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Escherichia coli, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance: A review

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Abstract

Avian colibacillosis, caused by *Escherichia coli,* is one of the major bacterial diseases in the poultry industry worldwide, and along with salmonellosis, it is the most common avian disease communicable to humans. The organism is a normal inhabitant of the intestinal tract of birds and can survive in a wide range of temperature. Certain strains *viz.,* avian pathogenic *E. coli* (APEC), however, could spread to various internal organs and cause colibacillosis characterized by fatal systemic disease. Faeco-oral route is the main mode of infection, though vertical transmission is also possible. The disease occurs in various forms in poultry: colisepticemia and acute septicemia, air sac disease, pericarditis, perihepatitis, Mushy chick disease (yolk sac infection), peritonitis, panopthalmitis, synovitis, salpingitis, bumble foot, cellulitis, swollen head syndrome, infectious asthenia, and Hjarre's disease. Increased cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) due to *E. coli* infection affect the absorption of sodium as well as chloride and water balance ultimately producing watery diarrhea and death. APEC isolates also are of potential concern for public health professionals. Infected persons usually manifest diarrhea which may be complicated by other syndromes depending on the serotype. Diagnosis is based on isolation and growth characteristics of the organism in wide variety of bacteriological media, biochemical tests, serological assays, enzyme linked immunosorbent assay (ELISA), molecular tools of polymerase chain reaction (PCR) and its various versions, phylogenetic analysis and other techniques. The disease must be differentiated from a wide variety of other bacterial diseases. Antibiotic sensitivity test is useful to select proper antibiotic but plasmid mediated resistance do occur for which vitamin as well as probiotic and bacteriophage therapy are gaining much attention nowadays. Live and inactivated mutant vaccines are available. Proper hygiene and sanitation along with good hatchery management are the prerequisites to prevent the occurrence of disease. Different disease manifestations caused by APEC, insights into this economically important avian pathogen, epidemiology, trends and advances in diagnosis, prevention and control, novel and emerging therapeutic regimens, and the associated public health concerns envisage the topic of discussion in the present review.

Keywords: *Escherichia coli*; poultry; avian colibacillosis; chronic respiratory disease; colisepticemia, diagnosis; treatment; zoonosis

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Introduction

Enormous economic and social strain is put on society because of contamination of food. Particularly in developing countries, around one-third of the world population is affected by food-borne pathogens per year. Treatment of food-borne diseases results in loss of billions even in developed nation like the United States (US) and there are reports of more than 48 million illness due to such diseases making the food-borne illness a great public health concern (Scallan et al., 2011; Centers for Disease Control and Prevention, 2013; Dhama et al., 2013a).

Avian colibacillosis is considered as one of the major bacterial diseases afflicting poultry industry worldwide, and along with salmonellosis, it is the most common avian diseases communicable to humans (Gross, 1994; Barnes et al., 2003, 2008; Kabir, 2010; Singh et al., 2011). Despite being known for over a century, avian colibacillosis remains one of the major endemic diseases of poultry resulting in decrease in productivity, mortality and economic losses (Otaki, 1995). Avian colibacillosis is a disease syndrome caused by *Escherichia coli*, a Gram-negative bacterium that belongs to family *Enterobacteriacae*. Colibacillosis in mammals is primarily a enteric disease whereas in poultry it causes typical localized or systemic disease occurring mostly secondarily when host defense have been impaired. It is characterized in its acute form by septicemia, resulting in death while in its subacute form by pericarditis, airsacculitis and perihepatitis, reproductive tract infection like salpingitis and/or peritonitis resulting in huge mortality (Landman and Cornelissen, 2006; Ozaki and Murase, 2009). *E. coli* infections have also been described in turkeys, geese, and ducks, and cause significant economic losses (Landman and Cornelissen, 2006).

E. coli infections affects all the systems in a bird, resulting in a complex syndrome characterized by lesions in multiple organ including digestive, respiratory and reproductive tract viz., colisepticemia, omphalitis, respiratory tract infection - air sac disease, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, pericarditis, perihepatitis, peritonitis, panopthalmitis, synovitis, cellulitis and salpingitis, bumble foot, infectious asthenia alone or in association with other pathogen Barnes et al., 2003, 2008; Singh et al., 2011). The organisms are present in the intestine as normal bacterial flora and pathogenic strains of avian pathogenic *E. coli* (APEC) produces disease conditions (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2003). Thus both commensal and pathogenic isolates co-exist. Multiple serogroups are associated with disease, especially O1, O2 and O78 along with many others (Barnes et al., 2008). Nowadays, advances in diagnosis and control have provided molecular diagnostic tools and novel therapeutic modalities for rapid detection and efficiently treating the disease conditions caused by *E. coli* in poultry (Kataria et al., 2005; Barnes et al., 2008; Dhama and Mahendran, 2008, 2011, 2013a,b,c,d; Kabir, 2010; Singh et al., 2011; Mahima et al., 2012; Tiwari et al., 2011, 2013).

A better understanding of *E. coli* and its disease manifestations, epidemiology, transmission and spread, diagnosis, and prevention and control measures would help in reducing and eliminating avian colibacillosis from the poultry flocks, thereby reducing economic burden to poultry producers and the potential hazards posed to the public health (Gross, 1994; Barnes et al., 2003, 2008; Dhama and Mahendran, 2008; Kabir, 2010; Singh et al., 2011; Dhama et al., 2013a,b). The present review describes in detail the different disease manifestations of *Escherichia coli* in poultry, insights into this economically important avian pathogen, epidemiology and diseases transmission, trends in diagnosis, prevention and control, newer therapeutic regimens, and the associated public health concerns.

