

Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview

Ayman A. Swelum *,^{†,1} Ahmed R. Elbestawy, [‡] Mohamed T. El-Saadony, [§] Elsayed O. S. Hussein, ^{*}
Rashed Alhotan, ^{*} Gamaleldin M. Suliman, ^{*} Ayman E. Taha, [#] Hani Ba-Awadh, ^{*}
Khaled A. El-Tarabily   and Mohamed E. Abd El-Hack  ^{**}

*Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; [†]Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt; [‡]Poultry and Fish Diseases Department, Faculty of Veterinary Medicine, Damanhour University, El Beheira 22511, Egypt; [§]Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; [#]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Edfina 22578, Egypt; ^{|||}Department of Biology, College of Science, United Arab Emirates University, Al-Ain 15551, United Arab Emirates; [¶]Harry Butler Institute, Murdoch University, Murdoch, 6150, Western Australia, Australia; and ^{**}Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

ABSTRACT On the commercial level, the poultry industry strives to find new techniques to combat bird's infection. During the first week, mortality rate increases in birds because of several bacterial infections of about ten bacterial species, especially colisepticemia. This affects the flock production, uniformity, and suitability for slaughter because of chronic infections. *Escherichia coli* (*E. coli*) causes various disease syndromes in poultry, including yolk sac infection (omphalitis), respiratory tract infection, and septicemia. The *E. coli* infections in the neonatal poultry are being characterized by septicemia. The acute septicemia may cause death, while the subacute form could be characterized through pericarditis, airsacculitis, and perihepatitis. Many *E. coli* isolates are commonly isolated from commercial broiler chickens as serogroups O₁, O₂, and O₇₈. Although

prophylactic antibiotics were used to control mortality associated with bacterial infections of neonatal poultry in the past, the commercial poultry industry is searching for alternatives. This is because of the consumer's demand for reduced antibiotic-resistant bacteria. Despite the vast and rapid development in vaccine technologies against common chicken infectious diseases, no antibiotic alternatives are commercially available to prevent bacterial infections of neonatal chicks. Recent research confirmed the utility of probiotics to improve the health of neonatal poultry. However, probiotics were not efficacious to minimize death and clinical signs associated with neonatal chicks' bacterial infections. This review focuses on the causes of the increased mortality in broiler chicks during the first week of age and the methods used to minimize death.

Key words: bacterial infection, broiler, pathogenic *Escherichia coli*, first week mortality

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INTRODUCTION

The commercial poultry industry depends on raising birds in large quantities at high stocking densities, especially in broiler production systems. According to the

European standards, [Yassin et al. \(2009\)](#) postulated that when the daily cumulative mortality rates are too high, the farmer should reduce the number of broiler chicks in the next cycle. However, owing to the highly intensive management systems with overcrowded barn environments, infectious diseases are inevitable. Hence, minimizing the mortality in a flock is crucial to gain profit in subsequent cycles. Notably, the first week of life is very important for the chick as its entire life is transforming during this period. Starting from a conditioned life in the hatchery, they move on to an independent life in a barn where they have to adjust to new feed,

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¹Corresponding author: aswelum@ksu.edu.sa

water, thermoregulation, and competition and fight infections at the same time (Vieira et al., 1999). Moreover, as their immune systems are not fully developed at hatch (Sedeik et al., 2019; El-Shall et al., 2020), the chick is susceptible to various infections, including bacterial, viral, and parasitic. Out of these infections, bacterial infections occur worldwide in a similar pattern causing massive economic losses to the poultry industry (Nhung et al., 2017).

Infections caused by harmful bacteria, particularly during the first week of birds' lives, result in severe mortalities, poor weight gain, and poor flock uniformity. This leads to economic losses for producers. Using the prophylactic and in-feed growth-promoting antibiotics was the preventative strategy for a long time to face the on-going problems. However, various public health concerns, such as the emergence of antibiotic-resistant bacteria in the environment and antibiotic residues in food, have raised questions regarding this practice (Cervantes, 2015; Mehdi et al., 2018). The poultry industry worldwide is choosing antibiotic-free production because of rapidly increasing customer demand resulting from public health points of view (Cervantes, 2015). Several bacterial infections were proved to cause high mortalities in chicks (first-week mortality [FWM]). As a major cause of high FWM, *Escherichia coli* infections lead to various disease syndromes in baby chicks, including yolk sac infection (YSI) or omphalitis, enteritis, swollen head, respiratory tract infection, cellulitis, and septicemia (Nolan et al., 2020). The acute form of *E. coli* infection results in septicemic lesions and mortality. In the subacute form, pericarditis, airsacculitis, and perihepatitis are the prominent lesions (Lutful Kabir, 2017; Nolan et al., 2020). Many *E. coli* isolates commonly associated with commercial broiler chickens belonged to serogroups O₁, O₂, and O₇₈ (Gomis et al., 2001; Ewers et al., 2004). Pathogenic *E. coli* isolates were categorized into intestinal pathogenic *E. coli*, or extraintestinal pathogenic *E. coli* (ExPEC), depending on the location of the infection. The intestinal *E. coli* includes enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteroaggregative *E. coli*. In this respect, the study of Russo et al. (2000) identified several traits for distinguishing the 3 pathotypes of ExPEC, including avian pathogenic *E. coli* (APEC), neonatal meningitis *E. coli*, and uropathogenic *E. coli*. Ewers et al. (2003) reported that *E. coli* pathogenicity was generally enhanced or initiated by several influencing factors: environmental factors, viral infections, mycoplasma infections, and immune suppression. Generally, young birds are more susceptible to severe infections than adults (Rodriguez-Siek et al., 2005a). The horizontal infections with *E. coli* occur by the contact with other birds, in addition to fecal and oral routes. On the other hand, the *E. coli* vertical transmission was reported from breeders through eggshell contamination (Nolan et al., 2020). Similar to *E. coli*, paratyphoid *Salmonella* species cause YSI and septicemia in newly hatched chicks triggering increased FWM which causes economic losses to the poultry industry (Yassin et al., 2009; Kemmett et al., 2014). The

present review article highlights the various bacterial infections that cause the FWM with special reference to *E. coli* as a major and main participant in FWM of commercial broiler chicks.

FWM in Broilers

The FWM is an essential indicator of the chick quality as well as welfare. The ideal FWM rate in a poultry flock is 0 to 1% (Heier et al., 2002). As FWM highly contributes to the total mortality of the operation, it is important to control it. Multiple factors contribute to increasing the rate of FWM, including breeder factors such as age, strain, genetic line, and egg weight (Lighter eggs cause more chick mortality) (Sedeik et al., 2019). Also, hatchery, feed, and environmental or housing climate (excess ammonia or dust) and season are included factors (Yassin et al., 2009). A study carried out in Taiwan reported that broiler chicks' cumulative mortality depends on the type of ventilation, flock size, distance of shipping the chicks, and delivery route (Chou et al., 2004). The infectious causes contributed to 50% of FWM, while dehydration and nephropathy associated with visceral gout contributed as the other causes in layer chicks (Olsen et al., 2012).

