Avian Pathogenic *Escherichia coli* (APEC) - an update on the control

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Escherichia coli is a common inhabitant of the gastrointestinal tracts of humans and animals. Commensal *E. coli*, designated as nonpathogenic, are considered to be the majority. This microorganism can be divided into two large groups: pathogenic and commensal *E. coli.* The pathogenic group is characterized by two more pathotypes: diarrheagenic *E. coli* (DEC) (with eight subpathotypes) and extraintestinal pathogenic *E. coli* (ExPEC) (with six subpathotypes). ExPEC leads to disease in humans and animals. Avian colibacillosis is the main disease caused by ExPEC and has led to millions of dollars in losses to the worldwide poultry industry. Another problem associated with ExPEC strains is the high rates of antibiotic resistance. The indiscriminate use of antibiotics in animal production may have contributed to the resistant strains of ExPEC. *E. coli* strains of animal origin have been reported in humans. Herein we discuss the major diseases associated with *E. coli* and the problems of resistance to antibiotics in humans and the poultry industry, as well as alternative methods to control colibacillosis in poultry.

Keywords: *Escherichia coli*; APEC; control; methods

1. Introduction

Escherichia coli (*E. coli*) was first described by Theodore von Escherich in 1885, which he named *Bacterium coli commune*[1]. Castellani and Chalmers renamed the bacteria as *Escherichia coli* in 1919[2]. The majority of *E. coli* strains are considered non-pathogenic and known as commensals. By acquiring specific virulence traits, a group of these bacteria developed the ability to survive in different organisms and cause clinical features related to intestinal and extra-intestinal diseases[3,4]. *E. coli* are a common pathogen of the human digestive tract and a leading microorganism associated with severe features of enteric diseases[5]; additionally, they are harbored within food animals. *E. coli* outbreaks leading to food contamination are often associated with ground beef, and more than 75% of *E. coli* outbreaks are related to beef[6]. Recently, retail chicken products have been found to carry *E. coli* strain O25b:K1:H4-B2-ST131[7,8]. *E. coli* can be divided into two main groups: commensals and pathogenic (Figure 1a). The pathogenic group is divided into two other subgroups, known as extraintestinal pathogenic *E. coli* (ExPEC) and diarrheagenic *E. coli* (DEC) (Figure 1b), which has been implicated in gastrointestinal diseases. The ExPEC pathotype is characterized by six main subpathotypes: uropathogenic *E. coli* (UPEC), sepsis/newborn meningitis assciated *E. coli* (NMEC), avian pathogenic *E. coli* (APEC)[9], sepsis-associated pathogenic *E. coli* (SePEC)[10], mammary pathogenic *E. coli* (MPEC)[11], and endometrial pathogenic *E. coli* (EnPEC)[12]. The DEC pathotype is divided into eight other subpathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC)[13]. There are two other emerging subpathotypes: adherent invasive *E. coli* (AIEC) and Shiga-toxin producing enteroaggregative *E. coli* (STEAEC)[14].

APEC, the etiological agent of extra-intestinal infections in birds, is a pathotype that belongs to the ExPEC group. Extraintestinal infections caused by APEC are known as colibacillosis and characterized by fibrinous lesions around visceral organs[15], such as septicaemia, enteritis, granulomas, omphalitis, sinusitis, airsacculitis, arthritis/synovitis, peritonitis, pericarditis, perihepatitis, cellulitis, and swollen head syndrome[9]. APEC infections also lead to reduced yield, quality, and hatching of eggs. The potential for zoonotic transmission must be considered, since poultry serves as the main host for APEC and the consumption of undercooked poultry may infect humans, which can serve as a reservoir of this pathotype[16].

Another problem with ExPEC is the use of antimicrobials for therapeutic purposes. The frequent administration of antibiotics in animal production has provided severe antibiotic resistances to many drugs. This fact leads to great concerns regarding animal and human health[17]. Cephalosporins, quinolones, tetracyclines, and trimethoprim-sulfamethoxazole are the main antibiotics used in poultry farms, so they have become the most resisted among ExPEC[18]. Resistance against these antibiotics has already been reported[19]. Since animal manure might be used as fertilizer in farms, it is important to establish the antibiotic resistance of environmental *E. coli*[16]. Some virulence genes found in APEC isolated from poultry are also present in human pathogenic *E. coli*. Nevertheless, foodborne urinary tract infections (FUTIs) may also be related to APEC[16].

The purpose of this chapter is to elucidate the control measures against avian colibacillosis and approach the main alternatives to minimize the spread of APEC in the poultry production environment.

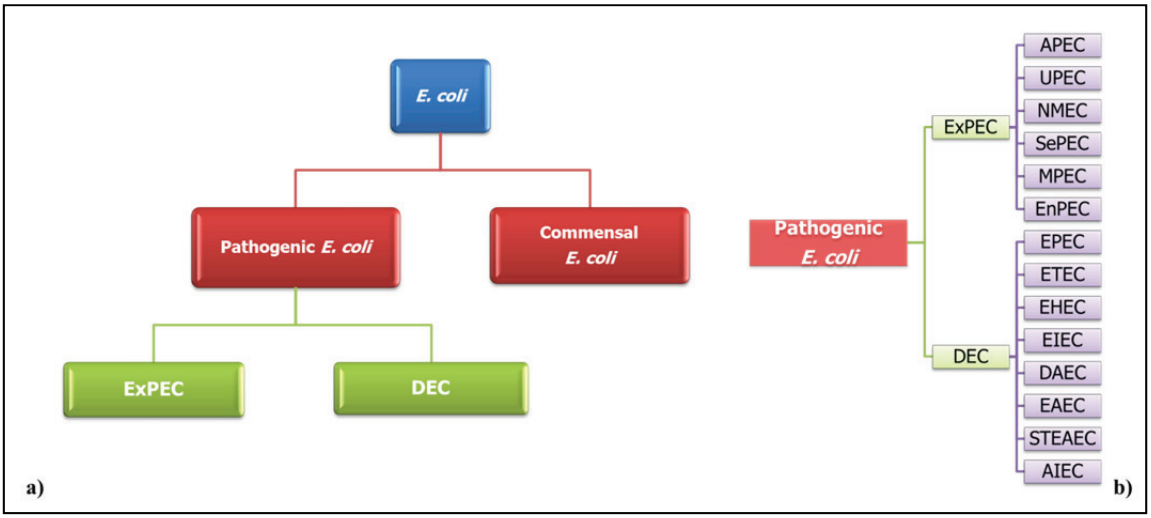

Fig. 1 a) Representation of *E. coli* groups and associated pathotypes b) Representation of pathotypes and subpathotypes associated with pathogenic *E. coli*. (L.S. Cavalli – IPVDF).

2. Zoonotic risk

Campylobacter, *Listeria*, and *Salmonella* are the main pathogens involved in food contamination[20]. Such bacteria are disseminated through meat and eggs[21]. The similarities between avian and human ExPEC regarding their virulence genes and phylogenetic backgrounds are related to a great concern of zoonotic risk[22]. The genome sequencing of APEC strain O1:K1:H7 revealed that it is highly similar to human UPEC and NMEC[23]. Another work has shown that ExPEC from human and chicken diseases, although belonging to different pathotypes, presented overlapping traits and might have a zoonotic potential[24].

Previous studies have demonstrated that healthy poultry and poultry meat from retail markets can be a source of ExPEC for human infections[22, 25]. Some specific strains—ST95 and ST23—might cause diseases in humans and chickens[26, 27]. Otherwise, not all ExPEC strains have zoonotic potential.

The association of ExPEC with zoonotic potential is evidenced by the fact that human ExPEC cause diseases in chicken models of colibacillosis and animal models of human infections that are infected with ExPEC display clinical signs[27, 28]. Another study showed that UPEC and APEC pose the same virulence gene pattern associated with colibacillosis lesions in poultry and appear to have the same potential to cause human UTIs in murine models[28].

It was recently[29] shown that *E. coli* belonging to the phylogenetic group B2 isolated from meat and chicken intestine might cause clinical signs of human UTI in a murine model. In addition, B2 *E. coli* from different sources (*e.g.*, humans with UTI, poultry meat and healthy chicken) possessed high virulence profile[29].

APEC plasmids could be a source of virulence genes for other ExPEC strains[30, 31]. Some common virulence genes in UPEC and APEC are associated with large transmissible plasmids of APEC[32], leading researchers to believe that the zoonotic risk of ExPEC is related to the large plasmids they harbour[30, 31].

Some virulence genes associated with APEC plasmids, such as aerobactin, salmochelin, and *sit* operons, are also present in UPEC plasmids[33]. Additionally, some ColV plasmid-associated virulence genes are present in APEC and NMEC[24], and APEC plasmids can contribute to the pathogenicity of UPEC in mice[34] and NMEC in rats[35].

