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by Freshinta Jellia Wibisono

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Occurrence, antimicrobial resistance, and potential zoonosis risk of avian pathogenic *Escherichia coli* in Indonesia: A review

Freshinta Jellia Wibisono¹, Mustofa Helmi Effendi², and Freshindy Marissa Wibisono³

1. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Surabaya, Indonesia; 2. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia; 3. Postgraduate Student in Veterinary Public Health Study, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

Corresponding author: Mustofa Helmi Effendi, e-mail: mheffendi@yahoo.com

Co-authors: FJW: freshinta.uwks@gmail.com, FMW: freshindymw@gmail.com

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Abstract

Avian pathogenic *Escherichia coli* (APEC) causes avian colibacillosis or colibacillosis and is a major endemic disease of poultry worldwide, including in Indonesia. It is characterized by a black proventriculus and can damage other organs, leading to pericarditis, perihepatitis, water sacculitis, mesenteritis, and omphalitis. The APEC strain is one of the six main sub-pathotypes of the extraintestinal pathogenic *E. coli* (ExPEC) prototype. The relationship between APEC and infection in humans is questionable. The previous studies have suggested poultry products, including meat and eggs, as a potential source of infection for the transmission of ExPEC disease to humans. Due to the absence of reporting of disease incidents and the lack of literature updates on this disease, it seems as if APEC does not exist in Indonesia. Since bacterial resistance is a growing problem in Indonesia, and globally, the World Health Organization issued a statement regarding the importance of assessing related factors and their control strategies. Antimicrobial resistance, especially multidrug resistance, presents a challenge when treating infectious diseases. In Indonesia, the incidence of resistance to several antimicrobials in cases of avian colibacillosis is high. In addition, avian and human extraintestinal *E. coli* infections present a potential zoonotic risk. Furthermore, a relationship exists between antibiotic resistance to foodborne bacteria and the occurrence of antibiotic resistance in humans, so the use of antibiotics in the poultry industry must be controlled. Therefore, the One Health strategy should be implemented to prevent the overuse or misuse of antibiotics in the poultry industry. This review aimed to increase awareness of people who are at risk of getting Avian pathogenic *Escherichia coli* (APEC) from poultry by controlling the spread of APEC by maintaining a clean environment and hygienic personnel in poultry farms.

Keywords: antimicrobial resistance, avian pathogenic *Escherichia coli*, extraintestinal pathogenic *Escherichia coli*, human health, zoonosis.

Introduction

The poultry industry is the main economic sector in Indonesia [1, 2] and the largest food supplier to the global population. Poultry is preferred over other types of animal food products because it is comparatively cheaper and has a relatively high proportion of animal protein [3–7]. The development of the poultry industry in Indonesia has increased and is driven by the high market demand for poultry commodities. For the majority of the Muslim community, prices that are able to meet purchasing power, fulfillment of animal protein nutrition requirements at all socioeconomic levels, maintenance management, environment, and health are factors that support the success of poultry farming in Indonesia [1, 8, 9]. The relationship among these associated factors must be in balance, and an imbalance of

one factor can give rise to a disease. Infectious diseases involve a causative agent and host, as well as environmental factors. Disease is a risk factor that is often faced by all livestock businesses, particularly poultry farming. Therefore, knowledge and information on disease incidence and efforts to prevent, control, and eradicate them are essential [7, 10–13].

Avian pathogenic *Escherichia coli* (APEC), which causes avian colibacillosis in poultry, is considered a major pathogen for the poultry industry. The disease is often named colibacillosis or coli disease by poultry farmers in Indonesia. Serogroups O2 and O78 are the most common in outbreaks of avian colibacillosis, representing 80% of disease cases worldwide [14–18]. Avian pathogenic *E. coli* often occurs in Indonesia [12, 19, 20], and cases of colibacillosis in the field are categorized as secondary diseases that can occur in farms with poor sanitation or secondary infections from respiratory diseases and other immunosuppressive diseases in poultry, such as swollen head syndrome, chronic respiratory disease, and Newcastle disease [3, 21–23].