Bacterial Infections as a Cause of FWM

Bacterial infections are a leading cause of FWM (Karunarathna et al., 2017). Despite the scarcity of informative studies, Kemmett et al. (2014) reported their findings on the significant contribution of *E. coli* on chicks' mortality within the first 48-72 h of placement. In their study, approximately 70% of the dead chicks displayed colibacillosis signs, and thirty different virulence profiles were identified in the *E. coli* isolates. In another study on 48 layer flocks, the average FWM was about 1.4%. More than half of the flocks showed FWM due to infectious causes, YSI followed by septicemia being demonstrated in 94% of the flocks. *Salmonella* is the next frequently isolated genus from YSI with high FWM in broiler and layer chicks, followed by many other bacterial species as *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Proteus*, *Klebsiella*, *Enterococcus*, *Corynebacterium*, *Citrobacter*, *Aeromonas*, *Bacillus*, *Clostridium*, *Micrococcus*, *Yersinia*, *Enterobacter*, *Aerobacter*, *Achromobacter*, and *Alcaligenes* leading to several economic losses (Shivaprasad, 2000; Cortés et al., 2004; Khan et al., 2004; Olsen et al., 2012). *E. coli* and *Enterococcus faecalis* were the most frequently isolated organisms from those infections (Olsen et al., 2012). In a study in Ethiopia during 2010-2011, a prevalence of 33.1% YSI was recorded in the FWM predominantly among 3- to 5-day-old chicks. *E. coli* was isolated as the most prevalent bacteria from these cases, followed by *Staphylococcus aureus* (Amare et al., 2013). Moreover, Rai et al. (2005) reviewed the YSI prevalence data in the previous studies. They reported that YSI is the most common cause of FWM

in chicks. The various reports concluded that the incidence of YSI varied between 5.1 and 20.7%, and mortality has been up to 31.5%. However, Karunaratna et al. (2017) reported the incidence of *Enterococcus* (29.71%) and *E. coli* (19.46%) isolated from dead chick embryos during the incubation.

E. coli Infections in Chickens

E. coli is the species of the genus *Escherichia* that belongs to the family Enterobacteriaceae. It is a gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus. Most of the *E. coli* strains have peritrichous flagella and therefore are motile. They are able to grow in both aerobic and anaerobic environments. They can grow on nutrient media at 18 to 44°C, fermenting glucose and producing gas. The *E. coli* produce diffused turbidity in both cultures, and on blood agar, colonies are usually 1 to 3 mm. The colony morphology may vary with rough colonies larger with irregular margins and smooth colonies raised, wet-looking with well-demarcated margins (Nolan et al., 2020). Generally, the recorded *E. coli* serotypes are more than 1,000 but fewer have been implicated in poultry diseases (Awad et al., 2020). *E. coli* is commonly present in the intestinal tract, mucosal surfaces, bird's skin, and feathers. Chickens' intestine contains about 10^6 colony-forming units of *E. coli* per gram feces. These strains always belong to both pathogenic and nonpathogenic types (Awad et al., 2020). Nevertheless, only these strains have specific virulence factors contributing to the ability to cause diseases in birds and are known as APEC. These infections are mostly extraintestinal, with a majority being respiratory and systemic infections. As a result, APEC has been categorized as ExPEC that share similar virulence to the *E. coli* strains that cause human urinary tract infections, sepsis, and newborn meningitis (Manges et al., 2012; Mellata, 2013) (Figure 1). Fecal contamination of eggs leads to YSI, which results in late embryonic mortality or early chick mortality up to 3 wks after hatching (Nolan et al., 2020). It was reported that APEC's egg transmission is common, leading to high FWM with consideration of vertically transmitted fluoroquinolone-resistant *E. coli* from clinically normal breeders (Rosario et al., 2004; Giovanardi et al., 2005; Petersen et al., 2006). Based on the infection's acquired time, embryonic, or at hatch, mortality could start as soon as hatching or as late as 20 h after hatch. Early studies on APEC strains showed that O₁, O₂, O₁₅, O₃₅, and O₇₈ serotypes were mostly associated with colibacillosis outbreaks (Dho-Moulin et al., 1999). A later study by Nolan et al. (2020) confirmed the presence of O₁₈, O₈₁, O₁₁₅, O₁₁₆, and O₁₃₂ serotypes, which were signals for the emergence of new pathogenic serotypes. In addition, Younis et al. (2017) and El-Sawah et al. (2018) showed wide antigenic diversity among APEC strains worldwide.

Serogroups, Antigenic Structure, and Virulence Factors of APEC

In *E. coli*, O antigen determines the serogroup and K (capsular) or H (flagellar) antigens determine the serotypes (Fratamico et al., 2016). O somatic antigen comprises the antigenic portion of the lipopolysaccharide (LPS) in the cell wall (Figure 2). Owing to the O antigens' high variability, it is used to differentiate serogroups as an epidemiological tool (Stenutz et al., 2006). There are 180 O, 60 H, 80 K, and 17 F (fimbrial or pillus-P) antigens (Stenutz et al., 2006). The immune response in poultry birds is mainly directed toward the O antigen of *E. coli*. According to serotyping, the most common serotypes that have been associated with poultry infections are O₁, O₂, O₁₈, O₃₅, O₃₆, O₇₈, and O₁₁₁ (Nolan et al., 2020). Out of those, O₁, O₂, and O₇₈ are the most commonly isolated from cases (Gomis et al., 2001; Ewers et al., 2004). K1 capsular antigen is frequently associated with APEC strains, and it is associated with resistance to serum bactericidal effects (La Ragione and Woodward, 2002; Nakazato et al., 2009). Flagellar (H) antigen, which projects out of the cell, is rotated to provide motility. In *E. coli* and several other species, the central region of the flagellum is variable and consists of H-serotype-specific epitopes (Wang et al., 2003).

Virulence Factors of Pathogenic *E. coli* Isolated From Birds

Protections or Serum Resistance Genes Serum resistance virulence genes (VGs) allow the bacteria to survive in the exterior of the gastrointestinal tract and overcome the host's defense mechanisms involving complement and antimicrobial peptides (Schouler et al., 2012). Mellata et al. (2003) reported that the capsular K1 and somatic O₇₈ LPS increased serum resistance of the APEC, leading to bacteremia. Nilsson et al. (2014) showed a strong correlation between the APEC pathogenicity and 4 serum resistance VGs such as increased serum survival (iss), structural genes of colicin V operon (cvaC), surface exclusion protein, and outer membrane protein A. This was significantly associated with APEC compared with avian fecal *E. coli*. However, the individual existence of the iss gene or whether this iss gene was a marker gene for the presence of the plasmids correlating with APEC pathogenicity was not confirmed. The determination of APEC resistance to heterophils and macrophages is another important trait that promotes the successful infection. It may be related to complement resistance. Kottom et al. (1997) noticed that a complement-sensitive mutant *E. coli* (C3 sub-units) bound and phagocytized is more significant than the wild-type APEC strain from which it had been derived. The presence of type 1 and P fimbriae, O₇₈ antigen, and the 0-min chromosomal region contributed to the protection of APEC against the bactericidal effect of phagocytes, in particular, heterophils (Mellata et al., 2003). Certain APEC strains can survive within macrophages and cause their destruction through apoptosis

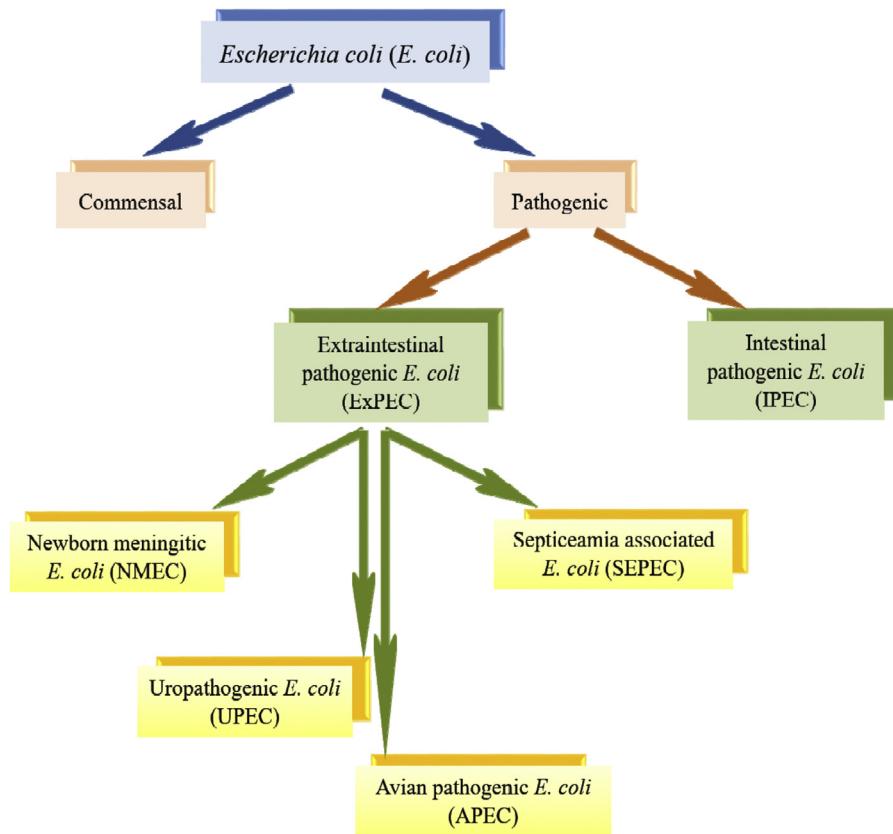


Figure 1. Prevalence and possible transmission of extraintestinal pathogenic *Escherichia coli* (ExPEC).