The bacteria

E. coli is a gram negative, non-acid-fast, non-spore forming bacterium variable in size and shape usually 2-3 x 0.6 µm. It normally inhabits the intestinal tract of all animals and birds. There are a number of different strains and many are species-specific. Most strains are motile with peritrichous flagella. *E. coli* can grow both aerobically and anaerobically, and uses simple carbon and nitrogen sources. The bacterium grows on ordinary nutrient media at temperature of 18-44°C. On nutrient agar plates, incubated for 24 hours at 37°C, colonies are low, convex, smooth and colorless. It rapidly produces turbidity in broth culture. Regarding biochemical characteristics, in sugar fermentation tests acid and gas is produced with glucose, maltose, mannitol, glycerol, rhamnose, sorbitol, and arabinose. It produces indole, and it is positive for methyl red (MR) and negative for VP Voges-Proskauer (VP) reaction. It is a lactose fermenting bacterium (Ewing, 1986; Bettelheim, 1994; Montgomery et al., 2005).

E. coli possesses three types of antigens: 'O' (somatic), 'H' (flagellar) and 'K' (capsular). 'O' antigen is a heat resistant endotoxin liberated on lysis of bacteria and is a major antigen responsible for classification of *E. coli*. 'K' antigen is associated with virulence (Mellata et al., 2003). 'H' and 'K' antigens are heat labile. Sixty per cent of the cases are caused by group 'O' (Ewers et al., 2004). Pathogenic and non-pathogenic *E. coli* can be differentiated by Congo red dye where pathogenic isolates produce red colonies. They grow in presence of low concentration of iron and cause hemolysis on blood agar, show adherence with pili to epithelial cells and demonstrate pathogenicity in

3 week chicks. Around 10-15% of the intestinal coliforms belong to pathogenic serotypes (Wray and Davies, 2001; Rahman et al., 2004). The disease causes decreased growth rate, depressed feed conversion efficiency, elevated flock mortality, downgrading and subsequent low performance in infected birds (Barnes et al., 2008).

E. coli is a member of the normal microflora of the poultry intestine, but certain strains - avian pathogenic *E. coli* (APEC), spread to various internal organs and cause colibacillosis characterized by systemic fatal disease. APEC forms certain serogroups, particularly O93, O92, O78, O1, and O2 and to some extent O15 and O55 are the predominant serogroups (Antao et al., 2009; Wang et al., 2010a). APEC probably does not cause intestinal diseases (Janben et al., 2001). Nevertheless, enterotoxigenic *E. coli* (ETEC) are occasionally isolated from poultry suffering from diarrhea, and diarrhea was also experimentally induced after intramuscular inoculation of APEC (Dho-Moulin and Fairbrother, 1999). Enteropthogenic *E. coli* (EPEC) were isolated from clinically healthy chickens. Isolates of certain O-types are very much heterogenous (both in terms of phenotypes as well as genotypes) although they are detected more frequently in APEC than in commensal *E. coli* (Achtman et al., 1986; Blanco et al., 1997; Carvalho de Moura et al., 2001; Chansiripornchai et al., 2001). The geographical localisation of the flock moreover influences the prevalence of certain serotypes (Blanco et al., 1998). Infectious agent is moderately resistant to environment but is susceptible to disinfectant and to temperature of 80^0C. It is interesting to note that APEC is most frequent in breeders followed by broilers and layers respectively and is responsible for both embryo as well as early chick mortality in breeders (Khoo et al., 2010).

Epidemiology and Disease Transmission

Avian colibacillosis is one of the prime causes of morbidity, mortality and decrease in productivity associated with heavy economic losses to the poultry industry, by its association with various disease conditions, either as primary or as a secondary pathogen (Barnes et al., 2008; Singh et al., 2011). It affects birds of all ages. Faeco-oral route is the main route of infection following ingestion of contaminated feed and water. Intestinal tract of animals, including poultry, is the most important reservoir of *E. coli.* Transmission of pathogenic *E. coli* through egg is common and can result in huge mortality in chicks. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from which they hatched suggesting rapid spread after hatching (Adesiyun et al., 2005). The most important source of egg infection seems to be faecal contamination of the egg surface with subsequent penetration of the shell and membranes (Chousalkar et al., 2010). Coliform bacteria can be found in litter and faecal matter. Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (Ozaki and Murase, 2009).

Litter and fecal material are the source of coliforms, but *E. coli* forms the minor group (Nandi et al., 2001). In the flock environmental isolates constitute a separate and distinct population (Jeffrey et al., 2004). Dust may contain 10^5 - 10^6 *E. coli* per gram and the organism can be isolated from the environment even from the height of 40 feet outside the poultry house (Davis and Morishita, 2005). Contamination of feed as well as feed ingredients can introduce new serotype in the flock (Martins da Costa et al., 2007). Rodent droppings may also be an important source. Gene transfer to susceptible strain from the resistant ones occurs in the mouse intestinal tract that provides suitable environment and is accelerated by the exposure to antibiotic (Hart et al., 2006). Contaminated well water may also act as an important vehicle of transmission (Morabito et al., 2001).

E. coli infection is considered as a multifactorial disease. Environmental factors and immunosuppressive viral infections further influence the outcome of the disease. Virulence of *E. coli* along with host factors like initiation of egg production may be associated with the occurrence of colibacillosis in poultry (Someya et al., 2007).

Increasing infection pressure in the environment increases the risk for colibacillosis. Infectious bursal disease (IBD), mycoplasmosis, coccidiosis, Newcastle disease or infectious bronchitis, as well as nutritional deficiencies all predispose the birds to this disease (Barnes et al., 2008; Singh et al., 2011; Gowthaman et al., 2012). The main reasons for the flare up of this commensal bacterium into a disease causing pathogen in poultry are unfavorable housing climate like dry and dusty conditions, poor ventilation, overcrowding, contaminated water, inclement weather conditions and stress on the affected birds leads to economic losses. Other risk factors are the duration of exposure, strain virulence, breed, and bird's immune status. Damage to the respiratory system like with an excess of ammonia or dust causing deciliation of the upper respiratory tract, renders the birds more susceptible to APEC infections. Distance between poultry farms and the hen density are also important risk factors (Landman and Cornelissen, 2006).

