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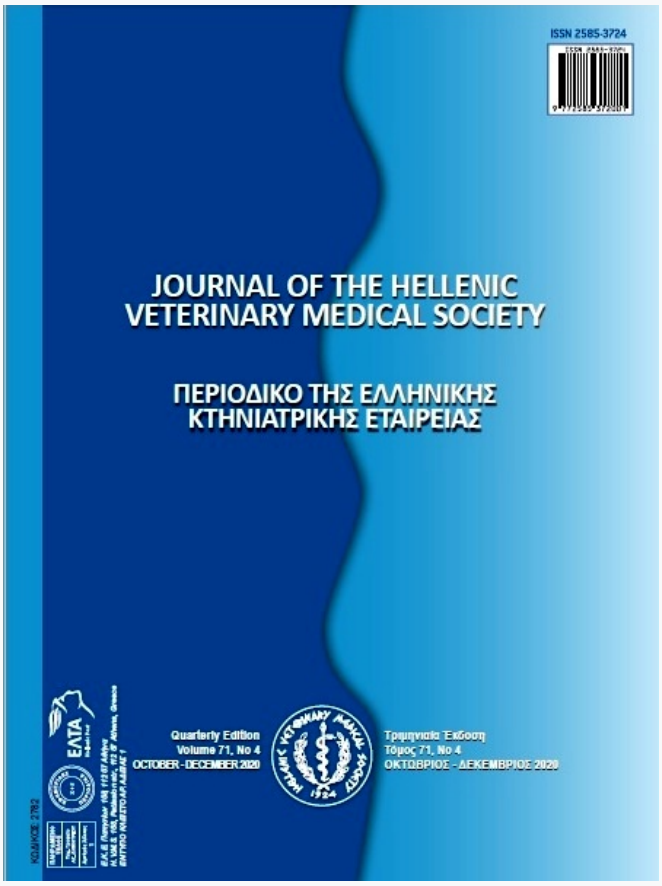

Colibacillosis in poultry: A disease overview and the new perspectives for its control and prevention

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Review article
Ανασκόπηση

Colibacillosis in poultry: A disease overview and the new perspectives for its control and prevention

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ABSTRACT: *Escherichia coli* (*E.coli*) is a common bacterium that can be naturally found in the intestinal tract of birds and as a result in their environment. However, it can cause clinical disease called colibacillosis which is regarded as one of the most common and important diseases in poultry. Strains of *E.coli* that have the ability to cause clinical disease are described as Avian Pathogenic *Escherichia Coli* (APEC). Colibacillosis can affect birds of all ages and different types of poultry production including broiler and commercial layers and breeders. The ability of *E.coli* to cause colibacillosis is not always the same; that is why its role as primary or secondary pathogen triggered by various predisposing factors is contradictory and differs from case to case. Antibiotics have been used as the main tool against colibacillosis for many decades. However, the emergence of increased antibiotic resistance has posed the need of alternative treatment to colibacillosis as well as emphasizing on preventive measures to avoid disease. The scope of this article is to assess recent scientific literature data on avian colibacillosis emphasizing on disease characteristics and recent data on prevention and control of the disease.

Keywords: antibiotic resistance, APEC, colibacillosis, epidemiology, incidence, poultry, vaccination

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INTRODUCTION

Escherichia coli (E.coli) is a bacterium that causes disease in different animal species, mainly localized in the intestine of mammals, but also they can cause septicemic or special type disease like 'Oedema disease' in pigs and 'watery mouth in calves' (Quinn et al., 2011; Tseng et al., 2014; Bashahun and Amina, 2017; Luise et al., 2019). Poultry is also affected by *E.coli* which causes serious clinical disease named colibacillosis. Those *E.coli* strains are called avian pathogenic *Escherichia coli* (APEC) and more often they can cause disease outside of the intestinal tract in poultry (extraintestinal APEC) (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2009).

Escherichia coli is described as the most frequent bacterial agent that causes general health and production problems in poultry (Barnes et al., 2008; Kabir, 2010; Zhuang et al., 2014; Landman and van Eck, 2015).

Avian pathogenic *E.coli* strains seem to have restricted role in causing clinical disease in humans. However, some human uropathogenic *E.coli* strains are found to share similar genetic factors with APEC indicating a relationship between them (Skyberg et al., 2006; Ewers et al., 2007; Zhu Ge et al., 2014; Stromberg et al., 2017; Jorgensen et al., 2019). Furthermore, APEC antibioresistance can be a source of resistance for human pathogens (Singer and Hofacre, 2006; Johnson et al., 2007; Mellata, 2013; Singer, 2015).