3. *E. coli* groups

E. coli belongs to the *Enterobacteriaceae* family. It is a facultative, anaerobic, rod-shaped bacterium that ferments lactose by producing acid and gas when incubated at 44°C[36]. It is Gram-negative, not esporulated, and approximately 1.1–1.5 μm in diameter and 1.0–6.0 μm in length. *E. coli* is normally motile due to the presence of flagella. It is usually fimbriated[37] and colonizes the gastrointestinal tracts of both humans and birds.

E. coli can be divided into two main groups: commensal *E. coli* (*e.g.*, harmless intestinal dwellers) and pathogenic *E. coli*. Pathogenic *E. coli* are divided into two main pathotypes: diarrheagenic strains and extraintestinal pathogenic *E. coli*, from genetic and clinical perspectives.

3.1 Commensal *E. coli*

The gastrointestinal microbiotas of most mammalian hosts, including humans, are colonized by commensal *E. coli* strains[38]. The presence of *E. coli* in this tissue has already been associated with digestion and defense mechanisms

against enteric pathogens. The major mechanism of defense by *E. coli* is competition with other pathogens, by avoiding the colonization of harmful pathogens and producing vitamin K[39]. Diseases caused by this pathotype have only been reported in immunocompromised hosts, through the colonization of the colon's mucous layer[4]. *E. coli* A and B1 are the main phylogenetic groups of human origin, and no virulence factors are found in them[40].

3.2 Diarrheagenic *E. coli* (DEC)

This group is associated with many health problems in animals, mainly mammals and humans[41]. According to their virulence factors, the harshness of their clinical implications, and their prognosis, the intestinal *E. coli* can be grouped into eight major subpathotypes: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli*, (EIEC), diffusely adherent *E. coli* (DAEC)[42], shiga toxin (Stx) producing enteroaggregative *E. coli* (STEAEC), and adherent-invasive *E. coli* (AIEC)[14]. DEC are mainly found in phylogenetic groups A, B1, and D[43].

3.2.1 Enteropathogenic *E. coli* (EPEC)

This pathotype is the most important and prevalent among pathogens that infect children worldwide due to their high prevalence in the community and hospital settings. One of the mains characteristics of EPEC is their efficacy in *attaching and effacing* (A/E) lesions[42]. The lesions occur due to the attachment of the *E. coli* to the host cell membrane, which disrupts the cell surface and leads to the effacement of microvilli. The *intimin*, an outer membrane protein, mediates the intestinal cell attachment[44].

3.2.2 Enterohemorrhagic *E. coli* (EHEC)

In this group, serotype O157:H7—the main source of foodborne *E. coli* outbreaks—is responsible for causing bloody diarrhea and hemolytic uremic syndrome (HUS) in humans worldwide. This strain produces a toxin and is known as verocytoxin-producing *E. coli* (VTEC), also known as Shiga-toxin (Stx) producing *E. coli*, which is responsible for causing outbreaks worldwide. An EHEC strain colonizes the human intestine, produces the Stx that binds to the endothelial cells, and gains access to the bloodstream, spreading the toxin out to other organs[45]. The epithelial or endothelial cells are killed by the Stx of the EHEC, which cleaves the ribosomal RNA and disrupts protein synthesis[4].

3.2.3 Enterotoxigenic *E. coli* (ETEC)

Associated with common causes of infectious diarrhea in the whole world, ETEC are disproportionately represented in cases of severe diarrheal illness as well as in deaths due to diarrhea among young children in developing countries. The heat-labile (LT) and/or heat-stable (ST) enterotoxins are responsible for the clinical signs of diarrhea. The toxin affects the intestinal epithelial cells, stimulating the production of cyclic nucleotides and activating the cystic fibrosis transmembrane regulator (CFTR), which will result in a net efflux of fluid into the intestinal lumen[46].

3.2.4 Enteroaggregative *E. coli* (EAEC)

EAEC is responsible for causing diarrhea in children and adults, and contaminated food and water play an important role in its transmission, which occurs via the fecal-oral route. Once ingested, EAEC can bind to the mucosa of the small and large intestines and trigger inflammatory mediators that produce cytotoxic effects involving the intestinal mucosa[47].

3.2.5 Enteroinvasive *E. coli* (EIEC)

This pathotype possesses some biochemical characteristics of *E. coli* and causes dysentery through the same method of invasion used by *Shigella* sp. The most frequent signs related to EIEC is watery diarrhea, which is similar to infection by other *E. coli* pathogens. Other observations related to this pathotype are invasive inflammatory colitis and occasionally dysentery. The pathogenesis of EIEC includes the invasion of the epithelial cells and lysis of the endocytic vacuole, followed by intracellular multiplication, directional movement over cytoplasm, and migration to the adjacent epithelial cells[4].

3.2.6 Diffusely adherent *E. coli* (DAEC)

Children older than 12 months of age are affected by DAEC. About 75% of this strain produces a fimbrial adhesion that inhibits the activity of the complement system against the bacteria. A characteristic cytophatic effect is the development of long cellular extensions that wrap around the adherent bacteria[4].

3.2.7 Shiga toxin (Stx) producing enteroaggregative *E. coli* (STEAEC)

The DNA of the newly emerged outbreak strain O104:H4 is very similar to that of EAEC strain 55989. This new strain contains the protease involved in colonization (Pic) on the chromosome and a pAA-like virulence plasmid that encodes

the aggregative adherence fimbriae (*AAF*), *AggR*, *Pet*, *ShET1* and *dispersin* genes. Another plasmid related to virulence encodes multiple antibiotic resistances. These virulence factors also contain a prophage integrated in the wrbA locus that can produce Stx2. The outbreak strain has also acquired the IrgA homologue adhesin (*Iha*) and a tellurite resistance cluster, which are common features of EHEC strains. According to a study preview, the STEAEC pathotype does not has new virulence factors, but a combination of virulence factors from two other pathotypes. The great morbidity and mortality of this strain might be related to the sturdier adherence of EAEC compared with EHEC, providing more toxins to be transferred[14].

3.2.8 Adherent invasive *E. coli* (AIEC)

AIEC do not cause diarrhogenic infection but are associated with Crohn's disease (CD) lesions in ileal, neo-terminal ileal, and colonic specimens. AIEC strains show a genetic relationship with ExPEC and appear to have acquired novel virulence-specific features, whose genetic basis remains unknown. Infection by AIEC is characterized by the colonization of the intestinal epithelium via type I pili binding to a specific receptor (CEACAM6) overexpressed in the ileal mucosa of CD patients. AIEC appear to require the secretion of outer membrane vesicles (OMVs) in order to invade intestinal epithelial cells. AIEC is able to replicate within the phagolysomes of infected macrophages in the lamina propria, leading to increased TNFα secretion, which may result in the inflammation associated with CD[14].

3.3 Extraintestinal pathogenic *E. coli* (ExPEC)

The ExPEC pathotype is found in the intestinal microflora but does not colonize this tissue, although it causes diseases in other organs. By passing the intestinal epithelium, ExPEC can effectively colonize other organs, causing diseases in both humans and animals. In humans, ExPEC cause urinary tract infection (UTI infections (UTIs) and septicemia or meningitis in newborns. Some animals can also develop UTIs and systemic disease.

Strains of *E. coli* that have been isolated from infections in other organs are grouped as ExPEC, *e.g.*, uropathogenic *E. coli* (UPEC), sepsis/newborn meningitis-associated *E. coli* (NMEC), septicemic pathogenic *E. coli* (SePEC)[48], endometrial pathogenic *E. coli* (EnPEC)[12], mammary pathogenic *E. coli* (MPEC)[11], and avian pathogenic *E. coli* (APEC).

Differently from diarrheagenic strains, ExPEC are characterized by possessing many virulence genes and can vary widely between strains. The most virulent factors are related to bacterial colonization, invasion, iron acquisition, serum resistance to complement phagocytosis, and toxic activity[49, 50, 51, 52].

3.3.1 Uropathogenic *E. coli* (UPEC)

This pathotype is related to major UTI problems, such as cystitis in the urinary bladder, pyelonephritis in the kidneys, and bacteriuria when present in urine. The groups at risk include pregnant women; elderly patients; infants; people with diabetes, multiple sclerosis, or urinary catheters; and immunocompromised individuals[53]. The bacteria adhere to the host tissue, colonize the epithelium, avoid the immune system's defense mechanism, and injure the tissue[54].

The *E. coli* must adhere to the uroepithelium using fimbrial adhesin FimH from type 1 fimbriae, which allow the bacteria to adhere to the host tissue[55]. Haemolysin, aerobactin, serum resistance, and encapsulation are expressed after adhesion occurs[4]. An uropathogenic strain present in the intestine may start urinary tract infections[4].

