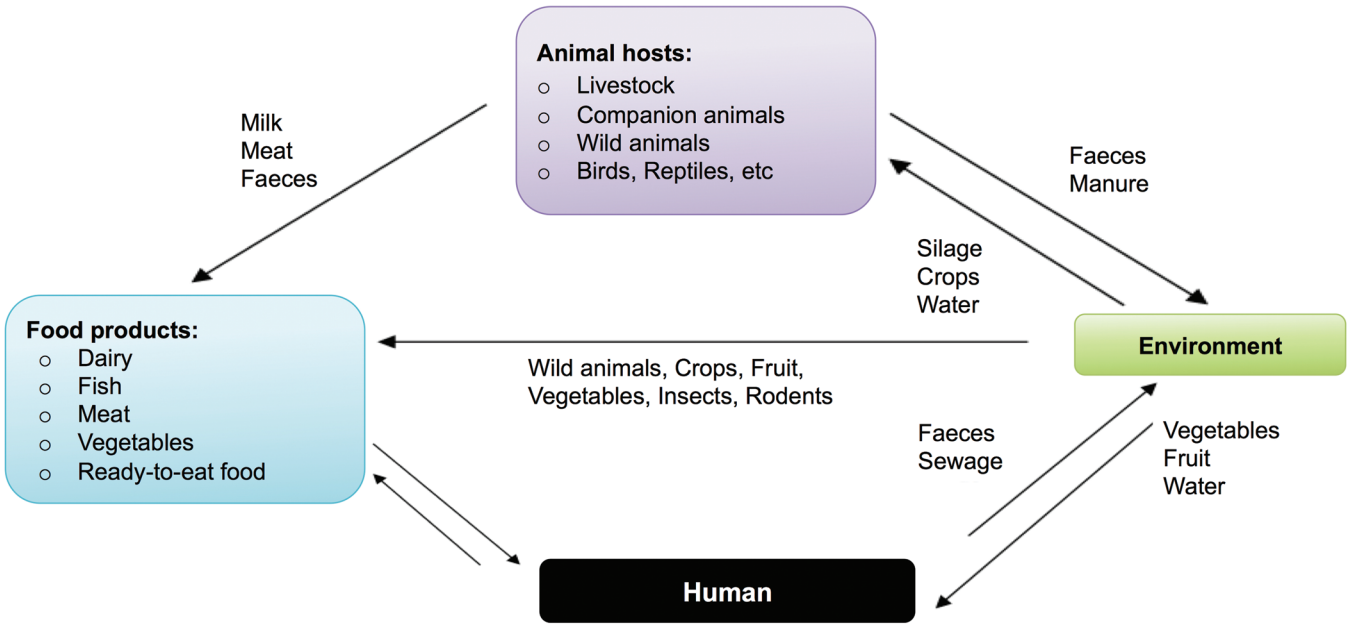


Figure 2 Transmission dynamics of listeriosis involving human and animal hosts. Potential transmission pathways of *Listeria* species are indicated by arrows, and vehicles are represented by colored boxes.

Listeria result from the ingestion of contaminated food such as raw milk, meat, and fish; ready-to-eat meals; unpasteurized dairy products; and uncooked vegetables in humans and from contaminated silage or other sources of feed in animals. The cycle of infection is completed with the liberation of *Listeria* into the environment in the feces. *Listeria* is a saprophyte when it is found in the environment but makes a transition to a physiological state that promotes bacterial survival and replication in host cells (24). *Listeria* has evolved a number of mechanisms to invade and exploit host processes to multiply, spread from cell-to-cell, and evade the immune system without damaging the host cell. The intracellular infectious cycle consists of distinct stages: (i) invasion of the eukaryotic host cell, (ii) escape from the intracellular vacuole, (iii) intercellular proliferation, (iv) actin filament-based intercellular spread, and (v) dissemination to adjacent cells (Fig. 3) (34).

As indicated, *Listeria* is an intracellular pathogen that spreads from cell to cell, and *L. monocytogenes* has evolved mechanisms to evade exposure to the host innate immune defenses. One example of immune evasion mechanisms in *L. monocytogenes* is the *O*-acetyltransferase (*oatA*), which encodes for *O*-acetylation of muramic residues contained in the peptidoglycan. OatA increases the bacterial cell wall resistance to antimicrobial compounds such as lysozyme, thereby favoring bacterial persistence in macrophages and virulence *in vivo* (34). However, the host has evolved innate and acquired immunity mechanisms, including the induction of apoptosis and the generation of cytotoxic T-cells that recognize and lyse listeria-infected cells, releasing bacteria into the extracellular space and rendering them susceptible to infiltrating phagocytes (35). (For a more complete description of the bacterial mechanisms underpinning the pathogenesis and virulence of *Listeria*, the following references 33 and 36–39 provide additional information.)

LISTERIOSIS IN FOOD-PRODUCING AND OTHER ANIMALS AND IN HUMANS

Listeria species have been isolated from more than 50 animal species, both domestic and wild (Table 2), with

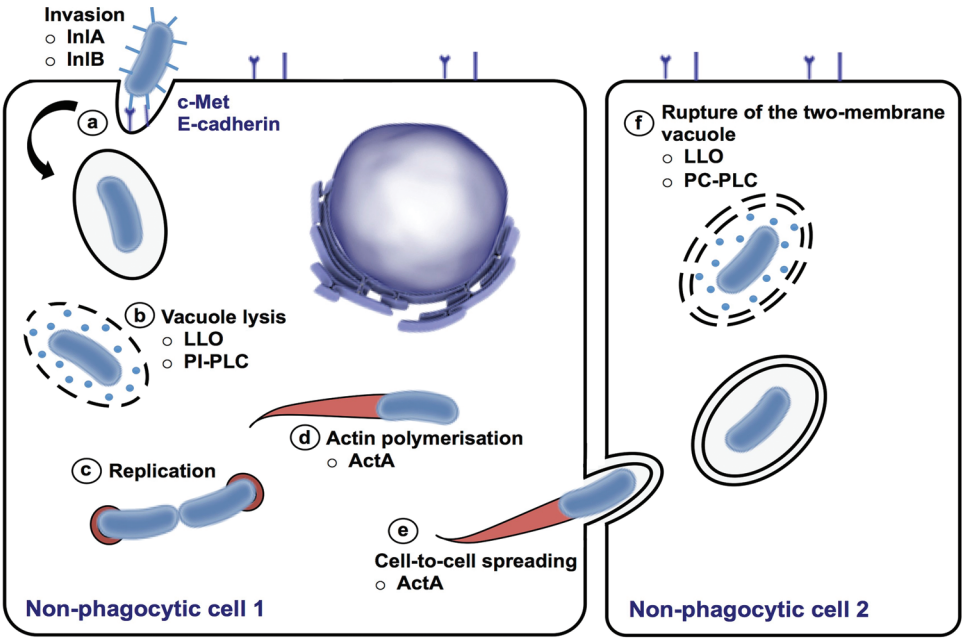


Figure 3 L. monocytogenes *intracellular life cycle*. (a) *Listeria* invades the host cells via a zipper mechanism, by the interaction of surface internalins InlA and InlB with the host cell surface receptors E-cadherin and Met, respectively. (b) *Listeria* escapes from the phagosome before the fusion with the lysosome occurs, by the action of the secreted proteins, the pore-forming toxin LLO, and phosphatidylinositide phospholipase C (PI-PLC). (c) *Listeria* may replicate in the cytosol, and (d) it spreads by actin polymerization, which propels the bacteria unidirectionally, (e) promoting cell-to-cell spreading of *Listeria*. (f) Rupture of the two-membrane vacuole is mainly mediated by the action of LLO and phosphatidylcholine-specific phospholipase C (PC-PLC).

Table 2 Mammals, birds, and other species from which *Listeria* species have been isolated

Mammals	Cats, cattle, chinchillas, deer, dogs, ferrets, foxes, goats, guinea pigs, horses, humans, jackals, lemmings, mice, mink, monkeys, moose, pigs, rabbits, raccoons, rats, sheep, skunks, and voles
Birds	Canaries, chaffinches, chickens, cranes, doves, ducks, eagles, geese, hawks, lorikeets, owls, parrots, partridges, pheasants, pigeons, seagulls, turkeys, white grouse, whitethroat grouse, and wood grouse
Other	Ants, crustaceans, lizards, fish, flies, frogs, snails, ticks and tortoises

presentation of disease varying according to the host (Table 3). Most infections in animals are subclinical, but invasive listeriosis can occur either sporadically or as part of an outbreak (12). Clinical disease is seen most often in domestic ruminants and humans, though occasional cases have been reported in poultry, pigs, rabbits, and other species.

Listeriosis in Food-Producing Domestic Ruminants

In ruminants, infection with *Listeria* and disease are predominantly due to the consumption of spoiled silage contaminated with *L. ivanovii* or *L. monocytogenes*. *Listeria* species can replicate in poorly fermented silage with pH values above 5.5, and these bacteria can reach numbers of up to 10^7 colony-forming units/kg. Other contamination sources apart from silage include barn equipment such as bedding, water, and feeding troughs (40), where bacteria can survive within biofilms. Depending on the number of bacteria ingested, the pathogenic properties of the individual bacterial isolate, and the immune status of the host, listeriosis may present as meningoencephalitis, abortion, or septicemia. It is uncommon for different clinical manifestations to be observed during the same outbreak. The following subsections provide a brief synopsis of the clinical features associated with these presentations.

Meningoencephalitis

Meningoencephalitis (rhombencephalitis) is caused by *L. monocytogenes* and is the most characteristic clinical manifestation of listeriosis in adult ruminants. Indeed, it is among the most common causes of neurological disease in ruminants. A naturally occurring case of ovine meningoencephalitis due to *L. innocua* has also been reported (41). The incubation period is usually 2 to 3 weeks, and the course of the infection is more acute and frequently fatal in sheep and goats (death may occur within 2 to 3 days after the onset of symptoms) but is subacute to chronic in cattle. Once these bacteria cross the intestinal barrier they can gain access to the central nervous system (CNS) via the blood stream. An alternative route to the CNS is through damaged oral, nasal, or ocular mucosas, where the bacterium can track along the trigeminal nerve terminals with subsequent intraneural direct spread to the brain stem and to further areas of the brain via axonal pathways (42). Clinical presentation depends on the localization and distribution of lesions in the nervous system (30). Early and nonspecific symptoms such as depression and anorexia are followed by neurologic signs, often unilateral, such as facial and tongue paralysis with profuse salivation, nystagmus, absence of palpebral reflex, and dropped ear. The syndrome is known as "circling disease" because it is characterized by a lack of coordination, leaning against objects, head deviation sometimes with head tilt, involuntary torticollis, and walking aimlessly in circles as a result of brainstem lesions. In a further development of the infection, prostration is followed by paddling movements and convulsions before death. The course of the infection is short, extending from 1 to 4 days in sheep and from 1 to 2 weeks in cattle.

Abortion

L. monocytogenes and *L. ivanovii* can gain access to the developing fetus via hematogenous penetration of the placental barrier. Vaginal transmission can also occur. The depression of cell-mediated immunity during advanced pregnancy is thought to play an important role in the development of listeriosis (33). The incubation period can be as short as 1 day, and the outcome of the fetal infection is mainly abortion, though cases of stillbirths have been reported. Abortion occurs in the last trimester of gestation (after 7 months in cattle [approximately 9 months gestation] and 12 weeks in sheep and goats [approximately 20 weeks gestation]),

Table 3 *Listeria* species, hosts, and forms of disease

Species	Host	Forms of disease
L. monocytogenes	Cattle, sheep, goat	Encephalitis, abortion, septicaemia, ocular form
	Cattle	Mastitis (rare)
	Cats, dogs, horses	Abortion, encephalitis (rare)
	Pigs	Encephalitis, abortion, septicaemia
	Birds	Septicaemia
L. ivanovii	Cattle, sheep	Abortion
L. innocua	Sheep	Encephalitis (rare)

and abortion storms are more commonly reported in sheep and goats. In both species, the rates of abortion in a group are low but may reach as high as 15%. Mothers have no other clinical signs, with the exception of fever and anorexia, and it is not known yet why these animals never develop CNS infection or overt septicemic disease (33). If the placenta is retained, metritis may result.

Septicemia

The septicemic form of listeriosis is relatively uncommon in adult ruminants and generally, but not invariably, occurs in the neonate as a consequence of *in utero* infection. It is marked by weakness, depression, inappetence, fever, and death. In this clinical form, multiple *foci* of necrosis in the liver and, less frequently, the spleen may be noted at necropsy.

Other presentations

Mastitis has rarely been associated with *L. monocytogenes* infection and is not as frequent as with *Brucella* species, *Mycobacterium bovis*, *Escherichia coli*, *Staphylococcus* species, or *Streptococcus* species. However, subclinical *L. monocytogenes* mastitis is also reported (43). Gastrointestinal infections can occasionally occur in sheep and goats (44), with dullness, inappetence, pyrexia, diarrhea, and death within 24 hours of clinical signs. Unilateral uveitis and keratoconjunctivitis (ocular listeriosis) are also reported in cattle (45) and are attributed to direct ocular contact with contaminated silage.

In addition to the clinical cases of listeriosis, a significant number of animals within a herd may be subclinically infected and excrete *Listeria* species in their feces, a feature that represents a strong risk factor for contamination of the environment and a means by which the bacterium could gain access to the slaughterhouse (46).

Listeriosis in Other Animals

Birds

Birds are usually subclinical carriers of *L. monocytogenes*. Secretions and excretions from colonized birds are rich sources of these bacteria, and they can play a major role in transmission and spread of *Listeria* species to both humans and animals via the ingestion of contaminated food, feed, water, litter, and soil (13). Outbreaks of listeriosis are occasionally observed in the young animals. Predisposing factors include immunosuppression, wet/damp conditions, moist litters, and cold (47). The most frequent form is septicemia occurring with symptoms including depression, listlessness, diarrhea, and emaciation. Peracute deaths occur sometimes without other clinical signs (13). The encephalitic form of listeriosis in birds, meningoencephalitis, is a rare occurrence. However, an outbreak of encephalitic listeriosis in red-legged partridges between 8 and 28 days of age has been recently documented (48). Meningoencephalitis in birds is characterized by incoordination, torticollis, stupor, and paresis or paralysis (49). In young geese, both meningoencephalitis and septicaemia can be seen concurrently. Salpingitis is observed in hens during the acute systemic phase (49).

Rabbits

In rabbits, *L. monocytogenes* usually causes abortion during late pregnancy or sudden death. Encephalitis is rare. *L. monocytogenes* was first isolated by Murray et al. as the etiological agent of septicemia in laboratory rabbits and guinea pigs in England in 1926 (9).

Swine

Swine listeriosis is caused by *L. monocytogenes*, but it is uncommon. The primary manifestation is septicemia in young piglets, with death within 3 to 4 days. Encephalitis in adults and abortions are also seen occasionally.

Others

Septicemia and neurologic signs resembling rabies have been reported in dogs, and *L. monocytogenes* has also been isolated in canine cutaneous infection (15). Rare cases of encephalitis or septicemia occur in cats, with typical nonspecific symptoms including depression, inappetence, abdominal pain, vomiting, and diarrhea. In horses, there have been reports of meningoencephalitis, abortions, intrauterine infection, septicemia, and kerato-conjunctivitis associated with *L. monocytogenes* (16). Occasionally, septicemia disease is the usual form of listeriosis in other species, but abortions can also occur.

Listeriosis in Humans

L. monocytogenes is the most commonly reported etiological agent in cases of human listeriosis, though adult meningitis caused by *L. seeligeri* (31), a fatal bacteremia caused by *L. innocua* (32), and various cases of listeriosis caused by *L. ivanovii* (50) have also been reported. The disease mainly affects pregnant women and immunocompromised individuals by either physiological means—due to an immature (in newborns and young children) or suppressed (in the elderly) immune system—by infectious means (arising from immunosuppressive viruses and advanced systemic pathologies),

or by iatrogenic means (associated with postchemotherapy or pretransplant immunosuppression). Currently, listeriosis is regarded as a foodborne disease of serious public health concern with a high mortality rate (25 to 30%) and a variable incubation period from 3 to 70 days.

In pregnant women, listeriosis is most commonly noted in the third trimester, but cases have been reported at all stages of pregnancy. The main clinical symptoms documented include mild flu-like symptoms, bloody vaginal discharge, and septicemia, and these may result in premature delivery, abortion, or stillbirth (33). A perinatal listeriosis infection occurs when *L. monocytogenes* crosses the placenta and elicits an infection in the fetus, but cross-infection during delivery is also possible (51). Perinatal infection is classified on the basis of the clinical symptoms observed: an early onset of listeriosis occurs in the fetus or neonate within the first week after delivery and is characterized by a serious septicemia (granulomatosis infantisepticum). Late onset occurs within the 2nd to 4th week of life and is manifested as meningitis with hydrocephalus as sequela (52). In immunocompromised adults, listeriosis can manifest as a rare but severe form of infection including septicemia or encephalitis (meningoencephalitis or rhombencephalitis), with symptoms appearing after 2 to 10 weeks of infection (33, 53).

Healthy individuals (who are immunocompetent) rarely develop clinical signs after infection, though a milder gastroenteritis with fever, abdominal and back pain, headache, and myalgia can occur. The incubation period is reported to extend from 8 to 48 h, and symptoms are typically self-limiting and resolve within 1 to 3 days. Direct transmission of *L. monocytogenes* from animals to humans is reported among farmers, veterinarians, and animal handlers who are in frequent direct contact with colonized animals or healthy carriers. A cutaneous form characterized by a nonpainful, nonpruritic pyogranulomatous rash has been reported in individuals who handle infected newborns, fetuses, or aborted cows or who perform necropsies on septicemic animals (54), while cases of conjunctivitis have been reported in workers in poultry processing plants who handled listeria-positive chickens (13).

