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Antimicrobial Susceptibility in *Escherichia coli* isolates from Guinea Fowls (*Coturnix coturnix japonica*) and Quails (*Numida meleagris*) in Ibadan, Oyo-State, Nigeria

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Abstract

Poultry are reared for meat, eggs and feathers. Poultry production is classified into four sectors which are chickens, ducks, guinea fowl, turkey and geese based on the marketing of poultry products and the level of biosecurity. Intensive poultry farming falls under chickens, ducks, guinea fowl and turkey characterized by moderate to high levels of biosecurity, while geese pertain to the 'backyard', 'village' or 'family' poultry system, with few or no biosecurity measures. Avian colibacillosis is an infectious disease of birds caused by *Escherichia coli* which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen. This study determined the prevalence and antibiotic susceptibility of *Escherichia coli* from guinea-fowl (*Numida meleagris*) and quail (*Coturnix coturnix japonica*) in Ibadan. A total of 180 cloacal sample was collected and bacteriologically analysed for the presence of *Escherichia coli*. For bacteriological analysis samples were pre-enriched in peptone broth and incubated overnight at 37°C, followed by a selective isolation on MacConkey agar supplemented with ampicillin (100mg/L). Ampicillin-resistant isolates were subcultured on MacConkey agar supplemented with cefotaxime (1mg/L). Biochemical tests of all the cefotaxime resistant isolates was performed. Double disc diffusion test specific for Extended Spectrum Beta Lactamase producing *E. coli* (ESBL *E.coli*) was performed. Antibiotic sensitivity testing was done using Mueller-Hinton agar. Twenty-six isolates were analysed using disc diffusion method. Eighty-four (84) isolates were lactose fermenters, 49 isolates were Ampicillin-resistant *E. coli* and 26 isolates were Cefotaxime-resistant *E. coli* and none was positive for ESBL-*E. coli*. Gentamycin has the highest sensitivity of 100% followed by ciprofloxacin of 96.2% of *E. coli* isolates while amoxicillin-clavulanic

(96.2%), tetracycline (96.2%) and sulphamethaxazole (92.3%) has the highest rate of resistance. This present study investigated antimicrobial susceptibility of *Escherichia coli* isolated from guinea fowls and quail in Ibadan, Nigeria.

Key words: Quails, Guinea fowl, *Escherichia coli*, Antimicrobial Susceptibility, Ibadan.

Introduction

Development of poultry production is one of the solutions for increasing animal protein production, especially in developing countries. To increase the daily food quota, it is imperative to identify the best means to stimulate local agricultural and animal production (Nobo et al., 2012). Raising of these poultry species is of high socio-economic importance for the population. Guinea fowls and quails are raised according to the scavenging system, using local surrogate domestic fowl (*Gallus gallus*) hens for incubation and hatching of the guinea fowl eggs (Mold 2010; Boni et al., 2010; Santos et al., 2011).

Guinea fowl and quail meat and eggs have high quality and biological protein value with low calories (Chindo and Olowoniyi, 2006). For suitable control and preventive programme in quail and guinea fowl industry, appropriate knowledge about the diseases affecting these is necessary. Poor breeder farm management and hatchery hygiene, as well as use of floor egg are some of the predisposing factors to early mortality in quail and guinea fowl chicks. Transmission of infection through the

blood stream and contamination of yolk sac have been reported as the route of infections (Jahantigh and Nili, 2010)

The most common infectious diseases that occur in production are colibacillosis, salmonellosis, ulcerative enteritis, fowl cholera, bronchitis, Newcastle disease (Dozier et al., 2010). Various microorganisms causing infections are transmitted through the yolk sac, *Salmonella* species and *Escherichia coli* are the most important factors in yolk sac infection. *Escherichia coli* bacteria can penetrate through the oviduct and they are common egg shell contamination.

In most cases, bacterial diseases are treated with antimicrobial agents. These agents are also administered in feed and water as prophylactic measure to forestall outbreak of infections. However, overdependence on antimicrobials has placed selective pressure on the pathogenic bacteria leading to the emergency and spread of antimicrobial resistance as commonly encountered in the treatment of bacterial diseases (WHO, 2014a). In the poultry industry, It has been observed that more and more bacterial diseases of poultry

are becoming resistant to commonly used antimicrobial (Gross, 1994; Kilonzo-Nthenge et al., 2008; Ogunleye et al., 2008).