Although known for more than a century, avian colibacillosis remains one of the main endemic diseases of poultry worldwide, including in Indonesia. Avian

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colibacillosis is a contagious disease in poultry caused by pathogenic *E. coli* bacteria and leads to fairly high economic losses. In Indonesia, this amounts to a loss of 13.10% of the total assets of poultry either directly (weight loss, reduced egg production, and increased total mortality) or indirectly (cleaning, disinfection, and labor compensation) in the event of disease. The proportion of losses incurred to total livestock assets is an indicator of how important it is for the disease to be controlled or overcome [11, 21, 23]. *Escherichia coli* is one of the causes of foodborne zoonoses; it is infectious and produces toxins [19, 24–27].

The incidence of colibacillosis in livestock has led to the use of antibiotics for disease treatment and prevention, and poultry farming is one of the sources of antimicrobial resistance (AMR). The AMR of *E. coli* is a global public health threat that requires action across all sectors of government and society. Uncontrolled use of antibiotics can lead to antibiotic resistance in layer [28, 29] and broiler [30–32] farms. The high use of antibiotics without a prescription is due to the perception of farmers that their use has no side effects and is a low-cost effort to prevent disease [33]. However, bacterial resistance is a growing problem worldwide, and the World Health Organization issued a statement regarding the importance of assessing the factors associated with this problem and strategies to control the incidence of resistance [34–37]. Antibiotic resistance in one country will now become a problem for all countries [38].

When the incidence of AMR increases, antimicrobial drugs become ineffective, and the infection persists in the body and increases the risk of spreading to the next host. The absence of reporting of disease incidents and the lack of literature updates on this disease make it seem as if APEC does not exist in Indonesia. This review discusses APEC in general, the incidence of APEC in Indonesia, resistance caused by APEC, and the potential risk of APEC as a zoonosis.

Avian Pathogenic *E. coli*

Poultry can act as an APEC reservoir which is spread through eggs and meat of chickens and other poultry [39–42]. The digestive tract of a day-old chicken (DOC) broiler is a reservoir of potentially pathogenic *E. coli* [43]. Avian pathogenic *E. coli* is a disease in poultry caused by extraintestinal pathogenic *E. coli* (ExPEC) that causes abnormalities in organs outside the gastrointestinal tract, such as thickened air sacs covered with fibrin fluid (airsacculitis), fibrinous pericarditis, peritonitis, salpingitis, ophthalmia, synovitis, and septicemia. Omphalitis occurs in chicks [17, 44–46]. Avian pathogenic *E. coli* strains cause primary and secondary diseases in chickens and other poultry breeds [44, 47–49]. In their review article, Filho *et al.* [50] presented a summary chart of the relationships between pathotypes and sub-pathotypes of *E. coli*. In this chart, *E. coli* is divided into two main groups, namely, commensal (nonpathogenic) and

pathogenic *E. coli*. Commensal *E. coli* exists as normal flora in the digestive tract of humans, mammals, and birds and helps the host digest food. It is also involved in competing against other pathogenic bacteria in the digestive tract. Phylogenetically, commensal *E. coli* belongs to *E. coli* Group A and Group B1 and does not possess any virulence factors [50–52].

The APEC strains of *E. coli* produce verotoxin (VT), also known as Shiga toxin (Stx) [19, 53–56]. This cytotoxin is produced by some enteropathogenic *E. coli* (EPEC) strains [57]. Some APEC strains have either VT1 (Stx1) or VT2 (Stx2, airsacculitis) genes or both [54]. Strains with only VT2 (Stx2) are more pathogenic than strains with only VT1 (Stx1) or both [54, 55]. *Escherichia coli* isolates that produce VT1 and VT2 can cause disease in poultry and humans [53].