CHEMOTHERAPEUTIC OPTIONS FOR THE TREATMENT OF ANIMAL AND HUMAN LISTERIOSIS

Cell-mediated immunity is the main host defense strategy against *Listeria* species and is largely dependent on the action of cytotoxic T-cells—hence the association between listeriosis and conditions involving impairments of cell-mediated immunity (55). The intracellular lifecycle of this bacterium confounds the efficacy of antimicrobial chemotherapy together with the lack of development of anti-*Listeria* immunity by the host postinfection. However, an anti-listeriolysin O (LLO) neutralizing monoclonal antibody has been reported to increase host resistance to infection in mouse models (56). Anti-LLO antibodies have been shown to help arrest the growth of *Listeria* species within macrophages, and this approach can be used for the serodiagnosis of these infections in humans and animals (57, 58), though it cannot discriminate between a current or previous infection (59), and furthermore, it provides no indication of the duration of this immunity (46).

Antimicrobial chemotherapy is the only viable option for the treatment of listeriosis. For an antibiotic such as a penicillin-based agent to be effective against this bacterium, it must penetrate into the host cell, maintain a high intracellular concentration, and bind to the penicillin-binding protein 3 (PBP3) expressed by *Listeria* species (60). High drug doses and early treatment are essential to treat animals with encephalitis, though if signs of encephalitis are severe, death usually occurs despite the intervention. Treatment in sheep and goats usually has little value soon after the appearance of neurological signs or in chronic cases. According to *The Merck Veterinary Manual* (54), in cattle, chlortetracycline given at a dose of 10 mg/kg body weight per day and administered for 5 days intravenously is effective in the treatment of encephalitis cases. If penicillin G is used, it should be given intramuscularly at 44,000 U/kg body weight per day for 1 to 2 weeks, and the first injection should be accompanied by the same dose given intravenously. Time durations for antimicrobial treatment may vary according to the level of infection. In severe cases, it is often recommended to continue treatment for up to 1 week after the clinical symptoms have disappeared. Supportive care, including the provision of clean housing along with fluids and electrolytes, is an important part of therapy. High-dose dexamethasone (1 mg/kg, given intravenously) at first examination is considered beneficial by some but is controversial and will cause abortion during the last two trimesters in cattle and after day 135 in sheep. Gentamicin has been found to be effective in the treatment of bovine genital listeriosis (61). Other drugs of choice to treat cases of listeriosis in livestock include erythromycin and trimethoprim/sulfamethoxazole. In birds, penicillin, tetracycline, erythromycin, gentamicin, and trimethoprim/sulfamethoxazole may be used successfully to treat the septicemic forms of infection,

while treatment of the encephalitic form is usually unsuccessful.

Strategies for vaccine development are currently under study, and these are urgently required to tackle the infection in sheep, though advances in these protocols remain to be validated (12).

In humans, there are specific antimicrobial chemotherapeutic strategies to treat listeriosis depending on the individual and the corresponding diagnosis. Ampicillin or penicillin G combined with an aminoglycoside, classically gentamicin, is the most commonly prescribed treatment for listeriosis (62, 63). However, the combination of trimethoprim/sulfamethoxazole can be used as an alternative treatment. A review by Janakiraman et al. (63) provides information on different treatment options for listeriosis in humans.

RESISTANCE OF *LISTERIA* SPECIES TO DIFFERENT ANTIMICROBIAL AGENTS

Antimicrobial agents have been used in a wide range of settings to eliminate or inhibit bacterial growth. Most of these compounds target unique bacterial cell features including cell wall synthesis, the bacterial membrane, particular stages of protein synthesis, DNA and RNA synthesis, and folic acid metabolism, and depending on the nature of the drug, these can result in bacterial cell death or the inhibition of growth (64).

Bacteria possess two types of resistance: intrinsic, or naturally occurring, resistance and acquired resistance via mutations in chromosomal genes and by horizontal gene transfer (65). Intrinsic resistance usually arises as a result of inherent structural or functional characteristics of the microorganism. An example of intrinsic resistance arises due to the lack of affinity of the antimicrobial compound for its bacterial target in *L. monocytogenes*, and this is found in two cases of β-lactam-based compounds, monobactams and broad-spectrum cephalosporins. In these cases, intrinsic resistance is caused by the low affinity of theses drugs for PBP3, the enzyme that catalyzes the final step during cell wall synthesis. Although a few intrinsic resistance mechanisms in *Listeria* species have been described, most cases of resistance to antimicrobial compounds in this bacterium are due to acquired mechanisms, such as mobile genetic elements including self-transferable plasmids and conjugative transposons (66, 67).

Listeria species are well known for their susceptibility to a wide range of antimicrobial agents. Nonetheless, they possess intrinsic or natural resistance to a select number of antimicrobial compounds. Although natural resistance differs among members of the genus *Listeria*, all of the *Listeria* species tested, including *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. grayi*, and *L. seeligeri*, were susceptible or intermediately resistant to aminoglycosides, carbapenems, cefotiam, cefoperazone, first- and second-generation cephalosporins (cefaclor, cefazolin, loracarbef), chloramphenicol, dalfopristin/quinupristin, glycopeptides, lincosamides, macrolides, penicillins (except for oxacillin), and tetracyclines (68). Troxler et al. (68) demonstrated that *L. monocytogenes* and *L. innocua* were naturally resistant to fosfomycin and fusidic acid, whereas other *Listeria* species were found to be naturally resistant only to fusidic acid (Table 4) (68). *Listeria* species differ in their natural susceptibility to co-trimoxazole, fluoroquinolones, fosfomycin, fusidic acid, rifampicin, and trimethoprim (Table 4), while *L. innocua*, *L. ivanovii*, and *L. seeligeri* elaborated a natural resistance to the fluoroquinolones, enoxacin, and sparfloxacin (Table 4) (68).

Listeria species are rarely reported to develop acquired resistance to antimicrobial compounds. Nonetheless, selective pressure imposed in the past following the overuse use of various antimicrobial agents has resulted in members of this genus acquiring target gene mutations and resistance-encoding genes on mobile genetic elements (69). Transfer of resistance genes

Table 4 Intrinsic or natural antibiotic susceptibility and resistance of *Listeria* species

Listeria spp.	Naturally susceptible	Naturally resistant
L. monocytogenes	Trimethoprim	Fosfomycin
	Co-trimoxazole	Fusidic acid
L. innocua	Trimethoprim	Fosfomycin
	Co-trimoxazole	Fusidic acid
		Enoxacin
		Sparfloxacin
L. welshimeri	Trimethoprim	Fusidic acid
	Co-trimoxazole	
L. ivanovii	Fosfomycin	Enoxacin
	Fusidic acid[a]	Fusidic acid[a]
	Trimethoprim	Fleroxacin
	Co-trimoxazole	Pefloxacin
		Sparfloxacin
L. seeligeri	Trimethoprim	Enoxacin
	Co-trimoxazole	Fleroxacin
		Pefloxacin
		Sparfloxacin
		Fusidic acid
L. grayi	All Fluoroquinolones	Fusidic acid
		Trimethoprim
		Co-trimoxazole

[a]Both fusidic acid-susceptible and -resistant *L. ivanovii* isolates occur naturally in the environment.

between *Listeria* species and other bacteria, such as *Enterococcus* and *Streptococcus*, by self-transferable plasmids has also been demonstrated (70).

Multidrug resistance was first documented in *L. monocytogenes* in 1988 in France (71). Since then, other *Listeria* species that are resistant to one or more compounds have been isolated, and several antimicrobial-resistance genes have been identified and characterized (62).

The following section provides a summary of the resistance-encoding genes that have been identified and mutations known to be associated with resistance to different classes of antibiotics, biocides, and heavy metals in *Listeria* species.

Antibiotics

Since the discovery of penicillin in 1929, antibiotics have been commercially used for the treatment of a wide variety of clinical diseases and have also been used for animal production purposes as additives in animal feed (72, 73). Over this period of time, selective pressure arising from the overuse of antimicrobial compounds has led to the emergence of bacteria expressing resistance to one or more of these agents. Similarly, members of the genus *Listeria* have developed mutations in the chromosome and/or acquired resistance genes carried on mobile genetic elements. In contrast and in comparison to other foodborne pathogens of human health importance, *Listeria* species do not exhibit these same features. Thus, data describing antimicrobial resistance in *Listeria* species is limited. Similar to other bacteria, *Listeria* species can express innate resistance to certain classes of antimicrobial compounds. In addition, several resistance genes have been reported to be located on mobile genetic elements such as conjugative transposons and plasmids. Therefore, members of the genus *Listeria* may play a role in the dissemination of resistance genes among bacteria.

Multidrug resistance has been documented in various isolates of *Listeria*, and several antimicrobial-resistance-encoding genes have been identified by means of molecular-based approaches. In the subsections below, a summary of the known mutations and genes associated with resistance to the different classes of antimicrobial agents is provided. The location of resistance genes on mobile genetic elements and the colocation of other markers is discussed.

Resistance to quinolones and fluoroquinolones

Quinolones and fluoroquinolones represent a class of antimicrobial agent which is important for the treatment of severe and invasive infections in animals and humans, with special interest for public and animal health. Quinolones were introduced into clinical use in 1962 in the form of nalidixic acid, and fluoroquinolones were first licensed for veterinary use in several countries in the 1980s (74). Subsequently, their use was followed by the reported emergence of antimicrobial resistance in bacteria. These bacteria were isolated from humans and food-producing animals (69, 75). Resistance to ciprofloxacin is mainly attributed to target gene mutations within the quinolone-resistance-determining regions of genes encoding the bacterial topoisomerase enzymes (76). The presence of plasmid-mediated quinolone-resistance (PMQR)-encoding genes can also contribute to the ciprofloxacin-resistance phenotype (77). Although these PMQR genes confer only low-level resistance to fluoroquinolones, the presence of PMQR (particularly *qnr* genes) may provide a selective advantage for bacteria exposed to fluoroquinolones and facilitate the subsequent development of high-level chromosomal quinolone resistance. In some *Listeria* isolates, the quinolone-resistance-determining region of DNA gyrase subunit A was altered; the deduced amino acid sequences revealed substitutions including Ser84 → Thr and Asp/Glu88 → Phe, both representing amino acid changes at hot spots commonly associated with resistance to these agents (78, 79). Another mechanism, involving efflux pumps such as Lde, MdrL, and FepA (see "Concluding Remarks"), has also been associated with resistance to fluoroquinolones in *Listeria* species (67, 80, 81). Macrolide-based antibiotics and cefotaxime, as well as heavy metals and ethidium bromide, are also exported by MdrL (80).

Resistance to penicillins and cephalosporins

Penicillins and cephalosporins are β-lactam-based antibiotics that inhibit bacterial cell wall synthesis. *Listeria* species are usually susceptible to penicillins, with the exception of oxacillin, monobactams, and broad-spectrum cephalosporins including cefetamet, cefixime, ceftibuten, ceftazidime, cefdinir, cefpodoxime, cefotaxime, ceftriaxone, and cefuroxime, to which this bacterium is naturally resistant (68). Only, the *penA*-encoding gene, a known PBP first identified from *Neisseria meningitides*, has been associated with resistance to penicillin G in *L. monocytogenes* (82). Some of the previously described mechanisms associated with the innate or natural resistance to cephalosporins most often include cell wall-acting gene products, two-component systems, and efflux pumps. The PBPs are enzymes that are responsible for extending the glycan chains in peptidoglycan and cross-linking the peptides between chains. Several PBPs were identified in *Listeria* and function to

confer a natural resistance phenotype to modern cephalosporins (83). The gene *oatA* encodes an *O*-acetyltransferase, which catalyzes the acetylation of muramic acid in peptidoglycan, thus conferring resistance to cefotaxime and gallidermin. OatA is important for pathogenesis in mice, probably via protection from macrophage killing (84). Other mechanisms also confer reduced susceptibility to cephalosporins, including the two-component systems CesR and LiaSR, and efflux pumps (MdrL and AnrAB) (85).

Resistance to aminoglycosides

Aminoglycosides inhibit protein synthesis by binding to the 30S ribosomal subunit. Although more than 170 aminoglycoside-resistance-encoding genes have been described in bacteria, these can be grouped into three major classes: acetyltransferase-acting modifying enzymes, nucleotidyltransferases, and phosphotransferases (86). The streptomycin-resistance gene, *aad6*, which encodes for 6-*N*-streptomycin adenylyltransferase, has been identified in *L. monocytogenes* and *L. innocua* (69, 70). To date, no other aminoglycoside-resistance genes have been described in *Listeria* species.

Resistance to tetracyclines

Tetracycline resistance is the most frequent resistance phenotype detected in *Listeria* species. Tetracyclines inhibit protein synthesis by binding to the 30S ribosomal subunit. More than 50 tetracycline-resistance-encoding genes have been described, and five of these—*tet*(A), *tet*(K), *tet*(L), *tet*(M), and *tet*(S)—have been reported in *Listeria* species. Two known mechanisms of resistance to tetracyclines have been reported in *Listeria* species. These are mechanisms that involve efflux of the drug mediated by proton antiporters, conferring resistance to tetracycline only [including the genes *tet*(A), *tet*(K), and *tet*(L)] and ribosome protection proteins, conferring resistance to both tetracycline and minocycline [encoded by *tet*(M) and *tet*(S)]. Two types of mobile genetic elements, conjugative plasmids and transposons originating from *Enterococcus-Streptococcus*, are thought to be responsible for the emergence of resistance to tetracycline in *Listeria*. The *tet*(M) gene is often associated with the conjugative transposon Tn*916*, while *tet*(S) and *tet*(L) are more often carried on plasmids (87).

Resistance to phenicols

The molecular basis of bacterial resistance to chloramphenicol and its fluorinated derivative florfenicol has been reviewed by van Hoek et al. and Schwarz et al. (88, 89). In *Listeria* species, enzymatic inactivation of this drug by type A chloramphenicol acetyltransferases (Cat) and the export of chloramphenicol/florfenicol by specific efflux proteins are the dominant resistance mechanisms. A *cat* gene (type A-8) has been detected in *Listeria*, located on a plasmid (89, 90). The *floR* gene is associated with the export of florfenicol in *L. monocytogenes*, and 50% of *floR*-positive isolates may also confer resistance to chloramphenicol (82). Florfenicol and chloramphenicol coresistance conferred by *floR* has also been reported in Gram-negative bacteria such as *Pasteurella piscicida* and *E. coli* (82).

Resistance to macrolides (macrolides-lincosamides-streptogramin)

The macrolides (macrolides-lincosamides-streptogramin B [MLS_B]) inhibit protein synthesis through binding to the 50S ribosomal subunit of bacteria. The resistance determinants responsible include rRNA methylases that modify the 23S ribosomal target sites, ATP-binding cassette (ABC) transporters, and efflux proteins of the major facilitator superfamily (MFS), as well as factors such as the *ere*-encoding genes that function as inactivating enzymes. The most common mechanism of MLS_B resistance is due to the presence of rRNA methylases, encoded by the *erm* genes. These enzymes methylate an adenine base, which prevents drug binding to the 50S ribosomal subunit, resulting in MLS_B resistance. There are currently 92 MLS_B resistance genes recognized in bacteria (88), but only *erm*(A), *erm*(B), and *erm*(C) have been reported in *Listeria* species (91, 92). Plasmid pDB2011 from *L. innocua*, isolated from prepackaged sprouts in Switzerland, contained three antibiotic genes: *spc* (spectinomycin-adenyltransferase), *erm*(A) (erythromycin-methylase), and *dfrD* (trimethoprim-dihydrofolate); *spc* was located together with *erm*(A) on the transposon Tn*554* (93). In Gram-positive bacteria, resistance to 14- and 15-membered-ring macrolides, such as erythromycin, can also be mediated by efflux pumps belonging to the ABC transporter family, such as *msr*(A) found in *Staphylococcus* species, or to the MFS *mef*(A) found in *Streptococcus pneumoniae* (69, 92). Although several studies have investigated the possible presence of *mef*(A) and *msr*(A) in *Listeria* species, further studies are necessary to extend our knowledge of the possible role of these efflux pumps in *Listeria*.

Resistance to trimethoprim

Trimethoprim is a folate pathway inhibitor, and folic acid is essential for the synthesis of adenine and thymine, two of the four bases that are involved as structural components of nucleic acids. Trimethoprim resistance has been described in *L. monocytogenes* due to the *dfrD*

resistance-encoding gene. High-level trimethoprim resistance in *Listeria* is mainly due to the replacement of a trimethoprim-sensitive dihydrofolate reductase by a plasmid-, transposon-, or cassette-borne version. To date, more than 40 trimethoprim-resistant dihydrofolate resistance-mediating reductase (*dfr*) genes have been identified (94). Initially, the *dfr* genes were classified into two major families according to their amino acid structure, *dfrA* and *dfrB*, and currently, six plasmid-mediated families can be distinguished in Gram-positive bacteria, including *dfrC*, *dfrD*, *dfrG*, and *dfrK* (88).

In *Listeria*, only two of these—*dfrG* and *dfrD*—have been reported. The *dfrD* gene was found to be encoded on the plasmid pIP823 (95), while the *dfrG* gene was recently found in Tn*6198*, a Tn*916*-like element associated with a trimethoprim-resistance gene (96).

Biocides

Biocides have been widely used to control the microbial ecology of various niches for centuries. European Union regulation no. 528/2102, covering biocidal products (97), divides biocides into four categories: disinfectants, preservatives, pest control agents, and other biocidal products. These compounds are used in a wide variety of settings including the food and cosmetic industries, for personal hygiene, and for applications in veterinary practice and farming (98). Currently, there is a diverse range of chemicals including alcohols, biguanides, bisphenols, chlorine-releasing agents, iodophors, peroxides, and quaternary ammonium compounds (QACs), among others, that constitute biocides. Reduced susceptibility to these agents has been recognized for decades and is increasing (98, 99).

This section provides a summary of the known mechanisms of resistance or tolerance of *Listeria* species.

