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Phenotypic and Resistance Patterns of Avian Pathogenic *Escherichia coli* Isolated from Commercial Poultry Farm

N. P. V. T. Timur[a,e], Y. P. Kristianingrum[b], I. W. Suardana[d], & M. H. Wibowo[c,*]
[a] Veterinary Science Doctoral Study Program, Faculty of Veterinary Medicine, Universitas Gadjah Mada
[b] Department of Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada
[c] Department of Microbiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada
Jl. Fauna No. 2, Karang Gayam, Sleman, Special Region of Yogyakarta, 55281, Indonesia
[d] Veterinary Public Health Department, Faculty of Veterinary Medicine, Universitas Udayana
Jl. Kampus Bukit Jimbaran, Badung, Bali, 80361, Indonesia
[e] Polytechnic Agriculture Development Manokwari
Jl. SPMA Reremi, Manokwari, West Papua, 98312, Indonesia
*Corresponding author: mhwibowo@ugm.ac.id
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ABSTRACT

Colibacillosis in poultry is induced by avian pathogenic *Escherichia coli* (APEC). No relevant studies have been extensively published in Indonesia. Colibacillosis can affect various poultry species, resulting in significant economic losses to Indonesia's poultry industry. The efficacy of colibacillosis treatment depends on the antibiotic sensitivity of the causative pathogen. This study aims to determine the phenotypic traits, pathological pictures, and antibiotic susceptibility of APEC bacteria isolated from colibacillosis cases in commercial farms in the Special Region of Yogyakarta and Central Java. Organ samples were collected from broiler and layer chickens suspected of having colibacillosis. The results of cultured, biochemical tests, pathological examinations, pathogenicity, and hemolysis testing confirmed 21 APEC isolates. The antibiotic susceptibility of the isolates was determined against nine different antibiotics. Pathological examination revealed severe macroscopic changes, including polyserositis (perihepatitis, pericarditis, and air sacculitis), accompanied by microscopic evidence of necrosis and widespread heterophilic inflammatory cell infiltration across the lungs, cardiac pericardium, and ovaries. The resistance patterns of the samples to nine antibiotics, ranked from highest to lowest, were as follows: amoxicillin, enrofloxacin, streptomycin, trimethoprim-sulfamethoxazole, nalidixic acid, oxytetracycline, tetracycline, gentamicin, and neomycin. The highest resistance was observed against β-lactam antibiotics. The APEC isolates displayed high pathogenicity, characterized by typical gross and histopathological lesions of colibacillosis, including polyserositis. Crucially, the high prevalence of multidrug resistance (MDR) against nine tested antibiotics (71.43%), particularly to amoxicillin, poses a serious challenge to effective colibacillosis treatment in the studied regions and necessitates a strategic shift in antibiotic usage policies.

Keywords: antibiotics resistance; avian pathogenic Escherichia coli; colibacillosis; pathology; phenotypics

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) is an extraintestinal pathogenic *E. coli* (ExPEC) that can cause colibacillosis in chickens, which can impact egg and meat production from poultry. APEC employs a variety of virulence mechanisms and pathogenic traits in poultry, leading to colibacillosis (Kathayat *et al*., 2021). Among them are adhesins, invasins (Sora *et al*., 2021), fimbriae or pili, outer membrane proteins, and toxins, including cytotoxins, hemolysins, and endotoxins (Meena *et al*., 2021). The severity of APEC induced disease depends on the host's health status, the virulence factors expressed by the specific strain and other predisposing factors. It often manifests as extraintestinal infection, with clinical signs such as air sacculitis, perihepatitis, pericarditis, peritonitis, salpingitis, coligranuloma, omphalitis, cellulitis, swollen head syndrome, and osteomyelitis (Nolan *et al*., 2020).

Food security and poultry welfare (Jørgensen *et al*., 2019) are global issues, resulting in huge economic losses in the poultry industry due to high morbidity and mortality rates (Mehat *et al*., 2021). Colibacillosis inflicts enormous losses on Indonesia's poultry industry, with an estimated average loss of IDR 14.20 trillion per cycle for broilers and approximately IDR 13.40 trillion per month for layers, amounting to 13.10% of the country's total poultry assets (Wibisono *et al*., 2018).