Cats and dogs are often affected by UPEC, with dogs more commonly affected than cats. The clinical features are characterized by cystitis, but urethritis, pyelonephritis, and prostatitis may also occur. Notwithstanding, swines can be affected by UPEC strains. Urinary infections in pigs lead to puerperal diseases, post-weaning infertility, reduced weight gain in piglets, and increased death rate. Urinary problems are responsible for 50% of sudden deaths of females in production and are the main cause of mortality in adult animals. The appearance of urinary infections depends on the interaction of many variables, such as microorganisms, management, food, and plant and animal condition. Urinary problems mostly occur in adult animals[56].

3.3.2 Neonatal meningitis *E. coli* (NMEC)

Newborns meningitis worldwide is related to this pathotype, which is a common inhabitant of the gastrointestinal tract. To cause the clinical signs of the disease, bacteria must cross the intestine, gain access to the bloodstream, and cross another barrier after that—the blood–brain barrier—to gain access to the central nervous system. At the central nervous system, the bacteria cause meningeal inflammation and pleocytosis in the cerebrospinal fluid. The colonization begins soon after the newborn's birth and is transmitted by the mother. Transcytosis occurs through entereocytes into the bloodstream. The disease depends on high levels of bacteremia: higher than 10^3 colony-forming units (CFU) per mL of blood. The *E. coli* can survive in the serum due to a mechanism that defends against the host's immune system. These mechanisms are mediated by an antiphagocytic capsule made up of polysialic acid and serum resistance mediated by outer-membrane protein A (OmpA). Other tools of defense by NMEC include inhibiting the maturation of dendritic cells invasion of immune cells, such as macrophages and monocytes. In the macrophage and monocyte cells, the bacteria avoid apoptosis and chemokine release, while replicating and disseminating into the bloodstream. The FimH of

type 1 pili and OmpA play a role in the bacteria becoming attached to the blood-brain barrier, through the binding of CD48 on the surfaces of brain microvascular endothelial cells. Invasion of the central nervous system is mediated by the actions of Ibe proteins, FimH, *ompA*, and cytotoxic nectrotizing factor 1 (CNF1). FimH and OmpA mediate the attachment to brain microvascular endothelial cells; OmpA interacts with its receptor and FimH mediates the increase of intracellular Ca^{2+}, stimulating actin rearrangements and CNF1-stimulated myosin rearrangements, which are involved in the invasion of NMEC. NMEC will cause oedema, inflammation, and neural damage by obtaining access to the central nervous system [57].

3.3.3 Sepsis-associated pathogenic *E. coli* (SePEC)

E. coli is the most commonly involved microorganism in cases of sepsis. To promote sepsis in the host, the bacteria must be present in the bloodstream and proliferate. Septicemia is a serious, life-threatening disease caused by bacterial infection in the bloodstream and is also known as systemic inflammatory response syndrome (SIRS). Dysfunction and organ failure are signs observed in severe sepsis cases. UTIs and respiratory disease may lead to septicemia in humans. Beyond UTIs, the infection of other organs, such as the intestine, which leads to peritonitis, the skin, causing cellulitis, and lungs, developing into pneumonia, can also result in sepsis. The main risk groups for developing septicemia are immunocompromised, infant, and elderly patients. Septicemia also causes significant economic losses in animal productionIndustrial poultry infection with *E. coli* can cause features of colisepticemia, a complex of diseases. The main serogroups of *E. coli* involved with septicemia are O1, O2, O8, O35, and O78[58].

3.3.4 Mammary pathogenic *E. coli* (MPEC)

Mastitis is a common disease in women who breastfeed and dairy cows. Mastitis is an inflammatory response of the mammary gland due to bacterial infection. In dairy cattle, mastitis leads to a problem of high economic losses worldwide. The main pathogen involved in mastitis in dairy herds is *E. coli*. However, there are still not enough studies to show where the main serotype of *E. coli* is involved in cases of mastitis, or which virulence factors are involved. In contrast, due to the high genetic similarity between the dairy herd and the high diversity among strains of *E. coli*, like other strains of ExPEC, the *E. coli* involved in mastitis may have different virulence factors that provide different clinical features of mastitis. Then, according to these speculations, *E. coli* involved in cases of mastitis can be allocated as a new putative pathotype, mammary pathogenic *E. coli* (MPEC). Because this strain is not involved in cases of intestinal pathogenic diseases, it can be inserted in the group of ExPEC pathotypes[11].

3.3.5 Endometrial pathogenic *E. coli* (EnPEC)

Pelvic inflammatory disease (PID) or endometritis in women is the result of an ascending infection of the upper female genital tract by Gram-negative bacteria. Such infections are observed the presence of neutrophils and macrophages that lead to the accumulation of pus in the uterus. Such infections by Gram-negative microorganisms are a major cause of PID and infertility in both women and dairy cows[12]. Most of the cattle, after parturition, are affected by ascending infections by Gram-negative bacteria[12]. In cattle, the infectious process begins with infection of the endometrium by the *E. coli*. There is evidence that the endometrium could be pre-colonised by commensal strains or unidentified *E. coli* that is pathoadapted in order to develop PID. In mice, these bacteria colonise the endometrium and cause PID. These *E. coli* strains do not have the same virulence genes such as the ferric yersiniabactin uptake gene (*fyuA*) and endotoxin LPS, which stimulate the inflammatory response. Thus, it is suggested that the EnPEC is adapted to be a pathotype of the endometrium and capable of causing PID[12].

3.3.6 Avian pathogenic *E. coli* (APEC)

This pathotype is the etiologic agent of extra-intestinal infections in broiler chickens and laying hens, and these are collectively known as colibacillosis[8]. Colibacillosis is responsible for significant economic losses in many countries[20], including the United States, Brazil, and China[20]. It affects all cycles of production and all sectors of the poultry industry. It causes high morbidity and mortality in broiler chickens and laying hens[8]. The losses are related to the condemnation of carcasses in slaughterhouses, mortality, and severe decreases in egg production[59, 60]. The main entrance route of *E. coli* is the upper respiratory tract. By gaining access to this tract, the bacteria spreads to other organs, such as lungs, air sacs, liver, lung, heart, and spleen, resulting in a generalised infection[61]. An *E. coli* strain can be designated as APEC when isolated from birds with characteristics of colibacillosis lesions and birds that were killed by this bacterium. *E. coli* designated as APEC must possess some virulence genes such as encoding adhesins, iron-scavenging systems, protectins, and other virulence traits[33, 51, 63]. Control methods based only on predisposing factors were not effective in preventing colibacillosis. Another important point is that the APEC isolates are becoming increasingly resistant to antibiotics, making control of colibacillosis difficult[51].

3.4 Serotyping

Currently, there are between 150 and 200 serotypes or serogroups for classifying strains of *E. coli* in O:H:K. The somatic antigen is related to the serogroup O[58]; K1 is related to capsular antigen[60]; flagella, designated as H antigens; and fimbriae antigens are termed F, as fimbriae type 1 (F1A), P (F11), as well as the curli fimbriae[63, 64]. There is no exact definition of how many serogroups exist as of now, but some authors state that there are more than 177 O antigens, 100 K antigens, and 56 flagellar H antigens[65], others say there are 167 O antigens, 74 K antigens, 53 H antigens and fimbriae antigens[66], and more recently 180 O, 60 H, and 80 K antigens[67].

The serogroups of type O that are more frequent in the APEC strains are O1, O2, O3, O4, O6, O8, O11, O15, O18, O21, O35, O36, O50, O64, O71, O74, O75, O78, O87, O88, O95, O100, O103, O109, O115, O119, O132, O141, and O152[58, 59, 68]. And serogroups more observed in *E. coli* isolated from colibacillosis features are O1, O2, O8, O35 and O78[58]. Notwithstanding, in Brazil, the most frequent serotypes observed are O78, O88, and O45[8].

The adhesion of the *E. coli* cell surface is related to the F antigen. Fimbriae more found in APEC are fimbriae type 1[64, 69, 70] and curli fimbriae.

The H antigen, flagellin, encodes the flagella of the *E. coli*[60]. This antigen can be found in APECs, and its presence is related to motility. This antigen is not related to the virulence of *E. coli*[60].

The capsular antigen K is widespread among APEC strains. This antigen is directly associated with the virulence of the strain. Extra-intestinal infections have been associated directly with this antigen[71].

Serotypes with zoonotic potential belonging to the same human clonal group of *E. coli* isolated from neonatal meningitis, urinary tract infections, and septicemia are O1:K1, O2:K1 and O18:K1[72]. Nevertheless, the serotype O25b: H4, an ExPEC strain, which circulates both in humans and animals, has already been detected in retail chicken[7] and recently in Brazil[8].