The emergence of antimicrobial resistance in pathogenic bacteria constitutes a serious problem in the control of infectious diseases. Many infections which were hitherto successfully treated based mainly on the clinician's past experience are increasingly becoming more refractory to traditional therapy (OIE, 2008). This may necessitate a longer period of therapy, an increase in the dose or change of drug. The consequences of these are reduction in the level of production, increase in the cost of production and a threat to availability of animal protein to human population. Antimicrobial resistance therefore could be a major limitation to the growth of the poultry industry. In this study we aimed to determine the antimicrobial susceptibility in *Escherichia coli* isolated from guinea fowls and quails in Ibadan, Oyo-State, Nigeria.

Materials and Methods

Sample collection

A total of One hundred and eighty (180) cloacae swab samples were collected from guinea fowls and quails. Ninety (50.0%) cloacae swab samples were collected from quails at the Department of Veterinary Anatomy, University of Ibadan; 67 guinea fowl's cloacae swab samples (37.2%) were collected from Shasha in Akinyele local Government area, Ibadan; 23 guinea fowls cloacae swab samples (12.8%) were collected from Bode, Molete in Ibadan South East Local Government area. An adequate precaution was taken to avoid contact between cotton swab tips and the surrounding of the anus to avoid cross-contamination. All samples were properly labelled and transported on ice to the Research Laboratory of the Department of Veterinary Microbiology, University of Ibadan, for bacteriological analysis.

Isolation and identification *Escherichia coli*

Samples were inoculated into buffer peptone water (BPW) and incubated at 37° C overnight for pre-enrichment. A loop full of the pre-enrichment broth culture was streaked onto MacConkey agar plates incubated at 37° C for 24 hours. Rose pink colonies on MacConkey agar (putative *E. coli*) were selected for further characterisation.

Isolation and identification of ampicillin resistant isolates

One rose pink colony from each MacConkey agar plate was inoculated and streaked onto MacConkey Agar supplemented with ampicillin at 100 g/L for the isolation of ampicillin resistant bacteria and incubated at 37° C for 24 hours.

Biochemical identification of *Escherichia coli* isolates

Rose pink colonies on MacConkey agar were selected for colonial morphology, microscopy following Gram staining and biochemical test. All the suspected *Escherichia coli* isolates were selected for oxidase and catalase production. Motility test, indole test, urease test, citrate test, sugars utilization test and other biochemical test were performed. Results of the biochemical test were interpreted using Cowan and Steel manual for identification of medical bacteria (Barrow and Feltham, 2005)

Isolation and identification of cefotaxime resistant isolates

One colony from each MAC- AMP-100 plate were inoculated onto separate MacConkey Agar supplemented at 1 g/L (MAC- CTX-1) and incubated at 37° C for 24 hours for isolation of C-R strains. One distinct colony of C-R isolate was selected from each MAC CTX- 1 plate. Selected isolate was preserved on nutrient Agar slant for further analysis.

Phenotypic detection of ESBL producing enterobacteria

All C-R isolates were tested for production of ESBL using the combination disc kits (Oxoid, Basingstoke) containing cefpodoxime (CPD, 10µg) and cefpodoxime-clavulanic acid (CD 01,10/1µg).

The bacterial suspensions were prepared using an inoculum level of 0.5 McFarland suspension on Mueller-Hinton agar. This was spread evenly on Mueller Hinton Agar (MAH) and the disc was placed firmly on the agar. The inoculated MAH was incubated at 37° C for 16 hours. The difference in the zone of inhibition around the two discs was determined.

Isolate that produce differences equal to or greater than five millimeters in the diameter of zone of inhibition between cefpodoxime and cefpodoxime-clavulanic acid discs were identified as phenotypic ESBL producers. (CLSI, 2018).

Pathogenicity Testing for Cefotaxime Resistance Escherichia coli isolates.

One colony from each MAC- CTX-1) plate was inoculated onto separate 7% sheep agar plates and incubated at 37° C for 24 hours for detection of pathogenic isolates of C-R strains.

Antimicrobial Susceptibility Testing

Isolates identified as C-R were tested for Susceptibility to selected antimicrobial with kirby Bauer disc diffusion method according to the guideline of the clinical laboratory standard Institute (CLSI,2018).

The following antibiotics were tested: Tetracycline(TET30g),sulfamethoxazole (SXT 25ug), Ceftazidime (CAZ,30ug), Chloramphenicol(CHL, 30 ug), Gentamicin(GEN120ug), Ciprofloxacin (CIP,5ug), Amoxicillin (AMX 30ug). A suspension of test organism was prepared

in normal saline. The suspension was spread evenly on Mueller Hinton agar using a sterile cotton swab. The antimicrobial disc was placed firmly on the inoculated agar and incubated at 37° C for 18-24h. The diameter of the zone of inhibition around each disc was measured and interpreted according to (CLSI, 2018).