Quaternary ammonium compounds

QACs are amphoteric surfactants with a broad spectrum of antimicrobial activity and are widely used as disinfectants in clinical, domestic, and environmental settings (100). The most commonly used QAC is benzalkonium chloride (BC), also known as alkyl dimethyl benzyl ammonium chloride, which targets cytoplasmic membrane permeability functions, causing potassium leakage, osmoregulation disruption, enzyme inactivation, and protein denaturation (101, 102). Tolerance to BC was first described in *L. monocytogenes* and *L. innocua* isolates in 1998. BC tolerance may be conferred by transposons or efflux pumps encoded on the chromosome or on plasmids. One such tolerance mechanism previously described in *L. monocytogenes* was associated with the *bcrABC* operon, which can be encoded in the chromosome or on a putative IS*1216* composite transposon harbored by the large plasmid pLM80 (103, 104). The *bcrABC* cassette is composed of a TetR-like transcriptional regulator (*bcrA*) and two small multidrug resistance (SMR) efflux pump-encoding genes (*bcrB* and *bcrC*) (103, 104). Another resistance mechanism identified in *L. monocytogenes* is the chromosomally integrated transposon Tn*6188*, which is related to other Tn*554*-like transposons. Transposon Tn*6188* is a 5,117-bp structure found integrated within the *radC* gene, and it consists of three transposase genes (*tnpABC*), along with genes encoding a putative transcriptional regulator and QacH, an SMR transmembrane protein associated with the export of BC (105, 106). The *qacH* gene also confers tolerance to a wide range of QACs including BC, benzethonium chloride, cetylpyridinium chloride monohydrate, cetyltrimethylammonium bromide, domiphen bromide, dodecyltrimethylammonium bromide, ethidium bromide, and the sanitizer Weiquat, resulting in higher MIC values being recorded as well as in increased expression of the transporter (106). Although Tn*6188* has been widely identified in *L. monocytogenes*, Tn*6188* has not been identified in other *Listeria* species to date. The putative EmrE SMR-related efflux pump located within Listeria genomic island 1 (LGI1) in *L. monocytogenes* can also confer tolerance to BC and QAC-based sanitizers (107). There are two MFS efflux pumps, MdrL and Lde, that have also been reported to be partially responsible for BC tolerance in *L. monocytogenes* (108–110).

Triclosan

Triclosan is a chlorinated aromatic compound with a broad spectrum of antibacterial and antifungal activity, which has been used for over 40 years as an antiseptic, disinfectant, and preservative in clinical and cosmetics settings (111, 112). It is bacteriostatic in nature at low or sublethal concentrations, acting to inhibit a specific enzyme, FabI, an enoyl-acyl carrier protein reductase linked to fatty acid biosynthesis, (113–116). Inhibition of FabI results in reductions in lipid biosynthesis (117). At high or bactericidal concentrations, triclosan has a more general membrane-disrupting action, consistent with potassium leakage from the cell (118, 119). It has been shown that point mutations and overexpression of the *fabI*-encoding gene conferred tolerance to triclosan in some bacteria, but not in *Listeria* species (116, 120, 121). Exposure of *L. monocytogenes* to sublethal concentrations of triclosan can cause cross-resistance to several aminoglycosides and exhibit two types of colony morphology: normal-size and pinpoint colonies.

This study demonstrated that adaptation to triclosan may be due to point mutations in several genes including ferrochelatase (*hemH*) or glutamyl tRNA reductase (*hemA*) (122).

Heavy Metals

From a saprophytic bacterium (all *Listeria* species) to intracellular pathogen (*L. monocytogenes* and *L. ivanovii*), maintaining heavy metal homeostasis is central to the survival of *Listeria* species across a broad range of environmental niches. Many heavy metals, such as iron and zinc, are an essential component of cellular function, and bacteria compete to sequester them using a variety of mechanisms such as siderophores and ATP-transporters. As heavy metal concentrations increase, associated toxicity can lead to cell death. Many factors can lead to an increase in heavy metal concentration in niches associated with *Listeria* species. The use of fertilizers or industrial processing can contaminate soil or water environments with heavy metals to levels which can be toxic to bacteria. Similarly, for pathogenic *Listeria* species, both heavy metal scavenging and resistance are necessary for survival and infection. It is becoming clear that heavy metals are an integral part of the host immune defense, either through host sequestering to deny availability or by exploiting their toxicity to kill invading pathogens (123–125).

Balancing the intracellular concentration of heavy metals is thus a matter of life or death for bacteria such as *Listeria* species. Studies investigating heavy metal resistance (HMR) in *Listeria* largely focus on *L. monocytogenes*, although these resistance mechanisms are often found in other *Listeria* species (126, 127). While much progress has been made in identifying mechanisms utilized by *Listeria* in resistance to certain heavy metals (e.g., cadmium and arsenic), less is known about many others (e.g., mercury and lead).

Many genes for HMR in bacteria are contained on mobile genetic elements such as plasmids and transposons (127–129), and as such, they are often found in association with a variety of other genetic markers, some of which may confer resistance to other antimicrobial agents or environmental stressors. Perhaps the most significant implication of this is the possibility of one resistance phenotype coselecting for another (130). Although mobile genetic element-mediated antibiotic resistance has not been frequently reported in *Listeria*, the potential coselection of *Listeria* plasmids containing multiple resistance markers has been reported (127, 131). In particular, heavy metal-mediated coselection of environmental stress resistance markers may contribute to survival and persistence of *Listeria* species in various environmental niches. Coselection of HMR plasmids may be elicited by a number of factors, including the use of heavy metals in agriculture in fertilizers or supplemented into animal diets as growth promoters or therapeutics (132, 133), heavy metal discharge in industrial wastewater effluent (134), and global mining activities (135). In addition, genetic markers of HMR may be associated with genes coding for disinfectant-resistance determinants (136); as a result, the use of disinfectants in food processing environments, which are frequently colonized by *Listeria* species (137, 138), may also serve to coselect for HMR (139). A multipronged approach will thus be necessary in any effort designed to limit the maintenance and spread of HMR-mediating mobile genetic elements in bacterial populations including *Listeria* species and others.

Cadmium resistance

Cadmium is not essential for the growth or survival of *Listeria* species, and it can be toxic to bacteria at low levels (140). Of all the heavy metals, cadmium-resistance phenotypes and mechanisms are among the most extensively studied in *L. monocytogenes* (136, 139, 141, 142). Resistance to cadmium was first correlated with plasmid carriage, with a *cadAC* operon being subsequently identified as the resistance determinant (143, 144). Efflux of Cd^{2+} is driven by the *cadA*-encoded energy-dependent pump, while *cadC* serves as a negatively acting regulatory protein of the operon (145, 146). The *Listeria cadAC* genes were first identified in a plasmid-borne Tn*5422* transposon and were initially thought to be more prevalent among serotype 1/2 isolates (147). Recent studies have shown a more uniform distribution across serotype 1/2 and 4 isolates (148). Based on the frequency of plasmid carriage, plasmid profiling along with cadmium and arsenic-resistance phenotyping and serotyping has been suggested as a simpler means of subtyping *L. monocytogenes* isolates with acceptable discriminatory power (147). Variations of *cadA* described in *Listeria* include *cadA1*, the Tn*5422* determinant described above, the *cadA2* gene identified on plasmid pLM80 (149), *cadA3* harbored by the EGD-e strain (3, 150), and *cadA4* located on the chromosome of the outbreak strain ScottA (Fig. 4) (151).

Arsenic resistance

Like cadmium, arsenic is also a nonessential heavy metal for bacterial growth and metabolism but becomes toxic as concentrations increase. Arsenic contamination of soil and water environments can arise from a number of sources including mining, industrial processing, and geothermal activity (152). Organic

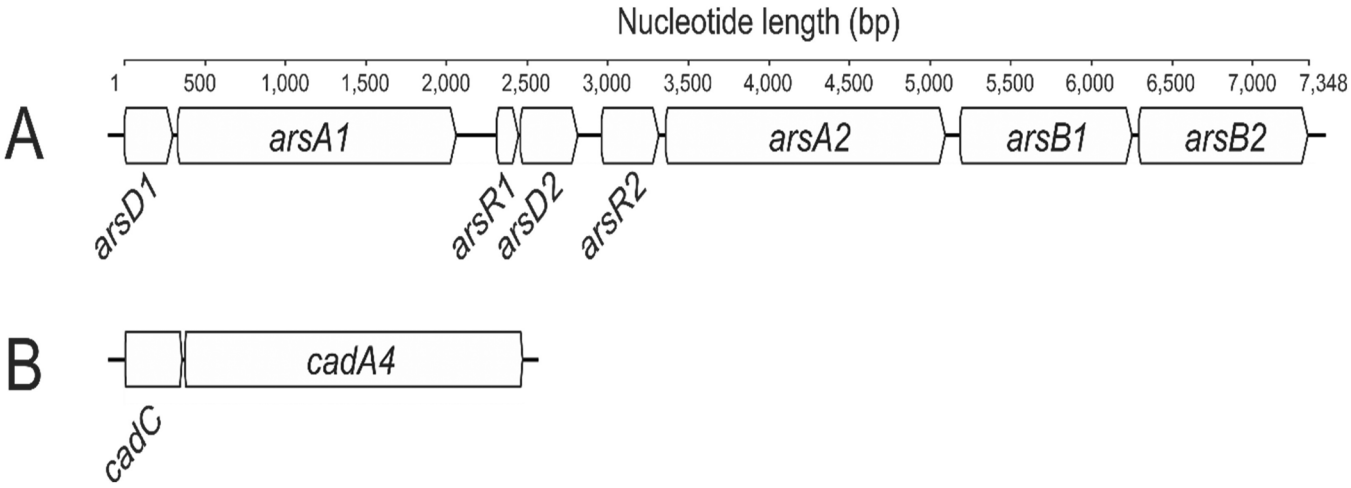


Figure 4 Heavy metal resistance operons in the *L. monocytogenes* strain ScottA. (A) Arsenic resistance operon. (B) *cadAC* cadmium resistance operon.

arsenic is less toxic than the inorganic form, and the use of arsenic has applications in medicine, pest control, and organic arsenic-based drugs. Organoarsenates have also been used as growth enhancers in food production systems (153, 154). Arsenic resistance in *Listeria* has been well characterized, and an arsenic-resistance cassette initially identified in *L. innocua* CLIP11262 on plasmid pLI100 has subsequently been identified in a number of *L. monocytogenes* isolates (Fig. 4) (127, 141, 151). Although harbored on plasmid pLI100, arsenic resistance in *L. monocytogenes* isolates to date typically show chromosomal association (141, 147). In contrast to cadmium resistance, which does not appear to have serotype-specific association, arsenic resistance has been primarily associated with serotype 4b isolates (139, 147). The use of organoarsenates in intensive poultry food production systems has also been implicated in selecting for arsenic resistance in *Listeria* species (139).

Zinc resistance

Unlike cadmium and arsenic, zinc is an essential micronutrient for *Listeria* species and is involved in diverse functions such as metabolism, cell division, and stress resistance (155). Two principle transport mechanisms responsible for sequestering zinc have been characterized in *Listeria*: the *zurLAM* and the *zinABC* operons (155). As with other heavy metals, however, zinc becomes toxic to *Listeria* species as the concentration increases. Elevated zinc levels are utilized in the host response to pathogen infection, and previous work has shown that this is associated with a bacteriostatic effect on *L. monocytogenes* (124, 156). Unlike other bacteria, *cadA* has not been shown to contribute to zinc resistance in *Listeria* species (128, 144).

Copper resistance

Although copper plays an important role in cellular function, *Listeria* species must maintain copper homeostasis to combat cytotoxic effects due to elevated concentrations (155). The *csoR-copA-copZ* operon confers copper resistance in *L. monocytogenes* (157). Other genetic markers implicated in copper resistance include the copper transporter CptA and a CutC homolog encoded by *lmo1018* (155, 157). Copper is used as a growth promoter in food animals and, as such, has implications for selection of copper-resistant *Listeria* species; future work should address the implications of this selection pressure on coselection of other resistance markers.

MOBILE GENETIC ELEMENTS ASSOCIATED WITH ANTIMICROBIAL RESISTANCE IN *LISTERIA* SPECIES

DNA elements that can transfer within a genome or to another genome are generally referred to as mobile genetic elements. Transposons and plasmids are among the most commonly described mobile genetic elements in *Listeria* species and have been identified as one of the most important driving forces underlying the evolution of the species (158). As knowledge of the diversity of these mobile genetic elements increases, it is becoming apparent that they are frequently associated with a plethora of resistance phenotypes. Their dissemination through the *Listeria* population raises a number of concerns for food safety and public health; mobile genetic

elements contributing to resistance of *Listeria* strains to disinfection regimes utilized in food processing have already been described, and reports are now emerging of resistance to clinically relevant antibiotics (96, 103, 105).

Transposons

Transposons are an important mechanism for the transfer of resistance markers through bacterial populations. A number of transposons and their insert sites (both chromosomal and plasmid) have been identified among *Listeria* species (129, 158), harboring resistance markers to different classes of antimicrobial agents, some of which include the transposon Tn*917*, which is implicated in cadmium resistance and is closely related to another plasmid-borne Tn*5422* cadmium-resistance transposon (144, 159), the Tn*554*-like transposon, which carries an arsenic-resistance operon (158), a Tn*6188* transposon (also a Tn*554*-like transposon), which carries the *qacH* gene and confers increased resistance to QACs, a *bcrABC* resistance cassette, which harbors an IS*1216* composite transposon, a Tn*916* to Tn*1545* family conjugative transposon associated with tetracycline resistance and which contains *tet*(M) (160), and a Tn*6198* multidrug-resistance transposon that confers resistance to tetracycline and trimethoprim (96).

Previous studies have noted the capacity for the transfer of chromosomal-located resistance determinants between *Listeria* species as well as other genera (96, 161), highlighting the potential for proliferation of antimicrobial resistance in *Listeria* species populations. The recent increase in whole-genome sequencing of *Listeria* genomes will undoubtedly expand our understanding of the repertoire of transposable elements present in *Listeria* species and provide insights into their putative transfer patterns. Such knowledge will be crucial in directing future control strategies aimed at tackling the issue of horizontal gene transfers among *Listeria* species.

Plasmids

Maintaining plasmids can impose a fitness cost on the host bacterium, which could lead to that host being outcompeted by other bacteria, in the absence of a selective pressure (162). However, resistance plasmid carriage is widespread in bacterial species, including subgroups of *Listeria* species (147, 163). Recent advances in understanding the factors underlying their ubiquity among bacterial populations have shown that very low levels of antimicrobial agent, often significantly below the MIC, can select for their carriage (164). This is further exacerbated by coselection, because plasmids often confer resistance to many different antimicrobial compounds, such as antibiotics, heavy metals, or biocides, and thus are maintained in bacterial populations by a multitude of selective pressures (103, 165).

Plasmids sharing replication mechanisms generally cannot exist together in the same host, which is often referred to as plasmid incomparability, and this feature forms the basis of incompatibility (Inc) grouping. Although commonly used for classification of Gram-negative plasmids, its use is less common among Gram-positive bacteria (166, 167). Studies relating to *Listeria* species plasmids suggest two distinct groupings based on their associated replicon and genetic diversity (127). Few instances of *Listeria* species harboring more than one plasmid have been reported (168), but more research is needed to understand the dynamics of plasmid carriage and incompatibility in *Listeria* species.

HMR markers are among the most commonly described with regard to *Listeria* plasmids, particularly cadmium-resistance genes (129, 144, 169). These plasmids frequently harbor other resistance markers associated with biocide and/or stress resistance, as well as efflux systems (127, 129, 136). These markers may contribute to the survival and persistence of *Listeria* species in food processing environments and, as such, present a significant concern for food safety and public health (127, 131). Another intriguing observation is the conservation of identical or similar plasmids among isolates from geographically diverse locations, suggesting that strong selective pressures are maintaining plasmid carriage among *Listeria* species (131).

Although the frequency of antibiotic resistance among *Listeria* species has been reported to be low (69, 170), plasmid carriage of resistance-encoding mechanisms to antibiotics including chloramphenicol, erythromycin, and tetracycline has been reported (71, 171, 172). In addition, a recently identified multidrug-resistance plasmid in *L. innocua* conferring resistance to both trimethoprim and spectinomycin exhibited a broad host range (93). The emergence of such resistance mechanisms highlights the need for increased surveillance efforts to attempt to minimize the risk of emergence of clinically relevant antimicrobial resistance in *Listeria* species and in *L. monocytogenes* in particular.

EFFLUX PUMPS ASSOCIATED WITH ANTIMICROBIAL RESISTANCE IN *LISTERIA* SPECIES

Efflux pumps are transmembrane-spanning protein complexes capable of transporting a broad range of chemically and structurally unrelated substrate molecules from inside the bacterial cell to the extracellular matrix

(173). Bacterial efflux pumps have been classified into five families: the ABC superfamily, the multidrug and toxic compound extrusion family (MATE), the MFS, the SMR family, and the resistance-nodulation-division superfamily (174). The most commonly identified efflux pumps found in Gram-positive bacteria are those represented by the MFS family, in contrast to those of the resistance-nodulation-division family being found in Gram-negative bacteria. The latter family is rarely reported in Gram-positive bacteria. All of these systems harness the proton motive force as an energy source, with the exception of the ABC transporters, which use direct ATP hydrolysis to drive the transport of substrates (Fig. 5) (175). In the past few years, several efflux pumps have been described in *L. monocytogenes*.

The first efflux pump to be described in *L. monocytogenes* was *cadAC*. Shortly thereafter, an MFS efflux pump, denoted MdrL, was described in *L. monocytogenes*, and it was reported to export cefotaxime, ethidium bromide, heavy metals, and macrolides (Table 5) (80). Several subsequent studies have also reported on the role of MdrL in the adaptation of *L. monocytogenes* to BC (108, 109). *L. monocytogenes* encodes two efflux pumps that are highly similar to QacA in *Staphylococcus aureus*, and they were identified as MdrT and MdrM. Both MFS efflux pumps confer resistance to cholic acid (176, 177). MrdT and MdrM also play a role during bacterial replication within the cytosol of infected cells, by secreting cyclic-di-AMP, which triggers the production of type I interferons, including beta interferon, which promotes *L. monocytogenes* virulence (178).

