






Escherichia coli Infections, and Antimicrobial Resistance In Poultry Flocks, in North Central Nigeria

Olatunde B. Akanbi^{1,2*} , Isaac D. Olorunshola^{3,4} , Peter Osilojo¹, Eunice Ademola^{2,4},
 G.O.A. Agada⁵, Julius O. Aiyedun⁶ , Christiana Ibiroke Odita⁷  and Shola David
 Ola-Fadunsin^{2,8} 

¹Department of Veterinary Pathology, ³Department of Veterinary Microbiology,
⁶Department of Veterinary Public Health and Preventive Medicine, ⁸Department of
 Veterinary Parasitology and Entomology

Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

²Laboratory Medicine Unit, Veterinary, ⁴Antimicrobial Resistant Sentinel Laboratory
 Veterinary Teaching Hospital, University of Ilorin, Ilorin, Nigeria

⁵Diagnostic Department, ⁷Veterinary Public Health and Preventive Medicine Division
 National Veterinary Research Institute, Vom, Plateau State, Nigeria

* Email. akanbi.ob@unilorin.edu.ng

ABSTRACT

To investigate *Escherichia coli* infections in poultry flocks, 291 tissue samples from 237 necropsied carcasses submitted from ninety-nine (99) poultry flocks in north central Nigeria were analysed. These flocks comprised layer chicken, broiler, pullet, cockerel, turkey, quail, guinea fowl and ducks. Tissue samples were pre-enriched in 10 mL buffered peptone water media and aliquots were inoculated into selective enrichment broth, sub-cultured on Eosin Methylene Blue (EMB) agar and MacConkey agar (MCA) and colonies of *E. coli* was examined based on cultural morphological characteristics. Layer poultry and laying quails exhibited reproductive lesions which correlates with history of disruption and reduction in egg production. Antibiotics resistance rate was significant ($p < 0.01$) with macrolide and penicillin classes being the most resistant antibiotics in layers and broiler, while quinolones and aminoglycoside were most significantly susceptible ($p < 0.01$). Multidrug resistance (MDR) was found in 56% of the *E. coli* isolates, with high prevalence in younger birds.

Keywords: AMR, *Escherichia coli*, Infections, Multidrug resistance (MDR), Poultry-flocks

INTRODUCTION

Pathogenic *Escherichia coli* belong to two major groups, which include the diarrheagenic *E. coli* (DEC) and the extraintestinal pathogenic *E. coli*

(ExPEC) (Cunha et al., 2017; Borgesa et al., 2017; Saka et al., 2019; Tonini et al., 2021). Gastrointestinal infections of *E. coli* are caused by the DEC strains, while septicaemias and extraintestinal

infections are caused by the ExPEC strains (Cunha et al., 2017). Avian pathogenic *E. coli* (APEC), an example of ExPEC strains is responsible for causing both localized and systemic *E. coli* infection known as avian colibacillosis in poultry flocks (Logue et al., 2017). The pathological manifestation of avian colibacillosis include septicaemia (colisepticaemia), pneumonia, yolk-sac infection, omphalitis, cellulitis, (Kabir, 2010), airsacculitis, osteomyelitis and peritonitis (Barnes et al., 2008; Dinidu et al., 2017), oftentimes leading to death, due to virulence gene content and antibacterial drug resistance (Laarem et al., 2017). Infection of poultry with *E. coli* causes economic losses by reducing productivity, egg production, and decreases hatching rates and meat condemnation during processing, in addition to costs of antibiotics use in treatment and prophylaxis (Guabiraba et al., 2015; Blyton et al., 2015; Geetha et al., 2018). Avian colibacillosis used to be easily treated with antibiotics, but antibiotic resistance is on the increase, and complicated (Stella et al., 2016), because of multidrug-resistant *E. coli* strains which has limited treatment options in both humans and animals (Oliveira et al., 2019).

Antibiotic resistance has become a global health problem in humans, animals, and the environment (Marshall et al., 2011; Faife et al., 2020). It is said that resistance is present in all currently used antibiotics in human and veterinary medicine (Marshall et al.,

2011; Faife et al., 2020). The prophylactic, anabolic and metaphylactic use of antibiotics, in addition to its therapeutic use in food animals has resulted in the emergence of antibiotic resistant bacteria of zoonotic and ingestible transmission (Todar, 2022). Sources of resistant strains of *E. coli* have included contaminated water and food (Cole et al., 2005). In addition, wild birds' intestinal microbiota has been implicated to contain multidrug-resistant (MDR) *E. coli* (Guenther et al., 2010; Blyton et al., 2015; Borgesa et al., 2017), and these birds may serve as reservoirs and mechanical vectors of antimicrobial-resistant ExPEC which can be transmitted to commercial poultry and humans (Hubalek, 2004). Previous studies have examined avian colibacillosis independent of antimicrobial resistance and vice versa. This present study co-examines colibacillosis manifestation in poultry species vis a vis the prevalence of antimicrobial resistance in poultry breeds and species in poultry flocks in north central Nigeria, and the multidrug resistant patterns in such flocks.

MATERIALS AND METHODS

Poultry flocks surveyed

Poultry flocks in some north-central states of Nigeria were surveyed of combined flock size of one hundred and sixty-one thousand and twenty-one

(161,021) poultry birds which comprised of commercial chicken layer, broiler, pullet, cockerel, turkey, quail, guinea fowl and ducks. The 99 poultry flocks were surveyed between 2007 and 2014 and located in the north-central states of Plateau (86), Bauchi (5), Niger (2), Nasarawa (2), Kaduna (1) and Abuja (3).

Postmortem Examination

Postmortem examination was carried out on two hundred and thirty-seven (237) poultry carcasses comprising of commercial layer chicken (94), pullet (52), broiler (41), cockerel (5), turkey (4), quail (34), guinea fowl (6) and duck (1). These carcasses were from ninety-nine (99) poultry flocks of combined flock size of one hundred and sixty-one thousand and twenty-one (161,021) poultry birds.

Samples for Bacteriological Isolation

Two hundred and ninety-one (291) tissue samples comprising of parenchymatous organs of heart, lung, liver, spleen, kidneys, yolk sac, intestine, and reproductive tracts from the 237 poultry carcasses sent for bacteriological isolation and identification.

Isolation and identification of *Escherichia coli*

Approximately 1 g of the tissue (liver, spleen, lungs, heart, kidney, intestine) samples were separately minced and placed in 10 mL of buffered peptone water (Oxoid, Basingstoke,

UK) as a pre-enrichment media and incubated at 37°C for 18 hours. Aliquots from pre-enrichment were inoculated into selective enrichment broth to make 1:10 sample to broth ratio in EC broth (*Escherichia coli* Broth) (Oxoid, Basingstoke, UK). A loop full of culture from EC Broth was subcultured by streaking on Eosin Methylene Blue agar (EMB) (Oxoid, Basingstoke, UK) and MacConkey agar (MCA) (Oxoid, Basingstoke, UK). The sub cultured plates were incubated at 37°C for 24 hours. The cultured plates were examined for the presence of typical colonies of *E. coli* based on cultural and morphological characteristics, that is, metallic sheen colonies on EMB and pinkish lactose fermenting colonies on MCA.

Purification of isolates

The isolates were subcultured onto EMB, MCA and nutrient agar (NA) (Oxoid, Basingstoke, UK) for isolation of pure culture and subsequent biochemical characterization.