(Bastiani et al., 2005). Caspases, enzymes essential for apoptosis, were activated by a strain of APEC (APEC17), which resulted in cytotoxicity within 8 h of infection (Bastiani et al., 2005).

Adhesions Bacterial adhesion is based on the recognition between bacterial surface components and specific receptors in the host tissues. Arp et al. (1980) concluded that piliated and motile *E. coli* were more

virulent to turkey poult. This indicates the importance of structures that provide adhesive properties (adhesins), increasing virulence in ExPEC. ExPEC strains encode many adhesions that promote the attachment of the bacteria to cell receptors and are very important for developing septicemia (Monroy et al., 2005). Type 1 fimbriae (F1 fimbriae) have been involved with the initial stages of upper respiratory colonization.

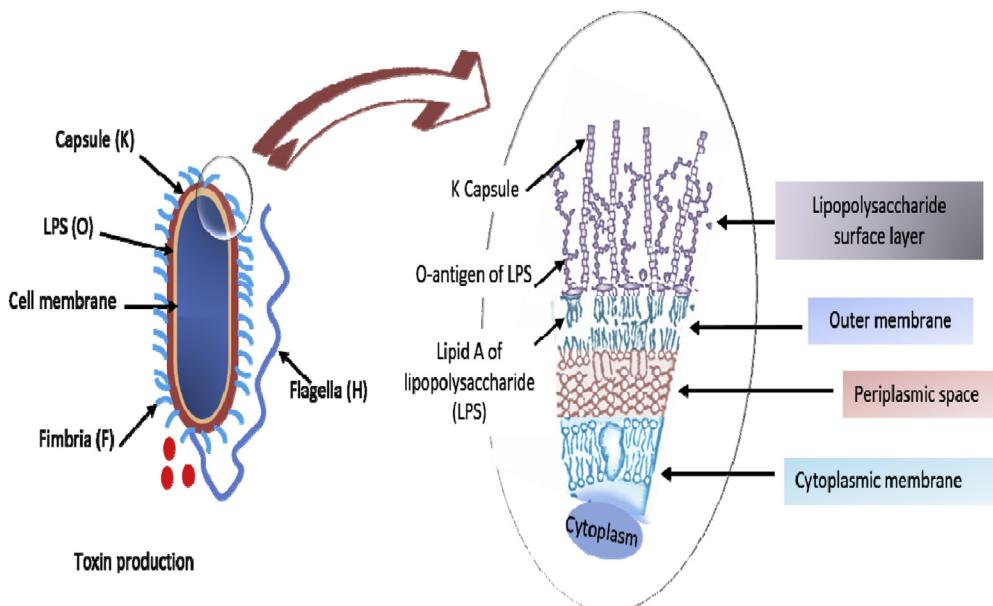


Figure 2. Antigenic structure of pathogenic *Escherichia coli* (note: K, capsule; O, antigen and lipid A of LPS; F, fimbriae; and H, flagella).

In contrast, the P fimbriae were involved in the colonization of the internal organs such as air sacs, lungs, kidney, blood, and pericardial fluid, depicting their involvement in later stages of infection (Wooley et al., 1998). The F1 fimbriae were encoded by 9 fim genes which include a major protein named FimA and minor proteins named as FimF, FimG, and FimH adhesions. Nevertheless, an earlier study of Arne et al. (2000) showed that the APEC FimH mutant strain failed to adhere to the chicken trachea epithelial cells *in vitro*. The P fimbriae are hemagglutinating fimbriae with mannose-resistant properties; they were found in *E. coli* strains producing human urinary tract infections as well as some APEC (Arne et al., 2000). Moreover, they were linked to the colonization of internal organs, which led to septicemia and lethality in one-day-old chickens. This is a temperature-sensitive hemagglutinin (tsh) gene that causes hemagglutination activity at 26°C and repression at 42°C (Provence et al., 1994). The tsh gene is an important virulence marker of APEC, having a strong association with internal organs colonization, septicemia, and lethality in one-day-old chicks (Ngeleka et al., 2002). At the same time, this protein's mucinolytic ability suggests that it must be playing an important role in the colonization of the tracheal mucosa (Kobayashi et al., 2010). P fimbriae were encoded by pyelonephritis-associated pili gene clusters (pap). This pap gene cluster involved 11 genes (papI, papB, papA, papH, papC, papD, papJ, papK, papE, papF, papG), for the biogenesis and synthesis of the P fimbriae (Dozois et al., 2000). Another type of adhesin in APEC, other *E. coli* strains, and *Salmonella enterica* is the curli fimbriae (Nakazato et al., 2009). They were identified and reported as thin coiled surface structures that consist of a single type of subunit called curli. It is associated with the bacterial binding to the extracellular matrix proteins such as fibronectin, laminin, fibrinogen, and H kininogen, enhancing the survival of such bacteria in the extracellular environment (Olsén et al., 1998). According to La Ragione and Woodward (2002), the genes accountable for curli fimbriae expression were encoded by 2 types of operons: csgBAC and csgDEFG. CsgA sequence was recognized in all APEC and recovered from chickens suffering from septicemia (Amabile de Campos et al., 2005). Additional adhesions recognized between APEC strains suggested to be involved in these strains' pathogenesis include type 1-like fimbriae, AC/1 fimbriae, Afa, Sfa, F17, and Eae fimbriae-related sequences (McPeake et al., 2005).

Toxins Cytotoxic activity in APEC was first studied in the early 1990s (Fantinatti et al., 1994). Several toxins were described in APEC strains but with unclear roles in pathogenesis. These involve cytolethal distending toxin, enterohaemolysin, cytotoxic necrotizing factor 1, cytoxin designated VT2y (Parreira et al., 1998), microcin ColV (cvaC), haemolysin, and secreted autotransporter toxin (Tóth et al., 2003). According to Ewers et al. (2005), the vacuolating autotransporter-type toxin coded on a pathogenicity island named VAT-PAI was found to have a role in APEC's virulence as it was

identified with a high frequency between APEC compared with avian fecal *E. coli*. The existence of enterohaemolysin, secreted autotransporter toxin, and cytotoxic necrotizing factor 1 genes has also been described in APEC strains (da Silva et al., 2017). However, their function in pathogenesis was not fully clarified. In addition, some of the toxins' genes (haemolysin, cytolethal distending toxin, and cvaC) have been associated with large transmissible plasmids, indicating that these VGs might be easily transmitted to other strains (Mellata et al., 2012). A recent study by Murase et al. (2016) suggested that hlyF, one of the ColV plasmid genes, is a molecular indicator for APEC. Moreover, this gene was directly included in the outer membrane vesicle production. Shiga toxin gene had been detected in APEC by PCR, but the proof of its expression was little. Lately, a strong mediator for apoptosis (caspase 3/7-induced) and cytotoxic action was described, after a 6-h infection assay using macrophage cell line by an APEC strain (Bastiani et al., 2005). Other toxins described in APEC strains involved the heat-labile enterotoxin and the heat-stable enterotoxin 1 (AstA), homolog of enteroaggregative *E. coli* (Janben et al., 2001).