The prevalence rate of colibacillosis is 22.2%, as reported in Indonesia (Panth, 2019). Antibiotic therapy remains a key strategy in managing colibacillosis. However, its overuse and misuse may accelerate antimicrobial resistance (AMR) by producing resistant strains (Naghavi *et al*., 2022). AMR is defined as a result of changes in microbial responses to antibiotics, leading to treatment failures and relapsing infections (Huemer *et al*., 2020). At the flock level, poultry disease outbreaks are often inadequately controlled when antimicrobials are used without accurately identifying the etiological agent and susceptibility testing (resulting in unmatched treatment) (Choisy *et al*., 2019). Factors contributing to AMR encompass inadequate treatment, utilization of unsuitable medications, and self-medication (Ahmed *et al*., 2024). Therefore, AMR surveillance or monitoring for APEC is essential to guide appropriate control measures and limit the dissemination of AMR.

The prevalence of colibacillosis in broiler and layer chickens caused by APEC has been well documented in various provinces of Indonesia. However, effective control and treatment strategies have not been developed, as there has been no recent data for over ten years. The purpose of this study is to find out the phenotypic traits of APEC bacteria that came from instances of colibacillosis in broiler and layer chickens from commercial farms in the Special Region of Yogyakarta and Central Java. It also wants to confirm the organ pathology shown in these case studies. This study also analyzes antibiotic sensitivity to provide real information about how to choose the best treatment for avian colibacillosis. The results should help improve diagnosis and enable more effective control of colibacillosis in both broiler and laying chickens.

MATERIALS AND METHODS

Ethical Approval

This research was approved by the Research Ethics Commission of the Faculty of Veterinary Medicine UGM with a Certificate of Ethics Number: 28/EC-FKH/int./2024.

Sample Collection and Bacterial Isolation

Samples were obtained from commercial broiler and layer farms in the Central Java and Special Region of Yogyakarta regions, where colibacillosis was suspected. Sampling occurred from October 2023 to August 2024 on a case-by-case basis. This research employed purposive and field-based sampling, consistent with case study methodology, rather than probabilistic sampling across regions. Chickens subjected to necropsy showed clinical symptoms of colibacillosis. A total of 66 samples from layer and broiler chickens were collected for necropsy.

Isolation and Identification of APEC

Microbiological testing, including purification, Gram staining, biochemical assays, and pathogenicity screening, was performed at the Department of Microbiology, Faculty of Veterinary Medicine, UGM. Bacterial isolation was conducted as per Bisping and Amtsberg (1988). Colony isolation was carried out based on colony morphology on EMBA after incubation at 37 ^{o}C for 24 hours and bacterial morphology on Gram staining and repeated until a pure culture was obtained. Then bacterial identification was conducted by biochemical tests (Bonnet *et al*., 2020; Buxton & Fraser, 1977; Bisping & Amtsberg, 1988). Confirmed *E. coli* isolates were further subjected to pathogenicity screening on Congo red agar (Berkhoff & Vinal, 1985). The ability of bacteria to perform hemolysis can be tested by using 5% sheep blood agar and observing the hemolysis zone around bacterial growth (Buxton, 2005).

Pathology and Histopathology Examination

Organs exhibiting pathological lesions were swabbed on selective media and fixed in 10% neutral buffered formalin. Tissues were processed routinely for histology, stained with hematoxylin and eosin, and examined histopathologically at the Department of Pathology, Faculty of Veterinary Medicine, UGM.

