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PREVALENCE OF MULTI-DRUG RESISTANT AVIAN PATHOGENIC *E. COLI* IN COLIBACILLOSIS-INFECTED BROILERS: A PROSPECTIVE STUDY

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ABSTRACT
Antibiotics remain the option for treating avian colibacillosis (AvCol), but the increasing emergence of antimicrobial resistant bacteria is negatively impacting treatment outcomes. To assist veterinarians in treating AvCol in Ghana, we evaluated the susceptibility of 40 avian pathogenic E. coli (APEC) obtained from 51 organs (lung, heart, spleen and liver) of 14 broilers diagnosed with colibacillosis, to six antibiotics using the Kirby Bauer disc diffusion method and further evaluated for colistin resistance using Chromagar COL-APSE. APEC was detected in at least one organ of each AvCol-diagnosed bird. The lungs showed the highest isolation frequency (91.7%). High resistance to tetracycline (100%), ampicillin (95.0%) and trimethoprim-sulphamethoxazole (77.5%) was observed. Susceptibility to gentamicin was 85.0%. About two-thirds (n=35, 87.5%) of isolates were multidrug-resistant with 40% (n=14) resistant to five antimicrobial classes. About a third (n=15/40, 37.5%) of APEC isolates from more than half (n=9/14, 64.3%) of the birds were resistant to colistin. APEC associated with AvCol showed high resistance to most antibiotics frequently used in the poultry industry but was susceptible to gentamicin. Isolates exhibited high MDR and moderate resistance to colistin. The findings highlight the need for enhanced surveillance systems on antibiotic use in poultry in the country.

Keywords: *Colibacillosis, avian pathogenic E. coli, antimicrobial resistance, colistin, Greater Accra region.*

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INTRODUCTION

Avian colibacillosis (AvCol) affects birds of all ages and is widely distributed in the global poultry industry (De Carli *et al*., 2015; Shtylla Kika *et al.*, 2023). Cases of AvCol, which are characterized by acute fatal septicemia or sub-acute fibrinous pericarditis, chronic respiratory disease, peritonitis, airsacculitis and salpingitis result in low productivity, increased mortality, increased carcass condemnation at slaughter, and an increase in veterinary care and medication costs affecting the profits of producers (Paudel *et al*., 2022). The identified primary cause of AvCol is the avian pathogenic *E. coli* (APEC) (Ewers *et al*., 2009).

There are a few potential vaccines identified for the prevention of colibacillosis but, antibiotics remain the option for treatment (Paudel *et al.*, 2024). Although effective, the constant use, misuse or abuse of antibiotics in the animal sector accelerates the development, selection and spread of antimicrobial-resistant bacteria. Studies have shown APEC isolates to be increasingly resistant to tetracyclines, amino-glycosides, fluoroquinolones, sulphonamides, β-lactams and extended-spectrum β-lactam antibiotics which represent key antibiotic classes employed for the treatment of poultry infections including AvCol, limiting treatment options for veterinarians (Mellata *et al*., 2010; Merati *et al*., 2020).

The increasing emergence of multidrug resistant (MDR) APEC isolates further presents a public health risk and disruption of environmental micro-stability.

Colistin, a polymixin, and beta-lactam antibiotics are key antibiotics used to treat MDR gram-negative bacteria infections mostly caused by *Enterobacteriaceae*. While colistin is a WHO reserve antibiotic with restrictive use in humans it is unrestrictive in the animal sector and constitutes key component of the cocktail of antibiotics used in the animals particularly, poultry. Interestingly, it was listed as part of the top five antibiotics sold in the European Union between 2010 and 2020 (EMA, 2021).

Poultry farmers in Ghana employ several antibiotics for prophylaxis and treatment during production, mostly without veterinary advice (Boamah *et al*., 2016). On the other hand, antibiotics of several classes, predominantly tetracyclines, penicillin, and sulphonamide, are reportedly prescribed by veterinarians at veterinary clinics (Adeapena *et al*., 2021; Turkson, 2023). These highlight potential challenges that similarly high frequency of antibiotic use by farmers and animal health professionals may contribute to AMR development.

A considerable number of AMR studies in animals in Ghana exist but these have focused mainly on commensals from apparently healthy poultry and their products, with little information on disease causing bacteria (Donkor *et al*., 2024). In this study, we sought to determine the susceptibility of APEC associated with AvCol, to frequently used antimicrobials in the poultry sector and further identify the proportion of isolates that harbour colistin resistance phenotypes.