4. ExPEC in poultry

4.1 Localised forms

4.1.1 Omphalitis and yolk sac infection

One of the most common causes of mortality in chickens during the first days (Figure 2a) of life is inflammation of the navel, or omphalitis. The presence of *E. coli* is the pathogen most commonly associated with this mortality[31]. Eggs are considered more susceptible to faecal contamination. However, bacteria can access the blood stream from the intestine. The umbilicus that is not yet healed serves as a gateway to an ascending infection with *E. coli* that can also contaminate the yolk sac. Presence of oedema, erythema, crusting in the navel area and/or the yolk sac, and swelling are clinical signs of omphalitis (Figure 2b). In severe conditions, the skin of the body can be lysed, and the chicks may look wet and dirty. Specific treatment for omphalitis disease in chickens does not exist. The best way to prevent disease is to control the temperature, humidity, sanitation of eggs during incubation, processing, and transport. Nevertheless, the birthplace of chicks should be well sanitised and disinfected[61].

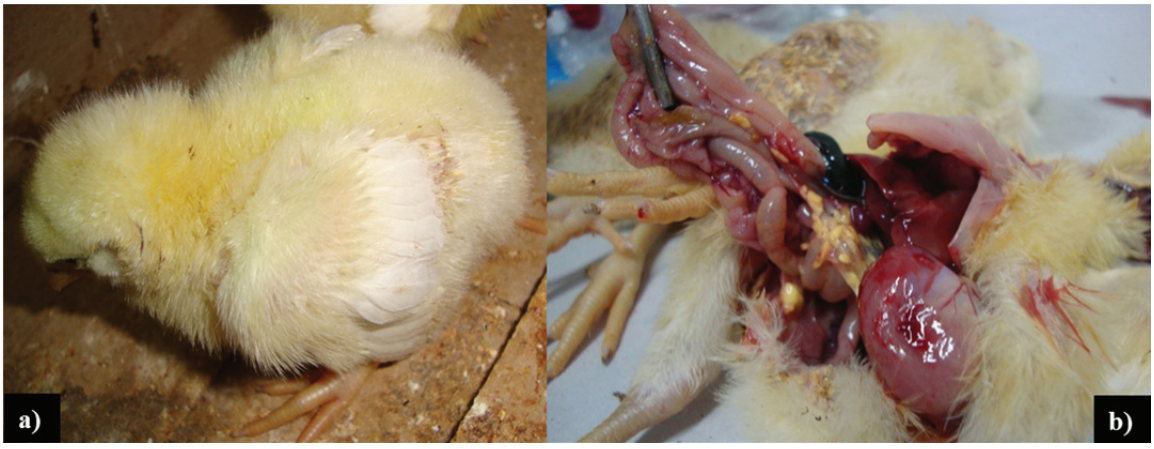

Fig. 2 Omphalitis. a) Five days-old chick with clinical signs of omphalitis. b) Presence of omphalitis and peritonitis. (K.C.T. Brito - IPVDF).

4.1.2 Cellulitis

Subcutaneous inflammation in the lower abdomen and thighs characterises the disease as cellulitis (Figure 3a). The microorganism implicated in this pathology is *E. coli*[61, 62]. Chickens with cellulitis have no obvious clinical signs, and the infection is only observed in slaughterhouses, resulting in housing condemnation[61]. This disease is characterised by superficial skin lesions due to close contact between birds and the quality of the bed[73] (Figure 3b). It is believed that the lesions in chickens as a result of cellulitis may be due to the weakness of their innate immune response, specifically heterophils[20, 74].

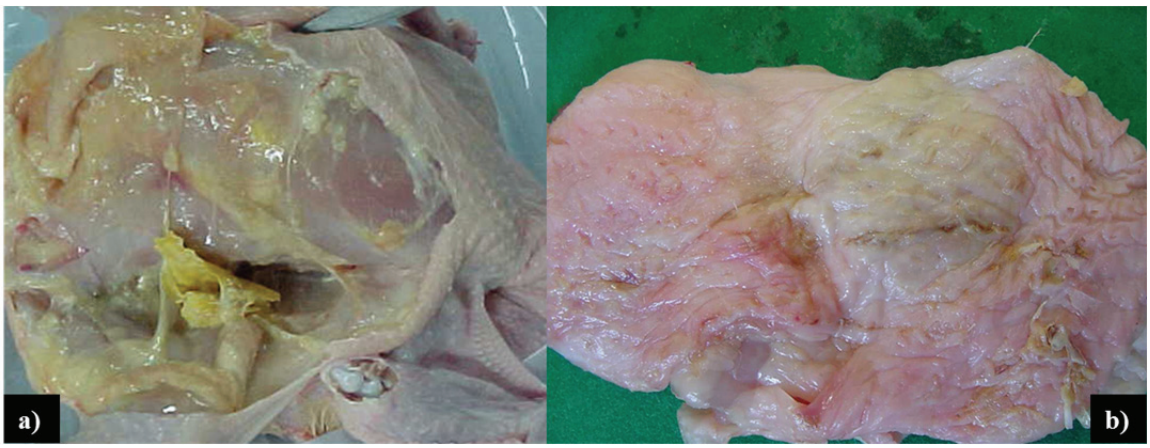

Fig. 3 Cellulitis. a) Presence of caseous exudate over the abdomen. b) Scratch area on the surface of the skin due to close contact between chickens and the litter. (B.G. Brito – IPVDF).

4.1.3 Swollen head syndrome (SHS)

It is characterised as an acute or subacute cellulitis that affects the periorbital region and adjacent subcutaneous tissue of the head (Figure 4a). The first report of SHS was described in South Africa and was associated with infection by *E. coli* and coronavirus. The accumulation of bacteria under the skin, usually *E. coli*, produces an inflammatory exudate (Figure 4b) that leads to swelling of the head, followed by viral infections such as avian pneumovirus and infectious bronchitis virus[61].

Fig. 4 Swollen head syndrome. a) Head swelling due to cellulitis. b) Presence of inflammatory exudate due to bacteria accumulation in the subcutaneous tissue. (K.C.T. Brito – IPVDF).

4.1.4 Acute vaginitis

This disorder is more associated with arrays of turkeys and causes an acute and fatal vaginitis soon after insemination. The *E. coli* infection usually occurs due to a perforation of hymen, which will result in vaginitis, sewage and bowel prolapse, peritonitis, egg binding, and internal laying[61].

4.1.5 Salpingitis/Peritonitis

Both chicken cutting and laying can be affected by salpingitis, which is characterised by the inflammation of the oviduct by *E. coli*[75]. The salpingitis may be related to systemic infection or an ascending infection of the oviduct and vent[76]. Asymptomatic infection may go unnoticed but brings a drop in egg production and increased embryonic mortality in the hatchery[20]. Peritonitis is characterised by the presence of caseous exudate in the body cavity with great presence of inflammation[61].

4.1.6 Orchitis and epididymitis

Similar to salpingitis in females, male genital tract infection will result in orchitis in the male. The *E. coli* infection occurs in an ascending route; the testicles increase in size and are firm, inflamed, and irregular[61].

4.2 Colisepticemia

Colisepticemia in broiler chickens or laying hens causes high morbidity and mortality[59, 77, 78]. The mortality rate is related to the presence of other secondary infectious agents such as virus or mycoplasma diseases. The most prevalent serotypes in the world are O1, O2, and O78, with O2 and O78 found in about 80% of cases of colibacillosis. Currently, a major problem of colibacillosis is high antimicrobial resistance, making treatment via antibiotics ineffective. However, currently available vaccines do not confer immunity sufficient to protect chickens against disease, so the laying hens, broilers, and turkeys can be easily affected. This fact causes huge losses to the poultry industry worldwide due to large losses or attempts at treatment[79]. The disease is characterised by the following steps: acute septicaemia and subacute and chronic granulomatous inflammation polyserositis[61].

Pericarditis is characterised by a prominently myocarditis vessels because of pericardial hyperaemia. The pericardium presents swollen and cloudy. Fluid and smooth masses of pale exudate accumulate in the pericardium, along with fibrin exudate[61].

4.2.1 Respiratory forms

Chicks between four and nine weeks of age are more predisposed to chronic respiratory disease (CRD) features that might evolve into septicaemia. The main entry site of APEC strains to the bloodstream is the respiratory tract, specifically the regions of gas exchange and lung air sacs[82]. The APEC can survive the action of phagocytic cells, heterophils, and macrophages due to adhesion to the epithelial cells. The high concentration of dust and ammonia inside the chicken house allows the deciliation of the upper respiratory tract of broilers, thus providing for the colonisation of this tract by *E. coli*[61]. *E. coli* enters the bloodstream after causing injury to the mucosa of the respiratory tract leading to a high mucus production and deciliation of the tracheal epithelium[61]. After the accession, *E. coli* spreads to the body of the infected bird. The inhalation of dusts contaminated with coliform has also been observed with a major infection of susceptible birds[61].