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was conducted using broth microdilution and disc diffusion methods at the Disease Investigation Center, Wates, Yogyakarta. Broth microdilution was performed automatically using the Thermo Scientific™ Sensititre™ AST Plate. The QC strains used in this study were *Escherichia coli* ATCC 25922. The disc diffusion method involved placing antibiotic-impregnated disks (oxytetracycline (30 μg), neomycin (30 μg), and streptomycin (10 μg) onto Mueller-Hinton agar plates inoculated with APEC suspensions standardized to 0.5 McFarland using a densitometer. The classification of antibiotic resistance categories was derived from CLSI guidelines, specifically VET01S, 7th edition (CLSI, 2024), and M100, 33rd edition (CLSI, 2023)

RESULTS

Isolation and Identification of APEC

Necropsy was performed on 66 chickens with suspected colibacillosis from various small- and large-scale poultry farms. The results of isolation and identification showed that 41 broiler chickens and laying hens (62.12%) of the samples showed culture in EMBA, Gram staining, and biochemical tests that had characteristics consistent with *E. coli*. The samples were further phenotypically screened to identify APEC-confirmed bacteria, 51.21% (21/41) were confirmed to be pathogenic based on their capacity to bind Congo red dye and exhibit alpha hemolysis activity.

Gross Pathology and Histopathology Examination

Gross pathological findings and histopathological analysis of samples infected with colibacillosis are detailed in Table 1 and illustrated in Figures 1 and 2.

Antibiotic Sensitivity Testing of APEC

Antibiotic sensitivity testing of the *E. coli* isolates was performed using the Sensititre system and the antibiotic disk diffusion method. The findings indicated varying levels of bacterial response, categorized as susceptible (S), intermediate (I), and resistant (R). The results of this study showed that the resistance levels, ranked from highest to lowest, were amoxicillin (66.7%, 14/21; 95% CI: 45.4–82.8), enrofloxacin (57.1%, 12/21; 95% CI: 36.5–75.5), streptomycin (52.4%, 11/21; 95% CI: 32.4–71.7), trimethoprim–sulfamethoxazole (52.4%, 11/21; 95% CI: 32.4–71.7), nalidixic acid (47.6%, 10/21; 95% CI: 28.3–67.6), oxytetracycline (47.6%, 10/21; 95% CI: 28.3–67.6), tetracycline (42.9%, 9/21; 95% CI: 24.5–63.5), gentamicin (42.9%, 9/21; 95% CI: 24.5–63.5), and neomycin (23.8%, 5/21; 95% CI: 10.6–45.1).

Statistical analysis using the Chi-Square test revealed a distinct antibiotic resistance profile between broiler and layer chicken APEC isolates from Central Java and the Special Region of Yogyakarta. Overall, isolates derived from broilers demonstrated

Table 1. The gross pathology and histopathological alterations of organs affected by colibacillosis in broiler and layer chickens (n= 21)

Organ	Gross pathology	n (%)	Histopathology lesions	n (%)
Lungs	Pale color	9 (42.86)	Inflammation in the parabronchi	4 (19.05)
	Exudate	3 (14.29)	Granulomatous inflammation	3 (14.29)
	Blackish red color	3 (14.29)		
Air sacs	Cloudy air sac	14 (66.67)	Thickening and inflammation	7 (33.33)
	Cloudy air sac with yellow foci	2 (9.52)		
Heart	Covered with a fibrin layer	20 (95.24)	Pericarditis	18 (85.71)
	Pale color	16 (76.19)	Myocarditis	6 (28.57)
	Fat in heart	2 (9.52)	Atrophy	3 (14.29)
	Yellowish exudate on the heart	4 (19.05)	Necrosis	1 (4.76)
Liver	Enlarged and brittle	20 (95.24)	Multifocal necrosis	11 (52.38)
	Multifocal grayish area	4 (19.05)	Inflammation	20 (95.24)
	Covered with a fibrin layer	9 (42.86)	Congestion and athropy	1 (4.76)
	Yellowish and brittle	11 (52.38)	Fatty degeneration	2 (9.52)
			Necrosis	1 (4.76)
Organs in the abdominal cavity	Coligranuloma	7 (33.33)	Erosion of the intestinal epithelium	3 (14.29)
	Mesenteritis	14 (66.67)		
	Omphalitis	7 (33.33)	Inflammation intestinal mucosa	2 (9.52)
	Exudate in the abdominal cavity	11 (52.38)		
Renal	Blackish red color	5 (23.81)	Inflammation	1 (4.76)
	Exudate on the surface	4 (19.05)	Congestion	1 (4.76)
			Nephrosis	3(14.29)
Ovary	Pale and undeveloped	4 (19.05)	Inflammation	2 (9.52)
	Balloning and caseous mass	1 (4.76)	Hemorrhage	2 (9.52)
	The ovaries smell foul	2 (9.52)		
Carcass	Bruised carcass	5 (23.81)		