Iron acquisition mechanisms In APEC, various iron-acquisition mechanisms or operons were found on large plasmids (siderophores, aerobactin or hydroxamate siderophore, yersiniabactin, sit, and iro systems) which are less common in commensal *E. coli* strains (Dozois et al., 2000; La Ragione and Woodward, 2002; Sabri et al., 2006; Johnson et al., 2006a, b; Caza et al., 2008; Tivendale et al., 2009; Caza et al., 2011). Similar to *S. enterica* serovar *Typhimurium* isolate (Zhou et al., 1999), this sit operon was described in APEC using genomic subtractive hybridization and signature-tagged mutagenesis (Li et al., 2005; Schouler et al., 2012). The sit operon encodes an ABC transport system involved in iron and manganese metabolism and resistance to hydrogen peroxide (Sabri et al., 2006).

Invasins The ibeA gene is found in chromosomal pathogenicity islands (PAIs) (APEC O1) of APEC, promoting the invasion of brain microvascular endothelial cells (BMECs) by neonatal meningitis ExPEC. The inactivation of ibeA significantly reduced the abilities of APEC strain BEN2908 to invade human BMEC, adhere to BMEC, and cause avian colibacillosis. The ibeA occurs in 14–20% of APEC (Rodriguez-Siek et al., 2005a,b; Germon et al., 2005; Kariyawasam et al., 2006; Cortes et al., 2008). Also, ibeA of APEC BEN2908 dominates the cellular endocytic pathways to invade cultured avian hepatocytes and human pneumocytes (Chanteloup et al., 2011).

Virulence Gene Traits in APEC Isolated From Chicks Worldwide

The pathogenicity of APEC in relation to the presence of certain VG patterns was studied. Several patterns have been suggested as diagnostic tools for rapid detection of APEC. These patterns include pentaplex pattern

containing hlyF, iutA, iroN, iss, and ompT genes (Johnson et al., 2008), the presence of 5 to 8 genes of the iss, tsh, papC, astA, irp2, vat, iucD, and cva/cvi genes (Kwon et al., 2008), and the presence of crl, fimH, and aer gene patterns (Ghanbarpour et al., 2011). Recently, the presence of one of 4 combination patterns of VGs—A [iutA+, P(F11)+], B [iutA+, P(F11)-, frzorf4+], C [iutA+, P(F11)-, frzorf4-, O78+], and D [iutA-, sitA + aec26+]—were reported (Schouler et al., 2012). Finally, at least 8 to 13 VGs were detected in chicken, whereas intermediate pathogenic isolates contained at least about 5 to 8 VGs (Wang et al., 2015).

In Egypt, 4 VGs (iucD, irp2, iss, and tsh) were detected using multiplex PCR in APEC serotype O27, while O78 and O86 had 2 VG (iucD and iss) and O115 had iss VGs gene only (Ammar et al., 2011). Helal (2012) found out iss and iucD VGs using multiplex PCR. Both genes were present in *E. coli* serotypes O1 and O146, while O114 and O119 serotypes only have iss gene. Regarding the VGs in APEC showing extended-spectrum beta-lactamase activity, AbdEl-Tawab et al. (2018) detected iss and outer membrane protein A VGs only in all examined isolates.

Pathobiology of Colibacillosis in Neonatal Chicks

In young chickens, *E. coli* mostly transmitted horizontally through the respiratory tract by the inhalation of contaminated fecal dust in hatchers or barn. Chicks also get infected in the hatcheries by APEC-contaminated or infected eggs (Nolan et al., 2020). These infections were suggested to happen by the entry of bacteria through the yolk sac. Sometimes, vertical transmission of *E. coli* is also possible from a hen with salpingitis caused by *E. coli*. During the incubation period, APEC causes YSI and embryo mortality. Posthatch infections happen 24 to 48 h after hatching, and mortality elevate up to 10 to 20% for 2 to 3 wks because of septicemia. In the initial part of the infection, lung congestion, edematous serous membranes, and splenitis were apparent. A few days later, acute, fibrinoheterophilic polyserositis appears involving the pericardium, pleura, air sacs, and perihepatic tissue. These chicks usually have retained yolk sacs and yolk abscesses, indicating the yolk sac entry of bacteria. Out of the survivors, up to 5% of the flock may be stunted, and the unaffected birds may grow naturally (Lutful Kabir, 2017; Nolan et al., 2020).

Bacterial Contamination of Poultry Feeds

Sanderson et al. (2005) reported that coliform bacteria, including *Escherichia* spp., *Klebsiella* spp., and *Enterobacter* spp., were the species contaminating animal feeds most. As such, they prompt zoonotic disease outbreaks and secondary infections in humans resulting in major hospitalization cases. Therefore, the ingredient quality control component of a poultry operation feed mill is an important first step in preventing birds'

contamination on the farm. Magwedere et al. (2015) indicated that foodborne and waterborne illnesses associated with *Salmonella* spp. were more commonly due to increased fecal pollution of feed resources. The study also reiterated that animal waste as fertilizer for crops or raw materials destined for producing animal feed is a common practice by some farmers. The *Salmonella* serotypes found in feed ingredients are often not the same as those commonly found in processed poultry. It is plausible that such raw materials may lead to subsequent contamination of the feed mill environment. Poultry feed, which comprises 68% of total production costs, is typically composed of maize and soybean meal mixtures, including several vitamins and minerals, and generally contains 2 or 3 medications (Jones et al., 2016). The microbial contamination of different animal feeds depend on the water activity, oxygen concentration, pH, and nutrient composition of the feed ration (Kim et al., 2007). Feed materials are usually inoculated during growing, harvesting, processing, storage, and dispersal of the feed (Maciorowski et al., 2004; Jones et al., 2016). A soil mixed with animal feces can contaminate standing crops either by direct deposition or fertilizer. Houseflies and cockroaches feeding on the fecal matter can act as both vectors and reservoirs for pathogens in the environment (Maciorowski et al., 2004; Kim et al., 2007). Most plant-based feeds provided to small animals have a difficulty to be treated or disinfected. As a result, the risk of foodborne diseases on animals and consumers will be possible (Maciorowski et al., 2007). Heat treatment is the most effective control method used to inactivate feed pathogens (Abd El-Hack et al., 2017; Saeed et al., 2017). Reductions in bacterial contamination by heat depend on the temperature, treatment time, and moisture content of the feed (Maciorowski et al., 2004). Chemical treatments, such as organic acids alone or formaldehyde, are sometimes used (Meeker and Meisinger, 2015). Also, pelleting is a process of pressing conditioned material with specific dimensions of openings and thickness. One of the supplementary benefits of pelleting is the destruction of pathogens and reduced total microorganisms due to the increased temperature during processing (Zimonja, 2009). Applications of steam and water in animal feed manufacturing have long been recognized as a good way to achieve high-quality pellets (Sredanović et al., 2005). Because poultry feeds are a main source of foodborne pathogens, on-farm control efforts are needed to decrease and prevent feed contamination (Sanderson et al., 2005; Maciorowski et al., 2007). Improving biosecurity measures for feed storage at the feed mill or on the farm would likely be a more cost-effective risk management strategy to lower pathogen proliferation in animal feeds.