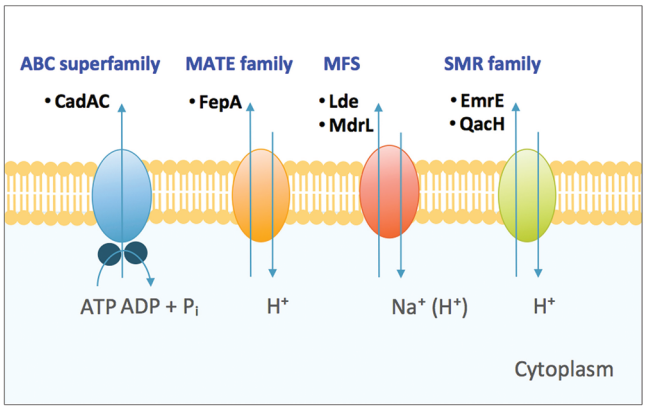


Figure 5 *Diagrammatic representation of the four families of efflux pumps in* L. monocytogenes. The ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic-compound extrusion (MATE) family, and the small multidrug resistance (SMR) family. Common examples of the individual proteins that form each class of efflux pump are shown.

Table 5 Multidrug efflux transporters characterzsed in *L. monocytogenes*

Gene	Efflux pump	Regulator	Substrates
lmo1409	MdrL	LadR	Ethidium bromide
			Macrolides
			Cefotaxime
			Heavy metals
			Benzalkonium chloride
lmo1617	MdrM	MarR	Rhodamine 6 G
			Cholic acid
lmo2588	MdrT	BrtA[a]	Tetraphenylphosphonium
			Cholic acid
			Bile
lmo2741	Lde	Unknown	Ciprofloxacin
			Norfloxacin
			Ethidium bromide
			Acridine orange
			Benzalkonium chloride
lmo1851	EmrE	Unknown	Benzalkonium chloride
lmo2089	FepA	FepR[a]	Norfloxacin
			Ciprofloxacin
			Ethidium bromide
lmo2115	AnrB	VirR	Nisin
			Bacitracin
			Gallidermin
			Cefuroxime
			Cefotaxime
			Ampicillin
			Penicillin
cadAC	CadAC	CadC	Cadmium
			Benzalkonium chloride[a]
			Arsenic
bcrABC	BcrBC	BcrA[a]	Benzalkonium chloride
qacH	QacH	TetR	Benzalkonium chloride
			Benzethonium chloride
			Cetylpyridinium chloride monohydrate
			Cetyltrimethylammonium bromide
			Domiphen bromide
			Dodecyltrimethylammonium bromide
			Ethidium bromide

[a]Belongs to the TetR family.

Two efflux pumps that confer resistance to fluoroquinolones have been characterized in *L. monocytogenes*, Lde and FepA. The former is encoded by the *lde* gene, whose protein product is a 12-segment transmembrane-spanning putative MFS efflux pump with 44% amino acid identity with PmrA from *S. pneumoniae*. Lde was characterized in *L. monocytogenes* CLIP 21369 by insertional inactivation of the *lde* gene, and these data demonstrated that this pump conferred resistance to ciprofloxacin and norfloxacin together with a reduced susceptibility to the dyes acridine orange and ethidium bromide (Table 5) (67, 179). Other studies have also suggested that *lde* may be partly responsible for BC tolerance (110). Lde has other interesting functions, because it may also cooperate with a eukaryotic multidrug resistance-related protein (MRP)-like efflux transporter to reduce the activity of ciprofloxacin, a substrate for both pumps, in J774 macrophages infected with *L. monocytogenes* (174, 180). Furthermore, the *L. monocytogenes in vivo*-induced virulence factor Hpt mediates uptake of fosfomycin in *L. monocytogenes*, thereby conferring a resistant phenotype *in vitro* and thus constituting an antibacterial *in vitro-in vivo* paradox, since the bacteria are resistant *in vitro* but susceptible to the drug *in vivo* (174, 181).

Another mechanism that may confer resistance to fluoroquinolones in *L. monocytogenes* is linked with the overexpression of a multidrug and toxic compound extrusion efflux pump, FepA. It has been demonstrated that a single point mutation in the FepA transcriptional regulator *fepR*, which belongs to the TetR family, produces a frameshift mutation that causes the introduction of a premature stop codon, resulting in an inactive truncated protein. This single point mutation was responsible for the overexpression of *fepA*, which confers resistance to ciprofloxacin, ethidium bromide, and norfloxacin, thereby confirming the role of FepR as a local repressor of *fepA* (Table 5) (81).

AnrB, an ABC transporter, confers innate resistance of *L. monocytogenes* to the lantibiotic nisin. The AnrB function was demonstrated by a nonpolar deletion mutation in the *lmo2115* gene, and the loss of this multidrug transporter increased susceptibility to bacitracin, β-lactams, gallidermin, and nisin (Table 5) (182).

In 2015, a novel putative efflux pump, EmrE (324 bp), was described within LGI1 in *L. monocytogenes* (107). An *emrE* deletion mutant demonstrated an increase in the susceptibility to BC along with QAC-based sanitizers but exhibited no effect on the MICs for acriflavine, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, tetracycline, and triclosan (Table 5) (107).

CONCLUDING REMARKS

Listeria species are ubiquitous throughout the food chain and the natural environment, and these microorganisms have been isolated from a wide variety of domestic and wild animals. Since 2009, nine new *Listeria* species have been discovered, and these remain to be further characterized.

Listeriosis may manifest in a mild form, presenting with gastroenteritis-like symptoms, or in a more severe form where *Listeria* crosses the blood-brain barrier and placenta, invading the CNS and the fetus. Antibiotic treatment strategies for listeriosis vary according to the infection type and the nature of the infected host. In humans, ampicillin or penicillin G in combination with an aminoglycoside is regarded as the front-line treatment (63). The primary antibiotics used to treat listeriosis in animals are chlortetracycline, penicillin, tetracycline, erythromycin, gentamicin, and trimethoprim/sulfamethoxazole (61). Although the incidence of antibiotic resistance in *Listeria* species remains low, multidrug-resistant *Listeria* species strains have been isolated from numerous sources including clinical isolates, retail foods, and the environment (69, 92, 183). Furthermore, the range of antibiotics to which resistance has been acquired is broad and includes compounds currently used to treat listeriosis, such as ampicillin, penicillin, trimethoprim, tetracycline, and erythromycin, among others (88, 183). Resistance to erythromycin, rifampicin, trimethoprim, and especially, to tetracycline has been commonly reported in *Listeria* species from Europe and North America and is consistent with the identification of *tet*-, *erm*-, *aad*-, and *dfr*-encoding genes (79). This development may be problematic for patients who are allergic to penicillin because the combination of trimethoprim and sulfamethoxazole is used for the treatment of listeriosis (63).

Multidrug resistance in *Listeria* species has also been linked to the presence of efflux pumps, which confer resistance to a wide variety of antimicrobials, including antibiotics, biocides, heavy metals, and other antimicrobial compounds. Lde and AnrB confer resistance to fluoroquinolones, whereas EmrE, BcrABC, and QacH confer tolerance to BC. Tolerance to biocides is of special concern in food-producing plants and farms, because these agents are used as sanitizers and their decreasing efficacy hinders the elimination of *Listeria* from these environments, increasing the risk of cross-contamination to the final product or animals. Efflux pumps and antimicrobial-resistance genes can be encoded chromosomally or on mobile genetic elements, e.g., plasmid pDB2011 from *L. innocua*, which contains three antibiotic resistant genes, *spc*, *erm*(A), and

dfrD, and confers resistance to spectinomycin, erythromycin, and trimethoprim, and Tn*6188*, which encodes for the *qacH* gene and confers resistance to QACs. Furthermore, *Listeria* can acquire antimicrobial genes from foreign sources by self-transferable plasmids or conjugative transposons from other bacterial species such as *Enterococcus* and *Streptococcus* (62, 170, 183).

Despite the ever-increasing threat of antimicrobial resistance to human health and the fact that *Listeria* is widespread in the environment and agriculture, relatively little research has been focused on this topic to date.

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References

1. **Orsi RH, Wiedmann M.** 2016. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Appl Microbiol Biotechnol* **100:**5273–5287.
2. **Feresu SB, Jones D.** 1988. Taxonomic studies on *Brochothrix*, *Erysipelothrix*, *Listeria* and atypical lactobacilli. *J Gen Microbiol* **134:**1165–1183.
3. **Glaser P, Frangeul L, Buchrieser C, Rusniok C, Amend A, Baquero F, Berche P, Bloecker H, Brandt P, Chakraborty T, Charbit A, Chetouani F, Couvé E, de Daruvar A, Dehoux P, Domann E, Domínguez-Bernal G, Duchaud E, Durant L, Dussurget O, Entian KD, Fsihi H, García-del Portillo F, Garrido P, Gautier L, Goebel W, Gómez-López N, Hain T, Hauf J, Jackson D, Jones LM, Kaerst U, Kreft J, Kuhn M, Kunst F, Kurapkat G, Madueno E, Maitournam A, Vicente JM, Ng E, Nedjari H, Nordsiek G, Novella S, de Pablos B, Pérez-Diaz JC, Purcell R, Remmel B, Rose M, Schlueter T, Simoes N, et al.** 2001. Comparative genomics of *Listeria* species. *Science* **294:**849–852.
4. **Orsi RH, den Bakker HC, Wiedmann M.** 2011. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *Int J Med Microbiol* **301:**79–96.
5. **Piffaretti JC, Kressebuch H, Aeschbacher M, Bille J, Bannerman E, Musser JM, Selander RK, Rocourt J.** 1989. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. *Proc Natl Acad Sci USA* **86:**3818–3822.
6. **Roberts A, Nightingale K, Jeffers G, Fortes E, Kongo JM, Wiedmann M.** 2006. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. *Microbiology* **152:**685–693.
7. **Ward TJ, Ducey TF, Usgaard T, Dunn KA, Bielawski JP.** 2008. Multilocus genotyping assays for single nucleotide polymorphism-based subtyping of *Listeria monocytogenes* isolates. *Appl Environ Microbiol* **74:**7629–7642.
8. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States: major pathogens. *Emerg Infect Dis* **17:**7–15.
9. **Murray EGD, Webb RA, Swann MBR.** 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). *J Pathol Bacteriol* **29:**407–439.
10. **Walland J, Lauper J, Frey J, Imhof R, Stephan R, Seuberlich T, Oevermann A.** 2015. *Listeria monocytogenes* infection in ruminants: is there a link to the environment, food and human health? A review. *Schweiz Arch Tierheilkd* **157:**319–328.
11. **Lyautey E, Hartmann A, Pagotto F, Tyler K, Lapen DR, Wilkes G, Piveteau P, Rieu A, Robertson WJ, Medeiros DT, Edge TA, Gannon V, Topp E.** 2007. Characteristics and frequency of detection of fecal *Listeria monocytogenes* shed by livestock, wildlife, and humans. *Can J Microbiol* **53:**1158–1167.
12. **Lopez J.** 2008. *Listeria monocytogenes*, p. 1238–1254. *In* OIE Biological Standards Commission (ed), *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, **vol 2**, 6th ed. World Organisation for Animal Health (OIE), Paris, France.
13. **Dhama K, Karthik K, Tiwari R, Shabbir MZ, Barbuddhe S, Malik SVS, Singh RK.** 2015. Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: a comprehensive review. *Vet Q* **35:**211–235.
14. **Gray ML.** 1958. Listeriosis in fowls: a review. *Avian Dis* **2:**296.
15. **Loncarevic A, Artursson, Johansson.** 1999. A case of canine cutaneous listeriosis. *Vet Dermatol* **10:**69–71.
16. **Revold T, Abayneh T, Brun-Hansen H, Kleppe SL, Ropstad E-O, Hellings RA, Sørum H.** 2015. *Listeria monocytogenes* associated kerato-conjunctivitis in four horses in Norway. *Acta Vet Scand* **57:**76.
17. **Weber A, Prell A, Potel J, Schäfer R.** 1993. Occurrence of *Listeria monocytogenes* in snakes, tortoises, lizards and amphibians raised as pets. *Berl Munch Tierarztl Wochenschr* **106:**293–295. (In German.)
18. **Arumugaswamy R, Gibson LF.** 1999. *Listeria* in zoo animals and rivers. *Aust Vet J* **77:**819–820.
19. **Bauwens L, Vercammen F, Hertsens A.** 2003. Detection of pathogenic *Listeria* spp. in zoo animal faeces: use of immunomagnetic separation and a chromogenic isolation medium. *Vet Microbiol* **91:**115–123.
20. **Yoshida T, Sugimoto T, Sato M, Hirai K.** 2000. Incidence of *Listeria monocytogenes* in wild animals in Japan. *J Vet Med Sci* **62:**673–675.
21. **Nowakiewicz A, Zięba P, Ziółkowska G, Gnat S, Muszyńska M, Tomczuk K, Majer Dziedzic B, Ulbrych Ł, Trościańczyk A.** 2016. Free-living species of carnivorous mammals in Poland: red fox, beech marten, and raccoon as a potential reservoir of *Salmonella*, *Yersinia*,

Listeria spp. and coagulase-positive *Staphylococcus*. *PLoS One* **11:**e0155533.

22. **Fenlon DR, Wilson J, Donachie W.** 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *J Appl Bacteriol* **81:**641–650.
23. **Fieseler L, Doyscher D, Loessner MJ, Schuppler M.** 2014. Acanthamoeba release compounds which promote growth of *Listeria monocytogenes* and other bacteria. *Appl Microbiol Biotechnol* **98:**3091–3097.
24. **Freitag NE, Port GC, Miner MD.** 2009. *Listeria monocytogenes*: from saprophyte to intracellular pathogen. *Nat Rev Microbiol* **7:**623–628.
25. **Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, Grohn YT, McDonough PL, Wiedmann M.** 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl Environ Microbiol* **70:**4458–4467.
26. **Oliver SP, Jayarao BM, Almeida RA.** 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathog Dis* **2:**115–129.
27. **Wilkes G, Edge TA, Gannon VPJ, Jokinen C, Lyautey E, Neumann NF, Ruecker N, Scott A, Sunohara M, Topp E, Lapen DR.** 2011. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. *Water Res* **45:**5807–5825.
28. **Esteban JI, Oporto B, Aduriz G, Juste RA, Hurtado A, Roberts A, Wiedmann M, Buncic S, Iida T, Kanzaki M, Nakama A, Kokubo Y, Maruyama T, Kaneuchi C, Skovgaard N, Norrung B, de Valk H, Vaillant V, Jacquet C, Rocourt J, Le Querrec F, Stainer F, Quelquejeu N, Pierre O, Pierre V, Desenclos J, Goulet V, McLauchlin J, Hall S, Velani S, Gilbert R, Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J, Carlier V, Ermel G, Nightingale K, Schukken Y, Nightingale C, Fortes E, Ho A, Her Z, Grohn Y, McDonough P, Wiedmann M, Gandhi M, Chikindas M, Fenlon D, et al.** 2009. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Vet Res* **5:**2.
29. **Weber A, Potel J, Schäfer-Schmidt R, Prell A, Datzmann C.** 1995. Studies on the occurrence of *Listeria monocytogenes* in fecal samples of domestic and companion animals. *Zentralbl Hyg Umweltmed* **198:**117–123. (In German.)
30. **Walland J, Lauper J, Frey J, Imhof R, Stephan R, Seuberlich T, Oevermann A.** 2015. *Listeria monocytogenes* infection in ruminants: is there a link to the environment, food and human health? A review. *Schweiz Arch Tierheilkd* **157:**319–328.
31. **Rocourt J, Hof H, Schrettenbrunner A, Malinverni R, Bille J.** 1986. Acute purulent *Listeria seelingeri* meningitis in an immunocompetent adult. *Schweiz Med Wochenschr* **116:**248–251. (In French.)
32. **Perrin M, Bemer M, Delamare C.** 2003. Fatal case of *Listeria innocua* bacteremia. *J Clin Microbiol* **41:**5308–5309.
33. **Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domínguez-Bernal G, Goebel W, González-Zorn B, Wehland J, Kreft J.** 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* **14:**584–640.
34. **Cossart P, Lebreton A.** 2014. A trip in the "New Microbiology" with the bacterial pathogen *Listeria monocytogenes*. *FEBS Lett* **588:**2437–2445.
35. **Appelberg R, Castro AG, Silva MT.** 1994. Neutrophils as effector cells of T-cell-mediated, acquired immunity in murine listeriosis. *Immunology* **83:**302–307.
36. **Carvalho F, Sousa S, Cabanes D.** 2014. How *Listeria monocytogenes* organizes its surface for virulence. *Front Cell Infect Microbiol* **4:**48.
37. **Cossart P, Lecuit M.** 1998. Interactions of *Listeria monocytogenes* with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling. *EMBO J* **17:**3797–3806.
38. **Pizarro-Cerdá J, Kühbacher A, Cossart P.** 2012. Entry of *Listeria monocytogenes* in mammalian epithelial cells: an updated view. *Cold Spring Harb Perspect Med* **2:**a010009.
39. **Camejo A, Carvalho F, Reis O, Leitão E, Sousa S, Cabanes D.** 2011. The arsenal of virulence factors deployed by *Listeria monocytogenes* to promote its cell infection cycle. *Virulence* **2:**379–394.
40. **Mohammed HO, Stipetic K, McDonough PL, Gonzalez RN, Nydam DV, Atwill ER.** 2009. Identification of potential on-farm sources of *Listeria monocytogenes* in herds of dairy cattle. *Am J Vet Res* **70:**383–388.
41. **Walker JK, Morgan JH, McLauchlin J, Grant KA, Shallcross JA.** 1994. *Listeria innocua* isolated from a case of ovine meningoencephalitis. *Vet Microbiol* **42:**245–253.
42. **Oevermann A, Di Palma S, Doherr MG, Abril C, Zurbriggen A, Vandevelde M.** 2010. Neuropathogenesis of naturally occurring encephalitis caused by *Listeria monocytogenes* in ruminants. *Brain Pathol* **20:**378–390.
43. **Hird DW, Genigeorgis C.** 1990. Listeriosis in food animals: clinical signs and livestock as a potential source of direct (nonfoodborne) infection for humans, p 31–39. *In* Miller AJ, Smith JL, Somkuti GA (ed), *Foodborne Listeriosis: Topics in Industrial Microbiology*. Elsevier, Amsterdam, The Netherlands.
44. **Clark RG, Gill JM, Swanney S.** 2004. *Listeria monocytogenes* gastroenteritis in sheep. *N Z Vet J* **52:**46–47.
45. **Starič J, Križanec F, Zadnik T.** 2008. *Listeria monocytogenes* keratoconjunctivitis and uveitis in dairy cattle. *Bull Vet Inst Pulawy* **52:**351–355.
46. **Ivanek R, Gröhn YT, Wiedmann M.** 2006. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. *Foodborne Pathog Dis* **3:**319–336.
47. **Kahn CM.** 2005. Listeriosis, p. 2240–2241. *In* Kahn CN, Line Scott (ed), *The Merck Veterinary Manual*, 9th ed. Merck Publishing Group, Rahway, NJ.
48. **Jeckel S, Wood A, Grant K, Amar C, King SA, Whatmore AM, Koylass M, Anjum M, James J, Welchman DB.**