The aim of this article is to provide important recent information concerning epidemiology, incidence, prevention and control of colibacillosis to anyone concerned in research or poultry production.

MICROBIOLOGY

Escherichia coli is a bacterium that belongs to the family of *Enterobacteriacae*. The family consists of many different important pathogenic genera like *Salmonella* and *Yersinia* and other members that can act like opportunistic pathogens (*Enterobacter*, *Citrobacte*r, *Proteus* etc) (Quinn et al., 2011). Bacteria of the *Escherichia* genus are Gram negative rods, with a flagella or not, that can grow under aerobic or anaerobic conditions. They have the ability to ferment different sugars like glucose and lactose; however, some strains do not ferment lactose, they are oxidase negative and indole positive (Barnes et al., 2008; Quinn et al., 2011).

Their colony morphology varies according to the medium that is used for culturing and the biochemical properties of the strain. For example, in MacConkey agar they have a pink appearance because of lactose fermentation and the production of acid, with the exception of a few non-lactose fermenting strains that are orange-pale strains (Filho et al., 2013).

The classification of various strains in different serotypes is based on 3 different antigens found in the bacterial cell of *Escherichia coli*: the O antigen (described as somatic antigen from the Greek word 'soma' that means 'body') that consists of polysaccharides (Stenutz et al., 2006), the H antigen that is cited on the flagella (flagellar antigen) and the K antigen (capsular antigen) (Brugere-Picoux et al., 2015). Up to now, about 180 somatic antigens, 60 flagellar and 80 capsular antigens have been reported.

The ability of certain *E.coli* bacteria to cause disease has been attributed to certain serogroups.

DISTRIBUTION

A big variety of serogroups/serotypes have been isolated from birds that were suffering from clinical colibacillosis with different patterns according to country/region using mainly the agglutination method and pulsed-field gel electrophoresis (PFGE). The agglutination method is a classical and simple method for serotyping *E.coli* strains (Orskov et al., 1977; Bettelheim and Thompson, 1987; Fratamico et al., 2016). The technique of PFGE is the most commonly used one to study the molecular epidemiology of infectious pathogens such as *Escherichia coli*, *Streptococcus spp., Staphylococcus spp., Neisseria meningitides, Vibrio cholerae, Bordetella pertussis* and *Campylobacter jejuni* (Ahmed et al., 2012; Natsos et al., 2019).

A big number of different serogroups were isolated from septicemic birds in Spain (Blanco et al., 1998). In a study conducted in Canada, 14 different serotypes were reported while most of the strains were untypeable (Allan et al., 1993). The O111 serogroup has been involved with polyserositis in layers in Italy (Zanella et al., 2000); furthermore, the O111 along with O166 and O64 were predominant isolates from egg peritonitis in layers in a study conducted in India (Srinivasan et al., 2013) while O111 with O78 were isolated from layer flocks in USA and Greece (Trampel et al., 2007; Koutsianos et al., 2017). A huge diversity of serotypes was revealed in a European study investigating the characteristics of APEC isolates from UK,

Italy and Germany (Cordoni et al., 2016). Heydel et al. (2019a) investigated the characteristics of *E.coli* strains isolated from broiler and layer chickens from Germany, Hungary, Belgium and the Netherlands. They reported that the majority of isolates (42.2%) belonged to various serogroups followed by O78 (27.5%), O2 (25%) and O1 (5.9%). A wide diversity of serotypes was also reported by Ali et al. (2019) for APEC strains of different geographical areas in Egypt. Different serotypes (O78, O1, O2, O86, O63, O125, O27, O169) were also found to be predominant in different country regions. In Brazil, the O6 serogroup was reported as the most prevalent serogroup in APEC isolates from broiler farms (Knobl et al., 2012) while the O2, O53 and O78 were found to be the predominant *E.coli* serogroups in a recent research project in Korea (Kim et al., 2020).

In general, the most described serogroups that are reported to cause clinical disease in poultry are O1, O2, O35, O36 and O78 (Barnes et al., 2008). The prevalence of 3 serogroups (O78, O2, O1) in birds suffering from colibacillosis has been reported in many studies (Ngeleka et al., 1996; Ewers et al., 2004; Mc Peake et al., 2005; Zhao et al., 2005; D'Incau et al., 2006; Camarda et al., 2008; Wang et al., 2010; Wun Jeong et al., 2012; Dou et al., 2016).