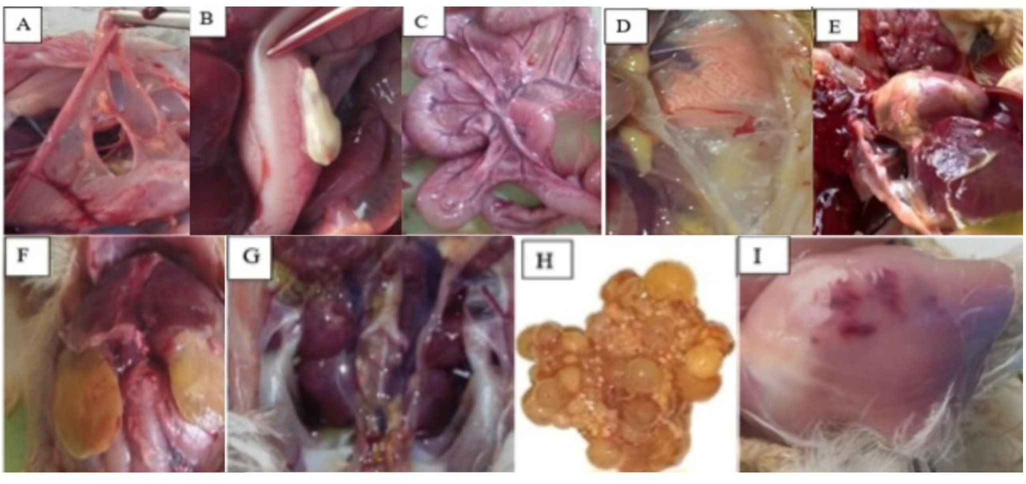

Figure 1. Gross pathological results in broiler and layer chickens: A. caseous masses in the peritoneum; B. granulomatous nodules in the intestine; C. congestion in the mesentery and tunica serosa of the intestine; D. cloudy and thickened air sacs with exudate; E. thickened pericardium coated with fibrin and perihepatitis; F. swollen, brittle, and hemorrhagic liver with fibrin and caseous masses on the surface; G. swollen, slightly brittle, and blackish-red kidneys with caseous deposits; H. Pale, hemorrhage, and atrophied ovarian follicle; I: ecchymosis hemorrhage in the thigh muscles.

a significantly higher and broader prevalence of resistance compared to those from layers, a trend particularly evident within the Special Region of Yogyakarta. Resistance rates to enrofloxacin (p=0.008), nalidixic acid (p=0.0012), gentamicin (p=0.049), and neomycin (p=0.0044) were significantly elevated in broilers from this region, ranging from 25% to 50%. In contrast, no statistically significant differences (p>0.05) were observed in the resistance patterns for amoxicillin, tetracycline, oxytetracycline, streptomycin, and trimethoprim-sulfamethoxazole between the two chicken types across both locations.

These results revealed that 15 of the 21 isolates (71.43%) exhibited MDR. MDR of 3 classes of antibiotics was 28.57% (6/21), 4 classes of antibiotics was 38.1% (8/21), and 5 classes of antibiotics was 4.76% (1/21). Further, 5 isolates (23.81%; 5/21) exhibited AMR, and 1 (4.76%; 1/21) was susceptible to all the tested antibiotics. The percentage data on the sensitivity of each antibiotic is presented in Table 2. The largest combination of resistance in this study was 9 types of antibiotics from 5 classes of antibiotics, and the combination of resistant antibiotics in each sample can be seen in Table 3.

DISCUSSION

The results of isolation and identification showed that the samples showed culture in EMBA, Gram staining, and biochemical tests that had characteristics consistent with *E. coli*. On EMBA, *E. coli* colonies appear with a green metallic sheen and greenish-blue spots (Basavaraju & Gunashree, 2023). In Gram staining, *E. coli* presents as pink, small rod-shaped structures, consistent with Gram-negative bacteria. The biochemical tests performed included triple sugar iron agar (TSIA), urea, citrate, methyl red, Voges-Proskauer (MRVP), sulfide indole motility (SIM), and indole tests (Bisping & Amtsberg, 1988). These isolates showed typical *E. coli* results in the indole, methyl red, Voges-Proskauer, citrate (IMViC) test, with SIM.