E. coli serotypes inhabit intestines of animals including humans and infect mammals, birds thus having a cosmopolitan distribution. *E. coli* inhabit intestines of poultry at concentration upto 10^6/gram. Higher numbers are found in younger birds. They are also commonly isolated from the upper respiratory tract, skin and feathers of the birds. Among normal

chicken 10-15% intestinal coliforms belong to potentially pathogenic serotypes (Gomis et al., 2001). Dust in poultry houses may contain 10^5-10^6 *E. coli* /gram. These persist for long periods particularly under dry conditions. Faecal contamination of egg may result in the penetration of *E. coli* through the shell and may spread to the chickens during hatching, often associated with high mortality rates, or leads to yolk sac infection. In a single bird a large number of different *E. coli* types are present, obtained via horizontal contamination from the environment, more specifically from other birds, faeces, water and feed. Rodent dropping also contain coliforms and thus are major source of infection to poultry (Antao et al., 2008).

Disease Manifestations

The incubation period in poultry ranges from 12-72 hours. The morbidity rate varies and mortality rate is around 5-20%. Maximum mortality occurs within 5 days of onset of disease. In birds, pathogenic *E. coli* infections may cause the following disease conditions (Singh et al., 2011).

Colisepticemia

It is the commonest infectious disease of farmed poultry seen worldwide in chickens, turkeys, etc. The bird gets infected mainly by inhalation of dust contaminated with faecal material. Infection can also occur by the oral route via water, feed and fomites, contaminated shell membranes or yolk. Young growing broilers (5-10 week) are mostly affected resulting in a mortality rate of 5-10%, occasionally can reach 50-100%. Air-sacculitis is seen in 0.5-2.5% cases, with thickened and cloudy appearance of the air sacs. Surviving chicks are weak and stunted. Diarrhoea, pasty vent, loss of appetite, depression, dyspnoea and sneezing are the clinical signs seen in colisepticemia (Ewers et al., 2003 Vandekerchove et al., 2004; Singh et al., 2011).

Respiratory tract infection - Air sac disease (chronic respiratory disease)

Air-sacculitis is observed at all ages. *E. coli* occurs as a secondary invader in infectious bronchitis (IB), Newcastle disease (ND) and Mycoplasma infections, where it aggregates to air-sacculitis. Due to stress and high ammonia concentrations in litter, deciliation of trachea occurs and organisms get entry via inhalation in a dusty and overcrowded environment, and causes air sac infection. It occurs in growers of 6-9 weeks of age. In this infection, the air sacs become cloudy, edematous, thickened and caseous deposition is observed. Histopathologically, single cell membrane becomes multilayered, edematous with heterophil infilteration (Ginns et al., 1998; Al Ankari et al., 2001; Barnes et al., 2008).

Pericarditis and Perihepatitis: These conditions are also found in colisepticemia. Such conditions occur as a sequele to septicaemia and organism gets settled in liver and heart. Heart shows thickening of pericardium, which appears cloudy and filled with thick yellow (milky) pericardial fluid. Liver shows thick fibrinous membranous covering which appears very prominent and can be easily peeled off (Barnes et al., 2008).

Mushy Chick Disease

It is also known as yolk sac infection, sleeping disease, omphalitis, responsible for heavy early chick mortality. Neonatal infection of chicks can occur horizontally from the environment, or vertically from the hen. The chick can be infected during or shortly after hatching. Retained infected yolk, omphalitis, septicemia and mortality of the young chicks up to an age of three weeks can be seen. This occurs worldwide in chickens, turkeys and ducks due to bacterial infection of the navel and yolk sac of newly hatched chicks as a result of contamination before healing of the navel. Chicks once born stagger about and look sleepy. They will be reluctant to move and tend to stay under the heat source. Chicks will show dejection, loss of appetite, swollen abdomen, vent pasting, and diarrhea. Postmortem lesions include enlarged yolk sac with congestion and abnormal yolk sac contents, viscid and thick yolk, which changes to yellowish green, sticky/watery emitting a very foul smell (Shah et al., 2004; Singh et al., 2011). Mortality follows with the worst of it occurring within the first couple of days of the chick's life. Chicks that survive the first few days will usually never be as strong or healthy as the rest of the flock. The infection is caused by contamination of a number of bacteria types that enter through the porous egg shell inside the hatchery incubator or before the egg is placed into the incubator. Incubation conditions (37^0C) are ideal for breeding bacteria as well as incubating eggs. Once the egg is infected, some of the harmful bacteria are capable of breaking down the yolk sac which causes secondary infection to move in (Montgomery et al., 1999).

Acute septicemia/infectious disease

It is seen in mature and growing chicken and turkeys. Septicemia also affects chickens of all ages, and is mainly described in broilers. It is the most prevalent form of colibacillosis, characterised by polyserositis and causes depression, fever and often high mortality. Several routes of infection are possible: neonatal infections, infections through skin lesions, infection of the reproductive organs, of the respiratory tract and even infection *per os*. When *E. coli* reaches the vascular system, the heart and internal organs are infected. Pulmonary congestion, green liver, congested pectoral muscles, small white foci in liver, enlargement of

spleen and liver and a tendency towards pericarditis and peritonitis is seen. The infection of the myocardium causes heart failure. Birds die acutely, and on post mortem good flesh and full crop is seen. Septicemia occasionally also leads to synovitis and osteomyelitis and on rare occasions to panophthalmia (Pourbakhsh et al., 1997a; Barnes et al., 2003, 2008; Singh et al., 2011).