Bacterial Contamination in Water Resources

Water composition varies with geographical region and environmental conditions. Water contamination

can occur if surface water drains into the well, especially if the water source is exposed. Several researchers have demonstrated a positive association between drinking water contaminations with *E. coli* O157:H7 and this organism's presence in poultry feces (Saha et al., 2009; Levantesi et al., 2012). Poor water quality may interfere with digestion and subsequent bird performance (Narita et al., 2004; Bain et al., 2014). The effectiveness of vaccines and medications administered through the water can be reduced when water quality is poor (Narita et al., 2004). Leaky water through nipples inside the poultry house will wet the litter and lead to increasing ammonia production and prompt microorganism's proliferation (Ashbolt, 2015). Therefore, control measures should be prioritized to prevent the occurrence of diseases that are spread through water and would certainly result in great economic losses (Kostyla et al., 2015). In this regard, water is an excellent transmission route for agents responsible for human and animal diseases, mainly those in which fecal-oral transmission occurs because contamination of water supplies still gradually increases because of urban and rural activities (Bain et al., 2014). In 2004, about 4 million people were still obtaining water from rivers, ponds, and springs, which were usually fecally contaminated and not treated. So, safe and sufficient water and sanitation would reduce animal mortalities and child deaths by 50% and prevent 25% of diarrhea (Momba et al., 2003a,b). Such waterborne outbreaks often lead to a considerable number of individuals being simultaneously affected, and in most cases, the outbreak subsides when the water supply is adequately treated (Pillsbury et al., 2010). Momba et al. (2006) reported the abundance of pathogenic *E. coli*, *Salmonella typhimurium*, and *Vibrio cholerae* in both surface and groundwater sources in South Africa. The presence of these pathogenic bacteria in drinking water sources poses a serious health risk to consumers. Eliminating *E. coli* O157:H7 from poultry drinking water may be a meritorious goal and an effective measure to reduce poultry drinking water contamination with this pathogenic bacterium (Bucher et al., 2007).

Fecal contamination of water bodies can lead to waterborne illnesses and is detrimental to human health, with microbial contamination being a major cause. Concentrated animal feeding operations (CAFOs) are a potential culprit for microbial water contamination. Human waste from these operations can end up in streams, rivers, and lakes, especially from rain events (Hribar, 2010). Fecal indicator bacteria such as *E. coli* have been used to detect and determine the level of fecal contamination in environmental waters to protect the general population from water-related pathogens (USEPA, 2009). However, owing to antibiotic use to protect animals from infection, CAFOs have consequently grown antibiotic-resistant bacteria, including *E. coli*, which also can end up in the environment from animal waste (Hribar, 2010). American agriculture has transitioned from family-owned small farming to large-scale corporate farming in the last century, with a few companies producing most of the food

animals (MacDonald et al., 2009). Today, these animals' production occurs in CAFOs, which are essentially large-scale industrialized agricultural factory farms. To qualify as a CAFO, a farming operation must first be considered as an animal feeding operation, which is defined as "a lot or facility where animals are kept confined and fed or maintained for 45 or more days per year, and crops, vegetation, or forage growth are not sustained over a normal growing period" (USEPA, 2009; Hribar, 2010).

CAFOs are classified by type and number of animals and how they discharge their animal waste into the nearest body of water. There are size thresholds in considering a CAFO to be small, medium, or large. For poultry, especially laying hens or broilers, the CAFO (which has a liquid manure handling system) is considered large with 30,000 or more chickens, medium with 9,000 – 29,999, and small with 9,000 or fewer chickens (USEPA, 2009). A large number of animals means a large amount of waste, where most environmental health issues arise. Poultry CAFO waste can have several contaminants, such as nutrients, pathogens, and antibiotics (Hribar, 2010). Previous studies have shown that poultry CAFOs have contaminated surrounding watersheds by runoff containing poultry litter (bedding contaminated with feces) (Campagnolo et al., 2002; Mallin and Cahoon, 2003). Poultry litter can lead to degradation of water quality through chemical and microbial pollution. A study conducted by Harden (2015) found that watersheds that had swine and poultry CAFOs exhibited significantly greater nutrient contamination, including ammonia, nitrate, and total N, than watersheds that lacked these operations. Previously, Stone et al. (1995) also found a stream that had both swine and poultry CAFOs and elevated nutrient concentrations during both dry and wet weather events than a nearby background stream that lacked these operations. Nutrients in poultry waste, such as nitrogen and phosphorus, can contribute to eutrophication, which is when there are excessive amounts of nutrients in a body of water, which leads to algal bloom growth which can be detrimental to local ecosystems (USEPA, 2009; Slonczewski, 2017).

A study conducted by Hurby (2015) in the United States found that some of these pathogens from poultry manure can survive weeks in soil, which means heavy precipitation events could also cause water contamination by collecting and discharging the slurry (soil and water mixture) into the nearest body of water. Antibiotics are found in residual levels in waste as they were used to ensure the animals can stay healthy in fighting off a potential infection and, until recently, to promote growth (Marshall et al., 2011). However, overuse of antibiotics has led to antibiotic resistance in pathogens such as *E. coli* because of selective pressure, causing some treatments for infection to be ineffective (Kaufman, 2000; Martínez-Antonio et al., 2008). In 2013, the Food & Drug Administration announced a plan to phase out certain medical antibiotics used in livestock to curb antibiotic resistance (USDA, 2013).

Tyson, the leading poultry producer in the United States, has curtailed antibiotic usage, and the company notably claimed it would eliminate antibiotics important to human medicine for raising its poultry by 2017 (Meyer, 2017). Monitoring *E. coli* and its potential for antibiotic resistance helps maintain water quality and subsequently, public health.

E. coli and Its Role in Water Quality

E. coli is a fecal coliform bacterium that is found in the intestines of humans and animals. Most strains are considered harmless to humans, but some strains produce shiga-toxin, causing hemorrhagic diarrhea, and the serotype O157:H7 is the one most related to food-borne illnesses (WHO, 2018). *E. coli* is commonly used as a fecal indicator organism (FIO) of human and/or animal fecal contamination in freshwater (Pitout et al., 2017; CDC, 2018). *E. coli* is monitored in water as its presence is indicative of other potential pathogenic microbes that could also be present (Edberg et al., 2000). For *E. coli* to qualify as an FIO, it should ideally meet criteria according to the Routledge Handbook of Water and Health (Bartram, 2015). While no one FIO currently satisfies all the criteria under all circumstances, many regulatory agencies and scientists still consider *E. coli* and members of the fecal coliform group as the best for microbial water quality testing (USEPA, 2009).

Owing to high volumes of antibiotics used in animal agriculture, monitoring antibiotic-resistant *E. coli* is also of interest. Some poultry CAFOs use antibiotics to prevent or treat diseases and, until recently, promote growth, but this can create potential antibiotic resistance in bacteria such as *E. coli* because of selective pressure (Martínez-Antonio et al., 2008). Antibiotic resistance occurs when the antibiotic kills most of the intended susceptible bacteria. Still, a small number naturally immune to this antibiotic can survive and reproduce, creating a population of predominantly antibiotic-resistant bacteria (CDC, 2018).

Antibiotic resistance has been an increasing public health issue in relation to CAFOs, as the use and overuse of antibiotics in animal feed have led to microbes becoming antibiotic-resistant (Kaufman et al., 2000). Animals such as poultry do not completely metabolize antibiotics and still exist in their waste (Hribar, 2010). One study tested antibiotic resistance of *E. coli* in fecal samples of turkeys and broilers, in farms and slaughterers, and found that there was significantly higher ($P < 0.005$) antibiotic resistance to ciprofloxacin, flumequine, and neomycin than laying hens that did not have high antibiotic usage (Bogaard et al., 2001). A related study found a high prevalence of antibiotic resistance but of *S. aureus* in turkey (79%; 22/28) and chicken (26%; 6/23) isolates. Antibiotic resistance is not confined to poultry CAFOs but is a concern across industrial food animal production facilities. In 2007, Sapkota et al. (2007) analyzed surface water samples downstream of a swine CAFO and found significant

antibiotic resistance of erythromycin ($P = 0.02$) and clindamycin ($P < 0.001$) in enterococci. Christenson and Stewart (2018) also found higher antibiotic resistance in *E. coli* isolated from surface water downstream of CAFOs than from background watersheds (19% vs. 6%). Some studies have also noted higher levels of contamination after precipitation events.

Prevention of Bacterial Infections in the Poultry Folks

Prevention of various infections that could be detrimental to poultry health is a significant responsibility in managing a poultry operation. Poultry producers, veterinarians, and other stakeholders have worked together in developing protocols applying strict biosecurity measures to prevent the introduction of infections at various stages of the production cycle.