E. coli pathovars can also be screened using the phenotypical Congo red binding assay (Saha *et al*., 2020). Twenty-one (51.21%) of the *E. coli* isolates were considered pathogenic based on Congo red binding, as they developed colonies with a red pigment (Ugwu *et al*., 2020). Similar observations were made in an earlier study on the Congo red binding positive APEC isolates

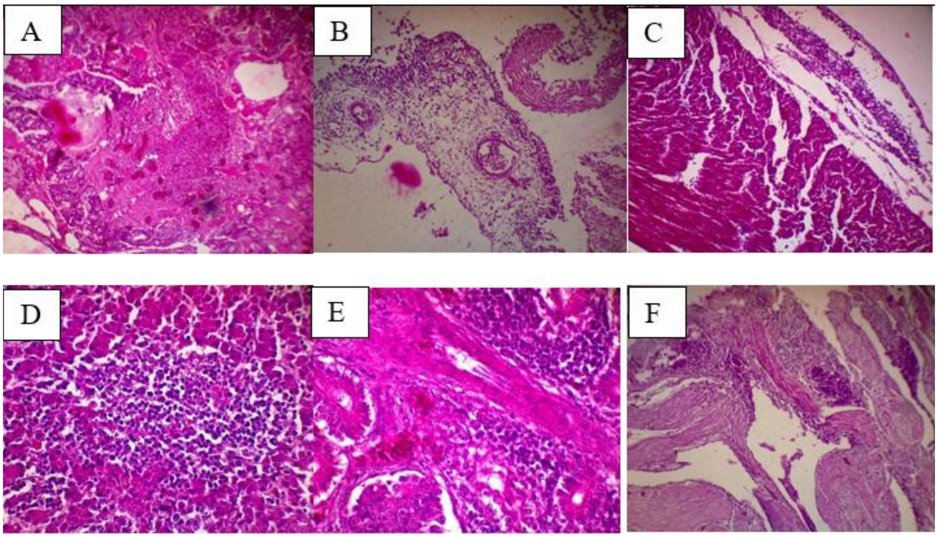

Figure 2. Histopathological findings in broiler and layer chicken organs. A. Lungs with granulomatous inflammation and giant cell formation; B. air sacs with connective tissue proliferation and infiltration of heterophilic inflammatory cells and lymphocytes; C. heart showing pericarditis; D. liver with multifocal necrosis in the parenchyma and perivascular inflammation; E. kidney with nephrosis in renal tubules; F. ovaries with hemorrhage and heterophilic infiltration in the ovarian stroma.

Table 2. Results of antibiotic susceptibility testing of avian pathogenic *Escherichia coli* isolated from broiler and layer chickens with colibacillosis, based on CLSI VET01S, 7th edition (2024), and M100, 33th edition (2023)

Antibiotic group	Types of antibiotics	Interpretation (%)		
		Susceptible	Intermediate	Resistant
β lactam	Amoxicillin	28.57	4.76	66.67
Tetracycline	Tetracycline	57.14	0	42.86
Tetracycline	Oxytetracycline	23.80	28.58	47.62
Fluoroquinolone	Enrofloxacin	14.29	28.57	57.14
Fluoroquinolones	Nalidixic acid	52.38	0	47.62
Aminoglycosides	Neomycin	71.43	4.76	23.81
Aminoglycosides	Gentamicin	57.14	0	42.86
Aminoglycosides	Streptomycin	0	47.62	52.38
Folate pathway antagonist	Trimethoprim-sulfamethoxazole	47.62	0	52.38