MATERIALS AND METHOD (METHODOLOGY)

Sample Collection and Processing

From December 2022 to February 2023, all broiler birds submitted by farmers to the Accra Veterinary Laboratory (AVL) for post-mortem examination were considered. Only birds clinically diagnosed by the pathologist with colibacillosis and coming from farms located within the Greater Accra region, were selected for the study. From these birds, lungs, liver, spleen and heart were harvested into separate zip lock bags and transported on ice to the Microbiology laboratory of the Council for

Scientific and Industrial Research-Animal Research Institute (CSIR-ARI). Before sampling, the study was explained to the farmer at AVL who subsequently signed or thumb-printed an informed consent. Permission for the study was obtained from the Head of the AVL.

Isolation and Identification of Apec Isolates

Using aseptic techniques, three incisions were separately made in each organ using a sterile pair of scissors and forceps. A sterile inoculating loop was used to take samples from the incisions, plated on sorbitol MacConkey agar and incubated at 37°C overnight. Using morphological characteristics, colonies suspected to be *E. coli* were selected and streaked on eosin methylene blue agar (EMBA). Suspected *E. coli* isolates were subjected to standard biochemical testing. Characteristics of negative sulphur, positive indole, positive motility and negative citrate utilisation were used to confirm *E. coli* isolates (Quinn *et al*., 2011).

The beta haemolysis test on blood agar containing 5% sheep blood was used to evaluate the pathogenicity or otherwise of *E. coli* (Saha *et al*., 2020). Avian pathogenic *E. coli* (APEC) isolates identified were subsequently purified on nutrient agar, harvested and stored at -20°C in sterile brain heart infusion broth containing 25% glycerol.

Antimicrobial Susceptibility Testing of APEC Isolates

The susceptibility of APEC isolates to antimicrobials was evaluated on Muller Hinton agar using the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The following antimicrobials (Oxoid, Basingstoke, Hampshire, England) belonging to six antimicrobial classes were used; ampicillin (10µg), tetracycline (30µg), ciprofloxacin (30µg), gentamicin (10µg) chloramphenicol (30µg) and sulphamethoxazole-trimethoprim (1.25/23.75µg).

Phenotypic Identification of Colistin Resistance of APEC Isolates

After overnight cultures on nutrient agar, APEC isolates were set to a 0.5 McFarland standard and a 10µl loopful was plated out on CHROMagar COL-APSE (CHROMagar, Paris, France) to screen for colistin resistance (Momin *et al*., 2017). *E. coli* ATCC 25922 was employed as a control for all procedures.

Data Analysis

Demographic data of the poultry sampled were collected from the AVL datasheet and entered into Microsoft Excel. Antimicrobial susceptibility results were entered into WHONET (version 5.6) for interpretation and analysis. Isolates showing intermediate results to a particular antimicrobial were ascribed a resistant status. Descriptive statistics were applied to categorical variables and presented as frequencies and percentages. SPSS version 21 was used.

RESULTS

Characteristics of Birds and Antibiotic Use

During the period, 14 broilers, each from a different farm, were clinically diagnosed with AvCol. The ages of the birds ranged from two to eight weeks, with a mean of 5.2 weeks. From the clinical treatment records, oxytetracycline or penicillin-streptomycin (combination) was prescribed for treatment after post mortem. Of the two, oxytetracycline was frequently prescribed (n=11, 78.6%).

APEC Isolated from Colibacillosis-Diagnosed Birds

A total of 51 organs comprising heart (n=13), spleen (n=12), liver (n=14) and lungs (n=12) were received for laboratory analysis. The number of organs collected per bird ranged from three (n=5, 35.7%) to four (n=9, 64.3%).

The liver comprised the highest proportion (n=14, 27.5%) of the organs received (Table 1). APEC isolates were detected in all the 14 birds and at least one of the organs of each bird. The frequency of APEC isolation varied among the four organs with the lungs showing the highest isolation frequency (91.7%) and the spleen the least isolation frequency (66.7%). The overall isolation frequency of APEC from the four organs was determined as 78.3% (Table 1).

Table 1. Rate of APEC Isolation from Colibacillosis-Diagnosed Birds

Organ type	Organs received n (%)	Organs with APEC n (%)	Frequency of isolation (%)
Heart	13 (25.5)	11 (27.5)	84.6
Spleen	12 (23.5)	8 (20.5)	66.7
Liver	14 (27.5)	10 (25.6)	71.4
Lungs	12 (23.5)	11 (28.2)	91.7
Total	51 (100)	40 (100)	78.3

Susceptibility of APEC Isolates to Antimicrobials

Forty APEC isolates (one per organ) were randomly selected and subjected to antimicrobial susceptibility testing. All the isolates exhibited resistance to at least one of the six antimicrobial agents used. The aminoglycoside, gentamicin, recorded the highest susceptibility of 85%. Nearly all APEC were resistant to ampicillin and a 100% resistance was observed against tetracycline.