Pathogenic *E. coli* is divided into two subgroups according to its predilection, namely, diarrheagenic *E. coli* (DEC) [54, 58], which is pathogenic in the digestive tract, and ExPEC [26, 59], which causes systemic infections [50]. Diarrheagenic *E. coli* is found in the intestinal tracts of both humans and animals and is classified into eight sub-pathotypes, including EPEC [60, 61], enterotoxigenic *E. coli* (ETEC) [62–64], enterohemorrhagic *E. coli* (EHEC) [65–67], enteroinvasive *E. coli* (EIEC) [68–71], diffusely adherent *E. coli* [72–76], enteroaggregative *E. coli* (EAEC) [75, 77–81], adherent invasive *E. coli* [82–85], and Shiga toxin-producing EAEC [65, 77, 81, 86]. Diarrheagenic *E. coli* is transmitted through food or water contaminated with animal or human feces and causes gastroenteritis and diarrhea. Diarrheagenic *E. coli* transmission by direct human-to-human contact can occur, but this is rare. Among the groups at risk of DEC infection, tourists who have just come to an area can be at greater risk of ETEC infection. Enteropathogenic *E. coli* and EIEC infections are common in children in developing countries, whereas EAEC infections are common in immunocompromised people. The Stx-producing *E. coli* (STEC) strain is also known as verotoxigenic *E. coli*, and the term EHEC is usually used to define STEC strains capable of causing disease in humans, especially bloody diarrhea and hemolytic uremic syndrome [58, 80, 87].

The ExPEC pathotype is divided into six main sub-pathotypes [50, 58], including uropathogenic *E. coli* (UPEC) [88–92], sepsis/newborn meningitis-associated *E. coli* (NMEC) [93–96], APEC [45, 97–102], sepsis-associated pathogenic *E. coli* (SePEC) [103, 104], mammary pathogenic *E. coli* [105–108], and endometrial pathogenic *E. coli* [109, 110]. The ExPEC strain was characterized as *E. coli* isolate containing two or more virulence factors. Known virulence factors include Type 1 (F1) and P (Pap/Prs) fimbriae for colonization, IbeA for invasion, iron acquisition systems, TraT and Iss for serum survival, K and O antigens for antiphagocytic activity, and temperature-sensitive haemagglutinin [49, 91, 94, 101, 111].

Occurrence of APEC in Indonesia

Avian pathogenic *E. coli* is an infectious disease in poultry caused by Gram-negative bacteria, including ExPEC strains [26], which lead to diseases outside the intestine, and includes strains that are pathogenic to animals and humans [44, 111]. Avian colibacillosis, characterized by a black proventriculus [112], is a disease that attacks poultry and often occurs in broilers and laying hens. It can infect all ages, from DOCs to the age when they are ready to harvest [40]. However, events in the field indicate that avian colibacillosis more frequently infects chickens at young ages [20, 113]. Colibacillosis is one of the main bacterial diseases that cause high morbidity and mortality in poultry. The most common presentation is a respiratory tract infection, often followed by septicemia [13, 45]. Transmission to healthy chickens occurs through direct contact of infected animals with contaminated drinking water, flies or insects as vectors, and litter or cage mats contaminated with feces from infected chickens. Avian pathogenic *E. coli* strains can survive for several months in chicken feces [114].

Information on the incidence of disease spread, phylogenetic relationships, host specificity, pathogenicity, and genes associated with the emergence of APEC disease in Indonesia is scarce, and it appears that APEC disease does not exist in Indonesia. We could only find a minimal number of scientific articles reporting APEC incidents in Indonesia [11, 18, 20, 113, 115–117], which indicates a lack of information regarding the incidence of avian colibacillosis in Indonesia, although this disease is known to occur in many Indonesian poultry farms. The high incidence of avian colibacillosis disease in the field has not been widely reported in scientific articles, although in terms of disease losses, it has a fairly high economic impact [22, 100, 111, 118, 119].