Table 3. Description of antibiotic resistance types in each sample

Sample code	Number of antibiotic resistances	Types of antibiotics
A1	4	AMX, ENR, NAL, STR
A4	6	AMX, ENR, GEN, TMP-SMX, NAL, STR
A7	4	AMX, ENR, GEN, TMP-SMX
A10	5	AMX, ENR, GEN, TMP-SMX, STR
A13	7	TET, AMX, ENR, GEN, NAL, OTC, STR
A15	6	TET, AMX, TMP-SMX, OTC, NEO, STR
A16	2	AMX, OTC
A17	5	TET, TMP-SMX, OTC, NEO, STR
A20	5	TET, AMX, TMP-SMX, OTC, STR
A21	9	TET, AMX, ENR, GEN, TMP-SMX, NAL, OTC, NEO, STR
A22	6	AMX, ENR, GEN, TMP-SMX, NAL, STR
A23	2	ENR, NAL
A28	3	NAL, OTC, NEO
A29	2	TET, AMX
A32	4	TET, AMX, TMP-SMX, OTC
A33	4	TET, ENR, NAL, OTC
A35	0	-
A39	6	AMX, ENR, GEN, TMP-SMX, NAL, STR
A42	4	AMX, ENR, GEN, NAL
A46	3	TET, ENR, OTC
A50	5	ENR, TMP-SMX, GEN, NEO, STR

Note: AMX= amoxicillin; ENR= enrofloxacin; GEN=gentamicin; NAL= nalidixic acid; NEO= neomycin; OTC= oxytetracycline; STR= streptomycin; TET= tetracycline; TMT-SMX= trimethoprim-sulfamethoxazole; A1-A50 = APEC bacterial sample code tested in this study.

(Saha *et al*., 2020). The variation of Congo red dye uptake among *E. coli* isolates may suggest differences in pathogenicity and virulence potential (Berkhoff & Vinal, 1985).

Hemolysis is often associated with *E. coli* pathogenicity, especially in strains isolated from blood (Daga *et al*., 2019). It has been reported that α-hemolysis (partial hemolytic activity) is observed in most pathogenic *E. coli* isolates (Salam *et al*., 2024). In this study, pathogenicity testing on whole blood agar media showed that the visible colonies produced incomplete hemolysis, or what is often called α-hemolysis.

Colibacillosis cases can be identified through necropsy, which typically reveals pronounced lesions of generalized polyserositis accompanied by various combinations of pericarditis, perihepatitis, air sacculitis, and peritonitis. Typical postmortem observations include the presence of fibrin, leftover egg yolk, or milky fluid within the peritoneal cavity and on the surfaces of several organs. In cases of peritonitis, a caseous exudate can accumulate in the body cavity, resembling clumped egg yolk, a condition referred to as yolk peritonitis (Nolan *et al*., 2013). Yolk accumulation in the peritoneal cavity can lead to yolk peritonitis in laying hens. In broilers, colibacillosis frequently occurs in conjunction with perihepatitis, pericarditis, and air sacculitis, with *E. coli* isolated from the affected organs (Apostolakos *et al*., 2021; Li *et al*., 2024). These results align with the postmortem findings in this study.

Anatomical pathological analysis conducted in this study revealed pulmonary hemorrhage and the presence of a caseous mass. Lungs exhibiting pathological alterations due to bacterial infection showed inflammation, which was characterized by a red-brown, meat-like coloration and increased tissue density (Meha *et al*., 2016). In APEC-infected chickens, Abalaka *et al.* (2017) also described perivascular and interstitial edema, pulmonary hemorrhage, granulomatous inflammation, and giant cells in the lungs. In infected broilers, histopathological examinations consistently showed marked pulmonary lesions characterized by heterophile infiltration and accumulation of eosinophilic exudates, as well as intravascular fibrin thrombi (Ghahramani *et al*., 2024). The endotoxins released during APEC infection may enhance vascular permeability and contribute to pulmonary edema, congestion, and hemorrhage, while inflammatory stimulation of vascular injury aggravates respiratory distress (Kathayat *et al*., 2021).