Table 2. Antimicrobial Resistance of APEC Isolated from Colibacillosis-Diagnosed Birds

Antibiotic (concentration)	Antibiotic class	Resistance n (%)
TCY (30µg)	Tetracycline	40 (100.0)
AMP (10µg)	Penicillin	38 (95.0)
SXT (1.25/23.75µg)	Trimethoprim-sulphamethoxazole	31 (77.5)
CHL (30µg)	Phenicol	22 (55.0)
CIP (30µg)	Fluoroquinolone	22 (55.0)
GEN (10µg)	Aminoglycoside	6 (15.0)

TCY= Tetracycline; AMP= Ampicillin; SXT= Sulphamethoxazole-trimethoprim; CHL= Chloramphenicol; CIP= Ciprofloxacin; GEN= Gentamicin

Characteristically, more than two-thirds (n=35, 87.5%) of the isolates were multidrug-resistant (MDR) (defined here as the resistance of a bacterium to three or more antimicrobial classes, Magiorakos *et al*., 2012). Most MDR isolates (n=14, 40.0%) resisted five antimicrobial classes and about 30% resisted three antimicrobials. Resistance pattern *AMP.TCY.CIP.CHL.SXT* was the commonest and accounted for 50% of antibiogram pattern of isolates exhibiting resistance to five antimicrobial classes. Three isolates of similar antibiogram were resistant to all six antimicrobials (Table 3).

Table 3. Antibiogram Patterns of Mdr APEC Isolated from Colibacillosis-Diagnosed Birds

Number of antimicrobial classes	Resistance pattern	APEC isolates n (%)
3	AMP.TCY.SXT	5 (14.3)
	AMP.TCY.CIP	4 (11.4)
	TCY.CHL.SXT	2 (5.7)
4	AMP.TCY.GEN.SXT	1 (2.9)
	AMP.TCY.CHL.SXT	4 (11.4)
	AMP.TCY.CIP.SXT	2 (5.7)
5	AMP.TCY.CIP.CHL.SXT	7 (20.0)
	AMP.TCY.GEN.CHL.SXT	1 (2.9)
	AMP.TCY.CIP.GEN.SXT	1 (2.9)
	AMP.TCY.CIP.CHL.SXT	5 (14.3)
6	AMP.TCY.CIP.GEN.CHL.SXT	3 (8.6)

AMP= Ampicillin; TCY= Tetracycline; SXT= Sulphamethoxazole-trimethoprim; CHL=Chloramphenicol; CIP= Ciprofloxacin; GEN= Gentamicin

Colistin Resistance Phenotypes of APEC Isolates

Phenotypically, about a third (n=15/40, 37.5%) of APEC isolates were resistant to colistin. These colistin resistant isolates identified were from more than half (n=9/14, 64.3%) of AvCol-diagnosed birds.

DISCUSSION

Colibacillosis in commercial poultry represents a key avenue of revenue loss and mortality to poultry farms worldwide. Resistance to antibiotics by APEC further complicates treatment for affected farms with limited treatment options and increased production costs. In this first study characterising the antimicrobial resistance profiles of avian pathogenic *Escherichia coli* in colibacillosis-affected broiler birds in the Greater Accra region of Ghana, we identified key resistances to essential antimicrobials used in both animals and humans in the country.

Analysis of affected organs in this study produced a 78.3% total isolation rate of APEC. This prevalence rate is similar to 78% from a survey in East Algeria (Aberkane *et al.*, 2023) on the livers of AvCol broiler birds from an abattoir. Identical to our isolation rate is 71% obtained from cloacal of chicken in Vietnam (Nguyen *et al.*, 2021). The prevalence of isolation observed in the present study is however higher than the 60% reported in another study in Vietnam in 2020 (Merati *et al*., 2020). The differences in these rates may be attributed to differences in sample types, laboratory methods and geographical regions. For instance, a study in Spain (Monroy *et al*., 2025) found slightly different APEC prevalences of 60.3%, 59.9% and 44.4% among breeders, broilers and layers highlighting the potential influence of bird type on acquiring avian colibacillosis and thus the corresponding APEC presence.

Out of four organs examined for the presence of APEC, the lungs yielded the highest number of APEC (91.4%), followed by the heart (84.6%), then the liver (71.4%), whereas the spleen demonstrated the least proportion of isolates. Most studies on APEC isolation in poultry-affected organs have been on the liver or lungs. Khanton and others found 87% and 73% APEC isolation rates in liver and lung samples respectively (Khaton *et al*., 2008). In contrast, no difference was obtained in percentage isolation from liver and spleen (50% each) from sick broiler chickens in Tiaret, Algeria (Merati *et al*., 2020). Despite the slight differences, it is worthy of note that the liver and lungs as well as the heart, as demonstrated by our study, present very high isolation frequency and could be considered key sample source(s) for laboratory isolation and identification of APEC strains, particularly in resource-limited regions.