Peritonitis

It occurs in laying hens and breeders. In abdominal cavity of birds, fibrin and free yolk is observed in this condition. Acute mortality occurs. Bacteria enters from intestine to oviduct by anti-peristaltic movements, grows in yolk material deposited in peritoneal cavity and produces yellowish fibrinous or fibrinopurulent (pus like) material in abdominal cavity (Landman and Cornelissen, 2006).

Panophthalmitis

It is an uncommon manifestation of *E. coli* septicaemia. In this condition, blindness may occur due to hypopyon and hyphema of eye. The eyes are swollen along with cloudy to opaque appearance with initial hyperemia. The presence of fibrinoheterophilic exudates as well as several bacterial colonies are characteristic. The inflammation may turn to granulomatous reaction gradually. There may be persistence of the organism in the affected eye for long with characteristic outcomes like retinal detachment as well as retinal atrophy and lysis of lens. Most birds die shortly after onset of lesions, some may recover. Histopathologically, heterophil infiltration and formation of giant cells around necrotic areas is observed (Nakamura and Abe, 1987; Barnes et al., 2003).

Synovitis

This is joint inflammation, occurs as a sequalae to colisepticemia and is experimentally produced by intravenous inoculation of *E. coli.* Many birds recover within a week but others can become chronically infected and emaciated (Droual et al., 1996).

Salpingitis

Inflammation of the oviduct, results in decreased egg production and sporadic mortality in laying chickens and others like duck and geese. The oviduct mau contain big caseous mass. Layers as well as broilers may suffer from acute or chronic salpingitis, resulting from an ascending infection from the cloaca or an infection of the left abdominal airsac (Landman and Cornelissen, 2006; Ozaki and Murase, 2009). Affected birds cannot produce and lay eggs. In chronic salpingitis, the oviduct has a yellowish-gray, cheese-like content, with a concentric structure. Hisopathologically, the tissue reaction in the oviduct is mild, consisting largely of multifocal to diffuse heterophil accumulations subjacent to the epithelium. In layers, salpingitis can cause egg peritonitis if yolk material has been deposited in the peritoneal cavity, characterized by a sero-fibrinous inflammation of the surrounding tissues. A laying hen suffering from *E. coli*-induced oophoritis or salpingitis may infect the internal contents of the egg before shell formation completes (Jordan et al., 2005; Timothy et al., 2008).

Bumble foot (ulcerative pododermatitis)

This is a bacterial infection and inflammatory reaction on the feet of birds and rodents and is much more likely to occur in captive animals than in those in the wild. It is caused by *E. venezuelensis* and *E. coli*. It is a common infection for domesticated poultry and waterfowl such as chickens and ducks. Due to constant walking on hard, rough, or sharp surfaces, birds can develop small wounds on bottom of their feet. Symptoms of bumble foot are limping and a large soft swelling on bottom of foot. Usually bumble foot is due to bruises or small wounds infected with bacteria. *E. coli* and *Staphylococcus aureus*, both produce this condition in chicks (Gross et al., 1994).

Necrotic dermatitis (cellulitis)

Broilers may be affected by necrotic dermatitis, also known as cellulitis, characterized by a chronic inflammation of the subcutis on abdomen and thighs (Kumor et al., 1998).

Swollen Head Syndrome (SHS)

It is an acute to sub-acute cellulitis involving the periorbital and adjacent subcutaneous tissues of the head. It is mainly a problem in broilers, causes oedema of the cranial and periorbital skin. Microscopic lesions include fibrino-heterophilic inflammation and heterophilic granulomas in the air-spaces of the cranial bones, middle ear and facial bones; lymphoplasmacytic conjunctivitis and tracheitis with formation of germinal centres. Affected birds show ocular discharge and conjunctivitis progressing to periorbital swelling. Terminally, eyes are closed and enlargement of the head is a prominent sign in severely depressed or recumbent broilers. Acutely affected birds may show tracheal hyperaemia and pulmonary congestion. Avian pneumovirus (APV), turkey rhinotracheitis (TRT) virus along with *E. coli* is associated with SHS, considered as a disease caused by usually followed by an opportunistic *E. coli* infection. SHS can cause a reduction in egg production of 2 to 3%, and a mortality of 3 to 4% (Gross, 1990; Van de Zande et al., 2001).

Infectious Asthenia: It is enteritis occurring in broilers (adults) and breeders of 10 weeks or greater age, occurring

as sporadic outbreaks. Great emaciation and weakness, sitting on haunches, inflammation of duodenum, wasting of muscles of breasts and legs is observed and birds show yellowish diarrhea (Jordan, 1990).

Hjarre's Disease (Coligranuloma)

It is a disease of adult chicken and turkey, characterized by nodular granulomas in liver, mesentery and walls of intestine, gizzard, duodenum, mesenteries, caeca, lungs and kidneys. Nodules are not observed in spleen. Coligranuloma is a relatively uncommon coliform disease, a rare form of colibacillosis, mostly seen at necropsy (1/10,000 cases), and may cause mortality as high as 75% in an individual flock. Pathological lesions includes coagulative necrosis and enlargement of liver, hard nodular granulomas in the mesentery, walls of intestine, particularly caecum, congested and swollen spleen with abnormal contours, congested viscera with atrophied bursa, normal peripheral nerves and bone marrow. Nodules are millet seed sized, like in tuberculosis (Shah and Qureshi, 2006; Ewers et al., 2007).