The air sacs became thickened due to hypertrophy of the connective tissue and infiltration of heterophilic inflammatory cells. Abdelhamid *et al*. (2024) described lung congestion and pneumonic lesions in their study of colibacillosis. Bhalerao *et al.* (2013) reported similar lesions, including congestion, edema, and foci of pneumonia. Pathological examination in this investigation revealed opacification of the air sacs, indicating air sacculitis. Birds with colibacillosis exhibit air sacculitis at all ages due to fibrin mass formation. It was most commonly observed in birds older than three weeks (Bhalerao *et al*., 2013; Taunde *et al*., 2021). Histopathology showed thickening and proliferation of connective tissue in the air sacs with heterophilic inflammatory cells.

Anatomopathology in this study showed thickened layers of pericardium with fibrin coating, as seen here. These changes cause the pericardium and epicardium to adhere through fibrinous connective tissue in their respective layers, a sign of pericarditis. Nolan *et al.* (2020) said pericarditis is colisepticemia, starting with tissue response edema or fluid accumulation in the pericardial sac and exudate, then fibrinous exudate. In chronic infections, the exudate becomes thickened and caseous. In long-standing cases, the pericardium becomes adherent to the epicardium. Microscopy showed infiltration of inflammatory cells (pericarditis and myocarditis) in the pericardium and interstitial tissues and muscle atrophy (muscle vacuolation). These findings are consistent with Nolan *et al.* (2020).

According to Taunde *et al.* (2021), the present study observed anatomical, pathological, and histopathological characteristics of the liver, describing a case of colibacillosis with fibrin exudate on the surface of Glisson's capsule. This is consistent with Abdelhamid *et al.* (2024) and Ghahramani *et al.* (2024), who noted that the liver capsule can be thickened and surrounded by fibrinous exudates, with heterophilic infiltration; further degenerative changes, such as fatty change, were also observed.

Colibacillosis in chickens causes renal congestion, with the kidney appearing pale and containing hard, pale areas of necrosis, as well as being congested and inflamed with infiltration by inflammatory cells (Abalaka *et al*., 2017). Chickens with colibacillosis showed kidney lesions such as hemorrhage and discoloration, as noted by Taunde *et al.* (2021), which is in line with the results of this study. The ovaries of the broilers in this study were underdeveloped, exhibiting atrophied follicles, irregular development, a soft texture, and a color change from pale yellow to white. These changes are in line with Ozaki *et al.* (2018), who described post-mortem findings of oophoritis and salpingitis in chickens with colibacillosis. In this study, the visible lesions were similar to those of colibacillosis, characterized by mostly inactive, yellowish, or pale follicular structures.

Excessive and improper use of poultry antimicrobials has driven the emergence of AMR, posing a significant public health threat (Abreu *et al*., 2023). Chickens are the most closely kept birds among humans across the poultry value chain, with an accompanying risk of a serious public health challenge (Ugwu *et al*., 2020). In this survey, amoxicillin (including enrofloxacin and trimethoprim-sulfadiazine) was used by farmers for the oral treatment of colibacillosis. While AMR can occur naturally, inappropriate use of antimicrobials in humans and animals has contributed to its increased emergence. Before 2018, antibiotic growth promoters were banned in Indonesia due to the Indonesian Regulation No. 14/PERMENTAN/PK.350/5/2017-Ministry of Agriculture regarding the classification of veterinary drugs.

This study revealed a high resistance to amoxicillin (66.67%; 14/21), a β-lactam antibiotic. This resistance rate is lower than that reported in China (81.7%) (Afayibo *et al.*, 2022) and Ukraine (78.4%) (Nechypurenko *et al.*, 2024). A significant prevalence of APEC resistance to clinically relevant antibiotics, notably β-lactams, poses a serious threat to public health (Osman *et al*., 2018) Antibiotic sensitivity tests also indicated a 57.14% (12/21) of this samples resistance to enrofloxacin and resistance to nalidixic acid was 47.62% (10/21) which is consistent with the resistance of 45% reported by Sgariglia *et al*. (2019). Fluoroquinolones inhibit bacterial gyrase and topoisomerase IV enzymes (Grabowski *et al*., 2022). Enrofloxacin is commonly used in broiler farms to treat colibacillosis (Temmerman *et al*., 2021).