2015. Outbreak of encephalitic listeriosis in red-legged partridges (*Alectoris rufa*). *Avian Pathol* **44:**269–277.

49. **Kurazono M, Nakamura K, Yamada M, Yonemaru T, Sakoda T.** 2003. Pathology of listerial encephalitis in chickens in Japan. *Avian Dis* **47:**1496–1502.
50. **Guillet C, Join-Lambert O, Le Monnier A, Leclercq A, Mechaï F, Mamzer-Bruneel M-F, Bielecka MK, Scortti M, Disson O, Berche P, Vazquez-Boland J, Lortholary O, Lecuit M.** 2010. Human listeriosis caused by *Listeria ivanovii*. *Emerg Infect Dis* **16:**136–138.
51. **Lecuit M, Nelson DM, Smith SD, Khun H, Huerre M, Vacher-Lavenu M-C, Gordon JI, Cossart P.** 2004. Targeting and crossing of the human maternofetal barrier by *Listeria monocytogenes*: role of internalin interaction with trophoblast E-cadherin. *Proc Natl Acad Sci USA* **101:**6152–6157.
52. **McLauchlin J.** 1992. Listeriosis. *BMJ* **304:**1583–1584.
53. **Goulet V, King LA, Vaillant V, de Valk H.** 2013. What is the incubation period for listeriosis? *BMC Infect Dis* **13:**11.
54. **Wesley IV.** 1999. Listeriosis in animals, p. 39–73. *In* Ryser ET, Marth EH (ed), *Listeria, Listeriosis, and Food Safety*, 2nd ed. Marcel Dekker Inc., New York, NY.
55. **Portnoy DA, Auerbuch V, Glomski IJ.** 2002. The cell biology of *Listeria monocytogenes* infection: the intersection of bacterial pathogenesis and cell-mediated immunity. *J Cell Biol* **158:**409–414.
56. **Edelson BT, Cossart P, Unanue ER.** 1999. Cutting edge: paradigm revisited: antibody provides resistance to *Listeria* infection. *J Immunol* **163:**4087–4090.
57. **Berche P, Bonnichon M, Beretti JL, Raveneau J, Gaillard JL, Veron M, Kreis H, Reich KA, Cossart P, Geoffroy C, Geslin P.** 1990. Detection of anti-listeriolysin O for serodiagnosis of human listeriosis. *Lancet* **335:**624–627.
58. **Low JC, Davies RC, Donachie W.** 1992. Purification of listeriolysin O and development of an immunoassay for diagnosis of listeric infections in sheep. *J Clin Microbiol* **30:**2705–2708.
59. **Baetz AL, Wesley IV, Stevens MG.** 1996. The use of listeriolysin O in an ELISA, a skin test and a lymphocyte blastogenesis assay on sheep experimentally infected with *Listeria monocytogenes*, *Listeria ivanovii*, or *Listeria innocua*. *Vet Microbiol* **51:**151–159.
60. **Hof H, Nichterlein T, Kretschmar M.** 1997. Management of listeriosis. *Clin Microbiol Rev* **10:**345–357.
61. **Chopra S, Sharma V, Shukla S, Nayak A.** 2015. Antibiogram of *Listeria* spp. isolated from reproductive disorders and livestock products of ruminants. *J Anim Res* **2:**187–190.
62. **Charpentier E, Courvalin P.** 1999. Antibiotic resistance in *Listeria* spp. *Antimicrob Agents Chemother* **43:**2103–2108.
63. **Janakiraman V.** 2008. Listeriosis in pregnancy: diagnosis, treatment, and prevention. *Rev Obstet Gynecol* **1:**179–185.
64. **Wright GD.** 2010. Q&A: Antibiotic resistance: where does it come from and what can we do about it? *BMC Biol* **8:**123.
65. **Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV.** 2015. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol* **13:**42–51.
66. **Charpentier E, Gerbaud G, Courvalin P.** 1999. Conjugative mobilization of the rolling-circle plasmid pIP823 from *Listeria monocytogenes* BM4293 among Gram-positive and Gram-negative bacteria. *J Bacteriol* **181:**3368–3374.
67. **Godreuil S, Galimand M, Gerbaud G, Jacquet C, Courvalin P.** 2003. Efflux pump Lde is associated with fluoroquinolone resistance in *Listeria monocytogenes*. *Antimicrob Agents Chemother* **47:**704–708.
68. **Troxler R, von Graevenitz A, Funke G, Wiedemann B, Stock I.** 2000. Natural antibiotic susceptibility of *Listeria* species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* strains. *Clin Microbiol Infect* **6:**525–535.
69. **Morvan A, Moubareck C, Leclercq A, Hervé-Bazin M, Bremont S, Lecuit M, Courvalin P, Le Monnier A.** 2010. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrob Agents Chemother* **54:**2728–2731.
70. **Lungu B, O'Bryan CA, Muthaiyan A, Milillo SR, Johnson MG, Crandall PG, Ricke SC.** 2011. *Listeria monocytogenes*: antibiotic resistance in food production. *Foodborne Pathog Dis* **8:**569–578.
71. **Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P.** 1990. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. *Lancet* **335:**1422–1426.
72. **Jones FT, Ricke SC.** 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. *Poult Sci* **82:**613–617.
73. **Castanon JIR.** 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult Sci* **86:**2466–2471.
74. **Robicsek A, Jacoby GA, Hooper DC.** 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* **6:**629–640.
75. **Lyon SA, Berrang ME, Fedorka-Cray PJ, Fletcher DL, Meinersmann RJ.** 2008. Antimicrobial resistance of *Listeria monocytogenes* isolated from a poultry further processing plant. *Foodborne Pathog Dis* **5:**253–259.
76. **Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, Meng J.** 2007. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella enterica* serovar typhimurium to fluoroquinolones and other antimicrobials. *Antimicrob Agents Chemother* **51:**535–542.
77. **Liu J-H, Deng Y-T, Zeng Z-L, Gao J-H, Chen L, Arakawa Y, Chen Z-L.** 2008. Coprevalence of plasmid-mediated quinolone resistance determinants QepA, Qnr, and AAC(6′)-Ib-cr among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. *Antimicrob Agents Chemother* **52:**2992–2993.
78. **Lampidis R, Kostrewa D, Hof H.** 2002. Molecular characterization of the genes encoding DNA gyrase and topoisomerase IV of *Listeria monocytogenes*. *J Antimicrob Chemother* **49:**917–924.

79. **Moreno LZ, Paixão R, Gobbi DDS, Raimundo DC, Ferreira TP, Moreno AM, Hofer E, Reis CMF, Matté GR, Matté MH.** 2014. Characterization of antibiotic resistance in *Listeria* spp. isolated from slaughterhouse environments, pork and human infections. *J Infect Dev Ctries* **8:**416–423.
80. **Mata MT, Baquero F, Pérez-Díaz JC.** 2000. A multidrug efflux transporter in *Listeria monocytogenes*. *FEMS Microbiol Lett* **187:**185–188.
81. **Guérin F, Galimand M, Tuambilangana F, Courvalin P, Cattoir V.** 2014. Overexpression of the novel MATE fluoroquinolone efflux pump FepA in *Listeria monocytogenes* is driven by inactivation of its local repressor FepR. *PLoS One* **9:**e106340.
82. **Srinivasan V, Nam HM, Nguyen LT, Tamilselvam B, Murinda SE, Oliver SP.** 2005. Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. *Foodborne Pathog Dis* **2:**201–211.
83. **Zawadzka-Skomial J, Markiewicz Z, Nguyen-Distèche M, Devreese B, Frère J-M, Terrak M.** 2006. Characterization of the bifunctional glycosyltransferase/acyltransferase penicillin-binding protein 4 of *Listeria monocytogenes*. *J Bacteriol* **188:**1875–1881.
84. **Aubry C, Goulard C, Nahori M-A, Cayet N, Decalf J, Sachse M, Boneca IG, Cossart P, Dussurget O.** 2011. OatA, a peptidoglycan O-acetyltransferase involved in *Listeria monocytogenes* immune escape, is critical for virulence. *J Infect Dis* **204:**731–740.
85. **Allen KJ, Wałecka-Zacharska E, Chen JC, Katarzyna K-P, Devlieghere F, Van Meervenne E, Osek J, Wieczorek K, Bania J.** 2016. *Listeria monocytogenes*: an examination of food chain factors potentially contributing to antimicrobial resistance. *Food Microbiol* **54:**178–189.
86. **Shaw KJ, Rather PN, Hare RS, Miller GH.** 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* **57:**138–163.
87. **Lancaster H, Roberts AP, Bedi R, Wilson M, Mullany P.** 2004. Characterization of Tn*916*S, a Tn*916*-like element containing the tetracycline resistance determinant *tet*(S). *J Bacteriol* **186:**4395–4398.
88. **van Hoek AHAM, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJM.** 2011. Acquired antibiotic resistance genes: an overview. *Front Microbiol* **2:**203.
89. **Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A.** 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* **28:** 519–542.
90. **Li L, Olsen RH, Shi L, Ye L, He J, Meng H.** 2016. Characterization of a plasmid carrying *cat*, *ermB* and *tetS* genes in a foodborne *Listeria monocytogenes* strain and uptake of the plasmid by cariogenic *Streptococcus mutans*. *Int J Food Microbiol* **238:**68–71.
91. **Roberts MC, Facinelli B, Giovanetti E, Varaldo PE.** 1996. Transferable erythromycin resistance in *Listeria* spp. isolated from food. *Appl Environ Microbiol* **62:** 269–270.
92. **Granier SA, Moubareck C, Colaneri C, Lemire A, Roussel S, Dao T-T, Courvalin P, Brisabois A.** 2011. Antimicrobial resistance of *Listeria monocytogenes* isolates from food and the environment in France over a 10-year period. *Appl Environ Microbiol* **77:**2788–2790.
93. **Bertsch D, Anderegg J, Lacroix C, Meile L, Stevens MJA.** 2013. pDB2011, a 7.6 kb multidrug resistance plasmid from *Listeria innocua* replicating in Gram-positive and Gram-negative hosts. *Plasmid* **70:**284–287.
94. **Huovinen P, Huovinen P.** 2001. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis* **32:**1608–1614.
95. **Charpentier E, Courvalin P.** 1997. Emergence of the trimethoprim resistance gene *dfrD* in *Listeria monocytogenes* BM4293. *Antimicrob Agents Chemother* **41:** 1134–1136.
96. **Bertsch D, Uruty A, Anderegg J, Lacroix C, Perreten V, Meile L.** 2013. Tn*6198*, a novel transposon containing the trimethoprim resistance gene *dfrG* embedded into a Tn*916* element in *Listeria monocytogenes*. *J Antimicrob Chemother* **68:**986–991.
97. **European Union.** 2012. Regulation (EU) 528/2012 of 22 May 2012 concerning the making available on the market and use of biocidal products. European Union Regul.
98. **Harbarth S, Tuan Soh S, Horner C, Wilcox MH.** 2014. Is reduced susceptibility to disinfectants and antiseptics a risk in healthcare settings? A point/counterpoint review. *J Hosp Infect* **87:**194–202.
99. **Ortega Morente E, Fernández-Fuentes MA, Grande Burgos MJ, Abriouel H, Pérez Pulido R, Gálvez A.** 2013. Biocide tolerance in bacteria. *Int J Food Microbiol* **162:** 13–25.
100. **McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P.** 2004. Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility. *Appl Environ Microbiol* **70:**3449–3456.
101. **McDonnell G, Russell AD.** 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* **12:**147–179.
102. **Goddard P, McCue K.** 2001. *Disinfectants and Antiseptics. Disinfection, Sterilization, and Preservation*. Lippincott Williams & Wilkins, Philadelphia, PA.
103. **Elhanafi D, Dutta V, Kathariou S.** 2010. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998–1999 outbreak. *Appl Environ Microbiol* **76:**8231–8238.
104. **Dutta V, Elhanafi D, Kathariou S.** 2013. Conservation and distribution of the benzalkonium chloride resistance cassette *bcrABC* in *Listeria monocytogenes*. *Appl Environ Microbiol* **79:**6067–6074.
105. **Müller A, Rychli K, Muhterem-Uyar M, Zaiser A, Stessl B, Guinane CM, Cotter PD, Wagner M, Schmitz-Esser S.** 2013. Tn*6188*: a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. *PLoS One* **8:**e76835.
106. **Müller A, Rychli K, Zaiser A, Wieser C, Wagner M, Schmitz-Esser S.** 2014. The *Listeria monocytogenes* transposon Tn*6188* provides increased tolerance to var-

ious quaternary ammonium compounds and ethidium bromide. *FEMS Microbiol Lett* **361:**166–173.

107. **Kovacevic J, Ziegler J, Wałecka-Zacharska E, Reimer A, Kitts DD, Gilmour MW.** 2015. Tolerance of *Listeria monocytogenes* to quaternary ammonium sanitizers is mediated by a novel efflux pump encoded by *emrE*. *Appl Environ Microbiol* **82:**939–953.
108. **Tamburro M, Ripabelli G, Vitullo M, Dallman TJ, Pontello M, Amar CFL, Sammarco ML.** 2015. Gene expression in *Listeria monocytogenes* exposed to sublethal concentration of benzalkonium chloride. *Comp Immunol Microbiol Infect Dis* **40:**31–39.
109. **Mereghetti L, Quentin R, Marquet-Van Der Mee N, Audurier A.** 2000. Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. *Appl Environ Microbiol* **66:**5083–5086.
110. **Romanova NA, Wolffs PFG, Brovko LY, Griffiths MW.** 2006. Role of efflux pumps in adaptation and resistance of *Listeria monocytogenes* to benzalkonium chloride. *Appl Environ Microbiol* **72:**3498–3503.
111. **Jones RD, Jampani HB, Newman JL, Lee AS.** 2000. Triclosan: a review of effectiveness and safety in health care settings. *Am J Infect Control* **28:**184–196.
112. **Schweizer HP.** 2001. Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiol Lett* **202:**1–7.
113. **Heath RJ, Yu YT, Shapiro MA, Olson E, Rock CO.** 1998. Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. *J Biol Chem* **273:**30316–30320.
114. **Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, Rock CO.** 1999. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *J Biol Chem* **274:** 11110–11114.
115. **Slater-Radosti C, Van Aller G, Greenwood R, Nicholas R, Keller PM, DeWolf WE Jr, Fan F, Payne DJ, Jaworski DD.** 2001. Biochemical and genetic characterization of the action of triclosan on *Staphylococcus aureus*. *J Antimicrob Chemother* **48:**1–6.
116. **Fan F, Yan K, Wallis NG, Reed S, Moore TD, Rittenhouse SF, DeWolf WE Jr, Huang J, McDevitt D, Miller WH, Seefeld MA, Newlander KA, Jakas DR, Head MS, Payne DJ.** 2002. Defining and combating the mechanisms of triclosan resistance in clinical isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **46:**3343–3347.
117. **Kampf G, Kramer A.** 2004. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clin Microbiol Rev* **17:** 863–893.
118. **Villalaín J, Mateo CR, Aranda FJ, Shapiro S, Micol V.** 2001. Membranotropic effects of the antibacterial agent triclosan. *Arch Biochem Biophys* **390:**128–136.
119. **Escalada MG, Russell AD, Maillard J-Y, Ochs D.** 2005. Triclosan-bacteria interactions: single or multiple target sites? *Lett Appl Microbiol* **41:**476–481.
120. **Bailey AM, Constantinidou C, Ivens A, Garvey MI, Webber MA, Coldham N, Hobman JL, Wain J, Woodward MJ, Piddock LJV.** 2009. Exposure of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to triclosan induces a species-specific response, including drug detoxification. *J Antimicrob Chemother* **64:**973–985.
121. **Nielsen LN, Larsen MH, Skovgaard S, Kastbjerg V, Westh H, Gram L, Ingmer H.** 2013. *Staphylococcus aureus* but not *Listeria monocytogenes* adapt to triclosan and adaptation correlates with increased *fabI* expression and *agr* deficiency. *BMC Microbiol* **13:**177.
122. **Kastbjerg VG, Hein-Kristensen L, Gram L.** 2014. Triclosan-induced aminoglycoside-tolerant *Listeria monocytogenes* isolates can appear as small-colony variants. *Antimicrob Agents Chemother* **58:**3124–3132.
123. **Appelberg R.** 2006. Macrophage nutriprive antimicrobial mechanisms. *J Leukoc Biol* **79:**1117–1128.
124. **Botella H, Peyron P, Levillain F, Poincloux R, Poquet Y, Brandli I, Wang C, Tailleux L, Tilleul S, Charrière GM, Waddell SJ, Foti M, Lugo-Villarino G, Gao Q, Maridonneau-Parini I, Butcher PD, Castagnoli PR, Gicquel B, de Chastellier C, Neyrolles O.** 2011. Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. *Cell Host Microbe* **10:**248–259.
125. **White C, Lee J, Kambe T, Fritsche K, Petris MJ.** 2009. A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. *J Biol Chem* **284:** 33949–33956.
126. **den Bakker HC, Cummings CA, Ferreira V, Vatta P, Orsi RH, Degoricija L, Barker M, Petrauskene O, Furtado MR, Wiedmann M.** 2010. Comparative genomics of the bacterial genus *Listeria*: genome evolution is characterized by limited gene acquisition and limited gene loss. *BMC Genomics* **11:**688.
127. **Kuenne C, Voget S, Pischimarov J, Oehm S, Goesmann A, Daniel R, Hain T, Chakraborty T.** 2010. Comparative analysis of plasmids in the genus *Listeria*. *PLoS One* **5:**5.
128. **Yoon KP, Silver S.** 1991. A second gene in the *Staphylococcus aureus cadA* cadmium resistance determinant of plasmid pI258. *J Bacteriol* **173:**7636–7642.
129. **Allnutt TR, Bradbury MI, Fanning S, Chandry PS, Fox EM.** 2016. Draft genome sequences of 15 isolates of *Listeria monocytogenes* serotype 1/2a, subgroup ST204. *Genome Announc* **4:**4.
130. **Wales AD, Davies RH.** 2015. Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics (Basel)* **4:** 567–604.
131. **Schmitz-Esser S, Müller A, Stessl B, Wagner M.** 2015. Genomes of sequence type 121 *Listeria monocytogenes* strains harbor highly conserved plasmids and prophages. *Front Microbiol* **6:**380.
132. **Mortvedt JJ.** 1996. Heavy metal contaminants in inorganic and organic fertilizers. *Fert Res* **43:**55–61.
133. **Yazdankhah S, Rudi K, Bernhoft A.** 2014. Zinc and copper in animal feed: development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. *Microb Ecol Health Dis* **25:**25.
134. **Barakat MA.** 2011. New trends in removing heavy metals from industrial wastewater. *Arab J Chem* **4:** 361–377.