Avian Pathogenic *E. coli* and AMR

Antimicrobials (antibiotics, disinfectants, and antiseptics) are substances that interfere with the growth and metabolism of microorganisms. The use of these drugs aims to inhibit (bacteriostatic) or kill (bactericidal) microorganisms without damaging the host tissue. Inappropriate antimicrobial use can lead to therapeutic failure and increase the risk of resistance or the occurrence of side effects [120–124]. Since the introduction of penicillin in the mid-20th century [125, 126], antibiotics have been used not only in human medicine but also in animal care [127]. Initially, antibiotics were used to treat sick animals, but with the intensification of agriculture, the utilization of antibiotics has expanded to include disease prevention and use as growth promoters [128, 129]. Excessive use of antimicrobials on livestock pollutes the environment and contributes to an increase in resistant microorganisms. This poses a threat not only to human health but also to animal health, animal welfare, and sustainable

livestock production, which has implications for food security and people's livelihoods [37, 130–132]. Antimicrobial abuse renders their use ineffective for both animals and humans because it leads to the development and appearance of AMR in disease-causing microorganisms [133–137]. Antimicrobial resistance prevents antimicrobials from killing or inhibiting bacterial growth, rendering antibiotic therapy for infectious diseases ineffective [121, 122, 138].

The presence of anti bacterial agents, the use of antibiotics, and farmer knowledge are some of the factors that can increase the incidence of antibiotic resistance. Antimicrobial resistance has become a global issue [122, 128, 131, 139–142]. The literature suggests avian colibacillosis as one of the factors that trigger the high level of antibiotic resistance. A high percentage of AMR to several antibiotics, such as ampicillin, enrofloxacin, tetracycline, erythromycin, trimethoprim-sulfamethoxazole, oxytetracycline, cephalosporin, chloramphenicol, and others, has been reported in cases of avian colibacillosis in Indonesia [20, 143–148]. Multidrug resistance (MDR) refers to the resistance of bacteria to more than 3 classes of antibiotics. The presence of strains with MDR in cases of avian colibacillosis on poultry farms has a high potential for antibiotic therapy failure [31, 141, 149, 150]. Antimicrobial resistance, especially MDR, is a difficult problem to overcome when treating infectious diseases [150].

Potential Risks of APEC as a Zoonosis

Avian pathogenic *E. coli* causes disease, particularly in poultry; however, the link between APEC and infection in humans is questionable. Some studies revealed chicken as an ExPEC reservoir for humans [39, 40, 42, 151]. Extraintestinal pathogenic *E. coli* strains include UPEC in humans and APEC in poultry [41, 45, 100, 111]. The genomic sequences of APEC strain O1:K1:H7 showed similarities with UPEC and NMEC strains in humans. These similarities allow APEC to cause disease in humans, and certain APEC strains have the potential to cause urinary tract infections in humans [17, 93, 152]. The phylogenetic analysis of ExPEC isolates revealed grouping according to genes related to virulence and plasmid replicon type. Extraintestinal pathogenic *E. coli* isolates were revealed as derived from the pathogenic strain APEC in poultry; NMEC, which causes neonatal meningitis in humans; and UPEC, which causes human urinary tract infections. This grouping implies that it is possible to observe the potential for transmission of certain ExPEC strains between humans and animals as a disease with a zoonotic risk [153, 154]. Poultry products, including meat and eggs, have been reported as a potential source of infection for the transmission of ExPEC disease to humans [41, 151, 155, 156].