Aminoglycosides such as streptomycin, gentamicin, and neomycin are all classified as aminoglycosides. The streptomycin resistance rate in this study was 52.38% (11/21), which is lower than the findings reported by Afayibo *et al*. (2022). The resistance rate to gentamicin was 42.86% (9/21), which is similar to the findings of Hardiati *et al*. (2021). Aminoglycosides are effective against Gram-negative species by binding to 30S ribosomal subunit, so bacteria can't produce protein. They can treat a wide spectrum of diseases (Dezanet *et al*., 2020). Sulfonamides, often combined with diaminopyrimidine compounds like trimethoprim, exert a synergistic antibacterial effect by inhibiting different stages of bacterial folic acid synthesis. This synergy reduces the dosages required for both agents, broadens antibacterial spectra, and decreases resistance (Stastny *et al*., 2024). The high resistance rate to trimethoprim-sulfamethoxazole observed in this study, 52.38% (11/21), was lower than that reported in previous studies conducted in Algeria and Jordan (Ibrahim *et al*., 2019; Aberkane *et al*., 2023).

The study's results showed that resistance to tetracycline antibiotics, specifically tetracycline (42.86%, 9/21) and oxytetracycline (47.62%, 10/21), was relatively low but not significantly different between the two drugs. Jahantigh *et al.* (2020) reported higher resistances in APEC compared to our study, although not as high as those reported by Nechypurenko *et al.* (2024). Tetracyclines are commonly used as cheap antibiotics in the poultry industry. Resistance can arise from excessive administration for prophylactic, therapeutic, or growth performance purposes (Jahantigh *et al*., 2020).

Denissen *et al.* (2022) explain that multidrug resistance (MDR) manifests when an individual acquires resistance to a minimum of three distinct classes of antibiotics. In this study, we found that 71.43% (15/21) of the tested APEC strains were MDR. This finding aligns with previous investigations in Iran (Jahantigh *et al*., 2020). *E. coli* significantly contributes to the issue due to its rapid transmission among humans and animals via the fecal-oral route (Galindo-Méndez, 2020). The antibiotic resistance genes can be spread through horizontal gene transfer from other bacterial strains or species (Larsson & Flach, 2022). Imprinted DNA can also persist in the environment long after resistant strains have died (Jian *et al*., 2021).

Guidelines emphasize administering antibiotics based on accurate diagnosis, choosing the most appropriate antibacterial agent, limiting last-resort antibiotics, and strictly following label instructions (Ungemach *et al*., 2006). Concerns arise that repeated low-dose antibiotic exposure in animals contributes to AMR. This is problematic in poultry production. The unrestricted availability of antibiotics to livestock farmers without prescriptions promotes their excessive use (Islam *et al*., 2024). Prudent antibiotic use requires optimized dosage regimens, including correct dosage, interval, initiation, and duration of therapy. Strategies that maximize antimicrobial pharmacokinetics and pharmacodynamics can help minimize AMR selection in pathogens, commensal bacteria, and the environment (Guardabassi *et al*., 2018). For the chicken's health and to reduce side effects, it is essential to have an accurate dosage schedule that includes the dose, the time between doses, and the length of treatment. This is especially important for antibiotics, which are necessary to keep AMR in check. It is essential to get the right amount of antibiotics at the right time. Antimicrobials must attain effective concentrations at the infection site and persist for adequate durations to facilitate recovery (Caneschi *et al*., 2023).

CONCLUSION

This study reported that the causative agent of colibacillosis from a commercial farm was APEC, which on EMBA, colonies appear with a green metallic sheen and the biochemical tests performed show *E. coli* characteristics, which could bind Congo red dye and exhibited incomplete hemolysis activity. Macroscopic changes observed were perihepatitis, pericarditis, air sacculitis, oophoritis, and carcass condemnation. Histopathological examination revealed hepatic necrosis and inflammation, heterophilic inflammatory cell infiltration in the lungs, thickened air sacs with heterophilic cell infiltrates, inflammatory cellular infiltration of the pericardium, nephrosis of the renal tubules, and hemorrhage in the ovaries. Resistance to β-lactams was the most common among the nine antibiotic types tested. MDR strains were found in 71.43% (15/21) of samples.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used Scopus AI to assist in finding references. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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