Biochemical characterization of *E. coli*

Characterization of the isolates were done using biochemical test methods described by Ibrahim et al. (2019) and Fredrick (2020). A 24-hour pure culture of each isolate was used to determine their Gram reaction. The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test, Voges-Proskauer test, urease test, sugar (trehalose, sucrose, inositol, glucose, dulcitol,

maltose, mannitol, melibiose, salicin, rhamnose and arabinose) fermentation test and motility test. Isolates were further characterized using Microbact 20 E (Oxoid, Basingstoke, UK).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was determined using a modification of the Kirby-Bauer disk diffusion method (CLSI, 2012). The antibacterial agents tested include: Chlor-Oxytetracycline (OTC) 10µg, Tylosin (TL) 10µg, Ciprofloxacin (CIP) 10µg, Conflox (CX) 10µg, Anicillin (AN) 10µg, Amoxicillin (AMX) 10µg, Gendox (GX) 10µg, Erythromycin (ERY) 10µg, Chloramphenicol (CHP) 10µg, Colistin (CLN) 10µg, and Furasol (FI) 10µg which were applied in the test. Eight (8) classes of antibiotics were used for the study, 1. Quinolones and Fluoroquinolones (Ciprofloxacin -Q and Conflox -Fq), 2. Penicillin-like antibiotics Beta lactam antibiotics (Amoxicillin and Anicillin), 3. Antimicrobial protein synthesis inhibitor (Chloramphenicol), 4. Tetracyclines (Chlortetracycline and Gendox- doxycycline), 5. Macrolide antibiotic (Tylosin and Erythromycin), 6. Polymyxin (Colistin), 7. Aminoglycoside antibiotics (Gendox-gentamycin), 8. Nitrofurantoin analogue (Furasol-furagin), were used for the antimicrobial susceptibility test.

Standardization of inoculum

Pure culture of identified *Escherichia coli* from an 18-hour plate culture was selected. Sterile wire loop was used to pick 2 to 3 colonies of each *Escherichia coli* and emulsified in a tube containing 5ml of sterile physiological saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic systems, UK) after calibration. Adjustment was made to 0.5 McFarland standard. Fifty microliter of the broth was transferred into 5 ml of Mueller-Hinton broth (Oxoid, UK) in a tube (CLSI, 2012).

Inoculation of test plates

A sterile cotton swab was dipped into the standardized suspension in Mueller-Hinton broth. The dried surface of a 20 ml Mueller-Hinton agar (Oxoid, UK) plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking the cotton swab over the entire sterile agar surface. The antibiotic discs were evenly dispensed onto the surface of the inoculated agar plate using disc dispenser (Oxoid, UK). The plates were inverted and incubated at 37°C for 24h. Each plate was examined after 24h of incubation. The diameters of the zones of complete inhibition were measured to the nearest whole millimeter, using a Vernier caliper.

Statistical method

The data obtained was visualized and descriptive statistics was performed using Microsoft Excel 2021

to determine the sum, mean, frequencies of the samples, perform graphical expression of both flock data and antimicrobial resistance. The data was further subjected to statistical analysis using Statistical Package for Social Sciences® (SPSS) (Armonk, US) and One-way analysis of variance (ANOVA) was used to analyze the relationship between each antibiotic and the different flock type. Statistical significance was set at $p < 0.05$. Openepi® software was used to calculate Chi square and P-value. The number in each flock type resistance or susceptible to each antibiotic was used in the analysis.

RESULTS

Poultry flocks survey

Ninety-nine (99) poultry flocks comprised of commercial layer chicken, broiler, pullet, cockerel, turkey, quail, guinea fowl and ducks (figure 1) were surveyed. The 99 poultry flocks were in the north-central states of Plateau (86), Bauchi (5), Niger (2), Nasarawa (2), Kaduna (1) and Abuja (3), and surveyed between 2007 and 2014. The breakdown showed that 56 commercial layer chicken flocks were surveyed, and this is the highest frequency of the species and breed investigated. The others are presented in figure 1.

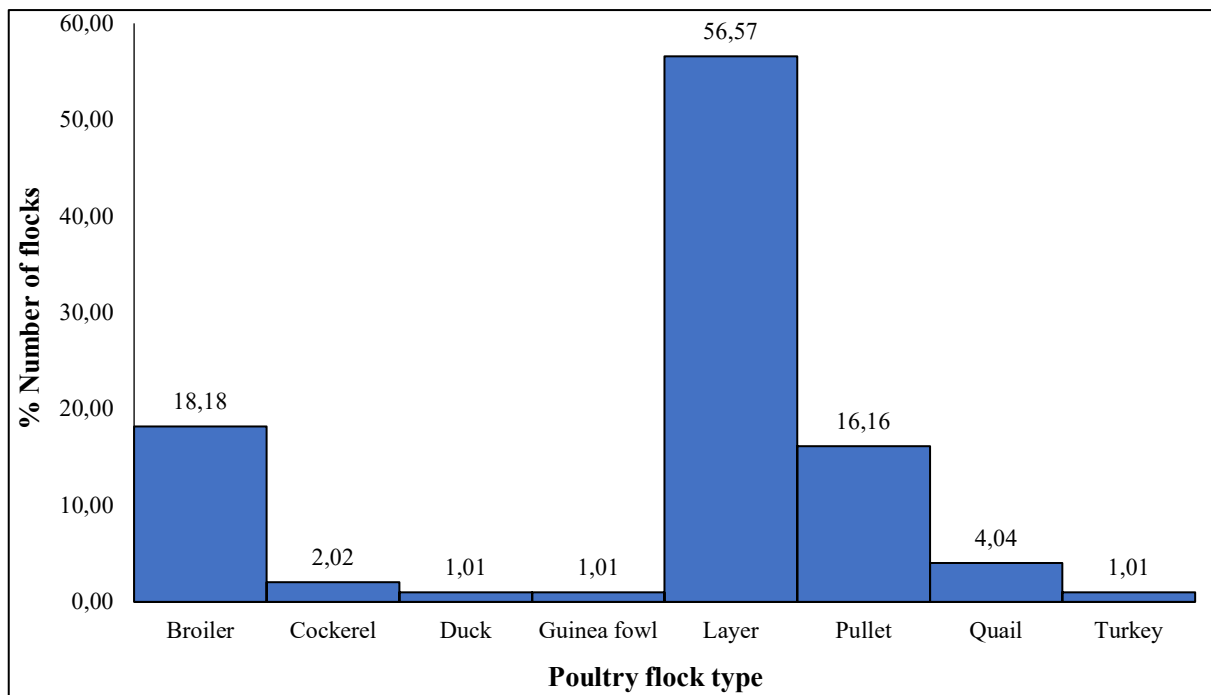


Figure 1. Poultry flock type sampled for *Escherichia coli* infections

Demographic presentation of the data

The combined flock size of the 99 poultry flocks sampled was one hundred and sixty-one thousand and twenty-one (161,021) poultry

birds, and this ranged from 10 birds to a maximum of 14,835 birds. Most of the flocks sampled (45), were from small sized poultry flocks, details are shown in figure 2.