Two different research projects performed in broilers with coliseptisemia in the USA, have referred the O78 as the most common serogroup among others isolated from samples (Ngeleka et al., 1996; Zhao et al., 2005).

A German study, using coliseptisemic birds (layers and broilers), found that 49.6% of the isolated *E.coli* strains belonged to 3 serotypes (O2:28.7%, O78:14.7%, O1:6%) (Ewers et al., 2004). A similar research project conducted in coliseptisemic broilers and layers in the UK, revealed that the majority of *E.coli* isolates belonged to the O78 serogroup (45.6%) while 14.9% belonged to the O2 serogroup (Mc Peake et al., 2005).

An Italian study that focused on colibacillosis in layers reported that the 3 most common serogroups of the isolates were O78 (49%), O88 (15%) and O2 (9%) (D'Incau et al., 2006). A second Italian survey in layers revealed a variety of 15 serotypes, with the O78, O2 and O129 being the most dominant while a 45.4% remained untypeable (Camarda et al., 2008).

A south Korean study reported O78 as the most common group (19.4%) for *E.coli* isolates coming from diseased poultry with colibacillosis (Wun Jeong et al., 2012) while Chinese researchers revealed that the two most common groups for *Escherichia coli* isolated from birds suffering from colibacillosis were the O78 (35.8%) and the O2:14.4% groups (Dou et al., 2016). Another survey that was conducted in Southern China in broilers suffering from colibacillosis identified *E.coli* isolates that belonged to 21 different serogroups. The most prevalent serogroups among strains were the O65 (27%), O78 (10%) and the O8 (9%) groups (Wang et al., 2010).

Finally, in a French survey, the most important *E.coli* serogroups isolated from colibacillosis cases in poultry were O78:17.6%, O2:17.3%, O18:9%, O1:6%, O5:4.5% and O8:2% (Schouler et al., 2012).

ROUTES OF INFECTION

Escherichia coli is a natural inhabitant of the poultry intestinal tract. It returns in the bird environment through droppings and can insert to other birds by the oral-feacal route (Dho-Moulin and Fairbrother, 1999). It seems that bird's intestine and environment are the most important reservoirs for *Escherichia coli* strains capable of causing infection (Ewers et al., 2009). This study reported that *E.coli* strains isolated from the intestine of healthy birds and from their environment are phylogenetically similar to APEC strains that have been isolated from coliseptisemic birds. Therefore, they have the zoonotic potential to cause colibacillosis in poultry (Ewers et al., 2009; Kabir, 2010). Vectors like darkling beetle (Goodwin and Waltman, 1996), flies, insects, mites (Wales et al., 2010), rats and wild birds can play a role in the spreading of *Escherichia coli* (Barnes et al., 2008). *Escherichia coli* can be transmitted either horizontally, directly or indirectly, or vertically from breeders carrying the organism in their reproductive tract to their progeny (Giovanardi et al., 2005).

The respiratory route seems to be also very important for the appearance of clinical disease, while the oviduct can also be another route of infection for *Escherichia coli* concerning layers and breeders (Antao et al., 2008; Ozaki and Murase, 2009; Landman et al., 2013). Finally, penetration of *E.coli* through the skin can produce an inflammation in the form of cellulitis (Norton et al., 2000).

VIRULENCE FACTORS

The ability of APEC to cause disease has been

identified in specific genes which are responsible for coding the production of specific components of the bacterial cell, the virulence factors. These factors can either increase the ability of *E.coli* to attach to the host cells, increase the bacterium ability to multiply and attack the bird cell or can protect *E.coli* against the immune response of the host (Dho-Moulin and Fairbrother, 1999).

The most important virulence factors described in *E.coli* bacteria cells are adhesins like the type 1-F adhesin (La Ragione and Woodward, 2002) and the P-adhesin , iron related factors, like aerobactin system consisting of iuc, iut factors (Dziva and Stevens, 2008), protectins like the K1 and the iss factor (Mellata, 2013), invasins like ibeA (Cortes et al., 2008; Flechard et al., 2012), toxins/bacteriocins like stx1,2, hlyD and hlyF and miscellaneous factors (Barnes et al., 2008).