Antibiotics use For years, prophylactic antibiotics have been used in the commercial poultry industry to prevent bacterial infections such as APEC, salmonellosis, and necrotic enteritis. Antibiotic growth promoters were introduced in the 1940s when feeding dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues was found to improve growth in animals (Castanon, 2007). The earliest report of the use of growth-promoting antibiotics ran to 1946 when streptomycin, sulfasuxidin, and streptomycin were used in the feed (Moore et al., 1946). Most commonly, ceftiofur (no longer in use), gentamicin, and lincomycin-spectinomycin were given *in ovo* at commercial hatcheries. Once they reached the barns, enrofloxacin (no longer in use), amoxicillin, penicillin-streptomycin, oxytetracycline, sulfamethazine, sulfaquinoxaline, tetracycline, and tetracycline-neomycin were used in water to prevent or treat neonatal diseases and bacterial septicemias. As for the in-feed antibiotics, the report indicates that penicillin, sulfatrimethoprim, tylosin, virginiamycin, bacitracin, and oxytetracycline were used mainly to prevent necrotic enteritis. Ionophorous and chemical anticoccidial drugs such as lasalocid, maduramicin, monensin, narasin, salinomycin, and nicarbazin were used in feed to prevent coccidiosis (Agunos et al., 2017).

Antibiotic Resistance and the Need for Alternatives Antibiotic resistance in APEC strains isolated from several regions, including Asia, Africa, the United States, Brazil, and Spain reported >70% resistance to ampicillin, amoxicillin, and tetracycline. Resistance against ciprofloxacin, neomycin, and chloramphenicol was 50-70% (Nhung et al., 2017). As the public health concerns of antibiotic resistance and the resulting withdrawal of antibiotics from prophylactic uses started happening in recent years, a dire need for alternative strategies has sparked in the production animal industry. Many researchers seek the potential of various natural and synthetic molecules in fighting bacterial infections, weighing their efficacy and cost-effectiveness on the scale. Probiotics, prebiotics, synbiotics,

postbiotics, organic acids, vaccinations, innate immune stimulation, and improving biosecurity are at the forefront of these alternatives. Although these strategies may not have shown consistent efficiency throughout research, combinations of 2 or more approaches have exhibited proficiency (Caly et al., 2015; Postma et al., 2015; Suresh et al., 2017).

Antimicrobial resistance has drastically increased because of the global misuse and overuse of antibiotics (Fletcher, 2015). This is prompted by the high prevalence of foodborne zoonotic pathogens such as *E. coli*, *Enterococcus* spp., *S. aureus*, and nontyphoidal *Salmonella* and *Campylobacter* spp. (Landoni and Albarellos, 2015; Abd El-Hack et al., 2021). Global monitoring epidemiology reported the resistance of *E. coli* and *Salmonella* strains to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (Hendriksen et al., 2011). They also confirmed that the application of antimicrobials in food animals might lead to the development of resistant strains of bacteria, which propagates to infect both animals and humans (Hendriksen et al., 2011).

Oguttu et al. (2008), on antimicrobial drug resistance of *E. coli* isolated from poultry abattoir workers at risk and broilers, reported antimicrobial usage in food animals to be increasing and resulting in the development of antimicrobial drug-resistant bacteria. It has been suggested that this resistant bacterial strain can be transferred to people working with such animals, for example, abattoir workers (Oguttu et al., 2008).

Antimicrobial drug resistance was investigated for *E. coli* from broilers raised on feed supplemented with antimicrobials and the people who carry out evisceration, washing, and packing of intestines in a high-throughput poultry abattoir. In addition to their specific effects, edible tissues of poultry might contain veterinary drug residues, which would cause hazardous health effects in humans, such as toxicological effects, hypersensitivity, allergic reactions, change of gut microflora, and increased bacterial resistance to antimicrobials (Beyene, 2016). Serious concerns are raised on the antimicrobial resistance in zoonotic enteropathogens (*Salmonella* spp., *Campylobacter* spp.), commensal bacteria (*E. coli*, enterococci), and bacterial pathogens of animals (*Pasteurella*, *Actinobacillus* spp.).

Alternative Strategies to Antibiotics

Since antibiotic use is diminishing because of concerns such as the emergence of antibiotic-resistant bacteria, alternative strategies have become valuable in the control of infections (El-Saadony et al., 2019, Abdelnour et al., 2020a,b; Abd El-Hack et al., 2020a,b; Akl et al., 2020; Ashour et al., 2020; El-Saadony et al., 2020a,b; Reda et al., 2020; Sheiha et al., 2020).

Probiotics, Prebiotics, Synbiotics, and Postbiotics The Food and Agriculture Organization and the World Health Organization collectively defined probiotics as “live microorganisms that grant a health

benefit to the host” (Bajagai et al., 2016). Several microorganisms are being used as probiotics for poultry species, such as bacterial and nonbacterial organisms. Some of the bacterial probiotics are several *Lactobacillus* species (Mookiah et al., 2014; Forte et al., 2018), *Bifidobacterium* (Khaksar et al., 2012; Pedroso et al., 2013), *Bacillus* (Abdelqader et al., 2013), and *Enterococcus* (Mountzouris et al., 2010). Yeast or fungal probiotics are added to feed such as *Saccharomyces cerevisiae* (Aluwong et al., 2013; Bai et al., 2013), *Aspergillus oryzae* (Daşkiran et al., 2012; Shim et al., 2012), *Candida pintolopsii* (Daşkiran et al., 2012), and *Saccharomyces boulardii* (Rajput et al., 2013) that caused positive effects on the performance and gut health.

There are also certain adverse effects reported contradictory to the positive effects (Philpot, 2015). However, multiple reports depict the positive enteric health effects of probiotics in improving intestinal integrity (Dalloul et al., 2003; Ritzi et al., 2014) and mentioning that they could be a great alternative to antibiotics in the poultry industry (Nava et al., 2005; Li et al., 2014).

According to Food and Agriculture Organization, prebiotics is defined as nondigestible food ingredients that benefit the host. They selectively stimulate the growth and activity of bacteria in the colon and thereby improve host health (Pineiro et al., 2008). They are supplying a substrate for beneficial microorganisms in the gastrointestinal tract. Although the previous definition has focused only on few carbohydrates, researchers have redefined prebiotics, including various oligosaccharides containing varying carbon lengths, and collectively designated them as nondigestible oligosaccharides. Different molecules such as fructooligosaccharides, galactooligosaccharides, mannan oligosaccharides, inulin, and isomaltooligosaccharide are among the nondigestible oligosaccharides that have beneficial properties as prebiotics (Ferreira et al., 2011; Ricke, 2018; Alyileili et al., 2020a,b). Reports suggested that certain nondigestible oligosaccharides such as fructose oligosaccharides, inulin-type fructans, and mannanoligosaccharides can modulate gastrointestinal microbiota by increasing the *Lactobacillus* population while reducing harmful pathogens such as *E. coli* and *Clostridium perfringens* (Kim et al., 2011; Pourabedin and Zhao, 2015). Alyileili et al. (2020c) studied the application of nondegraded date pits and degraded date pits using the cellulolytic fungus *Trichoderma reesei* as prebiotics in broiler's diets on the gut bacterial growth and growth performance. They demonstrated that the supplementation of 10% of degraded date pits in broilers' diet, partly replaced with dietary maize, served as a gut health enhancer and thus a growth promoter in the diet for broilers without the use of antibiotics (Alyileili et al., 2020c).