Pathogenesis

The mechanisms by which avian pathogenic *E. coli* cause infection are largely unknown. Pathogenic *E. coli* once invades the columnar epithelium of intestine, produces toxin, also known as enterotoxin leads to the activation of adenylate cyclase (in case of heat-labile toxin) and guanylate cyclase (in case of heat- stable toxin) which results into the production of increased cAMP and cGMP, respectively, which affects the absorption of sodium, chloride and water balance, ultimately produces watery diarrhea and death occurs due to dehydration and hypovolumic shock (Dziva and Stevens, 2008). It produces chicken lethal toxins (LT) – O2, O45 and O109. With pilus adherence it colonizes in the trachea and causes upper respiratory tract infections. Colicin V produced is important for its pathogenicity and invasion. *E. coli* is not killed by complement system. Capsular K polysaccharides and aerobactin siderophores helping in iron uptake of *E. coli* produce septicemia (La Ragione and Woodward, 2002; Zhao et al., 2009).

Along the side an acute inflammatory response takes place due to increased production of acute phase proteins *viz.*, liver acute phase proteins; cytokines: interleukins (IL) 1 and 6 and tumor necrosis factor because of exposure to non-specific indicator *i.e.* endotoxin (Nakamura et al., 1998; Chamanza et al., 1999; Xie et al., 2002). The effect of endotoxaemia in the acute phase includes reduced consumption of feed and efficiency of utilization; decreased tibial size and calcium content along with increased mortality. Liver weight along with ionized calcium in plasma and antibody responses increases. There is decrease in body weight and bone breaking strength due to increase in the amount of endotoxin in the circulation (Mireles et al., 2005).

Unambiguous makers of virulence have not been identified for APEC (Landman and Cornelissen, 2006; Antao et al., 2008) but the role of virulence factors like F (type 1) and P fimbrial adhesins, K1 Capsular antigen, curli, verotoxin-2 like toxin, heat-labile chick lethal toxin (CLT), coligenicity, outer membrane proteins, cytotoxins, the *tsh* gene encoding temperature sensitive hemagglutinin, *hylE, a* hemolysin gene, aerobactin siderophores, *stg* fimbriae, factors conferring resistance to serum and phagocytosis, resistance to immunologic defense and survival in physiologic fluids, cytotoxic effects and plasmid encoded specific fimbrial adhesins, like K88 and K99 produced by pathogenic *E. coli* strains are responsible for pathogenesis of the infection (Hacker, 1992; Pourbakhsh et al., 1997b; Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002; Ewers et al., 2003; Lymberopoulos et al., 2006; Dziva and Stevens, 2008; Kabir, 2010). Wild-type *E. coli* strains were reported to carry three or more fimbrial adhesin determinants normally and these fimbrial gene clusters could undergo phase variation and contribute significantly in the pathogenesis of *E. coli* (Hacker, 1992; Pourbakhsh et al., 1997). Recently, fimbrial adhesins *Yqi* have been identified to play role in the colonization of *E. coli* (Antao et al., 2009), and the role of *betA* gene harbouring APEC isolates was also suggested to be associated with virulence in ducks (Wang et al., 2010a, b). An auto tansporter adhesin located in the chromosome of highly virulent *E. coli* with possible role in the increased adherence was also identified (Pal and Singh, 2007a; Dai et al., 2010).

Public Health Concerns

Earlier, the avian strains of *E. coli* were considered as not causing any important disease in man and animals, so were not of much zoonotic significance. But as APEC share not only identical serotypes but specific virulence factors also with human pathogens, their zoonotic potential is now under consideration and is not under a single platform. However, colibacillosis is considered as food or water-borne zoonotic disease transmitted to humans via the fecal-oral route (Barnes et al., 2008; Kabir, 2010; Dhama et al., 2013a).

Recently on the basis of compiled data it has been suggested for avian colibacillosis that among poultry diseases transmissible to human, avian colibacillosis and avian salmonellosis are the leading anomalies (Kabir, 2010; Dhama et al., 2013a). Resistant strains from the gut readily soil the poultry carcasses at slaughter, and as a result poultry meats are often contaminated with multi-resistant *E. coli*. In a similar fashion eggs become contaminated during laying.

Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food chain. Poultry meat is still the primary cause of human food poisoning. The presence of pathogenic microorganisms in poultry meat and its by-products remains a significant concern for public health worldwide (Manges et al., 2007; Dhama et al., 2013a).

Humans with colibacillosis usually manifest diarrhea, which may be complicated by other syndromes depending on the *E. coli* serotype. These complications may include fever, dysentery, shock, and purpura (multiple small purplish hemorrhages in the skin and mucous membranes). In most cases, symptomatic treatment (fluids, anti-diarrheals) is all that is required to control the infection. In more severe infections, antibiotics such as tetracycline, chloramphenicol, ciprofloxacin etc. may be necessary (Chauvin et al., 2005 and 2007).

APEC isolates remain an important problem for poultry producers and a potential concern for public health professionals, as growing evidence suggests a possible role for APEC in human disease. *E. coli* of the O2:K1 serotype and O78 isolates isolated from human urinary tract infections and from septicemic chickens are phenotypically highly related indicating that chickens might be a source of septicemic human infections (Rodriguez-Siek et al., 2006). However, in contrast, few studies suggested that these avian isolates possessed very few of the attributes required to cause disease in humans. Reversely, human isolates can be pathogenic to day-old chicks after subcutaneous inoculation like of serotypes O1, O2, O18 and O78. It has been demonstrated that antimicrobial resistant, urinary tract infection (UTI) causing *E. coli* could have a food reservoir, possibly in poultry or pork. Uncontrolled, avian *E. coli* represents a serious animal welfare concern and risk to public health as it is a zoonotic organism with avian *E. coli* species known to adapt to humans. In human *E. coli* O157: H7 is an important enterohaemorrhagic pathogen producing Shiga toxin and chicken may get readily infected experimentally as well as naturally in different geographical locations. Along with this contamination of poultry meat with this particular organism is a serious public health concern (Guo et al., 1998; Heuvelink et al., 1999; Kabir, 2010; Dhama et al., 2013a). In extraintestinal habitats, APEC and uropathogenic *E. coli* (UPEC) establish infections and it has been found that APEC potentially can act as UPEC or as a virulence gene reservoir for UPEC (Zhao et al., 2009).