However, despite the fact that many specific virulence factors and their genes have been described, there is not a certain pattern attributed to APEC strains (La Ragione and Woodward, 2002). Different factors and combinations can be present in different avian pathogenic *Escherichia coli* strains. Ewers et al. (2004) reported a wide diversity of virulence genes combinations for isolates that belonged to O78, O1 and O2 serogroups, while strains that belonged to other serogroups had a higher variability. Schouler et al. (2012) reported that 13 virulence genes are more often present in APEC strains and 4 specific virulence gene combinations can be linked with the identification of APEC strains. However, it was found that 30% of the strains could not be confirmed as pathogenic because of the presence of different virulence factors that contributed in the strain pathogenicity. Guabiraba and Schouler (2015) also reported that although some virulence gene patterns can help us determine the pathogenicity of APEC strains, certain virulence gene combinations cannot be used in general to describe APEC strains. Furthermore, the presence of virulence factors patterns that are observed in APEC strains can also be present in nonpathogenic *E.coli* strains (Dziva and Stevens, 2008).

CLINICAL DISEASE

E.*coli* can infect and damage different bird systems causing various syndromes and localised or systemic disease.

In day old chicks, a very common type of *E.coli* infection is the yolk sack infection (omphalitis) which is characterized by inflammation of the yolk sack and high mortality (Dinev, 2007).

In birds of different ages, *E.coli* can cause severe infection of the upper or lower respiratory system through the nasal route or the trachea. In cases of localised infection of the upper respiratory system, facial oedema and sinuses swelling can be presented and described as swollen head syndrome. However, in case of lower respiratory infection, colibacillosis is characterized by the presence of fibrinous whitish yellow exudates in the air sacks and fibrinous pneumonia (Barnes et al., 2008). Another type of localized colibacillosis is cellulitis that is characterized by inflammation of the skin and presence of subcutaneous fibrinous exudates especially in broilers. Cellulitis is a very common reason for carcass condemnations in the slaughterhouse (Norton, 1997).

In layers and breeders, the most common type of colibacillosis is the infection of reproductive system; which is known as salpingitis, oophoritis and peritonitis (Jordan et al., 2005; Barnes et al., 2008; Brugere-Picoux et al., 2015). Regarding the macroscopic findings in necropsy, fibrin in different quantities can be present inside the peritoneum, the oviduct or around the ovaries (Dinev, 2007). Egg peritonitis syndrome caused by APEC is of great economic significance in layer production because of its high incidence, the induced flock mortality and drop in egg production (Landman et al., 2013). Furthermore, there is an added cost of flock antibiotic treatment. In males, *E.coli* infection can cause reduced fertility due to testicles inflammation (Barnes et al., 2008)

The septicemic or systemic form of colibacillosis is mainly found in broilers but can also be present in layers. It is characterized by polyserositis and presence of fibrin in various organs (pericarditis, perihepatitis, air-sacculitis, peritonitis) (Randall, 1985; Dinev, 2007). If birds survive the acute phase of coliseptisemia, colibacillosis can revert into a chronic localized type of disease. In such cases, coli infection can be present in unusual sites like brain, eyes, joints and bones (Barnes et al., 2008).

Another type of systemic colibacillosis is reported as Hjarre disease or coligranuloma. This type of colibacillosis is caused by specific *E.coli* strains and appears as multiple granuloma like lesions in various organs like liver, proventriculous and intestine. Coligranuloma is rarely seen but mortality rates in these

cases can be high (Brugere-Picoux et al., 2015). However, there is a new claim regarding a different aetiological agent responsible for granuloma disease in poultry. Landman and Van Eck (2017a) reported that in many case that granuloma disease was present, it was not possible to isolate E.coli or reproduce experimentally the granuloma lesions. They revealed that the role of a parasite, Tetratrichomonas gallinarum that has the ability to cause granulomatosis should be taken into consideration. This parasite has been isolated in cases of granuloma disease in poultry (Landman et al., 2019).

INTERACTION OF *E.COLI* WITH OTHER INFECTIOUS AND NON INFECTIOUS AGENTS

As it was mentioned before, *E.coli* is a bacterium can be related to other diseases. However, its role can differ from case to case, acting individually as a primary causative agent or act as a secondary pathogen complicating other viral, bacterial or parasitic disease as well as bad environmental management (Barnes et al., 2008).