Statistical Analysis

Data was analysed by using descriptive statistics, proportions and percentages and presented in form of tables and figures.

Results

One hundred and eighty cloacal swab samples were collected from quails (90) and guinea fowls (90) in Ibadan Table 1. Prevalence of 84 (46.7%) Escherichia coli was obtained from 180 cloacae swabs

Table 1: Showing the breakdown of the total samples collected and location

Location	Local Government Area	Number of sample collected from quails	Number of sample collected from guinea fowls	Total
University of Ibadan	Ibadan North	90	0	90
Bode, Molete.	Ibadan South East	0	67	67
Shasha	Akinyele	0	23	23
Total		90	90	180

samples collected. Also, 49(58.3%) of 84 (46.7%) were ampicillin-resistant while 26 (53.06%) of the 49(58.3%) were cefotaxime-resistant. None of 26(53.06%) was identified as Extended Spectrum Beta Lactamase (ESBL) *E.coli* producers (Tables 2). All the isolates were identified biochemically as *Escherichia coli*.

Three of 26 isolates of cefotaxime resistance *E.coli* showed pathogenicity

by showing complete clearing of the blood agar plates.

Detection of Ampicillin-Resistant *E. coli*

Out of the total of 84(46.7%) *E. coli* isolates, 49(58.3%) yielded Ampicillin-resistance *E. coli* isolates. Of the 49 Ampicillin-resistance isolates, 29(59.18%) was from Guinea-fowl, 20(40.82%) was from Quail (Table 2).

Table 2. Phenotypic Confirmation of Non-Esbl-Producing *E.coli*

Sample Source.	Number of Samples		Presumptive <i>E. coli</i> (MAC)		<i>E. coli</i> (AMP-RESISTANT)		<i>E. coli</i> (CTX-RESISTANT)		<i>E. coli</i> (ESBL PRODUCER)	
	Guinea Fowls	Quails	Guinea fowls	Quails	Guinea fowls	Quails	Guinea fowls	Quails	Guinea fowls	Quails
University of Ibadan.	00	90	00	27	00	20	00	11	0	0
Shasha	23	00	14	00	10	00	06	00	0	0
Bode	67	00	43	00	19	00	09	00	0	0
TOTAL	90	90	57	27	29	20	15	11	0	0
OVER			84		49		26		0	
ALL TOTAL	180									
			(46.70%)		(58.30%)		(53.06%)		(0%)	

Detection of Cefotaxime Resistant E. coli

Out of the total 84(46.7%) E. coli isolates, 49(58.3%) yielded Ampicillin-resistant isolates while 26(30.9%) were cefotaxime-resistant isolates. Of the 26 cefotaxime-resistant isolates 15(17.8%) was isolated from Guinea-fowl and 11(13.1%) from Quail. Table 2.

Phenotypic Confirmation on non-ESBL Producing E.coli

Of all the 26(53.06%) cefotaxime-resistant E. coli, none that is 0(0%) was confirmed to be ESBL producers. Table 2.

Antibiotic Susceptibility Testing

The 26 cefotaxime resistance E.coli isolates showed 100% susceptibility to

gentamycin followed by ciprofloxacin (96.2%), chloramphenicol (65.4%), ceftazidime (57.7%), while sulphamethoxazole/trimethoprim (07.7%) amoxicillin-clavulanic acid (03.8%) and tetracycline (03.8%) showed the least susceptibility. Seven resistant patterns of Escherichia coli were observed. Five of these seven groups could be classified as multidrug resistant due to their resistance to three different classes of antibiotics.

Table 3: Represent the summary of antibiotics resistance patterns of cefotaxime-resistant E.coli while Figure 1 illustrates antibiotic resistance rates of cefotaxime-resistant isolates.

Table 3: Summary of resistance pattern for cefotaxime resistant Escherichia coli isolated from guinea fowl and quail in Ibadan, Nigeria

Resistance pattern	Antimicrobial Agents Resistance	Number of strain showing
Double Resistance	AMC, TE	2
Double Resistance	AMC, SXT.	1
Triple Resistance	AMC, SXT, TE	8
Quadruple Resistance	AMC, C, SXT, TE	4
Quadruple Resistance	CAZ, AMC, SXT, TE	6
Quintuple Resistance	CAZ, AMC, C, SXT, TE	4
Quintuple Resistance	CAZ, CIP, C, SXT, R	1