Diagnosis

Diagnosis of colibacillosis in birds is based on the clinical features, lesions found during PM inspection and typical macroscopic lesions. Presence of cheesy material in the abdominal cavity, pericarditis, fibrinous hepatitis, air sacculitis, thickened yolk, inflammation of oviduct and egg peritonitis suggest very well the presence of *E. coli* infection. Confirmatory diagnosis is based on the isolation and identification of *E. coli* from lesions typical of colibacillosis. Material to be collected includes heart blood, and affected tissues like liver, spleen, air sacs, yolk, yolk sac, pericardial/peritoneal fluid, intestinal contents and bone marrow. Experimentally it has been shown that in acute cases, isolation is possible from six hours to three days after infection; in subacute cases, isolation is only possible, seven days after infection. Contamination from the intestines is rarely a problem, if fresh material is used and standard bacteriological procedures are applied. Care must be taken to avoid faecal contamination of samples. Bone marrow cultures are easy to obtain and are generally free of contaminating bacteria. Definitive identification of *E. coli* is based on the organism's characteristics. Suspected material should be streaked on Eosin methylene blue (EMB) or MacConkey agar or Tergitol-7 agar and observation of characteristic metallic sheen, bright pink and yellow colonies, respectively, confirms the pathogen. Selective media like drigalki agar can also be used for isolation. Identification of the isolated colonies is based on biochemical reactions viz. indole production, positive for MR and negative for VP, fermentation of glucose with gas production, presence of ß-galactosidase, absence of hydrogen sulphite production and urease, and the inability to use citrate as a carbon source. O-serotyping is a frequently used typing method. Pathogenicity testing of the *E. coli* isolate is also crucial for determining its virulence (Dhama and Mahendran, 2008). ELISA has been reported to be a more sensitive and repeatable test than indirect haemagglutination test, which is a common method for detecting antibodies against *E. coli.* ELISA, based on sonicated *E. coli*, has been developed for detection of antibodies against two important pathogenic serotypes of *E. coli*: O78:K80 and O2: K1, and another based on fimbrial antigen (Lymberopoulos et al., 2006). In order to detect F11 and F165 (P fimbrae) comparison of sandwich ELISA and PCR is a good combination (Bell et al., 2002; Pal and Singh, 2004). All currently known virulence-associated factors, detected in strains isolated from colibacillosis lesions, can also be detected in faecal isolates from clinically healthy chickens. For this reason, none of these traits can be used for APEC identification. For serotyping, the referral laboratory in India is National Salmonella and Escherichia Centre, Kasauli, Himachal Pradesh.

Restriction fragment length polymorphism (RFLP) and Pulsed field gel electrophoresis (PFGE) are considered to be the reliable molecular finger-printing techniques to differentiate *E. coli* isolates. ERIC–PCR and REP–PCR and random amplification of

polymorphic DNA (RAPD)–PCR have also been used to characterize *E. coli* isolates of poultry origin (Chansiripornchai et al., 2001; Radu et al., 2006). Molecular tools such as ribotyping and isoenzyme profile have also been used to evaluate the clonality of avian *E. coli*, with clones being specifically identified as APEC. Recently, to identify traits that predict APEC virulence, avian *E. coli* isolates of known pathogenicity and serogroup were subjected to virulence genotyping and phylogenetic typing (Someya et al., 2007). A multiplex PCR panel targeting five genes (strains iutA, hlyF, iss, iroN, and ompT) has been identified as being the most significantly associated with highly pathogenic APEC (Sylvester and Singh, 2002). When used to screen avian *E. coli* isolates it revealed that APEC isolates were clearly distinguished from the avian fecal *E. coli* isolates by their possession of these genes, suggesting that this pentaplex panel has diagnostic applications (Radu et al., 2001; Johnson et al., 2008a). IMT5155: an autotransporter adhesin gene of *E. coli* has been identified by suppression subtractive hybridization (Dai et al., 2010). Ewers et al. (2005) developed a multiplex PCR protocol, targeting genes for P-fimbriae (papC), aerobactin (iucD), iron-repressible protein (irp2), temperature-sensitive hemagglutinin (tsh), vacuolating autotransporter toxin (vat), cytotoxin, enteroaggregative toxin (astA), increased serum survival protein (iss), and colicin V plasmid operon genes (cva/cvi), *Crl* and *Csg-A* genes to detect avian pathogenic *E. coli* and differentiate them from nonpathogenic strains and those belonging to other pathotypes (Pal and Singh, 2002 and 2007b; Kumar et al., 2008; Singh et al., 2010). These studies also indicated contrary proof to the widely held belief that most APEC isolates are opportunistic pathogens. which may be useful in detecting APEC-like strains occurring in poultry production, along the food chain, and in human disease and may be helpful toward clarifying potential roles of APEC in human disease, ascertaining the source of APEC in animal outbreaks, and identifying effective targets of avian colibacillosis control (McPeake et al., 2005; Rodriguez-Siek et al., 2006).

Differential Diagnosis

Differential diagnosis of the disease should be made from infectious agents viz. virus, mycoplasma and other bacteria that cause lesions similar to those resulting from *E. coli* infection. Infection caused by various bacteria such as *Staphylococcus, Streptococcus, Enterococcus, Clostridia, Chlamydia, Salmonella, Klebsiella, Proteus* and *Pasteurella* needs to be differentiated. *Pasteurella*, *Salmonella* and *Streptococci* may cause acute septecaemic disease and additionally *Streptococci* can cause pericarditis and peritonitis. Not only this isolation of other organisms in association with *E. coli* such as: *Staphylococci* from chick embryos and yolk sac have also been reported for which differential diagnosis is of prime importance in case of avian colibacillosis (Shane, 2005; Barnes, et al., 2008; Singh et al., 2011).