Respiratory viruses such as paramyxovirus (Newcastle disease) (El Tayeb and Hanson, 2002), coronavirus (Infectious bronchitis) (Matthijs et al., 2003; Dwars et al., 2009), metapneumonivirus (Avian rhinotracheitis-swollen head syndrome) (Nakamura et al., 1997; Al-Ankari et al, 2001), herpesvirus (Infectious laryngotracheitis) (Nakamura et al., 1996), orthomyxovirus (Avian influenza) (Mosleh et al., 2017; Samy and Naguib, 2018) can trigger avian colibacillosis. Bacterial causes like *Mycoplasma gallisepticum* (Bradbury, 2005; Barnes et al., 2008) or *Mycoplasma synoviae* (Raviv et al., 2007) can also be complicated with *E.coli* creating serious respiratory syndromes with severe adverse effects on the birds. Those infectious agents can either damage the upper respiratory system cilia which is a significant defensive mechanism against *E.coli* penetration or act as immunosuppressive agents (Guabiraba and Schouler, 2015).

Immunosuppressive viruses as Birnavirus (Infectious bursal disease) and circovirus (chicken infectious anaemia) can predispose a secondary infection with *E.coli* (Gowthaman et al., 2012; Umar et al., 2017).

Apart from the aforementioned infectious agents, the environmental factors are well established to play a crucial role as a risk factor for horizontal disease transmission (Natsos et al., 2016). Some important non infectious factors that are predisposing to colibacillosis are shown in Table (1).

Table 1. Non infectious predisposing risk factors related to colibacillosis in poultry

RISK FACTORS	REFERENCE
Air quality/ammonia level/ventilation	Patterson and Adrizal, 2005
Water quality	Amaral, 2004; Dhillon & Jack,1996
Vaccination stress	Friedman et al., 1992; Nakamura et al., 1994; Matthijs et al., 2009
Bird density	Vandekerchove et al., 2004
Distance between poultry farms	Vandekerchove et al, 2004
Temperature/heat stress	Omer et al., 2010
Productive period/peak of lay	Zanella et al., 2000
Hatchery hygiene	Hill, 1994; Samberg and Meroz, 1995

TREATMENT USING ANTIMICROBIALS-ANTIBIOTIC RESISTANCE

Antimicrobials have been used for many decades as a tool against colibacillosis. However, *E.coli* like many other bacteria have the ability to create antibiotic resistance under the pressure of antibiotic usage (Aarestrup and Wegener, 1999). This resistance is created when antibiotics are used extensively on a prophylactic schedule or when antibiotic treatment is applied incorrectly using an underdosage (OIE, 2016).

The creation of antibiotic resistance is based on the ability of bacteria to change the composition of their outer membrane, to produce enzymes that damage antimicrobials or alter their metabolism (Quinn et al., 2011). Bacteria also have the ability to transfer genetic material that is regulating antibiotic resistance genes to other bacteria that infect animals or human, transforming susceptible bacteria to resistant and posing great concern for their health (Quinn et al., 2011; OIE, 2016). The transformation of a sensitive *E.coli* bacterium to a resistant one can occur either by genetic mutations, or transformation of genetic material like plasmids (Gyles, 2008; Fricke et al., 2009; Dheilly et al., 2013) between two bacterial cells horizontally.

The trends antibioresistance for *E.coli* strains vary from country to another country according to worldwide use of antibiotics and has become a field of research for many projects.

In a survey performed in Morroco, *E.coli* isolates from coliseptisemic birds revealed high resistance for sulphonamides, oxytetracycline, trimethoprim & sulphamethoxazole and chloramphenicol (Amara et al., 1995).

American scientists tried to investigate the antimicrobial patterns of avian *E.coli* strains isolated from broiler chickens suffering with colibacillosis (Zhao et al., 2005). It came out that the highest resistance levels were traced for sulfamethoxazole (93%), tetracycline (87%), streptomycin (86%) and gentamicin (69%). Furthermore, a high percentage of the isolates (92%) was proved to be multi-resistant (Zhao et al., 2005).

In a similar Chinese research project, the tested *E.coli* strains showed high levels of resistance to tetracycline (97.5%), nalidixic acid (82.3%), ampicillin (81.1%), sulphafurazole (80.7%), streptomycin (79.0%), trimethoprim (78.2%) and cotrimoxazole (78.2%). Most of these strains (80.2%) were resistant to more than 3 antimicrobial classes (Dou et al., 2016).