In this form of the disease are observed early signs of airsacculitis followed by a generalised infection in most organs due to septicemia[20]. An early sign of infection is airsacculitis, which is the result of inhalation of the bacterium[20]. The colonisation of air sacs by *E. coli* is readily achieved due to the absence of macrophages in the body[81]. Following the airsacculitis, there is widespread infection such as pericarditis (Figure 5a), perihepatitis (Figure 5b), and septicemia[20].
The most predisposing factors for infection are APEC infectious bronchitis virus (IBV), Newcastle disease virus (NDV) vaccine samples, mycoplasma, and high concentration rates of ammonia[61]. When infection occurs only for NDV or IBV, there is an increased susceptibility[61]. In turkeys, avian pneumovirus infection is also a factor which increases susceptibility to infection APEC[61]. Concomitant infections with IBV and *E. coli* are more severe than any other infection[61].

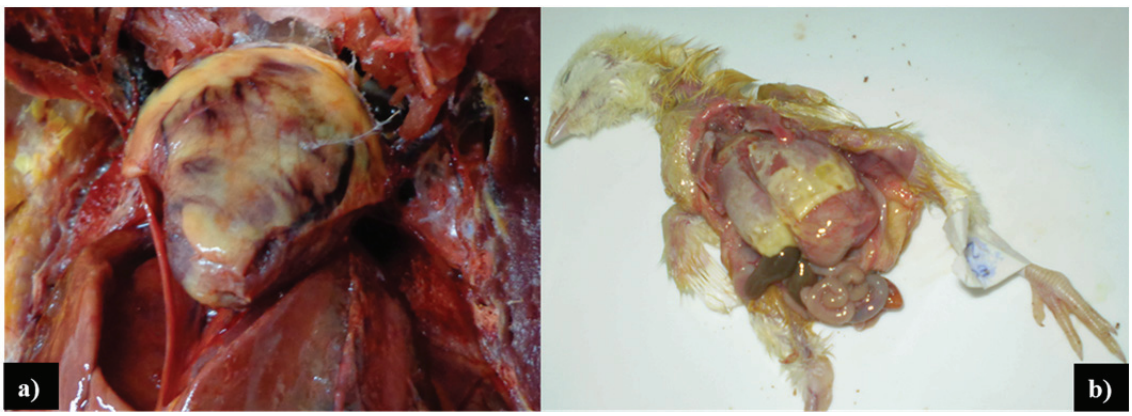

Fig. 5 Colisepticemia. a) Pericarditis: fibrosis and exudate within the pericardial sac. b) Perihepatitis due to widespread infection. (B.G. Brito – IPVDF).

4.2.2 Sequelae of colisepticemia

Colisepticemia can take the birds to death or leave them with sequelae[61]. If the birds do not die due to the infection, the *E. coli* can spread to other organs where the immune system is not very effective, such as eyes, brain, and bones/synovium[61]. After infection by the microorganism and elimination of the *E. coli*, the main findings that can occur are the presence of exudate in the pericardial sac, fibrosis in the liver, and ascites due to interaction of *E. coli*-IBV in the lung[61].

Osteoarthritis occurs due to synovitis and joint inflammation and leads to osteomyelitis. Growth retardation and lameness are clinical signs of this disease[61]. Osteomyelitis occurs due to an inflammatory response to bacterial invasion of the epiphyses of bones[61]. Dissemination into the joint and other tissues occurs due to invasion of bacteria to epiphyseal vessels[61]. The presence of osteomyelitis is seen most often in bones such as femur, thoracolumbar vertebrae, and humerus[61]. In the long bones, the most affected part is the proximal epiphysis. A characteristic of this disease is that the lesions are often seen in the same location as endochondral ossification[61]. Macroscopically, examination easily reveals osteomyelitis[61]. Arthritis occurs most often in bones such as hock, stifle, hip, wing joints, and joints free of thoracic vertebra[61]. Arthritis is usually accompanied by tenosynovitis[61]. In contrast, inflammation of adjacent tissues hardly occurs[61]. Presence of large amounts of exudate in superficial and deep pectoral muscles is an indication that the shoulder joint or the proximal humeri are also affected by inflammatory process[61]. Birds that show signs of progressive paralysis or paralysis may have inflammatory processes in articular joint spaces of the thoracic vertebra, leading to ankylosing spondylitis[61].

4.3 Coligranuloma (Hjarre's disease)

It is a rare form of systemic colibacillosis and can affect broiler chickens, laying hens, and turkeys[61]. The disease usually affects few birds, but when it spreads throughout the whole batch can cause high mortality rates, close to 75%[61]. The disease is characterised by the presence of granulomas in some organs such as liver, duodenum, cecum, and mesentery[61] (Figure 6). The characteristic lesions of coligranuloma are similar to leukosis tumors[61]. The presence of coagulation of the liver, few heterophils, and giant cells are observed[61]. In turkeys, the presence of pyogranulomas typhilitis and hepatitis was reported relating to coligranuloma[61].

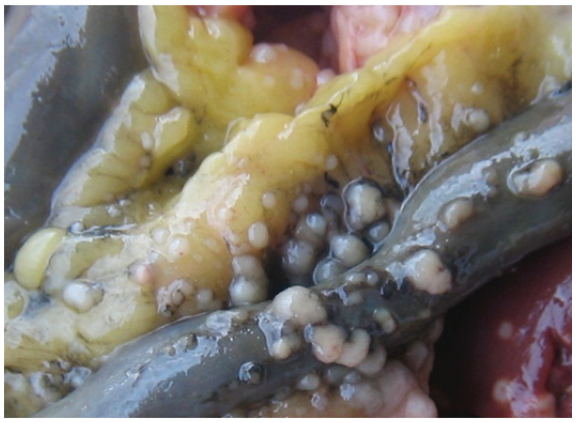

Fig. 6 Hjarre's disease. Presence of cecal and mesenteric granulomas. (B.G. Brito – IPVDF).

5. Diagnosis

5.1 Isolation and biochemistry

Many microorganisms to be cultivated in the laboratory must be cultured in media to allow it to grow in similar conditions as *in vivo*. Some culture media allow the microorganism to grow well and can be distinguished due to their biochemical characteristics. Nonetheless, some media allow some pathogenic microorganisms to grow and inhibit the growth of others[9].

The medium of choice for growth of intestinal pathogenic Gram-negative microorganisms is eosin–methylene blue agar (EMB)[83]. The dyes, eosin Y, and methylene blue are used as indicators of differentiation in response to fermentation of lactose and or saccharose in organisms that ferment lactose and those that do not. In addition, methylene blue acts as an inhibitor of Gram-positive organisms. Some enterobacteria or coliform groups may ferment saccharose more readily than lactose[84]. Colonies of *E. coli* usually present a dark centre and a greenish metallic sheen

due to the rapid fermentation of lactose, whereas other organisms such as *Salmonella*, which do not ferment lactose or saccharose, produce colonies that are non-coloured or have a transparent amber colour[9].

Not very different from EMB media, the MacConkey (MAC) agar is another selective medium that inhibits the growth of Gram-positive microorganisms, especially *Enterococcus* and *Staphylococci*. Growth inhibition is mediated by the presence of crystal violet and bile salts[9]. Bacteria grown on MAC are designated 'lactose fermenters'; the colonies are brick-red in colour and are surrounded by a zone of precipitated bile (Figure 7). The fermentation of lactose produces an acidic product, and these reactions provide the colour to the colonies. If the colonies grown on MAC are pink, the bacteria are Gram-lactose-fermenting[85].

Another way to identify the microorganisms is by their biochemical characteristics. *E. coli* and other pathogens respond to changes in the environment due to the presence of various enzymes they possess. The main change mechanisms are modulation, selective covalent modification, and inactivation[86]. *E. coli* has many biochemical characteristics, but the main biochemical characteristic of *E. coli* is the production of acid and gas after fermentation of glucose, maltose, mannose, mannitol, xylose, glycerol, rhamnose, arabinose, and sorbitol. Positive responses are observed in motility, lysine, and indole production. Negative results are expected in tests like oxidase, citrate utilisation, urea hydrolysis, gelatine liquefaction, and H_2S (hydrogen sulphide) production[86, 61]. Other tests like methyl red and Voges–Proskauer are expected to be positive and negative, respectively[60]. A basic characteristic of *E. coli* is the ability to ferment glucose with the production of acid and gas. The conversion of lactose to glucose and galactose takes place due to the presence of the enzyme *β*-galactose. This enzyme is used for the differentiation of *E. coli* from *Salmonella* spp. and *Shigella* spp[86].