135. **Guan Y, Shao C, Ju M.** 2014. Heavy metal contamination assessment and partition for industrial and mining gathering areas. *Int J Environ Res Public Health* **11:** 7286–7303.

136. **Xu D, Nie Q, Wang W, Shi L, Yan H.** 2016. Characterization of a transferable *bcrABC* and *cadAC* genes-harboring plasmid in *Listeria monocytogenes* strain isolated from food products of animal origin. *Int J Food Microbiol* **217:**117–122.

137. **Pritchard TJ, Flanders KJ, Donnelly CW.** 1995. Comparison of the incidence of *Listeria* on equipment versus environmental sites within dairy processing plants. *Int J Food Microbiol* **26:**375–384.

138. **Fox EM, Wall PG, Fanning S.** 2015. Control of *Listeria* species food safety at a poultry food production facility. *Food Microbiol* **51:**81–86.

139. **Mullapudi S, Siletzky RM, Kathariou S.** 2008. Heavy-metal and benzalkonium chloride resistance of *Listeria monocytogenes* isolates from the environment of turkey-processing plants. *Appl Environ Microbiol* **74:** 1464–1468.

140. **Khan Z, Rehman A, Hussain SZ, Nisar MA, Zulfiqar S, Shakoori AR.** 2016. Cadmium resistance and uptake by bacterium, *Salmonella enterica* 43C, isolated from industrial effluent. *AMB Express* **6:**54.

141. **Lee S, Rakic-Martinez M, Graves LM, Ward TJ, Siletzky RM, Kathariou S.** 2013. Genetic determinants for cadmium and arsenic resistance among *Listeria monocytogenes* serotype 4b isolates from sporadic human listeriosis patients. *Appl Environ Microbiol* **79:** 2471–2476.

142. **Xu D, Li Y, Zahid MSH, Yamasaki S, Shi L, Li JR, Yan H.** 2014. Benzalkonium chloride and heavy-metal tolerance in *Listeria monocytogenes* from retail foods. *Int J Food Microbiol* **190:**24–30.

143. **Lebrun M, Loulergue J, Chaslus-Dancla E, Audurier A.** 1992. Plasmids in *Listeria monocytogenes* in relation to cadmium resistance. *Appl Environ Microbiol* **58:**3183–3186.

144. **Lebrun M, Audurier A, Cossart P.** 1994. Plasmid-borne cadmium resistance genes in *Listeria monocytogenes* are similar to *cadA* and *cadC* of *Staphylococcus aureus* and are induced by cadmium. *J Bacteriol* **176:**3040–3048.

145. **Tynecka Z, Gos Z, Zajac J.** 1981. Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. *J Bacteriol* **147:**313–319.

146. **Endo G, Silver S.** 1995. CadC, the transcriptional regulatory protein of the cadmium resistance system of *Staphylococcus aureus* plasmid pI258. *J Bacteriol* **177:** 4437–4441.

147. **McLauchlin J, Hampton MD, Shah S, Threlfall EJ, Wieneke AA, Curtis GD.** 1997. Subtyping of *Listeria monocytogenes* on the basis of plasmid profiles and arsenic and cadmium susceptibility. *J Appl Microbiol* **83:**381–388.

148. **Ratani SS, Siletzky RM, Dutta V, Yildirim S, Osborne JA, Lin W, Hitchins AD, Ward TJ, Kathariou S.** 2012. Heavy metal and disinfectant resistance of *Listeria monocytogenes* from foods and food processing plants. *Appl Environ Microbiol* **78:**6938–6945.

149. **Nelson KE, Fouts DE, Mongodin EF, Ravel J, DeBoy RT, Kolonay JF, Rasko DA, Angiuoli SV, Gill SR, Paulsen IT, Peterson J, White O, Nelson WC, Nierman W, Beanan MJ, Brinkac LM, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Haft DH, Selengut J, Van Aken S, Khouri H, Fedorova N, Forberger H, Tran B, Kathariou S, Wonderling LD, Uhlich GA, Bayles DO, Luchansky JB, Fraser CM.** 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res* **32:**2386–2395.

150. **Mullapudi S, Siletzky RM, Kathariou S.** 2010. Diverse cadmium resistance determinants in *Listeria monocytogenes* isolates from the turkey processing plant environment. *Appl Environ Microbiol* **76:**627–630.

151. **Briers Y, Klumpp J, Schuppler M, Loessner MJ.** 2011. Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. *J Bacteriol* **193:**4284–4285.

152. **Sarkar A, Paul B.** 2016. The global menace of arsenic and its conventional remediation: a critical review. *Chemosphere* **158:**37–49.

153. **Henke KR, Atwood DA.** Arsenic in human history and modern societies, p 277–302. *In Arsenic*. John Wiley & Sons, Ltd., Chichester, United Kingdom.

154. **Chapman HD, Johnson ZB.** 2002. Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995 to 2000. *Poult Sci* **81:**356–364.

155. **Jesse HE, Roberts IS, Cavet JS.** 2014. Metal ion homeostasis in *Listeria monocytogenes* and importance in host-pathogen interactions. *Adv Microb Physiol* **65:** 83–123.

156. **Castillo Y, Tachibana M, Nakatsu Y, Watanabe K, Shimizu T, Watarai M.** 2015. Combination of zinc and all-trans retinoic acid promotes protection against *Listeria monocytogenes* infection. *PLoS One* **10:**e0137463.

157. **Corbett D, Schuler S, Glenn S, Andrew PW, Cavet JS, Roberts IS.** 2011. The combined actions of the copper-responsive repressor CsoR and copper-metallochaperone CopZ modulate CopA-mediated copper efflux in the intracellular pathogen *Listeria monocytogenes*. *Mol Microbiol* **81:**457–472.

158. **Kuenne C, Billion A, Mraheil MA, Strittmatter A, Daniel R, Goesmann A, Barbuddhe S, Hain T, Chakraborty T.** 2013. Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. *BMC Genomics* **14:**47.

159. **Camejo A, Buchrieser C, Couvé E, Carvalho F, Reis O, Ferreira P, Sousa S, Cossart P, Cabanes D.** 2009. *In vivo* transcriptional profiling of *Listeria monocytogenes* and mutagenesis identify new virulence factors involved in infection. *PLoS Pathog* **5:**e1000449.

160. **Bertrand S, Huys G, Yde M, D'Haene K, Tardy F, Vrints M, Swings J, Collard J-M.** 2005. Detection and characterization of *tet*(M) in tetracycline-resistant

Listeria strains from human and food-processing origins in Belgium and France. *J Med Microbiol* **54:**1151–1156.

161. Pourshaban M, Ferrini AM, Mannoni V, Oliva B, Aureli P. 2002. Transferable tetracycline resistance in *Listeria monocytogenes* from food in Italy. *J Med Microbiol* **51:** 564–566.

162. Humphrey B, Thomson NR, Thomas CM, Brooks K, Sanders M, Delsol AA, Roe JM, Bennett PM, Enne VI. 2012. Fitness of *Escherichia coli* strains carrying expressed and partially silent IncN and IncP1 plasmids. *BMC Microbiol* **12:**53.

163. Clewell DB, Weaver KE, Dunny GM, Coque TM, Francia MV, Hayes F. 2014. Extrachromosomal and mobile elements in enterococci: transmission, maintenance, and epidemiology. *In* Gilmore MS, Clewell DB, Ike Y, Shankar N (ed), *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. Massachusetts Eye and Ear Infirmary, Boston, MA. Available from https://www.ncbi.nlm.nih.gov/books/NBK190430/.

164. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI. 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog* **7:**e1002158.

165. Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *MBio* **5:**e01918–e14.

166. Jensen LB, Garcia-Migura L, Valenzuela AJS, Løhr M, Hasman H, Aarestrup FM. 2010. A classification system for plasmids from enterococci and other Gram-positive bacteria. *J Microbiol Methods* **80:**25–43.

167. Carattoli A. 2009. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother* **53:** 2227–2238.

168. Romanova N, Favrin S, Griffiths MW. 2002. Sensitivity of *Listeria monocytogenes* to sanitizers used in the meat processing industry. *Appl Environ Microbiol* **68:** 6405–6409.

169. Zhang H, Zhou Y, Bao H, Zhang L, Wang R, Zhou X. 2015. Plasmid-borne cadmium resistant determinants are associated with the susceptibility of *Listeria monocytogenes* to bacteriophage. *Microbiol Res* **172:**1–6.

170. Bertsch D, Muelli M, Weller M, Uruty A, Lacroix C, Meile L. 2014. Antimicrobial susceptibility and antibiotic resistance gene transfer analysis of foodborne, clinical, and environmental *Listeria* spp. isolates including *Listeria monocytogenes*. *MicrobiologyOpen* **3:** 118–127.

171. Poyart-Salmeron C, Trieu-Cuot P, Carlier C, MacGowan A, McLauchlin J, Courvalin P. 1992. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. *Antimicrob Agents Chemother* **36:** 463–466.

172. Hadorn K, Hächler H, Schaffner A, Kayser FH. 1993. Genetic characterization of plasmid-encoded multiple antibiotic resistance in a strain of *Listeria monocytogenes* causing endocarditis. *Eur J Clin Microbiol Infect Dis* **12:**928–937.

173. Piddock LJV. 2006. Multidrug-resistance efflux pumps: not just for resistance. *Nat Rev Microbiol* **4:**629–636.

174. Li X-Z, Nikaido H. 2009. Efflux-mediated drug resistance in bacteria: an update. *Drugs* **69:**1555–1623.

175. Webber MA, Piddock LJV. 2003. The importance of efflux pumps in bacterial antibiotic resistance. *J Antimicrob Chemother* **51:**9–11.

176. Crimmins GT, Herskovits AA, Rehder K, Sivick KE, Lauer P, Dubensky TW Jr, Portnoy DA. 2008. *Listeria monocytogenes* multidrug resistance transporters activate a cytosolic surveillance pathway of innate immunity. *Proc Natl Acad Sci USA* **105:**10191–10196.

177. Kaplan Zeevi M, Shafir NS, Shaham S, Friedman S, Sigal N, Nir Paz R, Boneca IG, Herskovits AA. 2013. *Listeria monocytogenes* multidrug resistance transporters and cyclic di-AMP, which contribute to type I interferon induction, play a role in cell wall stress. *J Bacteriol* **195:**5250–5261.

178. Schwartz KT, Carleton JD, Quillin SJ, Rollins SD, Portnoy DA, Leber JH. 2012. Hyperinduction of host beta interferon by a *Listeria monocytogenes* strain naturally overexpressing the multidrug efflux pump MdrT. *Infect Immun* **80:**1537–1545.

179. Marquez B, Pourcelle V, Vallet CM, Mingeot-Leclercq M-P, Tulkens PM, Marchand-Bruynaert J, Van Bambeke F. 2014. Pharmacological characterization of 7-(4-(Piperazin-1-yl)) ciprofloxacin derivatives: antibacterial activity, cellular accumulation, susceptibility to efflux transporters, and intracellular activity. *Pharm Res* **31:**1290–1301.

180. Lismond A, Tulkens PM, Mingeot-Leclercq M-P, Courvalin P, Van Bambeke F. 2008. Cooperation between prokaryotic (Lde) and eukaryotic (MRP) efflux transporters in J774 macrophages infected with *Listeria monocytogenes*: studies with ciprofloxacin and moxifloxacin. *Antimicrob Agents Chemother* **52:**3040–3046.

181. Scortti M, Lacharme-Lora L, Wagner M, Chico-Calero I, Losito P, Vázquez-Boland JA. 2006. Coexpression of virulence and fosfomycin susceptibility in *Listeria*: molecular basis of an antimicrobial *in vitro-in vivo* paradox. *Nat Med* **12:**515–517.

182. Collins B, Curtis N, Cotter PD, Hill C, Ross RP. 2010. The ABC transporter AnrAB contributes to the innate resistance of *Listeria monocytogenes* to nisin, bacitracin, and various beta-lactam antibiotics. *Antimicrob Agents Chemother* **54:**4416–4423.

183. Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell DA. 2001. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J Appl Microbiol* **90:**517–522.

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Antimicrobial Resistance in Nontyphoidal *Salmonella*

12

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BACKGROUND

Nontyphoidal *Salmonella enterica* (NTS) is a ubiquitous, motile, Gram-negative bacillus that is one of the most common bacterial causes of gastrointestinal disease worldwide. Salmonellae colonize the intestinal tract of a wide range of animal hosts, including pigs, cattle, poultry (1), and wildlife, as well as companion animals such as dogs, cats, birds, and reptiles (2). Humans acquire infection from the ingestion of contaminated foods. In the United States, most illnesses are associated with seeded vegetables, eggs, poultry, beef, and pork. Other sources include dairy, fruits, sprouts, and fish (3). Its ubiquity in nature and the variety of vectors mediating fecal-oral spread have made salmonellosis the most important foodborne bacterial zoonosis. For many decades, the cornerstone of *Salmonella* epidemiology has been the Kaufmann-White serotyping scheme (4). Based on antibodies to the three major surface antigens (somatic O, flagellar H, and capsular Vi antigens), over 2,500 distinct serovars are currently recognized. A comprehensive body of scientific information on *Salmonella* developed over the years makes it one of the best-understood bacterial pathogens. A great deal is known about *Salmonella* epidemiology and genetics, the various virulence factors, interaction of the bacterium with host cells, the host range of serovars, and the causes of antibiotic resistance.

Most human cases of acute NTS diarrhea are self-limiting and do not require treatment with antibiotics unless they are severe, invasive, or occur in the elderly, children, or those with underlying comorbidities (5). Cases that become invasive may result in life-threatening bloodstream infections. Antimicrobial resistance is especially problematic in these systemic infections, where antibiotic therapy can be life-saving. Efforts to limit the public health burden of salmonellosis, and the pressures leading to antibiotic resistance, have focused on farm practices, interventions in processing facilities, and consumer education on safe food handling practices.

In animals, *Salmonella* may cause overt signs of disease or may be carried asymptomatically and be shed into the environment (6). In food-producing animals, *Salmonella* is a continuous threat to animal health, especially in cattle, where infection typically presents as diarrhea with fever, anorexia, and dehydration (2). Less commonly, infection results in respiratory disease and death. *Salmonella* infections in dairy herds, where they may become endemic (7), result in decreased milk production and increased production costs, including the use of antibiotics. In addition to overt salmonellosis, a chronic asymptomatic carrier state may exist in food-producing animals. In both the carrier state and in cases of overt infection, exposure to antimicrobials promotes the evolution of resistant serovars that may be transmitted to humans. In the case of foodborne zoonotic bacteria, resistance spreads from food-producing animals to humans via the consumption of meat products derived either from treated animals or from foods cross-contaminated at processing or retail (8). Resistance also can spread by direct animal contact (as with pets) or environmental routes such as water or wildlife. The U.S. Centers for Disease Control and Prevention (CDC) classifies antibiotic-resistant *Salmonella* as a serious public health threat (9).

Bacterial resistance to antibiotics is a naturally occurring phenomenon that can be accelerated by selection pressures exerted through the use of antibiotics in medical and veterinary practice (10). Resistance is mediated by a limited number of mechanisms common to other pathogens. These include enzymatic modification, target protection, energy-dependent efflux, and permeability changes in the cell wall. The use and

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misuse of antimicrobials in both humans and animals result in antimicrobial resistance in both pathogenic and commensal bacteria within the treated host. Many classes of antimicrobial agents used in food-producing and companion animals are the same as those used in human medicine (11). Therefore, resistance developing in one drug use environment can compromise the efficacy of drugs used in other settings. For this reason, many countries are attempting to implement antimicrobial drug use monitoring.

The relationship between resistances in food animal bacteria and antibiotic use in animal agriculture is poorly defined. Very few countries can collect detailed information on the amounts and indications of antimicrobial compounds in different animal species over time. Only in Denmark is it mandatory for veterinarians to report (via VetStat) medicines used in their own practices. Like most countries, the United States and Japan collect data on total annual sales (in kilograms of active ingredient) by drug class. Japan collects sales data only for drugs used therapeutically (12). Most European Union countries also mandate collection of sales data (13). While usually the only practicable approach, bulk annual amounts of active ingredient sold is of limited utility and is not a reliable surrogate for actual drug use in the production environment (14). In July 2016, the U.S. FDA mandated that pharmaceutical companies report antimicrobial sales by the four major animal species: cattle, chickens, turkey, and swine, along with a combined "other" category (15). The goal of this provision is to better monitor drug use and better understand the drivers of antimicrobial resistance in *Salmonella* and other foodborne microorganisms.