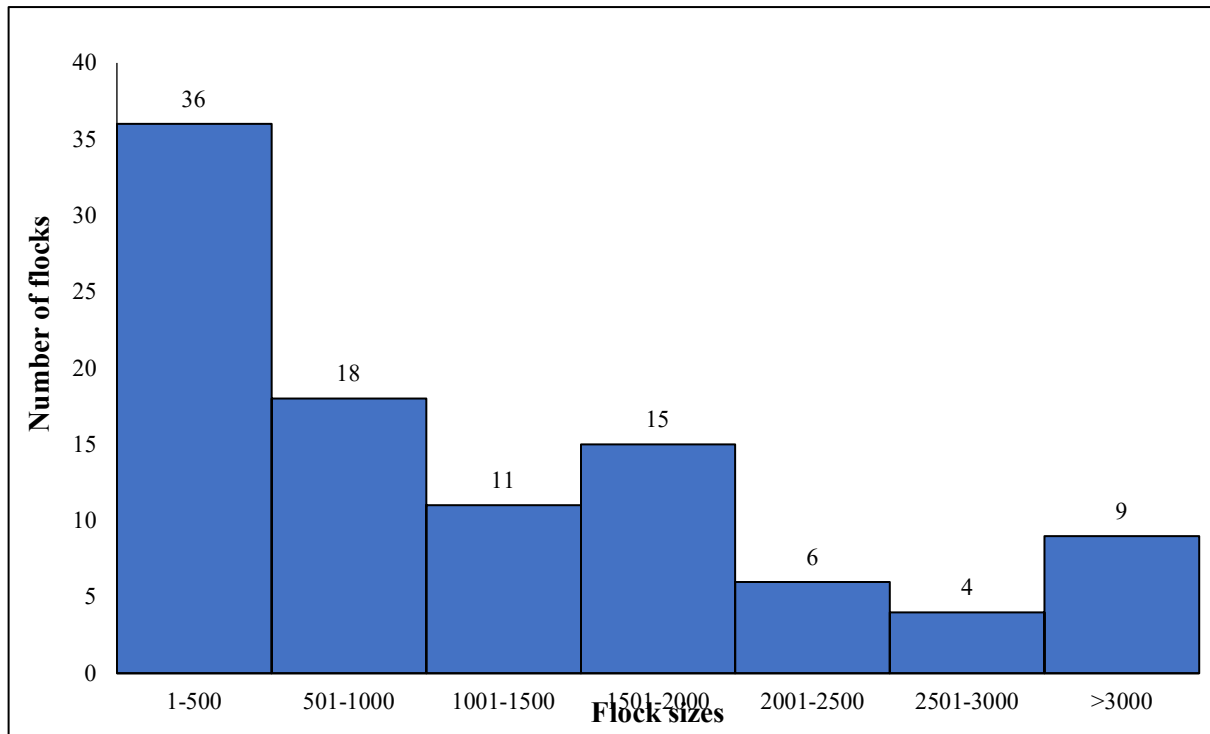


Figure 2. Distribution of poultry flock size range sampled for *Escherichia coli* infections

Postmortem findings

A total of two hundred and thirty-seven (237) carcasses (0.15%) from combined flock size of one hundred and sixty-one thousand and twenty-one (161,021) poultry birds from ninety-nine (99) poultry flocks, were submitted and necropsied. In the laying hens, ages 16 to 64 weeks, localized infection of the reproductive tract resulted in atresia of

ovarian follicles, oophoritis, oviduct rupture and haemorrhage follicular rupture, misshapen follicles, follicular congestion and haemorrhage, egg yolk peritonitis, soft and pedunculated follicles, soft shelled eggs, egg bounding, salpingitis (figure 3), and delay in egg forming time leading to multiple developing egg follicles in oviduct. The lesions in commercial

laying quail hens between the ages of 8 to 40 weeks were similar to the findings in laying chickens and were more localized to the reproductive tract in

addition to enteritis and pneumonia. The array of postmortem findings is listed in table 1.

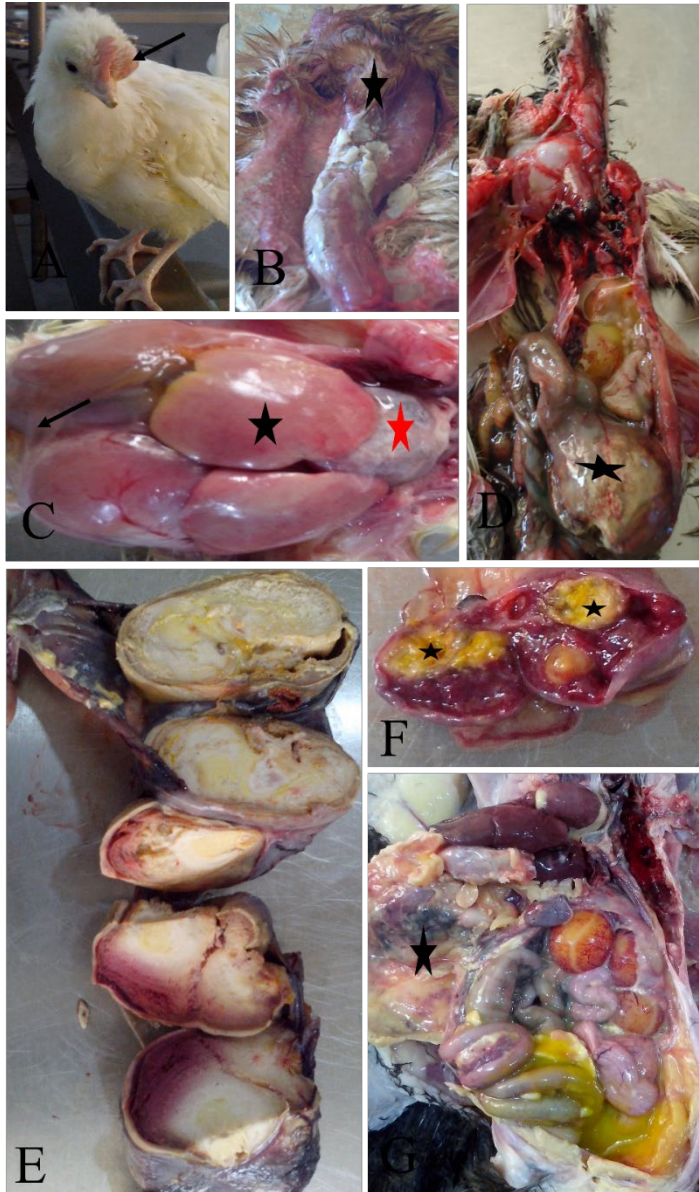


Figure 3. Postmortem examination gross findings, A. panophthalmitis (arrow), unilateral, broiler. B. cellulitis (star), suppurative, cervical, commercial layer. C. pericarditis (red star), perihepatitis (black star) and airsacculitis (arrow), broiler. D. Peritonitis, oophoritis, salpingitis (star) and egg-bounding (star), quail. E. egg-bounding numerous with salpingitis, commercial layer. F. coligranuloma, multiple, splenitis. G. peritonitis, caseous with egg-yolk rupture and adhesion.

Table 1. Pathological changes in organ systems of different poultry type infected with *Escherichia coli*.