The highest AMR observed was for tetracycline (100%) and ampicillin (95%). APEC from layer and broiler birds in Algeria, Bangladesh, and Pakistan similarly exhibited between 98% and 100% resistance to tetracycline and ampicillin (Aberkane *et al*., 2023; Azam *et al*., 2019; Ievy *et al*., 2020). The high resistance of APEC to these two antibiotics may be attributable to their long and frequent use for prophylaxis and treatment of poultry infections in Ghana. Boamah and colleagues (2016) observed that the tetracycline and penicillin classes of antibiotics were the first and third widely used antibiotics in the three major poultry-producing regions of the country including the Greater Accra region. Tetracycline, a broad-spectrum antibiotic is widely available and easily accessible in many parts of the world. It also has the added advantage of being relatively cheap, and easy to administer orally with very few side effects (Jahantigh *et al*., 2020). These attributes could account for their frequent use driving resistance.

The high tetracycline and ampicillin resistance could further be due to the presence, and persistence of common tetracycline (Kyselková *et al*., 2015) and penicillin-resistant microbes on the farm and subsequent spread of resistance genes on farms which could cause these resistances. It is a common practice of farmers in Ghana to keep birds of different ages on the farm making it impossible for farmers to practice all-in-all-out principle, thoroughly disinfection the farm premises and allow complete fallow periods on farms. These practices could support the persistence and spread of AMR bacteria on farms.

Although the present isolates are pathogenic *E. coli*, the resistance to tetracycline and ampicillin is similar to that reported for commensal *E. coli* from healthy poultry and meat products in Ghana (Mensah *et al.*, 2022; Ohene Larbi *et al*., 2021). This highlights the need to manage the use of antimicrobials prudently in commercial birds in the country.

APEC isolates recorded the least resistance to gentamicin (15%). Merati and colleagues also found a similar situation in APEC isolates of broiler birds in Algeria with a 12.1% resistance to gentamicin (Merati *et al*., 2020). Considering that gentamicin is rarely used in the management of infections by veterinarians in the country, the high susceptibility observed here affirms its efficacy and significance in infection treatment. It is worth noting that, although tetracycline recorded very high resistance, it was the most frequently prescribed medication after clinical diagnosis. No farmer however reported treatment failure and calls for probing into the efficiency of in-vitro and on-farm antimicrobial susceptibility.

We detected a high proportion (87.5%) of the APEC isolates to be multidrug resistant. This agrees with the similarly high MDR rates detected in previous studies on APEC in other countries ranging from 96.9% to 100% resistance (Ievy *et al*., 2020; Merati *et al*., 2020) and commensal *E. coli* in Ghana (Ayim-Akonor *et al.,* 2025; Donkor *et al*., 2012; Ohene Larbi *et al.*, 2021). A report by Boamah and colleagues (2020) has indicated that most medications used in the poultry sector in Ghana have between two and five different antibiotics. Therefore misuse of these agents could accelerate AMR development to the various classes of antibiotics leading to high MDR. In reviewing the molecular pathogenicity of APEC, Saha and others revealed that all plasmid-bearing APEC were also MDR (Saha *et al.*, 2020) and that these genes could be transferred to susceptible bacteria in healthy poultry and humans along the food chain through inappropriate meat processing or shed off into the environment. The development and emergence of high MDR microbes thus pose a challenge to human, veterinary and environmental health and require multi-sectorial intervention to reduce development.

As one of the last-line drugs for treating human infections caused by MDR bacteria, colistin is classified as a veterinary highly important antimicrobial and not critically important antimicrobial. Globally, colistin has been used in the animal sector for many years and forms a substantial component of medications used for prophylaxis and treatment in poultry in Ghana (Boamah *et al*., 2016; Paintsil *et al.*, 2021). The high colistin resistance observed in our study could be attributed to the frequent use of colistin-containing antimicrobials in the poultry sector of Ghana and threatens human and environmental health. To halt the development of colistin resistance, some countries like South Africa (Hassan *et al.*, 2024) have restricted the use of colistin-containing drugs in the poultry sector and have recorded a significant decline in the rate of colistin-resistant bacteria in their farm animals.

CONCLUSION

Colibacillosis remains a challenge to the poultry industry in Ghana. APEC-associated colibacillosis from birds raised in the Greater Accra region exhibit high resistance to multiple antibiotics frequently used in the sector and considerably high resistance to colistin which raises public health concerns.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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