While there are numerous small targeted surveys in the scientific literature describing antimicrobial resistance in *Salmonella* from different places and sources, an ongoing integrated national surveillance system is necessary to combat antibiotic resistance (16). The WHO defines integrated surveillance as "the coordinated sampling and testing of bacteria from food animals, foods, and clinically ill humans; and the subsequent evaluation of antimicrobial resistance trends throughout the food production and supply chain using harmonized methods" (1). It provides necessary data to identify emerging hazards, assess risks, monitor trends, measure interventions, and inform mitigation policies. The generation of robust and consistent data is important to the development and assessment of response measures used to combat antimicrobial resistance. In the United States, this work is conducted by the National Antimicrobial Resistance Monitoring System (NARMS). Similar systems are in place across the European Union, in Canada, across Latin America, and in some Asian and African countries. Most of these programs are focused on susceptibility surveillance of human clinical isolates. Active surveillance of animal and retail meat isolates operates to varying degrees in different countries (1). This article will review the latest available information on resistance in *Salmonella* (through test year 2014), with an emphasis on the situation in the United States and Europe.

THE BURDEN OF SALMONELLOSIS

NTS is recognized as one of the most common bacterial causes of foodborne diarrheal disease worldwide. Foodborne NTS is estimated to cause over 93 million cases of gastroenteritis annually and 155,000 deaths globally (8), resulting in about 4 million disability-adjusted life years (17). At-risk populations include children <1 year of age and adults ≥60, who are most vulnerable to infection and tend to have more severe disease (18). Extraintestinal or invasive NTS infections, often associated with certain serovars or phylogenetic clades, add to the global burden of illness, especially in resource-poor countries and immunocompromised patients (19). An estimated 3.4 million invasive infections occur annually, resulting in 681,000 deaths, with the young and old in Africa being most affected (20). The relatively high prevalence of highly invasive strain subtypes, such as those that have been identified among *S. enterica* serovars Enteritidis (21), Typhimurium (22), and Kentucky (23) may be expected to drive different antibiotic use practices to control disease in countries where invasive subtypes are endemic (24).

Despite sustained efforts to control NTS in animals and to educate the public on safe food-handling practices, the incidence of human salmonellosis has not changed significantly in the United States (25). The latest data from the CDC on microbial causes of zoonotic foodborne diseases ranked *Salmonella* first in incidence, at 15.5 cases per 100,000 inhabitants, resulting in over 2,100 hospitalizations and 32 deaths (18). In the European Union in 2014, the incidence of *Salmonella* infections (23 per 100,000) ranked well behind *Campylobacter* (71 per 100,000). In 2014, a total of 88,715 confirmed cases of salmonellosis were reported by 28 European Union member states and 4 nonmember states (26). In both the United States and the European Union (and in many other countries), *S. enterica* serovars Typhimurium and Enteritidis are consistently the most frequently isolated among confirmed human cases of disease (18, 26). In the United States and the European Union, poultry meat is the most commonly contaminated animal-derived food commodity (26, 27).

TREATMENT OF SALMONELLOSIS

The extent of antibiotic resistance in *Salmonella* varies by country and is influenced by antimicrobial use practices in humans and animals, as well as geographical differences in the epidemiology of *Salmonella* and regional serovar differences. In developed countries, drug resistance in *Salmonella* is driven largely by the use of antimicrobials in food-producing animals (28). In general, resistance profiles reflect the length of time an agent has been in use. Thus, irrespective of isolation source (humans, foods, food animals), the most frequent types of resistances are usually for older antimicrobials such as tetracycline, sulfamethoxazole, and streptomycin (26, 27, 29, 30).

Antimicrobial therapy is usually not indicated for uncomplicated infection. Therapy should be considered for populations at increased risk for invasive infection such as people >50 years of age with atherosclerosis, the immunocompromised, and those with cardiac disease. In these patients, the recommended antimicrobials include a fluoroquinolone such as ciprofloxacin, a third-generation cephalosporin such as ceftriaxone, trimethoprim/sulfamethoxazole, or amoxicillin. The recommended empiric antimicrobial therapy for bloody diarrhea in immunocompetent adults is either a fluoroquinolone or azithromycin, both of which show potent *in vitro* activity against *Salmonella* in the United States and the European Union. Additionally, most highly resistant strains of *Salmonella* are susceptible to carbapenem drugs (31, 32), making it a drug of last resort. Recommended empiric therapy for children includes a third-generation cephalosporin for those <3 months of age or azithromycin. Because fluoroquinolones, extended-spectrum cephalosporins, azithromycin, and carbapenems are critically important antibiotics for the management of salmonellosis, emerging resistance to these drug classes is a paramount concern (33).

ANTIMICROBIAL RESISTANCE IN *SALMONELLA* FROM THE UNITED STATES

The United States has extensive data back to 1996 on antimicrobial resistance in *Salmonella*, which is collected through NARMS (34). The NARMS program generates susceptibility data on *Salmonella* representing 5% of the nationally reported human clinical cases. These results are compared with data on *Salmonella* from 13 other sources that include (i) samples of retail chicken, turkey, pork, and beef collected in 14 states; (ii) cecal specimens from chickens, turkeys, cattle (dairy and beef), and pigs (hogs and sows) at slaughter; and (iii) carcass swabs, carcass rinses, and ground product from chickens, turkeys, cattle, and pigs at slaughter. A line listing of these data is freely available online (35), where information on nearly 160,000 isolates dating back to the start of NARMS can be downloaded and analyzed using standard spreadsheet and database software programs.

Few data are available from before 1996 to document the historical trends in *Salmonella* resistance. To address this gap, Tadesse et al. (36) conducted a retrospective study of 2,149 banked human clinical *Salmonella* strains and documented changing resistance patterns in strains dating back to 1948. Comparing data from pre-1960 with those from post-1989 for *S.* Typhimurium (which constituted most of the banked isolates) showed that resistance rose from 0% to 33% for ampicillin, 0% to 26% for chloramphenicol, 0% to 43% for streptomycin, 20% to 43% for tetracycline, and 0% to 37% for sulfamethoxazole. While recognizing the inherent limitations in directly comparing two disparate data sets, it is striking how the historical and modern data in juxtaposition show a monotonic increasing trend in resistance to older antimicrobial compounds (ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline) from pre-1960s up until the 1990s, followed by a decline to current levels approximating those of the 1970s (Fig. 1). This study examined just over 2,100 human clinical isolates collected over 6 decades, a number now tested annually in NARMS.

In NARMS, antimicrobial susceptibility testing is centralized at government laboratories of the FDA, the CDC, and the U.S. Department of Agriculture. All three laboratories employ identical media, methods, quality control parameters, and repeat testing criteria, along with a common drug panel from a single manufacturer. The compounds tested have many commonalities with the European Union testing design (see below) except that ceftriaxone is used in the United States as a class representative for extended-spectrum cephalosporins (Table 1), while in Europe either ceftazidime or cefotaxime is used. The United States currently also includes amoxicillin-clavulanate, cefoxitin, streptomycin, and trimethoprim/sulfamethoxazole, while the European Union member states test trimethoprim alone. Some European Union countries report resistance data for tigecycline and colistin.

Different breakpoints are used that result in more conservative interpretations in the European Union compared with the United States (Table 1). The European Union uses EUCAST epidemiological cutoff values (ECOFFs) as breakpoints where available (37), which are based on the highest MIC of the wild-type population. In contrast, the United States currently interprets

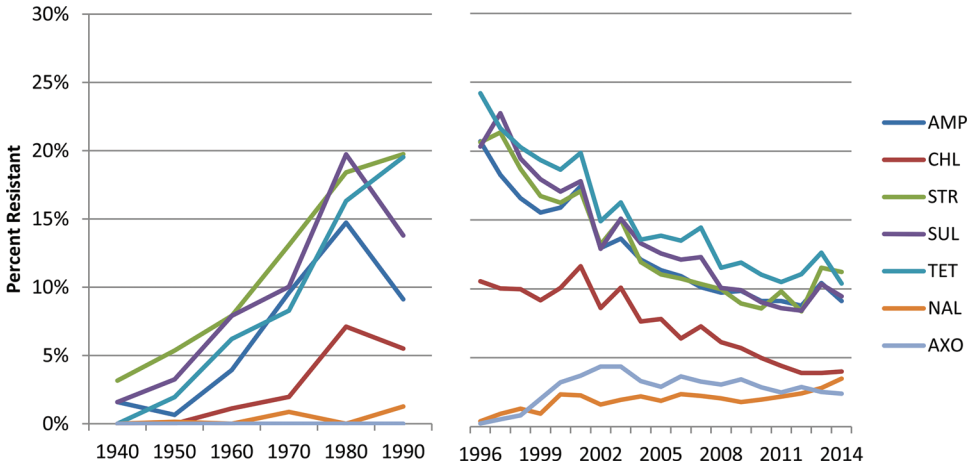


Figure 1 Temporal changes in resistance of clinical nontyphoidal *Salmonella* from the 1940s to 2014. AMP, ampicillin; CHL, chloramphenicol; STR, streptomycin; SUL, sulfonamides; TET, tetracycline; NAL, nalidixic acid; AXO, ceftriaxone.

susceptibility results in *Salmonella* based on the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints (for all agents except streptomycin). Clinical breakpoints also rely on data from MIC distributions, which are combined with pharmacological information and clinical outcome trials to set clinical breakpoints. In accord with WHO recommendations (1), NARMS MIC data are also presented as MIC distributions so that other interpretive criteria can be applied to allow direct comparisons (27). The presentation of MIC data also allows for increased power in detecting slight shifts in bacterial susceptibility to some antibiotics, giving users of the data increased ability to create predictive models of resistance based on policy interventions (38).

Table 1 Antimicrobials tested in the European Union and United States and criteria used to interpret microbiological and clinical resistance

	Breakpoints (μg/ml)		
	European Union[a]		U.S.[a]
Antimicrobial	Microbiological	Clinical	Clinical
Tetracycline (TET)	>8	>4[b]	>8
Sulfonamide (SUL)	>256	>256[b]	>256
Ampicillin (AMP)	>8	>8	>16
Chloramphenicol (CHL)	>16	>8	>16
Nalidixic acid (NAL)	>16	>16[b]	>16
Third-generation cephalosporin (CEP)[c]	>0.5	>1	>2
Gentamicin (GEN)	>2	>2	>8
Trimethoprim-sulfa (COT)[d]	>2	>2	>2
Ciprofloxacin (CIP)	>0.064	>0.064	>0.5
Azithromycin (AZI)	>16[e]	>16[e]	>16[f]
Colistin (COL)	>2		N/A

[a]European Union breakpoints are from EUCAST, and U.S. breakpoints are from CLSI unless otherwise noted.
[b]Derived from CLSI.
[c]In the European Union, isolates are tested against ceftazidime and cefotaxime.
[d]In the European Union, non-human isolates are tested against trimethoprim and sulfonamide separately. In the United States, isolates are tested against trimethoprim-sulfamethoxazole and sulfamethoxazole.
[e]Reference 150, 151.
[f]Reference 149.

The latest U.S. surveillance data (test year 2014) from NARMS comprised antimicrobial susceptibility results for 5,043 NTS isolates, including 2,127 from humans, 262 from retail meats, 1,579 from hazard analysis and critical control point samples (39), and 1,075 from animal cecal samples (27). The prevalence of *Salmonella* in U.S. retail meats in 2014 was 9.1% in chicken, 5.5% in ground turkey, 1.3% in pork, and 0.8% in beef.

A general overview of key antibiotic resistance trends in U.S. clinical isolates of *Salmonella* is shown in Fig. 2. Approximately 82% of *Salmonella* isolated from humans in 2014 had no resistance to any of the antimicrobial drugs tested, a trend that has improved since NARMS testing began in 1996. Multidrug resistance (MDR, resistant to ≥3 antimicrobial classes) was present in around 10% of isolates on average, appearing in 9.3% of human isolates in 2014. In 2014, resistance in human strains was most frequent to streptomycin (11.2%), tetracycline (10.4%), sulfamethoxazole (9.4%), and ampicillin (9.1%), followed by lower levels of resistance to chloramphenicol (4.0%), nalidixic acid (3.5%), ceftriaxone (2.4%), cefoxitin (2.2%), amoxicillin-clavulanate (2.1%), gentamicin (1.4%), trimethoprim-

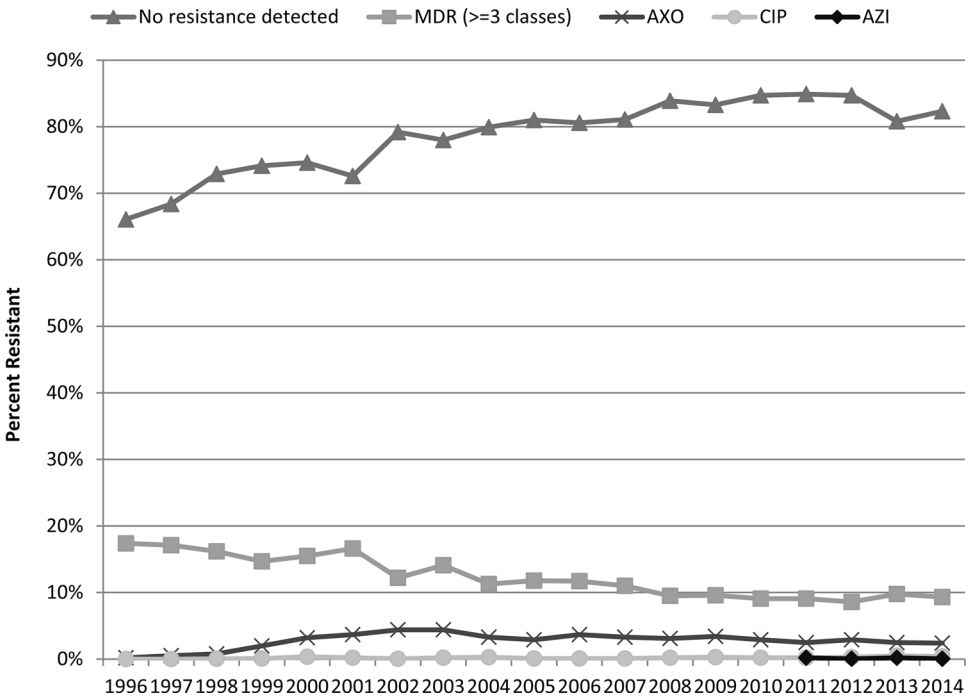


Figure 2 Resistances to critically important antimicrobials in human clinical *Salmonella* isolates from the United States. MDR, multidrug resistant; AXO, ceftriaxone; AZI, azithromycin; CIP, ciprofloxacin.

sulfamethoxazole (1.3%), ciprofloxacin (0.4%), and azithromycin (<0.1%) (27). Resistance to the three critically important drugs ceftriaxone, azithromycin, and ciprofloxacin was below 3% (Fig. 2). These three major findings were largely unchanged from the previous 10 years. The most common *Salmonella* serovars infecting humans in the United States in 2014 were Enteritidis (21%), Typhimurium (12%), and Newport (11%), followed by Javiana (6%), I 4,[5],12:i:– (5%), and Infantis (3.4%) (27).

Multidrug resistance

In general, resistance in human isolates of *Salmonella* has been fairly low and stable over the past decade in the United States. Because a substantial proportion of human infections are acquired from non-food-animal sources (3), however, it is not unexpected that resistance in animal isolates tends to be higher. By animal species, resistance to any of the 14 tested compounds was most frequent in turkey sources (approximately 60% to 80% resistant to ≥1 agent), followed by broilers (about 30% to 60%), and cattle (30% to 40%). These findings are consistent with other studies that show that *Salmonella* from poultry sources tend to be more resistant than *Salmonella* from cattle sources (38) and that swine isolates tend to be more resistant than those from cattle (40).

In human clinical strains, MDR is most frequent in *S. enterica* serovars Typhimurium (28.9%), I 4,[5],12:i:– (27.9%), and Heidelberg (7.6%). While MDR is declining in *S.* Typhimurium (see below), it has risen in *S.* 4,[5],12:i:–, from 5.5% in 2007 to 50% in 2014, with 47.3% exhibiting resistance to more than four drug classes. In animals at slaughter, MDR was most frequent in isolates from turkeys (47%) and hogs (20%), followed by broilers (15%), dairy cattle (10%), beef cattle (6%), and sows (6%) (27). In 2014, MDR was detected in 36% of retail ground turkey isolates and 20% of retail chicken meat. MDR is disproportionately high in broilers among serovars Kentucky (33% hazard analysis and critical control point to 58% cecal isolates) and Typhimurium (16% to 53% among hazard analysis and critical control point and cecal isolates, respectively).

Measuring MDR by the number of resistance phenotypes without regard to the importance of the drug classes involved is of limited value. To help overcome this limitation, NARMS has published new tools online that allow the user to investigate any combination of specific MDR patterns and their changes over time (31). This permits a more refined analysis of MDR patterns in assessing risks, for identifying specific resistance trends of higher public health importance, and to

better understand the specific drivers of resistance where coresistances are involved.

The percentage of *Salmonella* strains that are resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (the ACSSuT penta-resistant phenotype) has been tracked as a hallmark of the globally disseminated *Salmonella* Typhimurium DT104 for decades. The ACSSuT resistance pattern has declined steadily in all U.S. salmonellae from 34% in 1996 to 14.5% in 2014. Declining levels of both *S.* Typhimurium (27) and MDR *S.* Typhimurium (41) are the main drivers behind overall declining levels of MDR *Salmonella* in human isolates. The ACSSuT resistance in cattle isolates of *S.* Typhimurium declined sharply from 67% in 2009 to 7% in 2014. This highlights an important feature of *Salmonella* resistance, namely, the serovar-specific nature of some resistance patterns whose ascendancy and decline may be temporally associated with the prevalence of specific strains.

In the United States in recent years, the ASSuT (ampicillin, streptomycin, sulfonamide, tetracycline) MDR pattern has increased among human isolates of *S.* I 4,[5],12:i:–, where it climbed from 1.4% in 2009 to 43% in 2014. Outbreak investigations of this strain have pointed to swine as a possible source (42), backed by findings of increased *S.* I 4,[5],12:i:– in diseased pigs in Minnesota (40), one of the top five pig-producing states in the United States.

While MDR is not common in *S.* Enteritidis, it is driven by a higher resistance to nalidixic acid compared with other serovars. MDR is common in *S.* Newport, where the MDR-AmpC phenotype on an IncA/C or IncI backbone is a common feature of resistance (43).