Poultry type	Number of farms	Age (weeks)	Number of carcass(es)	System affected	Gross Pathological changes
Layer	55	16-68	94	General, Respiratory, cardiovascular, GIT, reproductive, renal, and immune	Pallor (3), emaciation (20), pasted vent (12), Airsacculitis (17), tracheitis (9), pneumonia (24), hydropericardium (2), pericarditis (12), enteritis (26), perihepatitis (6), hepatitis/hepatic necrosis (25), peritonitis (4), peri-splenitis (2), splenitis (16), caecaitis (9), oophoritis (3), follicular rupture (4), misshapen follicles (2), follicular congestion (3), follicular haemorrhage (2), egg yolk peritonitis (7), soft and pedunculated follicles (9), soft shelled eggs (6), egg bounding (12), salpingitis (2), oviduct rupture and haemorrhage (2), atretic ovarian follicle (8), multiple follicles in oviduct (1), nephritis (12).
Pullet	16	1-15	52	General, Respiratory, cardiovascular, GIT, renal and immune	Pallor (2), emaciation (3), pasted vent (3), Airsacculitis (1), tracheitis (2), pneumonia (5), pericarditis (3), enteritis (1), perihepatitis (2), hepatitis (1), hepatic necrosis (5), peritonitis (1), splenitis (2), caecaitis (1), nephritis (2), yolk sac infection (1)
Cockerel	2	1-9	5	General and GIT	Emaciation (3), enteritis (1), hepatitis (1), caecaitis (1)
Broiler	18		41	General, Respiratory, cardiovascular, GIT, renal, and immune	Pallor (1), emaciation (3), pasted vent (3), Airsacculitis (3), tracheitis (1), pneumonia (7), pericarditis (2), enteritis (7), perihepatitis (3), hepatitis (4), hepatic necrosis (3), peritonitis (1), splenitis (3), caecaitis (3), nephritis (1), yolk sac infection (2)
Turkey	1	5	4	Respiratory, GIT	Pneumonia (2), hepatitis (4), yolk sac infection (2)
Duck	1	8	1	General, Respiratory, cardiovascular, GIT	Airsacculitis (1), pericarditis (1), enteritis (1), caecaitis (1), hepatitis (1)
Guinea fowl	1	1	6	General	Emaciation
Quail	5	8-40	34	General, Respiratory, GIT, reproductive	Pasted vent (2), emaciation (1), pneumonia (3), hepatitis (3), enteritis (1), follicular rupture (1), egg bounding (1).
	99		237		

Antimicrobial susceptibility test result

Of the 99 *E. coli* strains isolated from poultry farms, resistance to macrolide antibiotic class (tylosin (63.6%) and erythromycin (49.1%) was high, followed by resistance to penicillin-like antibiotics Beta lactam antibiotics-Anicillin (52.7%), and Tetracyclines-Chlortetracycline (38.2%). Others are listed in table 2. The results (table 2) showed a high prevalence of multi-drug resistance (resistance to three or more antibiotic classes) in younger birds especially broilers and pullets' commercial flock. Tylosin, Anicillin and Erythromycin had the highest number of *E. coli* isolates resistance. Highest multidrug resistance (MDR) of 8MDR was observed in fewer *E. coli* isolates.

The total MDR resistance is 56 (56%), while MDR in layer flocks was 35 (62.5%), pullet flocks are 6 (37.5%) and in broiler flocks is 12 (66.6%). MDR in quail flocks is 3 (75%), one quail was not tested, while cockerel was 1 (50%), guinea fowl was 100 %.

The most sensitive antimicrobial agent (figure 4) for all poultry breeds and species is ciprofloxacin 85% (84/99), this was followed by conflax 71% (70/99), gentamicin 62% (62/99) and oxytetracycline 37% (37/99). The *Escherichia coli* isolates drug susceptibility pattern is presented in table 3, while figure 5 and 6, shows antibiotic and multidrug resistance. Isolates shows an upsurge in resistance to Anicillin, Conflux and Erythromycin.

Table 2. Prevalence of antimicrobial resistance of *Escherichia coli* isolated from different poultry species and breeds

Flock types	Flock number	AMX	ANI	CHP	CHT	CIP	COL	CON	ERY	GEN	FUR	TYL	OXY	P value	χ^2
Number Resistant (%) within flock types															
Layer	55	0 (0.0)	29 (52.7)	5 (9.1)	3 (5.5)	5 (9.1)	2 (3.6)	9 (16.4)	27 (49.1)	16 (29.1)	23 (41.8)	35 (63.6)	21 (38.2)	<0.01*	150.5
Broiler	18	1 (5.6)	12 (66.7)	1 (5.6)	1 (5.6)	3 (16.7)	0 (0.0)	6 (33.3)	10 (55.6)	7 (38.9)	8 (44.4)	13 (72.2)	9 (50.0)	<0.01*	59.15
Pullet	16	1 (6.3)	4 (25.0)	0 (0.0)	0 (0.0)	3 (18.8)	1 (6.3)	3 (18.8)	7 (43.8)	3 (18.8)	6 (37.5)	11 (68.8)	6 (37.5)	<0.01*	41.2
Quail	5	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)	2 (40.0)	2 (40.0)	3 (60.0)	3 (60.0)	3 (60.0)	0.05	20.0
Cockerel	2	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)	0.57	9.6
Duck	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	0.36	12.0
Guinea fowl	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0.36	12.0

Statistics is done within flock type

* = significant at <0.05

χ^2 = Chi square value, Openepi® software was used to calculate Chi square and P-value

AMX = Amoxicillin; ANI = Anicillin; CHP = Chloramphenicol; CHT = Chlortetracycline; CIP = Ciprofloxacin; COL= Colistin; CON = Conflox; ERY = Erythromycin; GEN = Gendox; FUR = Furasol; TYL = Tylosin; OXY = Oxytetracycline

Table 3. Prevalence of antimicrobial susceptibility of *Escherichia coli* isolated from different poultry species and breeds

Flock types	Flock number	ANI	CHP	CHT	CIP	CON	ERY	GEN	FUR	TYL	OXY	P value	χ^2
Number susceptible (%) within flock types													
Layer	55	23 (41.1)	1 (1.8)	6 (10.7)	46 (82.1)	43 (76.8)	21 (37.5)	37 (66.1)	20 (35.7)	3 (5.4)	19 (33.9)	<0.01*	175.6
Broiler	18	5 (27.8)	0 (0.0)	0 (0.0)	15 (83.3)	11 (61.1)	6 (33.3)	10 (55.6)	6 (33.3)	2 (11.1)	7 (38.9)	<0.01*	52.06
Pullet	16	8 (50.0)	0 (0.0)	0 (0.0)	13 (81.3)	12 (75.0)	5 (31.3)	9 (56.3)	6 (37.5)	1 (6.3)	6 (37.5)	<0.01*	52.27
Quail	5	1 (20.0)	0 (0.0)	0 (0.0)	5 (100.0)	2 (40.0)	3 (60.0)	2 (40.0)	1 (20.0)	1 (20.0)	2 (40.0)	0.04*	17.91
Cockerel	2	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	1 (50.0)	0.12	13.94
Duck	1	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.35	10.0
Guinea fowl	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0.35	10.0
Turkey	1	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0.35	10.0

Statistics is done within flock type

* = significant at <0.05

χ^2 = Chi square value, Openepi® software was used to calculate Chi square and P-value

ANI = Anicillin; CHP = Chloramphenicol; CHT = Chlortetracycline; CIP = Ciprofloxacin; CON = Conflox; ERY = Erythromycin; GEN = Gendox; FUR = Furasol; TYL = Tylosin; OXY = Oxytetracycline.