Treatment

Antibiotics, chosen on the basis of antimicrobial sensitivity testing and their pharmacokinetic properties can be used as therapy; however residues in eggs may occur (Barnes et al., 2008). Yet antibiotic therapy is widely used, although APEC are frequently resistant to a wide range of antibiotics. In affected birds, broad spectrum antibiotics such as tetracyclines, ampicillin, chloramphenicol, sulfa drugs, furazolidone, neomycin, gentamicin, etc. are commonly used to control the infection (McGruder and Moore, 1998; Barnes et al., 2008; Singh et al., 2011). Isolates of *E. coli* from poultry are frequently resistant to one or more drugs, especially if they have been widely used for longer periods. Therefore, drug sensitivity should be determined before starting a therapy (van den Bogaard et al., 2001). Vitamin B complex and C added in feed can reduce mortality (Hofstad et al., 1992).

It is of utmost importance to determine susceptibility of bacterial isolate while selecting antimicrobial for the purpose of treatment in order to avoid drugs that are ineffective. Avian pathogenic *E. coli* strains are often resistant to antimicrobials approved for poultry including cephradine, tetracyclines, chloramphenicol, sulfonamides, amino-glycosides and β-lactam antibiotics. Resistance to fluoroquinolones, streptomycin was also reported. Multiresistance commonly occurs in association with virulence factors (White et al., 2000; Vandemaele et al., 2002; Barnes et al., 2008). Organisms that are isolated from shell eggs are susceptible to most antibiotics and resistance often may be higher especially in commensal strains in comparison to APEC. By embryo lethality assay it has been proven that resistance to gentamicin is higher in chicken origin *E. coli* (Altekruse et al., 2002). Multiple antimicrobial resistance traits of APEC strains have also been associated with transmissible R-plasmids (Johnson et al., 2004).

As a potentially safe alternative to antibiotics, the utilization of bacteriophage to treat colibacillosis and prevent foodborne pathogens has been suggested (Huff et al., 2002, 2005; Johnson et al., 2008a; Tiwari et al., 2011, 2013). Vitamin E supplementation has both prophylactic as well as therapeutic benefits for *E. coli* infections but certainly the manner and timing of administration must be taken care of to avoid inefficient therapeutic use (Huff et al., 2004). Impact of experimental colibacillosis in chicken is reduced due to the use of aspirin (sodium salicylate) but certainly a

reverse response may happen if used either in high concentration or when combined with products that cause inflammatory response impairment (Likoff et al., 1981). Response to *E. coli* challenge can also be improved when yeast cell wall derived betaglucan product is fed but it must be noticed that unchallenged control growth is inhibited by such approach (Huff et al., 2006). Recently, it has been suggested that bacteriophage EC1, a lytic bacteriophage against *E. coli* O78:K80, which causes colibacillosis in poultry, is effective in vivo and could be used to treat colibacillosis in chickens (Johnson et al., 2008b; Lau et al., 2010). Huff et al. (2002, 2005) reported that aerosol spray of bacteriophage or intramuscular injection may be a potential alternative to the use of antibiotics in poultry production and could reduce the mortality associated with severe *E. coli* infection. But prior exposure to bacteriophage would limit the therapeutic efficacy of the bacteriophage due to immune interference (Huff et al., 2010; Tiwari et al., 2011). Probiotic supplementation has been shown to significantly enhance the intestinal immunity in poultry and play a vital role in providing resistance to enteric infections due to *E. coli*, *Salmonella* and *Campylobacter spp.* (Dhama et al., 2011a). Bacteriocin producing strain of *Lactobacillus plantarum* F1 or the purified bacteriocin when administered protects chicks against O2 APEC challenge. A mixture of fermented coarsely ground wheat and that of *L. plantarum* as well as *Pediococcus pentosaceus* eliminate *E. coli* completely provided pH remains less than 4.0 for a minimum of 24 hours (Moran et al., 2006). There is marked reduction in small intestine colonisation with *E. coli* when *L. johnsonii* is used for therapeutic purpose (La Ragione et al., 2004; Dhama et al., 2011a; Dhama et al., 2013b). Avian egg yolk antibodies (IgY) have also been explored recently for their inhibitory effect on *Escherichia coli* and inducing resistance in broiler chickens to respiratory/septicemic disease caused by APEC (Sunwoo et al., 2002; Kariyawasam et al., 2004; Yegani and Korver, 2007; Wilmar and Tambourgi, 2010; Dhama et al., 2011b).

Prevention and control

Management practices designed to minimize the exposure level of these types of organisms in the bird's environment are necessary in any preventive program. Avoiding overcrowding and providing proper ventilation along with good biosecurity measures, and appropriate sanitation and hygiene standards are highly effective in the control of colibacillosis in birds, and thereby reducing its zoonotic incidences (Barnes et al., 2008; Singh et al., 2011; Dhama et al., 2012). Prevention should be based on a good programme of hygiene and sanitation meticulously from the nest to the chick box e.g. clean nests, frequent collection, sanitation of eggs, exclusion of severely soiled eggs, separate incubation of floor eggs etc. Follow good hatchery management practices. Immunosuppressive agents like infectious bursal disease (IBD), chicken infectious anaemia and aflatoxins should be checked, as they can flare up and increase the incidences of *E. coli* (Dhama and Mahendran, 2008a; Gowthaman et al., 2013). Prevention of egg contamination by fumigating them within two hours after lay, and by removing cracked eggs or eggs soiled with faecal material is important step (Shane and Faust, 1996; Dhama et al., 2012).

Ultrasonic inactivation of *E. coli* followed by irradiation is one of the most efficient methods for preparation of effective vaccine against colibacillosis. BT-7, a piliated strain is effective as a live vaccine obtained from naturally occurring, non-pathogenic strain, used in chicken older than two weeks of age. Vitamins A, C, D, E along with selenium, β-carotene and iron can be given to improve the bird's immune status (Frommer et al., 1994; Shane, 2001).