Synbiotics are made by synergistically combining probiotics and prebiotics to provide beneficial effects to the host by promoting microbial feed supplements in the gastrointestinal tract (Andersson et al., 2001). Studies have shown the beneficial effects of synbiotics on reducing harmful bacteria such as *Campylobacter jejuni* (Baffoni

et al., 2012) and *Salmonella typhimurium* (Revollo et al., 2009). Markazi et al. (2018) recently reported that drinking water with synbiotic supplementation helps to influence a healthy microbiota and improved immune response in the intestines of laying hens during a *Salmonella* challenge. The most recent review by Micciche et al. also compiled study reports of synbiotics effect on improving gut health and weight gain other than reducing the harmful effects of *Salmonella* (Micciche et al., 2018). Markowiak and Slizewska (2018) published a review in 2018, compiling most of the studies that showed beneficial effects of synbiotic use in poultry birds and other production animals rather than using probiotics or prebiotics alone. Depending on the probiotic and prebiotic combination, the host's immunomodulatory and microbiome modification effects can vary, as observed via *in ovo* delivery (Dunislawska et al., 2017). An interesting study revealed that synbiotic treatment during heat stress could act as an effective management tool to minimize detrimental effects, particularly in hot regions of the world (Mohammed et al., 2018).

A new term called postbiotics is recently introduced into the poultry industry, referring to the soluble factors (products or metabolic byproducts) secreted by live bacteria or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids. It is characterized by their clear chemical structure, long shelf-life, safe usage, and dosage. Their properties including anti-inflammatory, immunomodulatory, antibesogenic, antihypertensive, hypcholesterolemic, antiproliferative, hepatoprotective, and antioxidant led to the improvement of host health through enhancing several physiological functions (Aguilar-Toalá et al., 2018). Postbiotics are mainly derived from *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Faecalibacterium* species (Konstantinov et al., 2013; Tsilingiri and Rescigno, 2013).

Vaccines against *E. coli* Vaccination is a common strategy used in the control of most viral infections. However, several vaccines have been developed to prevent diseases caused by bacterial pathogens such as *Salmonella*, *E. coli*, *Campylobacter*, *Pasteurella multocida* (fowl cholera), *Avibacterium paragallinarum* (infectious coryza), *Ornithobacterium rhinotracheale*, and *Bordetella avium* in poultry birds (Desin et al., 2013; Filho et al., 2013). Although several vaccine candidates such as whole organism and subunit vaccines have been experimentally tested, the protective efficacies of these commercial vaccines against salmonellosis and colibacillosis have been minimally studied.

Poultry researchers use different strategies from generating autogenous vaccines to modified live, inactivated, and subunit vaccines to help producers protect against *E. coli* infections (Ghunaim et al., 2014). There are currently 3 commercially available vaccines against colibacillosis in the market; Nobilis *E. coli* (MSD Animal Health, Summit, NJ) is an inactivated subunit vaccine consisting of fimbrial antigen F11 and a flagellar toxin (Gregersen et al., 2010; Mombarg et al., 2014).

Gregersen et al. (2010) conducted a study to test breeder vaccination's effect on broiler mortality and found no significant impact or benefit of this vaccine against colibacillosis in the progeny. However, they found a significant effect of this vaccine on the breeders against *E. coli* infection. The introduction of the live attenuated commercial vaccine Poulvac *E. coli*, (Zoetis, Florham Park, NJ) has tempted poultry producers to begin using it because of the reports of its protective effect (Galal et al., 2018). The current vaccine against colibacillosis in the market comprises live attenuated *E. coli* EC34195 serotype O78 with deleted *aroA* gene as the active substance (La Ragione et al., 2013). Researchers reported that the Enterobacteriaceae family members require *aroA* genes for the biosynthesis of aromatic amino acids to acquire the full expression of virulence (Galal et al., 2018).

Thus, the deletion of the gene and adding the aromatic amino acids would result in sufficient bacterial growth on minimal media to produce safe live-vaccine candidates (Hoiseth and Stocker, 1981). Studies revealed that coarse spray vaccination of one-day-old chicks with this vaccine could protect birds against homologous and some heterologous *E. coli* serotypes challenge (Filho et al., 2013; La Ragione et al., 2013; Sadeyen et al., 2015). At the same time, it was identified as safe (Mombarg et al., 2014). Sadeghi et al. (2018) reported that this vaccine was efficient against *E. coli* O78 and that the birds did not develop any lesions (Sadeghi et al., 2018).

Furthermore, it has been elucidated that APEC dominates the immune response induced by the vaccine in the early stage, diminishing by the end of 7 d. Cell-mediated immunity managed by CD 4+TCRV β 1+ on the mucosal surfaces producing immunoglobulin (IgA) and CD8 cells are hypothesized as the protective mechanisms of the vaccine-induced immune response (Filho et al., 2013). In Japan, a group of researchers reported that only the deletion of the *aroA* gene was insufficient to attenuate full virulence. They reported the mutation of the *crp* gene of O78 in APEC as the development of a live vaccine candidate (Nagano et al., 2012). They mentioned a vaccine containing this bacterial strain had been marketed in Japan since 2012. Their studies indicated that with this vaccine, survival rate, and egg-laying rate improved in egg layers (Uotani et al., 2017). The third commercially available vaccine is a live mutant called Nisseiken Avian Colibacillosis Vaccine CBL (Nisseiken Co., Ltd., Tokyo, Japan). This vaccine comprises a 10^7 – 10^9 colony-forming units/dose of AESN1331 O78 APEC strain, which has a deleted *crp* gene. A study by Abd El-Mawgoud et al. (2020) recorded the efficacy of this vaccine against homologous (O78) but not heterologous (O125) APEC challenge.

A recombinant multiantigen vaccine comprising a combination of common EXPEC surface proteins has been recently reported to produce significant levels of IgY against specific antigens and impose immune responses favorable for killing the bacteria. It has reduced bacterial growth of multiple APEC serotypes, reduced bacterial load in organs, and reduced lesions

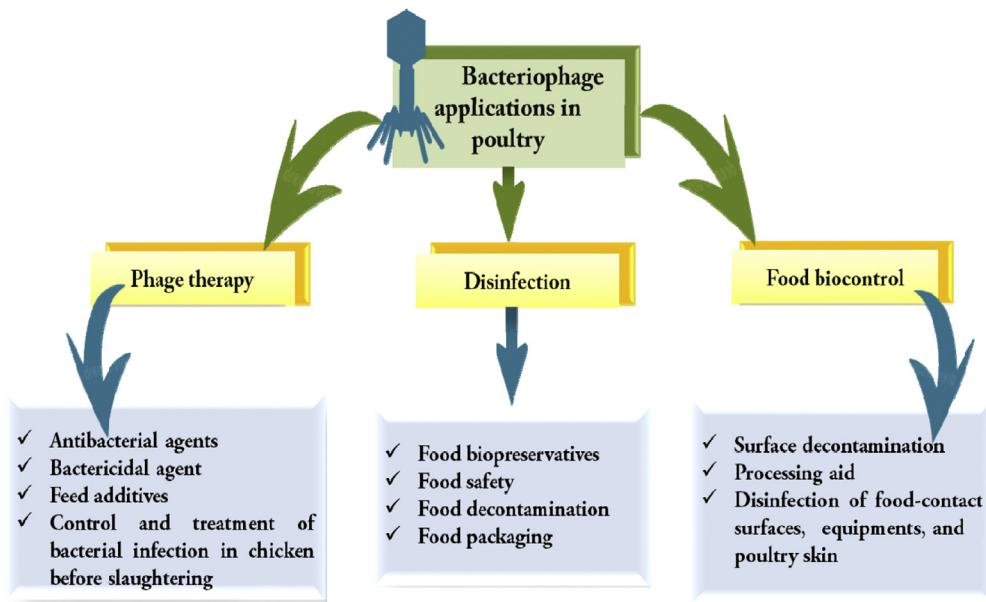


Figure 3. Applications of bacteriophage in the poultry.

(Van Goor et al., 2017). They demonstrated increased IgA and IgY and reduced air sac lesions against homologous challenge using this vaccine (Ebrahimi-Nik et al., 2018).