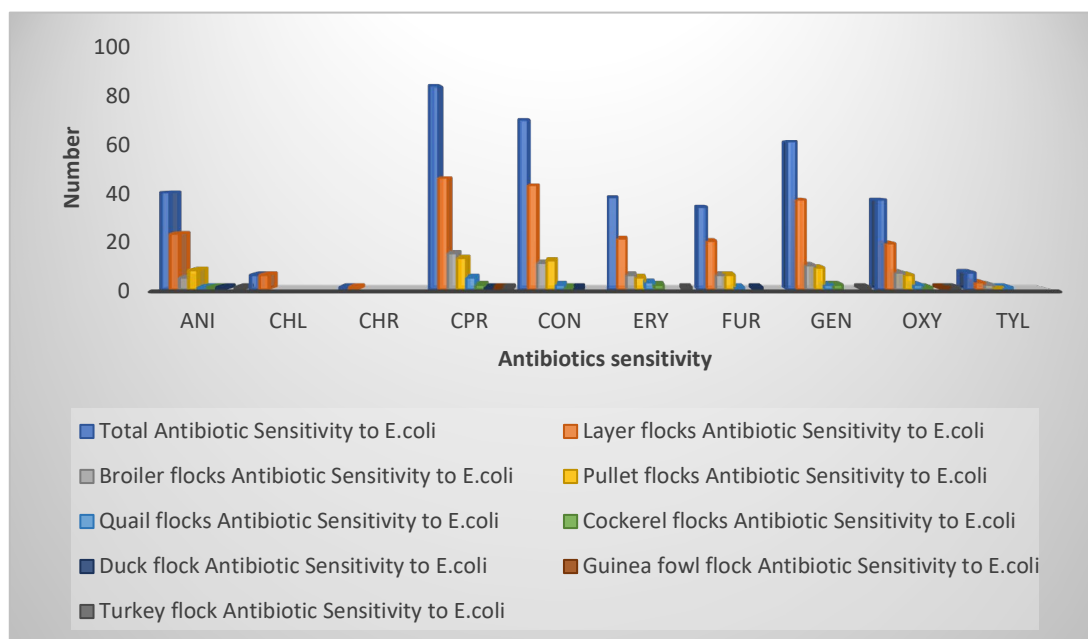


Figure 4. Number of *E.coli* isolates Susceptible to individual Antimicrobial Agents