Contamination with APEC from the environment must be controlled by reduction and control of intestinal infection, best achieved using competitive exclusion (CE), *i.e.*, inoculating day-old chicks with normal bacterial flora (probiotics) of healthy adult chickens. Birds also need to be protected against other pathogens that promote infections with APEC (McPeake et al., 2005). For this appropriate disease prevention and control programmes need to be followed like regular vaccinations for important poultry pathogens. The housing climate must be kept optimal for bird density, humidity, ventilation, dust and ammonia. The great diversity among APEC strains limits the possibilities of vaccination, and vaccines are not used on a large scale (Barnes et al., 2008).

Vaccination

Several vaccines based on killed or attenuated strains have been tested experimentally, but in general, they give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient. Hence, vaccination for colibacillosis is not widely practised because of the large variety of serogroups involved in field outbreaks. To check infection due to serotypes *viz.*, O2:K1 and O78:K80 effective inactivated vaccines have been produced (Trampel and Griffith, 1997). Autovaccines can prevent and protect against homologus *E. coli* serotypes (Landman and Cornelissen, 2006). Passive immunisation of young birds via the breeder hens is efficient for two weeks, if the birds are challenged with homologous strains. For chickens above 14 days of age live vaccine prepared from an *E. coli* strain that is non-pathogenic and piliated is efficacious It provided protection against both homologous as well as

heterologous strains (Frommer et al., 1994). J5: an *E. coli* strain that is mutant and lacks endotoxin thereby having the gram-negative core antigen was found to be safe, as well as effective to provide protection in chicks (Abdul Aziz and Sukhon, 1996 and 1998). Vaccines based on virulence factors like fimbriae, also give a good homologous protection, *i.e.*, against APEC possessing the same fimbriae. Autovaccines are often used as prevention because in practice effective protection is only achieved against homologous *E. coli* serotypes. Autologous bacterins provide limited serotype-specific protection. In a recent study, broilers vaccinated with Nobilis *E. coli* vaccine, mortality due to *E. coli* infections was only 8.2% in vaccinated birds compared with 24.6% in unvaccinated birds, although the overall mortality between the vaccinated and control flocks did not differ (Gregersen et al., 2010). Further investigations are needed to explain the protection observed and the impact on the genetic diversity of *E. coli*. Ducks can be protected by an inactivated O78 vaccine (Sandhu and Layton, 1985). Bacterial membrane vesicles can be incorporated for use in vaccine that stimulate production of antibody and activity of bacterial lysis possessed by complements; stimulates activity of T cells and cytotoxic T cell activities (Chaffer et al., 1997). Mutant vaccines are also gaining popularity. A mutant O2 serotype showing a *carAB* mutation is found to be stable as well as immunogenic and attenuated and can be used for oral vaccination (Kwaga et al., 1994). *cya* and *crp* gene deleted mutants *viz.,* O2 and O78 can be used for spray vaccination for broiler chicken immunization (Peighambari et al., 2002). The utility of outer membrane protein (OMP) vaccine against APEC in broiler chicks and its efficacy has also been reported (Sylvester et al., 2010). DNA vaccine has also been attempted for colibacillosis, gene encoding the enterotoxigenic *E. coli* K88 fimbrial protein induced good protection during challenge studies (Cho et al. 2004; Dhama et al., 2008).

Precautionary measures for checking public health hazards

The risk of colibacillosis can be reduced through simple precautions viz. thorough cleaning and sanitation of poultry houses, providing proper ventilation, chlorination of drinking water, washing hands before and after food preparation, thorough cooking of poultry and poultry products, proper packaging of meat to avoid drip contamination of other food products, storage of egg at 4^0C rather that at room temperature (Chousalkar et al., 2010; Kabir, 2010; Singh et al., 2011; Dhama et al., 2012, 2013a).

Conclusion and future perspectives

Escherichia coli infections in avian species have become an economic threat to the poultry industry worldwide and the diseases constitute a major public health burden and represent a significant loss of cost in many countries. The economic and public health burden of these diseases have made this topic burning at present time demanding immediate attention. Most avian species are susceptible to colibacillosis. Susceptibility and severity of infections are greatest in young birds including developing embryos. Low and high stress increases susceptibility to the disease. Birds with low antibody response are most resistant to bacterium. Birds acquire nonspecific resistance to colibacillosis following moderate stress. Non-specific resistance is short lived and can be suppressed by cold, stress and corticosteroid. Adaptation of proper hygiene, sanitation, ventilation, strict biosecurity measures, effective vaccination and treatment with antibiotics to protect poultry against coliform pathogens is also detrimental for the other microbial agents that predisposes them to colibacillosis, and thus greatly aid in preventing the occurrence of other disease conditions, and helps in raising healthy flock. Management and sanitation practices designed to minimize the exposure level of these organisms in the bird's environment are necessary in any preventive program. A wide variety of virulence factors have been identified in both APEC as well as strains isolated from chicken that are clinically healthy. APECs are diversified both phenotypically and genotypically hampering clear distinction of pathogenic and commensal isolates. For this reason further studies are needed to determine the role of newly identified putative virulence genes and genes with unknown functions as virulence markers of APEC to strengthen the current understanding of mechanisms underlying the pathogenesis of avian colibacillosis. Unraveling the molecular basis of virulence of avian pathogenic *E. coli* in their natural hosts would provide the basis for the development or improvement of strategies to control APEC infections in the food-producing avian species. Recent advances in molecular diagnosis, effective vaccines and application of novel therapeutic regimens would help control this economically important avian pathogen. More effective applications of existing diagnostics and control methods would greatly reduce the economic burdens posed to poultry producers as well as alleviate the public health hazards associated with this pathogen.

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