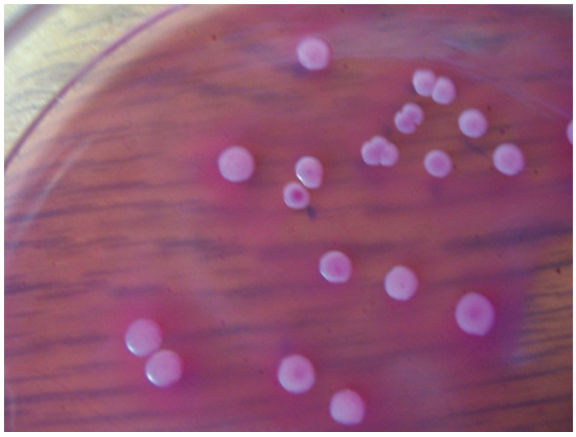

Fig. 7 Isolated brick-red coloured *E. coli* colonies surrounded by a precipitated zone on MAC agar. (K.C.T. Brito – IPVDF).

5.2 Molecular biology

E. coli can be classified into different phylogenetic groups according to genetic sub-structure. The phylogenetic classification groups may be demonstrated by triplex PCR[87], based on the detection of genes of the *chuA* and *yjaA* genes and a DNA fragment TspE4.C2. This PCR allowed the classification of *E. coli* into 4 major groups, A, B1, B2, and D. According to this technique, the ExPEC strains belong mainly to group B2, with some located in group D[43] and commensal to group A. Further studies made by the same author indicate that the presence of 8 phylo-groups is now recognised; 7 are designated A, B1, B2, C, D, E, and F; and one is *Escherichia* cryptic clade I[88]. The group with the highest prevalence and also increased virulence is B2, usually where infectious strains of ExPEC are allocated[20].

PCR multiplex panel targeting was described to detect five genes carried by plasmids, considering that these genes are associated with highly pathogenic APEC strains: *iutA*, *hlyF*, *iss*, *iroN*, and *ompT*[51] (Figure 8). This multiplex PCR that amplifies these five genes was used in 994 avian *E. coli* samples. The PCR distinguished APEC strains from the avian faecal *E. coli* isolates due to the presence of these five genes.

Another tool that may be useful to provide information about the APEC strains is the phylogenetic tree. In Brazil, a phylogenetic analysis was performed based on multilocus sequence typing (MLST) of seven housekeeping genes[52]. The phylogenetic tree assembled by them was able to cluster the different pathotypes together. An *in silico* virulence gene profile was also determined for each of these strains, according to the presence or lack of 83 well-known virulence genes described in pathogenic *E. coli* strains. APEC strains from Brazil and the United States and human strains from UPEC and DEC had genetic similarities, according to the MLST phylogeny and virulence profiles. These results show that APEC strains from poultry may be hazardous to humans[9].

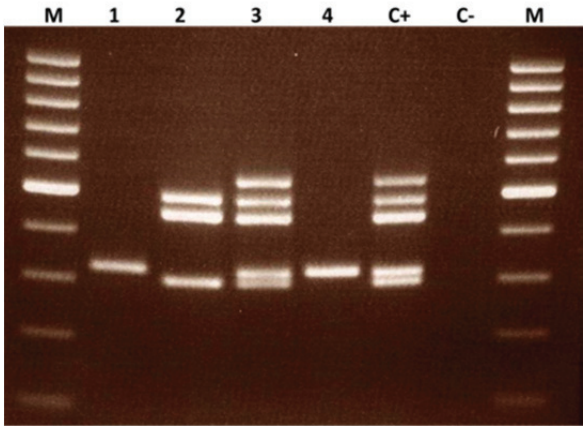

Fig. 8 Pentaplex PCR by Johnson et al. 2008, performed at our lab. **Lanes: M** DNA ladder 100 base pairs (bp) (Ludwig Biotec®). **1** and **4** amplicons of the *iss* gene (323 bp). **2** amplicons of the *iutA* (302bp), *hlyF* (450bp) and *ompT* (496bp) genes. **3** amplicons of the five genes: *iutA* (302bp), *iss* (323 bp), *hlyF* (450bp), *ompT* (496bp) and *iroN* (553bp). **C+** Positive control (BK325). APEC strain isolated from a celullitis lesion. **C-** Negative control (*Escherichia coli* ECO 51): lack of virulence genes. (H.C. Kunert Filho – IPVDF).

5.3 Pathogenicity test in day-old chicks

The virulence mechanisms related to the strains of APEC are still not well understood. Many researchers seek whether there are one or more genes related to clinical cases of colibacillosis. Even with advances in molecular biology techniques, it is not known which genes are directly related to the infection of birds by APEC. Because of this problem, the subcutaneous inoculation method of APEC in embryos or 1-day-old chicks is the gold standard test to confirm whether a strain of *E. coli* is virulent. To consider an APEC strain to be pathogenic to birds, it has to cause the death of the chick or the characteristic lesions compatible with colibacilosis followed by death[9].

6. Control

6.1 Management procedures

One of the most important forms of dissemination of *E. coli* among the flocks is through contamination of hatching eggs. Care to avoid contamination should be frequent. The measures to be adopted should be collecting eggs frequently to keep the material in the nest always clean, discarding floor eggs with obvious faecal contamination and cracks, fumigation or disinfection of eggs shortly after laying or no later than two hours[61]. Such measures help decrease the transmission of *E. coli*. Sanitising the shell surface may reduce or eliminate the presence of *E. coli*[89]. The use of sanitizers via electrostatic spraying enhances their effectiveness[90]. Another method that works effectively and does not interfere with incubation and does not affect the hatchability of eggs is irradiation by ultraviolet, which reduces or eliminates *E. coli* as well as other pathogens[91]. During incubation and hatching, handling and care should be the maximum possible, because if an infected egg breaks, it will be a source of contamination for the other chicks. The handling team as well as the hatchery equipment are also potential sources of contamination to other chicks[61]. The susceptibility period of eggs is up to the time of hatching. There are a few recommendations about methods of dissemination and prevention during incubation and hatching. Cross-contamination and losses can be minimised by ventilating the incubator to the outside and having a few batches of arrays if possible. Contamination within the hatchery takes place through people who are exposed to *E. coli* on the farms or in environments contaminated with this pathogen. Chicks that may be contaminated with *E. coli* should be kept warm and hand fed[61].

Birds that have a well-balanced diet rich in protein, increased selenium[92], and vitamins A and E[93] tend to have better survival. However, the excessive use of selenium in the diet may also be harmful because it favours cellulitis and colibacillosis and hinders the production of antibodies. The severity of colibacillosis is directly dependent on bird nutrition[61]. A feed system based on alternate days was shown to be more effective in *E. coli* challenges than birds fed normally[94].

To reduce the level of pathogenic *E. coli* in the intestinal tract much as in stool, certain factors should be considered: pelleted feed has fewer *E. coli* than mash, rodent dropping are a source of pathogenic *E. coli*, and contamined water can contain high numbers of the organism; these should not be overlooked[61]. The *E. coli* is eliminated by heat during the pelletizing process[95]. Another attempt to eliminate *E. coli* is the addition of egg yolk powder, about 5-10%, to feed[96]. The water is the source of contamination. To reduce the dissemination of the *E. coli* through water, the use of a nipple watering system and chlorination are recommended[63]. These measures reduce the levels of colibacillosis and condemnations by airsacculitis. Competitive exclusion may be another alternative to eliminate pathogenic strains of *E.*

coli[97] from the guts of chicks. Some methods for the competitive exclusion elucidated already provide the microflora of poultry chicks resistance, such as commercial products of competitive exclusion or *Bacillus subtilis* spores[98, 99].

To avoid contamination of the flock by colibacillosis, it is recommended to keep a good quality of the air and bed. The correct ventilation of avians maintains levels of dust and ammonia at low concentrations and reduces the level of exposure to bacteria[100]. High levels of ammonia and dust within the avian environment allow *E. coli* to adhere to these particles to be inhaled by the birds, thus reaching the respiratory tract, and can initiate an infection[61].

The *E. coli* may persist and proliferate in wet litter. To reduce litter moisture, correct air velocities should be provided, approximately 100 ft/min[61]. In this velocity, the litter remains dry and decreases the *E. coli* proliferation[101]. The personnel must be vigilant to the feeders and waterers because any water leakage from the waterers will humidify the litter and predispose the environment to proliferation of *E. coli*. Another point that the personnel must always take care for is the accumulation of dirty litter around the feeders and waterers, removing the soiled litter, and, if necessary, replace or covering wet litter with dry litter[61].

6.2 Vaccination

There is a wide variety of vaccines and different types of vaccines that are used to immunise chickens against colibacillosis[61]. There are live attenuated vaccines, inactivated, subunit, and recombinant. But the vaccines available in the market do not provide sufficient immunity to protect birds against APEC strains.