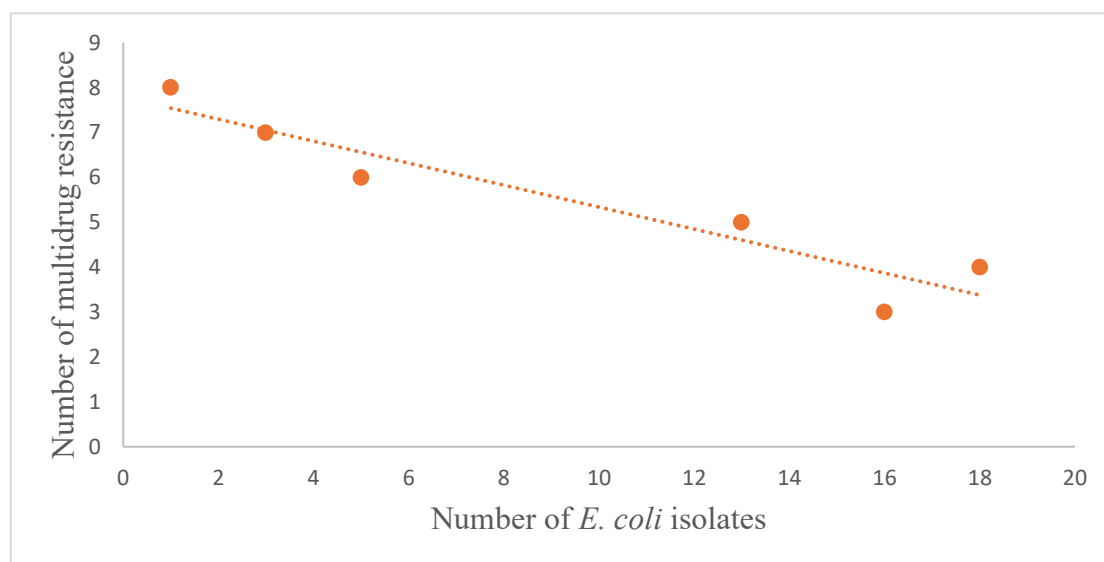


Figure 5. *Escherichia coli* isolates multidrug resistance rate

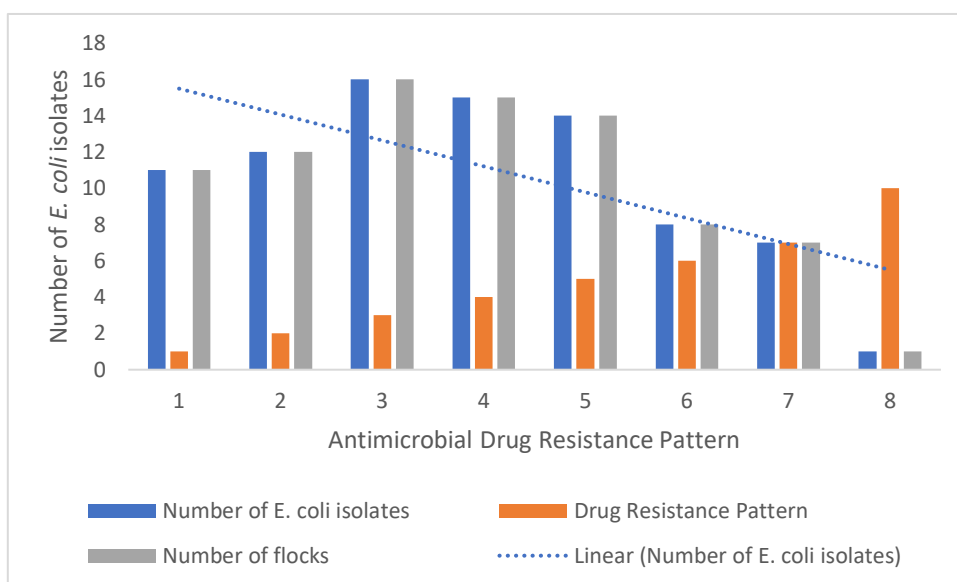


Figure 6. *Escherichia coli* isolates drug resistance pattern

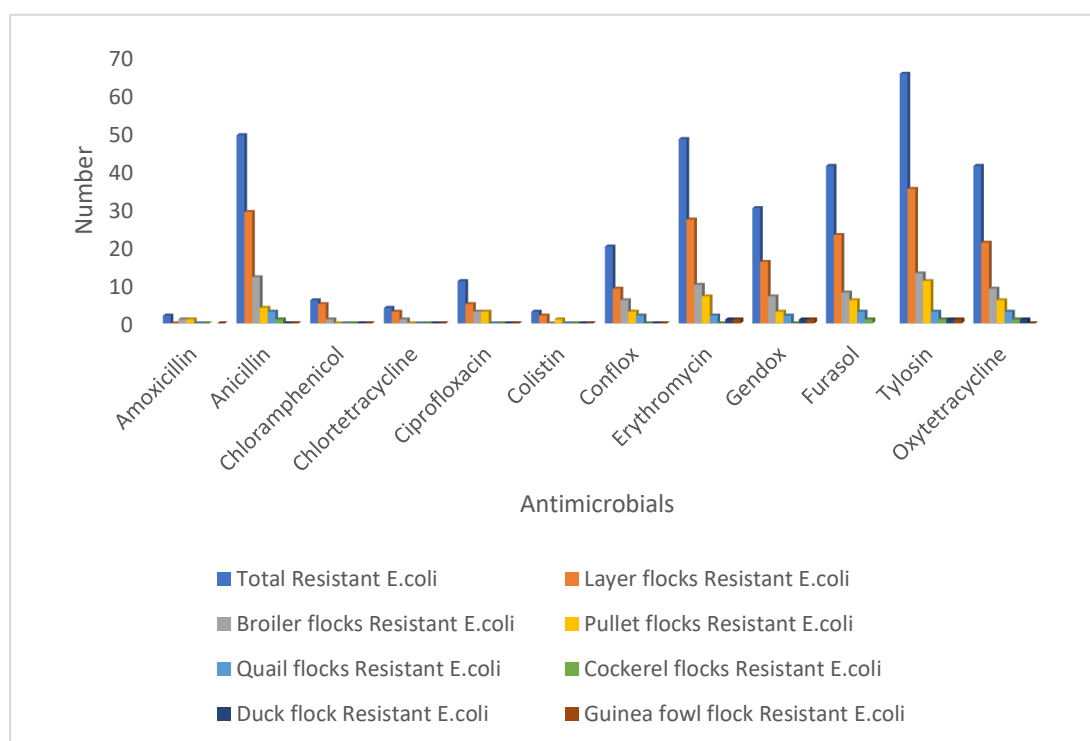


Figure 7. Number of *E. coli* isolates Resistant to individual Antimicrobial Agents

Statistical Analysis

There was a statistically significant difference ($p < 0.01$) in the resistance rate of antibiotics among the layers broiler, and pullets flock types with tylosin (63.6%) been the most resistant antibiotics, followed by anicillin (52.7%) and erythromycin (49.1%) in laying and broiler flock types. Tylosin (68.8%), erythromycin (43.8%), Furasol (37.5%), and Oxytetracycline (37.5%) were the most resistant antibiotics among pullet flocks. There was no statistically significant difference ($p > 0.05$) in the resistance rate of antibiotics among the quail, cockerel, duck, and guinea fowl flock types (Table 2). Ciprofloxacin, conflox, and gendox had the highest level of antibiotics susceptibility among the laying, broiler, and pullets flock types, and the difference was statistically significant difference ($p < 0.01$). There was a significant difference ($p = 0.04$) in the susceptibility rate of antibiotics among the quail flock type with ciprofloxacin (100.0%), and erythromycin (60.0%), being the most susceptible antibiotics. The susceptibility rate of antibiotics among cockerel, duck, guinea fowl, and turkey flock types were not statistically significant ($p > 0.05$) (Table 3).

DISCUSSION

The survey of *Escherichia coli* infections in poultry breeds and species (figure 1) in five (5) north-central Nigeria states investigated revealed small sized poultry flocks were mostly affected (figure 2), and the infection exhibited an array of pathological manifestations and multidrug resistance in commercial layer chicken, pullets, broiler, and quails. It was

more colisepticaemic, localizing in several systems including respiratory, cardiovascular, gastrointestinal, reproductive, urinary, and immunological as was previously reported (Barnes et al., 2008; Kabir 2010; Dinidu et al., 2017), most of the times leading to death. The reproductive tract lesions observed in commercial layer chickens and laying quails correlates with the history of disruption, reduction, and oftentimes total cessation in egg production. Although ninety-nine (99) *E. coli* isolates were recovered from the 99 sampled poultry farms of which 56 isolates were from 56 commercial laying chicken hens flock and 18 isolates from 18 broiler flocks while 16 *E. coli* isolates were recovered from 16 pullets' flocks which formed the bulk of the *E. coli* recovered, the flock size of the 5 quail flocks represents 5% of the entire flock size of the sampled poultry flocks and 14% of the entire carcasses examined. These array of lesions (figure 3) suggested a Pathogenic *Escherichia coli* i.e diarrheagenic *E. coli* (DEC) and extraintestinal pathogenic *E. coli* (ExPEC) infection. The DEC strains are known to be responsible for gastrointestinal infections and lesions, while ExPEC strains are responsible for the coli septicaemias and the extraintestinal lesions as previously reported (cunha et al., 2017). Avian pathogenic *E. coli* (APEC), of ExPEC strains is responsible for causing both localized and systemic *E. coli* infection known as avian colibacillosis in poultry flocks (Logue et al., 2017). In the other poultry breeds (pullets, cockerel, broiler) and species (turkey, duck, and guinea fowl), the lesions were more colisepticaemic affecting the respiratory, cardiovascular, gastrointestinal, renal, and immunological. Lesions such as enteritis,

airsacculitis, pneumonia, pericarditis, hepatic necrosis, and yolk sac infection in young chicks were predominant. Although, characterization of the *E. coli* isolates from these farms and their carcasses were not carried out due to limited resources, the pathological manifestation of the disease in the poultry flocks, their ability to cause death and their multidrug resistant properties suggests these *E. coli* isolates to be pathogenic *Escherichia coli* and not commensal. Based on the lesions caused, these pathogenic *Escherichia coli* strains may be classified as diarrheagenic *E. coli* (DEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Cunha et al., 2017; Borgesa et al., 2017; Saka et al., 2019; Tonini et al., 2021). Gastrointestinal infections of *E. coli* are caused by the DEC strains (Cunha et al., 2017), and the diarrhoea seen in the DEC is because of enteritis leading to malabsorption in the large intestine and increase intestinal motility, resulting in the clinical syndrome known as diarrhoea. All the poultry breeds and species except the turkey showed lesion of enteritis, hence were diarrhoeic. Extraintestinal pathogenic *E. coli* (ExPEC) is responsible for septicaemias and extraintestinal infections (Cunha et al., 2017), and the lesion in these poultry breeds and species suggests infection by ExPEC Avian pathogenic *E. coli* (APEC) strains, which is responsible for causing both localized and systemic *E. coli* infection known as avian colibacillosis in poultry flocks (Logue et al., 2017). ExPEC strains have been known to presents higher resistance profiles, when compared to commensal *E. coli* isolates (Cyويا et al., 2015).