Meanwhile, the careless use of antimicrobials in the developing countries, and the difficulty of discovering new antimicrobial therapies for resistant *E. coli*, led to the suggestion of using vaccines as the best choice to control *E. coli* infections in poultry farms. Multiple

trials have been conducted to evaluate the efficacy of using vaccination against *E. coli* infecting poultry. However, several difficulties hindered such efforts, including the vaccine's capability to induce cross-protection against various APEC serogroups, vaccine mass delivery methods, and vaccination (Ghunaim et al., 2014). Generally, studies revealed that the inactivated vaccines protected homologous challenges only (Roland et al., 2004). Meanwhile, research on live

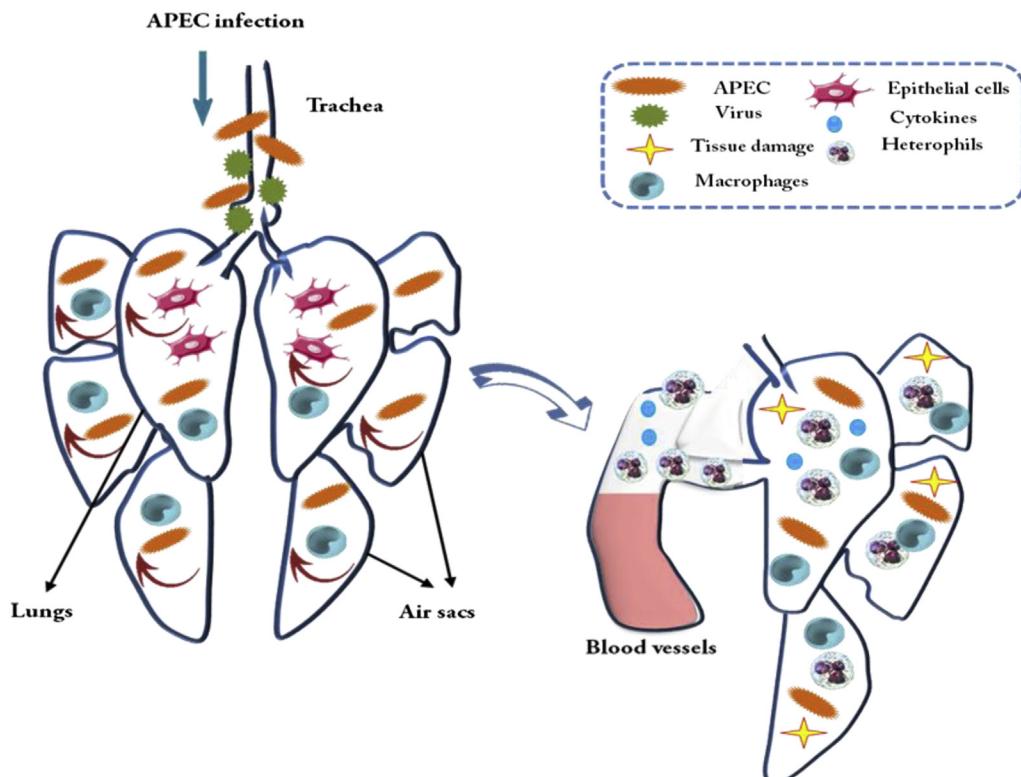


Figure 4. Inflammatory response against avian pathogenic *Escherichia coli* (APEC) in chicken respiratory tract. After inhalation of contaminated aerosol particles, APEC interacts with tracheal epithelial cells, where previous viral infection can facilitate APEC colonization.

attenuated *E. coli* vaccines resulted in the production of 2 commercial vaccines. Both vaccines are currently used in Egypt; however, their field efficacy against homologous and heterologous *E. coli* needs to be further evaluated (Galal et al., 2018). Although subunit vaccines demonstrated better immune response and better protection against homologous and heterologous challenges, large scale experiments are yet not conducted.

Other Strategies In addition to the aforementioned methods, poultry producers use various strategies to prevent bacterial infections in farms. Biosecurity remains a vital measure that is typically underrated in the field. Keeping strict biosecurity in segregation, traffic control, cleaning, and disinfection, helps prevent a large proportion of harmful bacteria and viruses from entering poultry barns (Segal, 2013). Apart from good management practices, there are many alternative approaches proposed and explored by researchers worldwide to overcome bacterial infections in birds. While immuno-modulatory agents will be discussed separately in this article, it is worth brushing over the other strategies here.

Bacteriophages and their lysins have been studies for a long time for their antibacterial effects as potential solutions (Figure 3). These viruses that specifically attack bacteria are currently being recognized for their ability to prevent infections by pathogens such as *E. coli*, *Salmonella*, and *Campylobacter* (Cheng et al., 2014). Huff et al. (2005) demonstrated that the administration of 2 bacteriophages as an aerosol spray and an intramuscular injection reduced mortality significantly against *E. coli* infection. However, owing to these phages' obligatory parasitic nature and their strict dependence on specific bacterial species, many precautions have to be taken during the phage preparation process. Despite the challenges, phage therapy (Figure 3) has lots of potential because of its natural presence, minimal harm, and the ability to adjust to its host bacterium's mutations (Rios et al., 2016).

Several plant extracts have been identified with antimicrobial properties and applied in poultry studies as alternatives to antibiotics (Cheng et al., 2014). Phytobiotics or phytogenic compounds are divided into several categories, such as herbs, spices, phenols, essential oils, alkaloids, and lectins. Adding these to animal diets has been identified to improve the quality of the animals' lives and the food derived from them (Windisch et al., 2008). Essential oils such as oregano, rosemary, and lavender oil used in broiler trials have shown efficient antimicrobial activity (Mathlouthi et al., 2012; Yang et al., 2015; Adaszynska-Skwirzyńska and Szczerbińska, 2018).

Immune Modulation as an Alternative to Antibiotics

Various immuno-modulatory strategies are hot topics as alternatives to antibiotics in food animal production.

Antibacterial vaccines are part of this category, although they are not able to control all bacterial infections because of their specificity to different bacterial agents. Nevertheless, the essence of immune-modulation is about modulating the host immune system or immune functions using the action of immuno-modulatory molecules such as toll-like receptor (TLR), ligands and agonists, hyperimmune antibodies, bacteriophages (Figure 3), probiotics, herbs, and essential oils (Lillehoj and Lee, 2012). Out of the agents, TLR ligands and agonists have been widely studied as strategies to control infections owing to their potent immunogenicity as stand-alone immune stimulants as well as vaccine adjuvants.

As opposed to vaccines, the other benefit of these TLR agonists is that they target the host rather than the pathogen. It is improbable that their repeated use would revert to any virulence or give rise to resistance (Mifsud et al., 2014). TLRs are a cluster of pattern recognition receptors that recognize pathogen-associated molecular patterns that comprise the invading pathogenic organisms such as cell wall components such as LPS, peptidoglycans, bacterial deoxyribonucleic acid, and double-stranded viral ribonucleic acid (Kannaki et al., 2010). Synthetic counterparts of CpG-oligodeoxynucleotides, polyinosinic:polycytidylic acid, and other molecules are promising analogs that are mostly being used in research as TLR agonists (Zhang et al., 2017).

Recognition of specific pathogen-associated molecular patterns by the TLRs results in the activation of signaling cascades leading to the expression of various innate immune responses (Figure 4) such as proinflammatory cytokine genes, reactive oxygen intermediates, and nitrogen intermediates as reported by Takeda and Akira (2005). Such immunomodulatory properties (Figure 4) have been acknowledged to use in vaccine production as antigens, adjuvants, and direct immune stimulators to prevent or fight pathogenic infections (Ahmad-Nejad et al., 2002; Barjesteh et al., 2014). As we discussed using immunomodulatory molecules as alternatives to antibiotics, it would be useful to review the organization of the avian immune system to understand their mechanisms of action.

CONCLUSIONS

In conclusion, minimizing bacterial infections, especially *E. coli*, during the first week of age in chicks is very critical. This will help to obtain high performance of broiler chickens (body weight, weight gain, homogeneity of the bird's weight, immune response to vaccination as well as meat quality) and consequently increasing the profitability. Different strategies should be used for decreasing mortality during the first week of life. The biosecurity, selection of good quality chicks, and immune modulation remain as the main factors to achieve the best results.

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DISCLOSURES

Authors declare no conflict of interests.

SUPPLEMENTARY DATA

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