Even today, there are many attempts to develop and find the ideal and immunogenic vaccine against *E. coli* for chickens. Once the initial studies used bacterins; nowadays, the trend is recombinant or subunit vaccines[59]. Regardless of whether the type of vaccine being used is recombinant, attenuated, inactivated, or subunit, the main problem is that this vaccine be capable of immunising chickens in three different ways. Initially, the vaccine has to confer cross-immunity against the different serotypes exist APEC, which may be administered by different routes (water, food, *in ovo*, and spraying). These methods allow the immunisation vaccine to be used in mass within a chicken barn. No less important than the factors mentioned before, another very important factor for APEC vaccination is that the vaccine must confer immunity to broilers when they reach 21 days, as this is the crucial age for infection with APEC strains[59].

6.2.1 Inactivated vaccines

This method was the first to be used in an attempt to immunise poultry against colibacillosis-caused strains. The first attempt to inactivate the vaccine was in 1957 when the serotypes O2:K1 and O78:K80 were used, inactivated by formalin[102]. In this study, it was observed that bacterial O78 was more protective against homologous *E. coli* strains than heterologous O2. In 1976, another study also found that inactivated vaccines confer better protection only against homologous strains[103]. In 1978, another study was done using bacterial strains O78:K80, but using different forms of inactivation, such as with alcohol-killed, heat-killed, acetone-killed, formalin-inactivated, and formalin-inactivated/alum-precipitated pathogens. Also, the application of different forms of routes were used, subcutaneous (SC), intramuscular (IM), and intraperitoneal (IP), in 3-week-old broiler chickens. The vaccine using formalin-inactivated/alum-precipitated formula conferred immunity to homologous strains in six-week-old chickens[103]. These previous studies indicate that O and K antigens are not the most effective to induce protective immunity against colibacillosis in broilers[104]. In 1985, another study was conducted using oil-emulsified bacterins containing the three most prevalent serotypes of APEC, O1, O2, and O78. It was observed that vaccination induces protection against the three serotypes groups of APEC via IM after two vaccinations at 1 and 14 days of age[105].

The methods used to inactivate the bacteria could influence the immunogenicity of the vaccine[104]. Then, a study was done using the serogroups O2:K1 and O78:K80 inactivated ultrasonically and mixed with aluminium hydroxide adjuvant. This study reported that vaccines confer better protection that bacterins used previously[106]. Not unlike the method used to inactivate the vaccine in 1991, in 1997 a vaccine was used that was made of membrane vesicles of ultrasonically inactivated members of serogroup O2:K1. This vaccine was effective in protecting turkeys when challenged with that serogroup[107]. These previous studies have shown that inactivated vaccines confer immunity only against similar serogroups or challenges. The effectiveness of an inactivated vaccine depends on various factors such as the serotype to be used, the type of adjuvant, the inactivation method, the route of administration, the number of doses to be used, and the age of the broiler to be immunized[104].

6.2.2 Live vaccines

Studies with live attenuated vaccines were conducted utilising virulent or avirulent strains[108]. Attempts have been made using non-virulent strains from chicken faeces which contained a living fimbriated *E. coli*. This strain was used to immunise chickens[109]. *E. coli* J5, a mutant strain which lacks the enzyme uridine diphosphate galactose-4-epimerase was used to immunise chickens. This strain conferred immunity against experimental challenge serogroup O78[110]. A mutant *E. coli* serotype O78 was live attenuated for use as a vaccine. This strain had a mutation in the *carAB* operon that encodes an enzyme essential in the metabolism of arginine and pyrimidine. This attenuated vaccine conferred immunity to broilers of one day when applied to SC[111]. Three mutants were used as serotype O78 attenuated

vaccines[112]. These mutants had deletions in the *galE* gene encoding the enzyme uridine diphosphate galactose-epimerase; chorismic acid, which encodes *aroA*; and *purA*, which encodes adenylosuccinate synthetase, which is required for purine biosynthesis[112]. None of these mutants were effective at inducing immunity in heterologous challenge strains[112]. Notwithstanding, another study conducted in 2012 using a strain of serotype O78:K80 with a deletion in the Δ*aroA* gene failed to induce immunity to homologous and heterologous challenge in immunised chicks at 1 and 14 days of age[113].

More recently, another attempt to create live attenuated mutant was made using the serogroup O78 of APEC. The AESN1331 mutant has a deletion in the *crp* gene, is missing the deamination activity of tryptophan, has no production of indole, does not ferment sugars, and does not harbour the four virulence-associated genes known (*iss*, *tsh*, *cvaA*, *papC*). The chickens immunised with this mutant and subsequently challenged with serotype O78 had decreased clinical signs of colibacillosis lesions[114]. Another mutant used was the E956 APEC wild strain with deletion in the Δ*tonB* gene and the mutant E956 with deletion in the Δ*fur* (ferric uptake repressor) gene. Both mutants conferred immunity against colibacillosis[115].

6.2.3 Subunit vaccines

The serotype O78:K80:H9 was used to produce antisera against iron-regulated outer membrane proteins (IROMPs). Turkeys receiving this antiserum had reduced bacteremia 96 h after challenge[116].

Four siderophore receptor proteins (SRPs) of serogroup O78 were grown under iron restriction and subsequently used to immunise turkeys. They have been tested for potential cross-protection. For this, turkeys were challenged with *Salmonella*. Turkeys withstood the challenge and reacted with Gram-negative bacteria other than *E. coli* such as *Salmonella*, *Pasteurella*, *Pseudomonas*, and *Klebsiella* spp[117]. Antibodies produced against receptor outer membrane (*iutA*) provided protection against respiratory and septicemic disease. Chickens survived the homologous and heterologous challenge, O78 and O1 and O2, respectively. Thus, it may be suggested that the IROMPs are good at inducing immunity and may be considered for use in vaccines[118].

Antibody IgY purified from either PapG or FimH was used to immunise broiler chickens at 11 days of age. The chickens were challenged with homologous and heterologous serogroups O78, O1, and O2, respectively, three days after immunisation. The chickens that received the antibody were protected against both challenges. But when challenged with a strain of APEC via air sacs, the antibodies did not confer immunity[119].

Another recombinant gene used to immunise chickens was the increased serum survival (*iss*) gene[120]. The vaccine from recombinant Iss was used to immunise broilers at 28 days of age against a respiratory form of colibacillosis[121, 122]. Vaccinated broilers had a reduction in clinical signs consistent with the disease. However, animals vaccinated with high concentrations showed more significant damage than those vaccinated with lower concentrations[121].

Subunit vaccines have been shown to be more effective against heterologous challenge than inactivated vaccines[104].

6.2.4 Passive immunisation

This immunisation method provides the elimination of bacteria from the blood stream due to an increase of resistance to challenge by aerosol[123]. Passive immunisation against challenges counterparts in chicks was observed by immunisation with inactivated vaccines[124]. Antibodies against IROMPs confer immunity in turkeys. The presence of bacteremia 96 hours after the challenge and the recovery of viable *E. coli* from air sacs were significantly smaller than in control groups[116].

Chickens immunised with serotype O78 and antibodies extracted from the yolk of their eggs conferred immunity to homologous strains of APEC O78. Immunisation with PapG or the IutA conferred partial protection against homologous serotypes O1 and O2 in challenged chickens[119].

6.2.5 Immunopotentiation

The great challenge of the use of recombinant vaccines is to find the highly immunogenic gene or use effective immunopotentiators[61]. The use of cytosine-phosphodiester-guanine (CpG) oligodeoxynucleotides subcutaneously or intramuscularly provides a reduction of cellulite lesions[125, 126]. In many bacterial DNA sequences, CpG motifs are found, and these improve the innate immune response[126, 127].

6.3 Bacteriophages

Viruses that infect and cause the death of bacteria are called bacteriophages. These viruses do not have pathogenic effects on animals or plants. Due to this characteristic, they can be used in the control of certain bacterial diseases in farm animals[128]. These viruses have been identified in various forms, and their genetic material may be either DNA or RNA and can be single or double stranded[129, 130, 131, 132]. Approximately 95% of bacteriophages are tailed, and only 3.7% are filamentous, polyhedral, or pleomorphic[132]. These tailed bacteriophages have linear double-stranded DNA ranging from 11 to 500kb in the order Caudovirales, which is further divided into 3 families based on tail morphology. The heads of these viruses have an icosahedral shape; those with the contractile phage are allocated to the *Myoviridae*

family; those with a long and noncontractile tail are in *Siphoviridae*, and those with short and noncontractile tails belong to the *Podoviridae* family[130, 131]. There is great interest in the study of the biology of bacteriophages due to their characteristic of antibacterial agents[133, 134, 135, 136].

In the search for alternatives to the use of antibiotics, bacteriophages are being considered as a therapy[137]. The great advantage of bacteriophages is that they exist in large quantities in nature and reside in the gastrointestinal tract of humans and animals and are safe to use in these individuals as an alternative to antibiotics[138]. Obligate lytic phages are self-replicating and at the same time self-limiting, meaning that when host cells are multiplying, the number of phages will amplify as well. When the host is eliminated, the phages will also be eliminated[138]. Their use as phage therapy may be effective if the phage reduces the number of bacteria in the individual and the immune response eliminates the remainder of infectious bacteria[139].