Since ExPEC in poultry and humans share several common phylogenetic groups and genes related to their virulence, great attention must be paid to the zoonotic

risk of APEC disease [157]. Most cases of ExPEC disease in humans show AMR. Extraintestinal pathogenic *E. coli* strains have the special ability to cause disease in internal organs in humans [25, 58, 158] and other extraintestinal organ infections. Extraintestinal pathogenic *E. coli* strains with AMR are transmitted through contaminated food [44, 155, 156, 159]. A new problem related to ExPEC transmission from food, especially poultry products, is the emergence of diseases with MDR, meaning that APEC creates economic problems as well as animal and human health problems [26, 155].

Avian and human ExPEC infections present a potential zoonotic risk. Extraintestinal pathogenic *E. coli* infections in humans can be systemic (urinary tract infection as a manifestation of UPEC and sepsis that progress to meningitis as a manifestation of NMEC and SePEC), whereas infection in poultry, namely, APEC, can cause airsacculitis, salpingitis, and cellulitis. Both animal and human infections can transfer genes related to virulence and antibiotic resistance, and here, the factors that play a role are poultry products as a source of disease [18, 26, 91, 99, 111, 115, 152].

Foodborne disease has the potential to disrupt animal and human health, including transmitting zoonoses or chemical residues found in foods of animal origin. The AMR of some foodborne bacteria has led to treatment failure of gastrointestinal infection in humans. Foodborne bacteria that show AMR can be transmitted to humans through the food chain or by direct contact. Since there is a relationship between the antibiotic resistance of foodborne bacteria and the occurrence of antibiotic resistance in humans, the use of antibiotics in the livestock industry must be controlled [23, 24, 33, 41, 45, 79, 145, 160–169].

One Health Approach for APEC

The use of antimicrobials is a cost-effective practice to reduce the incidence and mortality rate of avian colibacillosis. However, the overuse and misuse of antibiotics have exerted selective pressure for the emergence of AMR and MDR strains, leading to therapeutic failure and potential economic loss in the poultry industry worldwide. Apart from the spread of antimicrobial-resistant strains, AMR genes can be transferred and disseminated between food-producing animals and human pathogens, and this is a global public health issue [170]. For this reason, our discussion of the presence of APEC isolates may pose two threats: Poor antimicrobial treatment of poultry and the potential risks associated with consumer exposure to poultry products from the food chain. Thus, we also need to elucidate the presence of AMR genes in APEC isolates. This review clearly illustrates that in the context of One Health, genomic analysis can reveal opportunities for timely intervention and prevention of the spread of AMR. Another important One Health strategy is the prevention of infection and disease related to APEC and other poultry pathogens [32, 121, 171, 172] by organizing coordinated scientific and public health

efforts, ensuring effective surveillance, research, public education, communication, and new policymaking resolutions [173].

Conclusion

The poultry sector is the largest supplier of human food. Avian pathogenic *E. coli* is a poultry disease caused by ExPEC. The main endemic disease of poultry is APEC infection or avian colibacillosis. Poultry acts as an APEC reservoir that is spread to humans through eggs and meat. Many APEC incidents in Indonesia occur in the field, but the lack of reporting and literature updates on this disease makes it seem as if APEC does not exist in Indonesia. The presence of antibacterial agents, the use of antibiotics, and farmer knowledge are some of the factors that can increase the incidence of antibiotic resistance. The problem of AMR has become a global issue. There is a high incidence of AMR to several antibiotics in cases of avian colibacillosis in Indonesia. Overcoming AMR, especially MDR, is challenging when treating infectious diseases. Infection with avian colibacillosis and human ExPEC presents a potential zoonotic risk. Foods of animal origin have the potential to be hazardous to the health of animals and humans, including transmitting zoonoses. There is a relationship between the antibiotic resistance of bacteria present in food of animal origin and the occurrence of antibiotic resistance in humans. Thus, the One Health strategy should be implemented to prevent the overuse or misuse of antibiotics in the poultry industry.

Authors' Contributions

FJW: Conceived the idea and drafted and revised the manuscript. MHE and FMW: Reviewed the manuscript. FJW and MHE: Literature search and edited and reviewed the manuscript. All authors have read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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