A survey that was conducted in India, revealed high *E.coli* resistance to chlortetracycline (88.58%), streptomycin (85.72%), penicillin-G (82.86%), amikacin (82.86%), furazolidone (77.14%), ampicillin (74.29%) and tetracycline (74.29%) (Sahoo et al., 2012) while an Iranian survey revealed high resistance of broiler *E.coli* strains(>80%) for many different antimicrobials (Salehi and Bonab, 2006).

Antibioresistance was traced even also for antimicrobials that were not supposed to be used. Resistance against cephalosporines in broilers which are not allowed to be used in Belgium since 2000 was recorded in broilers (Smet et al., 2008).

EU has also set a monitoring program for controlling of *E. coli* infection in poultry, as all participating member countries must report their data especially for broiler chickens and turkeys. In Table (2), the antimicrobial resistance EU data for 2014 in broilers are shown in comparison to Greek data (Valkanou, 2016; EFSA, 2016).

Table 2. Antimicrobial resistance to different antimicrobials in broilers (EFSA journal, 2016)

TYPE OF ANTIMICROBIAL	%RESISTANCE EU	%RESISTANCE GREECE
Ciprofloxacin	65.7	89
Nalidixic acid	62.6	86
Ampicillin	58.6	69.8
Sulfamethoxazole	53.1	70.3
Tetracycline	50.1	68
Trimethoprime	40.6	61.6
Chlorapmhenicol	21.6	35.5
Gentamicin	11.6	12.8
Azithromycin	6.7	9.3
Cefotaxime	5.1	2.9
Ceftazidime	5.0	2.9
Colistin	0.9	0
Tigecycline	0	0
Multi drug resistance	54.6	49.1

* The European Union reported information includes data from 27 countries belonging to European Union plus Norway.

The table reports a big variation of resistance among different classes where high resistance is observed to some antimicrobials (ciprofloxacin, nalixidic acid, ampicillin, sulfamethoxasole, tetracycline and very low resistance to others (tigecycline, colistin). Resistance to cephalosporins was observed to be low in general, even though there were reports of higher levels of resistance in some countries (Cyprus, Latvia, Lithuania, Malta, Spain) (EFSA, 2016). *E.coli* strains with multidrug resistance reached the overall level 54.6% following a trend of increasing resistance. However, antibiotic resistance of indicator *E.coli* varied among different countries. For example, Scandinavian countries (Norway, Sweden, Denmark and Finland) revealed low resistance levels for most antimicrobial classes (EFSA, 2016).

Resistance to colistin, was reported to be restricted

(0.9%) in total while no resistance was observed in Greece. The discovery of the *mcr-1* gene, that is responsible for bacterial resistance to colistin, is located on a plasmid and can transfer to other bacteria has increased concern for colistin resistance spreading (Liu et al., 2015).

Since colistin is used as a last resort treatment for serious human diseases caused by Gram negative bacteria like (Enterobacteriacae, Acinetobacter, Pseudomonas) when other antimicrobial treatments prove to be ineffective, its sensitivity is crucial for human Medicine (Falagas and Kasiakou, 2005). Since 2014, EU has decided to apply a monitoring schedule for colistin resistance which was updated with a surveillance scheme for the *mcr-1* presence as well for bacteria like *Escherichia coli* and *Salmonella* (EFSA, 2016).

ALTERNATIVE CONTROL STRATEGIES

Since antibioresistance is increasing, it is necessary to adopt alternative measures and implement new tools and methodologies in order to control bacterial diseases. As many strains of *E.coli* are harboring in the bird's intestinal tract, compounds that can control the gut overpopulation with *E.coli* can be useful against colibacillosis. The term 'competitive exclusion' has also been introduced to describe their mechanism.

Probiotics and prebiotics proved their efficacy against colibacillosis (Patterson and Burkholder, 2003).