Quinolone Resistance

Overall, fluoroquinolone resistance has been consistently low among *Salmonella* isolated from all U.S. surveillance sources. In human isolates, it predominantly presents in serovar Enteritidis (47%) and is associated with travel (44). Since 2007, ciprofloxacin resistance has been detected in a total of only four cattle and six swine isolates in the United States. Ciprofloxacin resistance is not present (using CLSI breakpoints) in isolates from U.S. poultry *Salmonella*, where fluoroquinolones have not been used since 2005. In 2014, the first instance of ciprofloxacin-resistant *Salmonella* in meat was a single isolate from a retail pork sample which carried the *qnrS* gene. This was the first report of *qnr* genes present in retail meat *Salmonella* isolated in the United States (27).

While ciprofloxacin resistance is rare in U.S. salmonellae in general, decreased susceptibility to ciprofloxacin (DSC, MIC $\geq$ 0.125 μg/ml) has increased in humans and cattle strains as well as in swine strains. (Strains with this MIC would be considered microbiologically resistant according to EUCAST breakpoints.) Studies have reported extremely low quinolone resistance among *Salmonella* isolated from feedlot and beef cows as well as dairy cows (45, 46). However, decreased susceptibility is rarely assessed. Distribution of extrachromosomal *qnr* genes is thought to be the main reason why this phenotype is increasing in frequency. Because fluoroquinolones are widely, and often repeatedly, used to treat bovine respiratory disease, a common illness in cattle herds, there is a possibility that fluoroquinolone use may help to propagate an acquired resistance gene. Particularly concerning is the emergence of DSC in *S. enterica* serovar Dublin isolates from humans and cattle, where increasing cephalosporin resistance is also occurring. The incidence of human *S.* Dublin infections is relatively low, but it can cause invasive disease with more severe outcomes. *S.* Dublin also causes severe disease in cattle, mainly respiratory infections, and ranks among the top four serovars isolated from retail ground beef and cattle samples in NARMS and the top serovar among isolates derived from clinical specimens (40). As of the 2014 NARMS testing year, 57% (4/7) of DSC *S.* Dublin isolates from humans and 40% (18/42) of DSC *S.* Dublin isolates from cattle were also resistant to ceftriaxone. This combination of DSC and ceftriaxone resistance puts significant limitations on possible treatment options for human illness.

Cephalosporin Resistance

Among critically important human antibiotics, the temporal association of ceftiofur use in food animals and the emergence of ceftriaxone resistance in both animals and humans has been an area of concern in the United States, the European Union, and elsewhere. In the United States, ceftriaxone resistance was not detected in *Salmonella* prior to the approval of ceftiofur (36) and rose following its approval for use in the United States in livestock and poultry (Fig. 3). From 1996 through 2009, the percentage of NTS human isolates resistant to ceftriaxone increased from 0.2% to 3.4% (41). These rising trends have caused several countries, including the United States, to limit certain uses of cephalosporins in animal agriculture, with positive effects. A well-known example occurred in Canada when voluntary restrictions on ceftiofur use were followed by a rapid and significant decline in ceftriaxone-resistant *S.* Heidelberg (and *Escherichia coli*) in chickens, retail chicken meats, and human clinical isolates (47). In the United States, the FDA announced plans in 2008 to

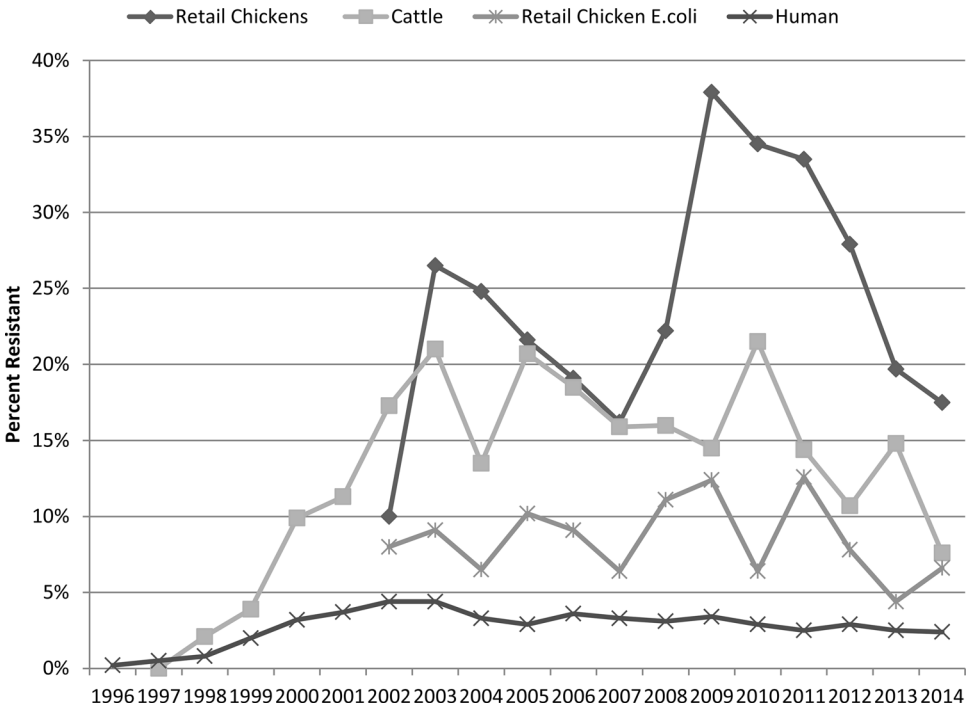


Figure 3 Trends in third-generation cephalosporin resistance in *Salmonella* from the United States.

restrict the use of some cephalosporins, which went into effect in 2012 (48). As of 2014, it appears that the restrictions may be producing the desired effect of reducing cephalosporin resistance in humans and select animal species. Ceftriaxone resistance has declined since 2009 in human (3.4% to 2.4%) and retail chicken (38% to 18%) *Salmonella* isolates (Fig. 3).

Changes in ceftriaxone resistance were most notable for *S.* Heidelberg, where resistance in human isolates declined to 8.5% in 2014, down from a peak of 24% in 2010 (27). Ceftriaxone resistance in retail chicken *S.* Heidelberg remained at 0% from 2011 to 2013 (down from a peak of 32% in 2009), but reappeared in 3/24 isolates in 2014. In retail ground turkey isolates in 2014, resistance continued to decline to 7% after peaking at 22% in 2011. In cattle *Salmonella* isolates in 2014, ceftriaxone resistance reached its lowest level (7.6%) since 1999 (31). Studies of diagnostic cattle isolates show varied results. While some studies show demonstrable decreases in cephalosporin resistance among dairy cattle since 2012 (46), others show continued increases (40) or even no resistance at all among healthy feedlot cattle (49). Many of these disparities are due to regionalization of serovar frequencies, as well as the types of samples analyzed (diseased versus healthy animals).

Ceftriaxone resistance in human strains is most common in the same serovars in which MDR prevails, namely, Typhimurium, Newport, Heidelberg, and I 4,[5],12:i:–, with the addition of Dublin, 11.5% of which were ceftriaxone resistant. Ceftriaxone resistance was also high in *S.* Dublin from cattle (34.6%) and ground beef (60%). In other nonhuman sources, ceftriaxone was disproportionately high in serovars Newport from cattle (66.7%), Kentucky from broilers (66%), Typhimurium from broiler meat (72%), and Heidelberg from fattening turkeys (60%).

NARMS data show that extended-spectrum cephalosporin resistance among U.S. isolates of *Salmonella* (and *E. coli*) is usually mediated by bla_{CMY} genes, whereas extended-spectrum beta-lactamases (ESBLs) have been rare (50). This appears to be changing. Examining NARMS strains from 2012 to 2014, 26 instances of ESBLs occurred, mainly conferred by members of the CTX-M family, along with 3 instances of $bla_{\text{SHV-12}}$ and one case of $bla_{\text{SHV-30}}$. Finding $bla_{\text{CTX-M-65}}$ in a 2014 retail chicken sample led to an expanded examination of U.S. human and animal strains and revealed that the gene had become widespread in a strain of serovar Infantis previously identified in Europe and South America (51).

ANTIMICROBIAL RESISTANCE IN EUROPE

Denmark established the world's first integrated antimicrobial resistance surveillance program in 1995, and

other members of the European Union have followed with their own national programs. In the European Union, antimicrobial resistance in *Salmonella* is tracked in data submitted by European Union member states and Norway and is reported jointly by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC).

The latest European Union data are from 2014 (26), when for the first time, all 28 member states along with Iceland and Norway submitted isolate-level data on poultry and poultry meat products. Therefore, the European Union report focuses on resistance in human and poultry sources of isolates. Countries submit both MIC data, which is interpreted using published ECOFFS, and susceptibility categories interpreted from disk diffusion. In 2014, 21 member states and Norway provided data on human *Salmonella* isolates. Twelve countries (Austria, Denmark, Estonia, Finland, Greece, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, and Romania) reported isolate-level results in the form of inhibition zone diameters or MICs, which allows for improved comparability between human and animal/food isolates. Ten countries reported categorical interpretations of susceptible (S), intermediate (I), or resistant (R) according to the clinical breakpoints. The number and types of antimicrobials reported varied by country, from 2 countries testing only three antimicrobials to 13 countries testing all 10 antimicrobials in the priority panel. This mixture of breakpoints and testing methods, sampling strategies, differences in serovar by country, and the incomplete nature of the isolate-level data mean that the results must be interpreted, and the country differences compared, with caution.

In 2014 in the European Union, a total of 14,412 *Salmonella* isolates from human infections were tested, constituting 16% of all confirmed human cases of illness. When examining all serovars (n = 247) and countries (n = 22) combined, the most common resistances in human *Salmonella* isolates were to tetracyclines (30.3%), sulfonamides (28.6%), and ampicillin (28.2%), followed by lower levels of resistance to trimethoprim-sulfamethoxazole (9.2%), ciprofloxacin (8.8%), chloramphenicol (6%), gentamicin (2.7%), and cefotaxime (1.1%) (26). The top three resistances mirror the order of resistance profiles in the United States (except for streptomycin, which is not reported by EFSA).

Although the United States and the European Union employ different criteria for surveillance reporting, these differences are absent or minor for most drugs. Small discrepancies are evident for extended-spectrum cephalosporins and gentamicin that have nugatory effect on reported rates of resistance. Breakpoints for ciprofloxacin have the largest affect, where application of the EUCAST criteria changes the U.S. resistance percentage in human isolate data from 0.4% to 4.3%. The EUCAST breakpoints were applied for the purposes of comparison below.

Monitoring data from food sources show some commonalities with the U.S. situation. An overall comparison between the European Union and the United States of resistance to the "older" antimicrobials of chloramphenicol, tetracycline, gentamicin, and ampicillin is shown for human isolates (Fig. 4) and broiler isolates (Fig. 5) of *Salmonella* spp. For human data, the U.S. surveillance data are comparable to Slovenia, which reports the lowest resistance levels for these drugs among the member states. Resistance data for broiler strains of *Salmonella* spp. show that the United States is slightly below the European Union average.

Other general features of resistance in both the European Union and the United States are evident. With some exceptions, *Salmonella* recovered from poultry meats is generally more resistant than isolates from human infections in both regions (26, 31). Among poultry isolates, resistance levels in the European Union were generally higher in turkeys than chickens. This is comparable to what was observed in the United States, where turkey isolates tended to be the most resistant, followed by those from chicken, swine, and cattle (27). One might presume that this pattern is the result of higher antimicrobial use in turkeys than in broilers, but there have been no on-farm studies that confirm a causal relationship (52). In general, fluoroquinolone resistance is higher in the European Union, and third-generation cephalosporin resistance is higher in the United States. Other resistance patterns are compared below.

Multidrug Resistance

In 2014, 10 member states tested *Salmonella* against 9 classes of antimicrobials (26). Overall, only 54.8% of human isolates were susceptible to all agents tested. MDR was high overall (26.0%) in human isolates from the European Union, with very high prevalence in some countries. Among poultry sources, MDR was low in laying hens; high in broiler meat, turkey meat, and broilers; and very high in turkeys. As with other types of resistance, MDR tends to be associated with certain serovars, generally being rare in *S.* Enteritidis. In Europe, MDR variants of *S.* Kentucky (74.6%), along with monophasic *S.* Typhimurium I 4,[5],12:i:– (69.4%) and *S.* Infantis (61.9%), are especially problematic in humans. As noted above, the United States also is witnessing a rise in MDR *S.* Typhimurium I 4,[5],12:i:–.

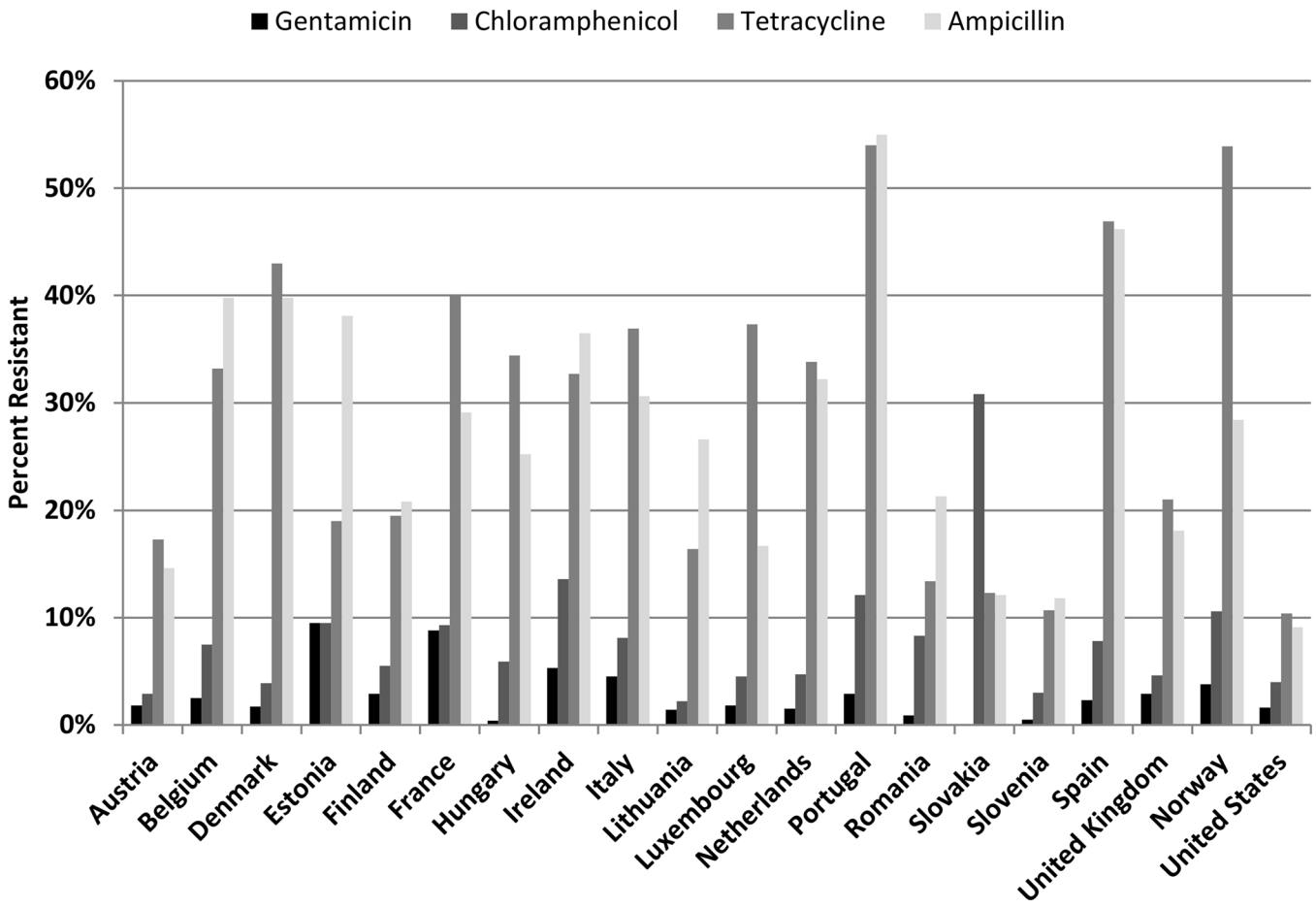


Figure 4 Resistance to gentamicin, chloramphenicol, tetracycline, and ampicillin in human *Salmonella* isolates from select European Union countries, Norway, and the United States. Breakpoints used for interpreting MICs were derived from the EUCAST.

While *S.* Kentucky rarely causes human disease in the United States, it commonly exhibits MDR.

Extended-spectrum cephalosporin-resistant *S.* Infantis (carrying the $bla_{\text{CTX-M65}}$ gene) has emerged in the United States as a public health concern. The circulating clone is similar to an Italian strain of *S.* Infantis carrying the $bla_{\text{CTX-M65}}$ gene (53). While MDR *S.* Infantis has not increased in poultry isolates collected for NARMS surveillance, EFSA reports that *S.* Infantis isolates from European Union broiler meat express very high levels of MDR (>70.0%). Isolates from Italian broilers exhibit exceptionally high levels of resistance to third-generation cephalosporins, which may be characteristic of the CTX-M clone. Particularly concerning was the detection of high-level resistance to ciprofloxacin in these isolates. The clone may be limited to chickens, because no resistance to third-generation cephalosporin was detected in fattening turkeys (26).

Much of the difference in resistance among poultry isolates from the United States and the European Union is likely due to the variation in serovar profiles of poultry isolates between the two regions. *S.* Infantis and *S.* Enteritidis are among the top three serovars in chickens and chicken meats in the European Union, but in the United States, serovar Kentucky predominates. Likewise, in European Union fattening turkeys, serovars Derby, Kentucky, and Newport account for 30% of *Salmonella* isolates, but in the United States, serovars Hadar and Reading round out the top two in turkeys and turkey meats (31).

Quinolone Resistance

A comparison of human isolates from European countries that reported data for both fluoroquinolones and extended-spectrum cephalosporins is shown alongside the U.S. data in Fig. 6. The average resistance to