Of the eight classes of antibiotics used, resistance to macrolide antibiotic class (tylosin (63.6%) and erythromycin

(49.1%)) were high, followed by resistance to penicillin-like antibiotics Beta lactam antibiotics- Anicillin (52.7%), and Tetracyclines- Chlortetracycline (38.2%). Antimicrobial use and abuse in the blind treatment of poultry bacterial infections is known to increase antibiotic resistance, of which multidrug resistance is a common place in *Escherichia coli* infections. Highly pathogenic and multidrug resistant strains (figure 5& 6) have been associated with APEC isolates, from several avian species, including broilers, laying hens, helmeted guineafowl, turkeys, and urban pigeons (Silveira et al., 2016; Silva et al., 2017; Borzi et al., 2018; Hoepers et al., 2018; Cunha et al., 2019). The sources of these pathogenic strains can be hypothesized, to limit the spread of multidrug resistance, in addition to the suggestion by (Chakraborty et al., 2015). Sources of resistant strains of *E. coli* have included contaminated water and food (Cole et al., 2015). Food animals and their production environments are known reservoirs of both resistant bacteria and resistance genes (Marshall and Levy, 2011; WHO 2021) due to the spread of animal waste on land (Heuer and Smalla, 2007; Gosh and LaPara, 2007). In addition, wild birds' intestinal microbiota has been implicated to contain multidrug-resistant (MDR) *E. coli* (Guenther et al., 2010; Blyton et al., 2015; Borgesa et al., 2017), and these birds may serve as reservoirs and mechanical vectors of antimicrobial-resistant ExPEC which can be transmitted to humans and commercial poultry (Hubalek, 2004). This study revealed that most of the *E. coli* isolates recovered from all poultry breeds and species were highly sensitive to the quinolone class of antibiotics, Ciprofloxacin (85%) and Conflox (71%), followed by aminoglycoside-gentamicin

(62%) and Tetracycline- Oxytetracycline (37%), as presented in figure 4. The resistance prevalence of *E. coli* to quinolone-ciprofloxacin in this study are 18.8% in broilers and 16.7 % in pullets, in fact, the most susceptible antibiotics to *E. coli* in our study is quinolone-ciprofloxacin. This finding is at variance with the report of Faife et al. (2020) in Mozambique, who found that resistance prevalence to ciprofloxacin was 70%, which was like the resistance prevalence of 70.4% reported by Ghodousi et al.(2015) and lower resistance of *E. coli* to ciprofloxacin in retail broiler chicken in Italy (88. 8%). Reich et al. (2013), found a resistance prevalence of 39% from German retail outlets selling poultry of Italian origin. Although, a study in the southwestern states of Nigeria (Adelowo et al., 2014), revealed a 42% resistance to ciprofloxacin, this finding still reflects a lower resistance to ciprofloxacin in Nigeria, which corroborates our finding of low ciprofloxacin resistance by *E. coli* isolates from northcentral Nigeria. Laarem et al. (2017), also reported a 72% *E. coli* resistance against ciprofloxacin (72%) contrary to our findings of less than 20%. Macrolide class- (Tylosin, and Erythromycin) and penicillin- Anicillin had the highest number of *E. coli* isolates resistance, as shown in figure 7. This study also found a 21% resistance against Oxytetracycline contrary to 96.6% resistance against tetracycline (Laarem et al., 2017). In this study the resistance against Amoxicillin was less than 10% contrary to the 65.5% reported by Laarem et al. (2017). Isolates shows an upsurge in resistance to macrolide (Tylosin and Erythromycin) and penicillin (Anicillin) antibiotics classes (figure 7). High multidrug resistance of 8DR (figure 4) was

seen in an *E. coli* isolates, and a high prevalence of MDR in younger birds especially broilers and pullets' commercial flocks. The total MDR resistance is 56 (56%), while MDR in layer flocks was 35 (62.5%), pullet flocks is 6 (37.5%) and in broiler flocks is 12 (66.6%). MDR in quail flocks is 3 (75%), one quail was not tested, while cockerel was 1 (50%), guinea fowl was 100%.

Antibiotic resistance in food animals especially poultry and its products present a more dangerous threat to human health. The poultry sub-sector is considered a potential reservoir of Gram-negative bacteria which can be acquired by humans contact or ingestion of contaminated meat (Leverstein van Hall et al., 2011). *E. coli* from poultry is a potential hazard to humans from the antimicrobial resistance standpoint (Leverstein van Hall et al., 2011; Diercke et al., 2014). Antibiotic resistance has become a global health problem in humans, animals, and the environment (Marshall and Levy, 2011;Faife et al., 2020). It is said that resistance is present in all currently used antibiotics in human and veterinary medicine (Marshall and Levy, 2011;Faife et al., 2020). The prophylactic, anabolic and metaphylactic use of antibiotics, in addition to its therapeutic use in food animals has resulted in the emergence of antibiotic resistant bacteria of zoonotic and ingestible transmission (Todar, 2022).

CONCLUSIONS

This study was able to show the pathological manifestation of *E coli* infection viz a viz the antimicrobial resistance in different poultry breeds and species from various poultry farms in

northcentral Nigeria states and demonstrates, the resistance significance level among the laying chickens, broiler, and pullets flock and show the importance of antimicrobial susceptibility tests to combat AMR in poultry. The pathological manifestation of *E. coli* infection viz a viz the antimicrobial resistance to macrolides antibiotics was significant for laying chickens, broiler, and pullets' flocks in north central Nigerian States.

ACKNOWLEDGEMENT

We gratefully acknowledge all laboratory staff at the Central diagnostic laboratory, National Veterinary Research Institute, Vom for the technical support.

REFERENCES

- Adelowo, O.O., E.F. Obasola and A. Yvonne. 2014. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. *J. Infect. Dev. Ctries*; 8:1103-1112.
- Barnes, H.J., L.K. Nolan and J.F. Vaillancourt. 2008. Colibacillosis. In Saif, Y.M., A.M. Fadly, J.R. Glison, L.R. McDougald, L.K. Nolan and D.E. Swayne, 12th Ed. *Diseases of Poultry*. Blackwell publishing. Ames.
- Blyton, M.D.J., H.Pi, B.Vangchhia, S. Abraham, D.J. Trott, J.R. Johnson and D.M. Gordon. 2015. Genetic structure and antimicrobial resistance of *Escherichia coli* and cryptic clades in birds with diverse human associations. *Appl. Environ. Microbiol*; 81:5123-5133.
- Borges, C.A., L.G. Beraldo, R.P. Malutab, M.V. Cardozo, K.B. Barboza, E.A. Guastallic, S. Kariyawasam, C. DebRoy and F.A. Ávila. 2017. Multidrug-resistant pathogenic *Escherichia coli* isolated from wild birds in a veterinary hospital. *Avian. Pathol*; 46:76-83.
- Borzi, M.M., M.V. Cardozo, E.S. Oliveira, A.S. Pollo, E.A.L. Guastalli, L.F. Santos and F.A. Ávila. 2018. Characterization of avian pathogenic *Escherichia coli* isolated from free-range helmeted guineafowl. Brazil. *J. Microbiol*; 49:107-112.
- Chakraborty, A., V. Saralaya, P. Adhikari, S. Shenoy, S. Baliga and A. Hegde. 2015. Characterization of *Escherichia coli* phylogenetic groups associated with extraintestinal infections in South Indian population. *Ann. Med. Health Sci. Res*; 5:241-246.
- Clinical and Laboratory Standards Institute (CLSI), CLSI document M100-S22. 2012. *Performance Standards for Antimicrobial Susceptibility Testing*; 22nd Ed. Wayne, Pennsylvania.
- Cole, D., D.J. Drum, D.E. Stalknecht, D.G. White, M.D. Lee, S. Ayers, M. Sobsey and J.J. Maurer. 2005. Free-living Canada geese and antimicrobial resistance. *Emerg. Infect. Dis*; 11:935-938.
- Cunha, M.P.V., A.B. Saldenberg, A.M. Moreno, A.J.P. Ferreira, M.A.M. Vieira, T.A.T. Gomes and T. Knöbl. 2017. Pandemic extraintestinal pathogenic *Escherichia coli* (ExPEC) clonal group O6-B2-ST73 as a cause of avian colibacillosis in Brazil. *PLoS One*; 12:1-11.
- Cunha, M.P.V., M.C.V. Oliveira, M.G.X. Oliveira, M.C. Menão and T. Knöbl. 2019. CTX-M-producing *Escherichia coli* isolated from urban pigeons (*Columba livia domestica*) in Brazil. *J. Infect. Dev. Ctries*; 13:1052-1056.