Probiotics are microorganisms that belong mainly to the Bacillus family (*Lactobacillus, Bacillus, Bifidus* and *Enterococcus*) and the yeasts/moulds family (*Aspergillus/Candida*). Probiotics proved their positive effect in improving the bird's health by minimizing pathogenic bacterial intestinal colonization, producing antagonist pathogen metabolites and stimulating the immune response (Kabir, 2009; Papatsiros et al, 2013; Wang et al., 2017). Jaiswal et al. (2019) showed that the use of *Lactobacillus reuteri* can reduce the pathogenic bacterial population including *E.coli* in broiler intestine. Similar findings of decreased caecal *E.coli* counts in broilers after the use of probiotics eas revealed by Salim et al.(2013). Two similar projects by Dong et al. (2019) and Cao et al. (2013) revealed the beneficial effect of a probiotic (*Enterococcus feacalis*) supplementation in broilers diet. The use of probiotic contributed in the reduction of *E.coli* numbers in broilers intestine, after challenge with *E.coli*-K88.

Prebiotics are nutritional substances that are not digested and are necessary for the survival of specific intestinal bacteria which help the normal functioning of intestinal microflora (Hazati and Rezaei, 2010). The two main categories of prebiotics are fructo-oligosaccharides and Mannan-oligosaccharides (Hazati and Rezaei, 2010). Prebiotics are adjusting the type and number of poultry beneficial microflora in comparison to pathogenic bacteria and also stimulate the bird immune system (Pourabedim and Zhao, 2015). Xu et al. (2003) managed to reduce the *E.coli* population in broilers intestine after the use of fructo-oligosaccharide in their diet. Kim et al. (2011) showed that the use of fructo-oligosaccharide (FOS) and mannan-oligosaccharide (MOS) in broilers' diet managed to decrease significantly the number of *E.coli* in the gut.

Some essential oils are also found to to reduce the number of *E.coli* in the gut (Hammer et al., 1999). Ebani et al. (2018) tested the antimicrobial activity of 16 essential oils against and reported good activity for at least 5 oils against *E.coli* isolates deriving from poultry. Another study from Iran tried to test the efficacy of different essential oils coming from plants extracts. Some of the tested essential oils were proved to have antimicrobial activity against *E.coli* (Habibi et al., 2018)

Acidifiers or organic acids can decrease the number of *E.coli* by changing the intestinal PH and consequently reducing the intestinal pathogens metabolism, as well as by adjusting the population of beneficial bacteria (Khan and Iqbal, 2016). Furthermore, it has been found that organic acids can trigger the birds' immunity (Khan and Iqbal, 2016). Emami et al. (2017) investigated the antimicrobial activity of organic acids after *E.coli* K88 challenge in broilers and showed that the use of 3 commercial organic acids managed to reduce the *E.coli* counts in ceca. Finally, another study revealed that the use of a product consisting of formic acid, acetic acid and propionic acid contributed in the reduction of *E.coli* that was resistant to ampicillin, tetracycline, ciprofloxacin and sulfamethoxazole (Roth et al., 2017).

Another tool for controlling E. coli infection is the use of viruses which destruct the bacterial cells or bacteriophages. Bacteriophages can be naturally found in the environment and found to have an efficacy in colibacillosis control (Barrow et al., 1998; Huff et al, 2002; Huff et al., 2003 and 2004; Brussow, 2005; Huff et al., 2005 and 2006).

Good hygiene practice in the hatchery reduces significantly the risk of new hatcher birds with omphalitis. Rejection of dirty eggs containing large number of coliforms on their outer shell is a first step for minimizing hatchery infections. Spraying hatching eggs with disinfectants reduces contamination of egg surface with *E. coli* (Barnes et al., 2008) and also using of ultraviolet light was found to limit the possibility of *E. coli* infection in chicks (Coufal et al., 2003).

Maintaining optimum environmental conditions inside poultry house (air quality, temperature or stocking density) maintaining a good health status as well as the application of a novel vaccination scheme minimize the risk of predisposing factors that are usually trigger colibacillosis outbreaks (Barnes et al., 2008).

Apart from all the above, vaccination against colibacillosis is the most interesting tool as an alternative mean of control of colibacillosis. Live, subunit and inactivated vaccines have been tested to prevent E. coli infection in poultry (Ghunaim et al., 2014). Live vaccines are used in commercial poultry and they are administrated by mass applying route like spraying and seem to offer a wider protection against different serotypes (Ghunaim et al., 2014). Subunit vaccines are applied by injection as they contain proteins (*E.coli* virulence factors) and seem to produce better heterologous immunity than inactivated vaccines. Inactivated vaccines also taken by injection and have strict homologous protection as they contain more than one strain of *E. coli* (Ghunaim et al., 2014). Vaccines mainly trigger a cell mediated immunity against *E.coli* challenge as this type of immunity plays the most important role in bird's protection when compared with humoral immunity and antibodies (Filho et al., 2013; Sadeyen et al., 2014 and 2015).