Chickens naturally or experimentally infected with bacteriophages showed success after treatment against infection by APEC strains[140, 141, 142]. The efficiency of a bacteriophage cocktail base was used experimentally via the trachea, oesophagus, and water in chickens against APEC strains[143]. There was no significant difference between treated and untreated groups[143].

By evaluating the treatment for gut biosafety for pathogenic *E. coli* with bacteriophage therapy, induction of diarrhoea was observed in chickens[144]. About ten bacteriophages were isolated from the faeces and sewage chickens with diarrhoea. Treatment with various bacteriophages, although diarrhoea, caused a weight gain, and saved the diet consumption as the utilisation rate of diet increased 11% compared with the control group[144].

The ØEC1 bacteriophage has been described as being effective for the control of colibacillosis in chickens when inoculated intratracheally[145].

Many factors are involved so that the bacteriophage therapy has promising results[145]. These factors depend on the specificity of adsorption of bacteriophage, virus gastric acid tolerance, the body temperature of the infected animal, and the stability and viability during preparation of the virus[145]. If the choice of bacteriophage is not adequate, it may diminish the therapy's effectiveness against target bacteria[145]. Notwithstanding, phages that are well characterised and show superior characteristics *in vitro* may not work efficiently *in vivo*[145].

6.4 Antibiotics

All livestock, cattle, dairy, pigs, sheep, goats, and poultry use antibiotics in all production phases. Antibiotics in animal production have diverse uses, such as therapeutic, prophylactic, and as growth promoters. But the uncontrolled use can cause problems both for animal health as humans. Due to this risk of problems for human health, in 2000 the EU began to set limits for the use of growth promoters and in 2006 completely banned the use of antibiotics as growth promoters. This measure adopted by the European Union has generated a great impact on the commercial poultry industry because it increased the presence of microorganisms such as *E. coli* and *Clostridium perfringens* and therefore framed colibacillosis and necrotic enteritis. The economic losses in poultry production are still high due to the worsening health of birds as nutrient uptake was impaired due to intestinal health.

The measure adopted by the European Union was effective against bacterial resistance in microorganisms isolated from animals, as they have reduced resistance. But this positive aspect did not extend to the resistance of antibiotics in human patients. Some researchers report that the habit of eating raw or undercooked meats can contribute to the spread of still viable agents and to the transferring of resistance. The proper cooking of food destroys bacteria, and thus they cannot transfer the genes for resistance to antibiotics utilised by humans.

Antimicrobials have always been used to treat outbreaks of colibacillosis, but the availability effective drugs decreased due to lack of new drugs. Before using an antimicrobial indiscriminately, susceptibility testing of the isolated strain should be done. This method avoids ineffective treatment and spread of resistance among the antibiotics.

The main antibiotics used in the commercial poultry industry are β-lactam (penicillin v, amoxicillin, ceftiofur); aminoglycosides (streptomycin, gentamicin, neomycin, spectinomycin); macrolides (erythromycin, tylosin, spiramycin, kitasamicina, tilmicosin); lincosamides (lincomycin); pleuromutilins (tiamulin, chloramphenicol, florfenicol); tetracyclines (tetracycline, oxytetracycline, chlortetracycline); folate inhibitors (sulfonamides, trimethoprim); polymyxin b; quinolones (oxolinic acid, nalidixic acid); fluoroquinolones (flumequine, enrofloxacin, danofloxacin, difloxacin).

6.4.1 Public health concern

The high mortality rates and prolonged hospitalisation stays are related to multidrug resistance (MDR) from ExPEC[146]. ExPEC strains were highly sensitive to antibiotics such as ampicillin and sulfamethoxazole trimethoprim (SXT), commonly used until the late 1990s[20]. However, ExPEC have become highly resistant to many drugs, such as cephalosporins, fluoroquinolones, and SXT, as of the end of the last decade[18]. Antibiotics such as fluoroquinolones and SXT were considered drugs of first choice for the treatment of cystitis and pyelonephritis in women, but UPEC strains are now resistant to these drugs[147]. Resistance to antibiotics as SXT has already exceeded 20% in the US, and this drug is no longer used for UTI treatment[148]. ExPEC infections have become a problem due to high rates of antibiotic

resistance of these strains[149], and due to this fact, antibiotics of last resort are being chosen, such as carbapenems, and consequently, resistance to these ATB by ExPEC is already being reported[20].

E. coli resistant to third-generation cephalosporins were responsible for 15,183 episodes, and these 2,712 were associated with death in Europe[150]. Based on prevailing trends, the number of sepsis cases caused by these *E. coli* is likely to rapidly increase, potentially outnumbering methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis cases in the near future[150].

High rates of spread of ExPEC strains producing "*β*-lactamases newer", such as plasmid-mediated, have been observed. Among class C cephalosporinases (AmpC), *β*-lactamases (*e.g.* cephamycinase [CMY] types), and extended-spectrum *β*-lactamases (ESBLs) have it (e.g., sulfhydryl variable [SHV]-, TEM-type[20], CTX-M, oxacillin-types)[18].

The intrapartum prophylaxis reduced *Streptococcus* neonatal cases in the USA, but predisposed an exchange of microorganisms as well as resistance, thus providing the increase of other Gram-negative bacteria, such as *E. coli*[151].

A resistance to antibiotics involved in neonatal sepsis was observed by *E. coli* in premature infants[152]. Of particular concern is the recent apparition of NMEC producing cefotaxime (CTX)-M-type or TEM-type extended-spectrum *β*-lactamase[20].

6.4.2 Resistance to antibiotics in poultry

Tetracyclines, fluoroquinolones, and sulfonamides are the most commonly used antibiotics to eliminate *E. coli* from broiler flocks. Because of this, high rates of resistance to these antibiotics have been reported[153]. Fluoroquinolones, nalidixic acid, tetracycline, and other eight antibiotics were resistant to more than 80% of 71 strains of *E. coli* isolated from the livers of broilers from 10 different barns in China[154]. In a study of APEC strains, 100% resistance to the antibiotics tetracycline and trimethoprim/sulfonamide was found, and 79% of these same strains showed resistance to chloramphenicol, ampicillin, ciprofloxacin, and enrofloxacin[155]. Nevertheless, in a study in Brazil with APEC strains of isolated from severe cellulitis resistance less than 30% was observed for most of the 15 antibiotics tested, except to tetracycline and sulphonamides, 70% and 60% respectively[156]. Another study in Brazil using 109 samples of *E. coli* from environmental sources revealed that 9-78% of the samples were resistant to one of the 14 antibiotics tested. Quinolones and tetracyclines showed the highest resistance, 75%, and the most sensitive were the amphenicols, 68.8%[157]. In this year, in another study from Brazil analysing 205 strains of *E. coli* isolated from carcasses, the strains were classified as ESBL and found resistance to tetracycline, 70.24%; nalidixic acid, 61.9%; and sulfamethoxazole trimethoprim, 58.33%[158].

Healthy broilers, without colibacillosis signs, were isolated APEC strains and faecal origin *E. coli* positive for ESBL genes[159,160]. The use of third-generation cephalosporins in broilers, particularly ceftiofur, used to control omphalitis, can be associated with the emergence of ESBL genes[161]. ESBL-producing *E. coli* were detected in chickens that were not treated with cephalosporins, so this assumption may not be the only explanation for the appearance of these resistant strains[160]. In addition, recent study has detected ESBL-producing *E. coli* in both the environment and wildlife, especially birds[162]. This fact emphasises that ESBL-producing *E. coli* can transfer from one ecosystem to the other the resistance genes to different environments[20].

The risk of spreading antibiotic resistance genes to humans should be considered when there is contamination of animal products, especially chickens, by bacterial strains resistant to antibiotics[163].

With the expansion of the global poultry industry, the use of antibiotics has been increasingly used to combat diseases or prevent them. Many countries use antibiotics via feed or water, as growth promoters, with the exception of EU countries. For many years, antibiotics have been allies of livestock because they propitiated health and welfare to the animals. Uncontrolled use of this product has generated a great public health problem, as there is great concern that the resistance genes can be transferred from the meat of the animals to humans via consumption. ExPEC strains resistant to antibiotic are speculated that originated through direct contact of birds and consumption of poultry products[22]. Retail chicken meat was considered as a major source of contamination rates for ESBL, and it is suggested that the same ESBL genes that is present in humans may originate from animals[164]. A clone associated with multiresistance to ciprofloxacin was found in humans and animals, thus confirming the potential risk of zoonotic avian isolates[165]. Many of these antibiotic resistance genes found in *E.coli* can be transferred to other bacteria. Samples of *Salmonella* Kentucky possess a plasmid APEC-like that confers resistance to streptomycin and tetracycline[166].

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