- Cyoia, P.S., G.R. Rodrigues, E.K. Nishio, L.P. Medeiros, V.L. Koga, A.P.D. Pereira, E.C. Vespero, S. Houle, C.M. Dozois, G. Nakazato and R.K.T Kobayashi. 2015. Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic *Escherichia coli* isolates from Brazil. *J. Infect. Dev. Ctries*; 9:1068-1075.
- Diercke, M., M. Kirchner, K. Claussen, E. Mayr, I. Strotmann, J. Frangenberg, A. Schiffmann, G. Bettge-Weller, M. Arvand and H. Uphoff. 2014. Transmission of shiga toxin-producing *Escherichia coli* O104:H4 at a family party possibly due to contamination by a food handler, Germany 2011. *Epidemiol. Infect*; 142:99-106.
- Dinidu, S.W., A.N. Chamings, R.N. Bushell, O' D. Rourke, M. Stevenson, M.S. Marena, A.H. Noormohammadi and A. Stent. 2017. Pathological and microbiological investigations into cases of bacterial chondronecrosis and osteomyelitis in broiler poultry. *Avian Pathol*; 46:683-694.
- Faife, S.L., Z. Tomas, J.O. Sekyere, U. Govinden, H.Y. Chenia, G.S. Simonsen, A. Sundsfjord and S.Y. Essack. 2020. β -lactam and fluoroquinolone resistance in Enterobacteriaceae from imported and locally produced chicken in Mozambique. *J. Infect. Dev. Ctries*; 14:471-478.
- Frederick, A. 2020. Incidence and antimicrobial susceptibility of *Escherichia coli* isolated from beef (meat muscle, liver and kidney) samples in Wa Abattoir, Ghana. *Cogent Food & Agric* 6: 1.
- Geetha, M and K.M. Palanivel. 2018. Avian Colibacillosis - A Mini Review. *Int J Pure App Biosci* 6:376-380.
- Ghodousi, A., Bonura, C., A.M. Di Noto and C. Mammina. 2015. Extended-spectrum β -lactamase, AmpC-producing, and fluoroquinolone-resistant *Escherichia coli* in retail broiler chicken meat, Italy. *Foodborne Pathog. Dis*; 12:619-625.
- Gosh, S and T.M. LaPara. 2007. The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. *ISME J*; 1:191-203.
- Guabiraba, R and C. Schouler. 2015. Avian colibacillosis: still many black holes. *FEMS. Microbiol. Lett.* 362:118.
- Guenther, S., M. Grobbel, A. Lübke-Becker, A. Goedecke, N.D. Friedrich, L.H. Wieler and C. Ewers. 2010. Antimicrobial resistance profiles of *Escherichia coli* from common European wild bird species. *Vet. Microbiol*; 144:219-225.
- Heuer, H and K. Smalla. 2007. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ. Microbiol*; 9:657-666.
- Hoepers, P.G., P.L. Silva, D.A. Rossi, J.E.C. Valadares, B.C. Ferreira, J.P. Zuffo, P.K. Koerich and B.B. Fonseca. 2018. The association between extended spectrum beta-lactamase (ESBL) and ampicillin C (AmpC) beta-lactamase genes with multidrug resistance in *Escherichia coli* isolates recovered from turkeys in Brazil. *Brit. Poult. Sci*; 59:396-401.
- Hubálek, Z. 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. *J. of Wild Dis*; 40:639-659.
- Ibrahim, R.A., Cryer, T.L., Lafi, S.Q., E. Basha, L. Good and Y.H. Tarazi. 2019. Identification of *Escherichia coli* from broiler chickens in Jordan, their

- antimicrobial resistance, gene characterization and the associated risk factors. *BMC Vet. Res*; 15: 159.
- Kabir, S.M.L. 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int. J. Environ. Res. Public Health*; 7:89-114.
- Laarem, M., B. Abouddihaj, N. Kaotar, A. Abdi, K. Zerouali, N. El Mdaghri and M. Timinouni. 2017. Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian *Escherichia coli* isolates from Algeria. *J. Infect. Dev. Ctries*; 11: 143-151.
- Leverstein-van Hall, M.A., C.M. Dierik, S.J. Cohen, G.M. Voets, M.P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A.C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M.J. Bonten and D.J. Mevius. 2011. National ESBL surveillance group. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect. Dis*; 17:873-880.
- Logue, C.M., Y. Wannemuehler, B.A. Nicholson, C. Doetkott, N.L. Barbieri and L.K. Nolan. 2017. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. *Front. Microbiol.* 8:283.
- Marshall, B.M and S.B. Levy. 2011. Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev*; 24:718-733.
- Oliveira, E.S., M.V. Cardozo, M.M. Borzi, C.A. Borges, E.A.L. Guastalli and F.A. Ávila. 2019. Highly pathogenic and multidrug resistant avian pathogenic *Escherichia coli* in free-range chickens from Brazil. *Braz. J. Poult. Sci*; 21:1-8.
- Reich, F., V. Atanassova and G. Klein. 2013. Extended-spectrum β lactamase- and AmpC-producing enterobacteria in healthy broiler chickens, Germany. *Emerg. Infect. Dis*; 19:1253-1259.
- Saka, H.K., N.T. Dabo, B. Muhammad, S. García-Soto, M. Ugarte Ruiz and J. Alvarez. 2019. Diarrheagenic *Escherichia coli* pathotypes from children younger than 5 years in Kano State, Nigeria. *Front. Microbiol*; 7: 34.
- Silva, K.C., M.P.V. Cunha, L. Cerdeira, M.G.X. Oliveira, M.C.V. Oliveira, C.R. Gomes, N. Lincopan, T. Knöbl and A.M. Moreno. 2017. High-virulence CMY-2- and CTX-M-2-producing avian pathogenic *Escherichia coli* strains isolated from commercial turkeys. *Diagn. Microbiol. Infect. Dis*; 87:64-67.
- Silveira, F., R.P. Maluta, M.R. Tiba, J.B. Paiva, E.A. Guastalli and W.D. Silveira. 2016. Comparison between avian pathogenic (APEC) and avian faecal (AFEC) *Escherichia coli* isolated from different regions in Brazil. *Vet. J*; 217:65-67.
- Stella, A.E., M.C. Oliveira, V.L.D.S. Fontana, R.P. Maluta, C.A. Borges and F.A. Ávila. 2016. Characterization and antimicrobial resistance patterns of *Escherichia coli* isolated from feces of healthy broiler chickens. *Arq. Inst. Biol*; 83:e0392014.
- Todar, K. 2020. Bacterial resistance to antibiotics. Available online: <http://textbookofbacteriology.net/resistance/antimicrobial.html>. Accessed 24 January 2022.
- Tonini da Rocha, D., S.F. de Oliveira, K.A. Borges, T.Q. Furian, V.P. do

Nascimento, H.L. de Souza Moraes and T.P.S. Carlos. 2021. Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC): characterization and comparison. J. Infect. Dev. Ctries; 15:962-971.

World Health Organisation (WHO). Tackling antibiotic resistance from a food safety perspective in Europe. Available online: http://www.euro.who.int/__data/assets/pdf_file/0005/136454/e94889.pdf. Accessed 12 December 2021.