Live vaccines to control *E. coli* infection in poultry have been tested in Germany (Heydel et al., 2019b), in Italy and USA (Alberti et al., 2019), in Taiwan (Lee Guo-Wei et al., 2019), in Japan (Nagano et al., 2012), in USA (Cookson et al., 2008), in the UK (La Ragione et al., 2013), in Morocco (Mombarg et al., 2014), in Iran (Sadeghi et al., 2018) and in Israel (Frommer et al., 1994) and the results revealed that this type of vaccine reduced mortality, macroscopic lesions of colibacillosis and in some cases diminished bacterial recovery after challenge with *E.coli* strains. Live *E.coli* vaccines have also been reported to reduce mortality and increase egg laying rates in layer breeders in Japan (Asano et al., 2019; Uotani et al., 2017) and in Thailand (Awaiwanont and Chotinum, 2019) where the *E.coli* vaccination also reduced the applied antibiotic treatment.

However, another study showed that live *E. coli* vaccines could not control colibacillosis after homologous or heterologous challenge (Kariyawasam et al., 2004).

Inactivated *E.coli* vaccines have already been reported to be beneficial in experimental trials. A Canadian study tested the efficacy of inactivated vaccines with different adjuvants and found reduced recovery of the challenging *E.coli* in comparison to unvaccinated birds (Gomis et al., 2007). Yaguchi et al. (2009) reported that the use of an inactivated liposomal E.coli vaccine managed to reduce clinical symptoms and bacterial numbers in chicken blood after *E.coli* challenge of specific pathogen free chickens.

Another survey tried to evaluate the benefits conferred by a commercial inactivated vaccine to broiler breeders and their progeny. A broiler breeder flock was divided in 2 groups of birds. Half of the birds were vaccinated with a commercial inactivated *E.coli* vaccine while the rest birds received no vaccination against *E.coli.* Even though, overall mortality between the 2 groups was similar, the vaccinated group was reported to have reduced losses due to colibacillosis in comparison to the unvaccinated group of birds (Gregersen et al., 2010).

Shehata et al. (2019) investigated the efficacy of a formalin inactivated *E.coli* vaccine that consisted of 7 different APEC serotypes, after challenge of SPF chicks. The efficacy was assessed by means of mortality, clinical signs and seroconversion. It was observed that the vaccinated birds were 100% protected when challenged with an O157:H7 and partially protected after an O125 challenge.

Autogenous vaccines are another type of vaccines that are used with increasing interest against *E. coli* infections. The term autogenous vaccine is used to describe vaccines that are produced specifically to protect a certain flock. Their production procedure uses bacterial isolates from a specific flock suffering from colibacillosis and produces a vaccine containing the homologous *E.coli* strains of the flock. Those vaccines may delay the clinical onset of colibacillosis and reduce mortality.

Landman and Eck (2017b) demonstrated complete protective effect of autogenous vaccines after aerosol homologous challenge with pathogenic *E.coli* in layer

hens. However, protection was not proved after heterologous challenge. The efficacy of an autogenous vaccine has also been reported after homologous intratracheal challenge in layer pullets in a Greek study (Koutsianos et al., 2019). Another Egyptian study showed reduced mortalities in *E. coli* challenged birds after vaccination with an autogenous vaccine (El Jakee et al., 2016). On the other hand, there are reports of incomplete protective efficacy of using autogenous vaccines against *E.coli* challenges (Li et al., 2016). The authors reported that the reasons for this may be the high infective dose that was used or the insufficiency of the stimulating humoral immunity to confer protection against *E.coli* challenge.

So, vaccines seem to be an interesting tool against colibacillosis and also contributing in the limitation of treatment with antimicrobials. However, there are still some cases with weak vaccine protection against colibacillosis. Further investigations related to avian *E. coli* vaccine production, and efficacy are in need.

CONCLUSIONS

Colibacillosis is the most common bacterial disease in poultry having great economic significance for the producers. Disease controlling using antimicrobials has led to the emergence of antibioresistance creating a big concern for animal and human health. It is crucial that judicious use of antimicrobials will be combined with a different approach for controlling colibacillosis including various preventing measures along with alternative treatments. Vaccination can have an important role in the control of the disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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