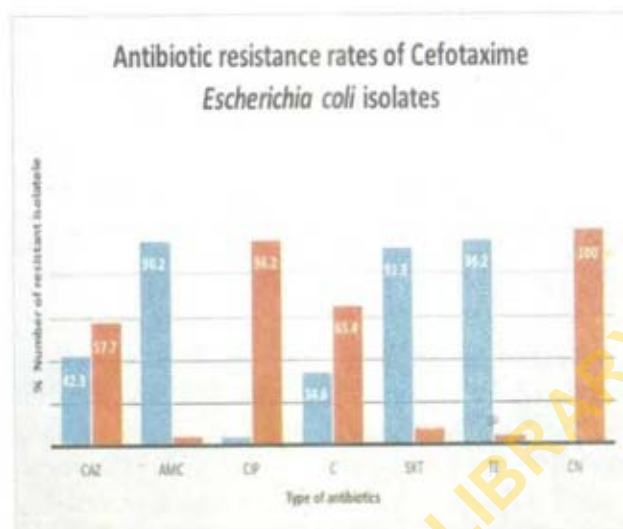


Figure 1.

Legend:



CAZ= Ceftazidime; AMC= Amoxicillin Clavulanic Acid; CIP= Ciprofloxacin; C= Chloramphenicol; STX= Sulphamethoxazole/Trimethoprim; TE= Tetracycline; CN= Gentamycin.

Discussion

This study revealed a high incidence of 46.7% *Escherichia coli* in cloacae swab of guinea fowls and quails in Ibadan, Nigeria. In this study none (0%) of the ESBL producing *E. coli* of the 53.06% cefotaxime-resistant *E. coli* were phenotypically proven to be non ESBL producing *Escherichia coli*. This value is lower than the 5% reported by (Ayeni

et al., 2016; Duru et al., 2013) reported a 22.2% of ESBL producing *Escherichia coli* in a study carried out in Owerri South-east Nigeria.

The findings in this present study however agree with the fact that antibiotics have not been abused in guinea-fowls and quails. High resistance rates among cefotaxime-resistant *E. coli* to first line antimicrobial therapy such

as tetracycline, sulphamethoxazole/trimethoprim, amoxicillin clavulanic acid were observed which is similar to previous report by (Kwoji et al., 2019) where antimicrobial susceptibility test revealed a high frequency of resistance of ESBL-producing *E. coli* to most of the similar antimicrobial agents.

Gentamycin has the highest sensitivity of 100% followed by ciprofloxacin of 96.2% of ESBL-producing *E. coli* isolates while amoxicillin-clavulanic (96.2%), tetracycline (96.2%) and sulphamethoxazole (92.3%) has the highest rate of resistance.

This high resistance to tetracycline is in agreement with the findings of Kibret et al., (2011).

ESBL-*E. coli* isolates of broiler chicken showed the highest resistance towards tetracycline (62.5%) followed by amoxicillin (56.3%) and ciprofloxacin (50%). Each of 18.2% of backyard isolates while 12.5% and 37.5% of broilers were resistant to gentamycin and cotrimaxazole. (Acharya et al., 2020). Of the total 26 (53.06%) cefotaxime-resistant *E. coli*, only 3 (3.6%) were pathogenic on blood agar. The implication of this is that the 3 cefotaxime-resistant *E. coli* is highly pathogenic and is infectious to humans.

The findings of this study would help in creating awareness about antibiotics stewardship so as to reduce the rate of antibiotics resistance. Detection of pathogenic *E. coli* in guinea-fowls and

quails in Ibadan emphasizes that they are reservoir and can disseminate it into the environment.

The detection of ESBL producing *Escherichia coli* is of public health importance as guinea-fowl and quail may serve as reservoirs of these resistant organisms which could be transferred to humans (Aworh et al., 2020) and also a means whereby human pathogens acquire these resistant genes (Akinlabi et al., 2008).

Overuse of antimicrobial agents, including third generation cephalosporin may be associated with emergence of ESBL-producing *E. coli* in small ruminants. (Bitruse et al., 2019).

The treatment with antibiotics does not improve the outcome of *Escherichia coli* infection and may increase the chances of developing haemolytic uremic syndrome as the resistance to the antibiotics used were variable, for Ceftazidime (42.3%), Augmentin (96.2%), Ciprofloxacin (3.8%), Chloramphenicol (34.6%), Sulphamethazole (92.3%), Tetracycline (96.2%), Gentamycin (0%) which implies that thorough bacteriological analysis and tests must be carried out to know the type of antibiotic to be used to prevent misuse of antibiotics and antibiotic multi-drug resistance. This is in agreement with the fact that commensal bacteria contribute to the distribution of persistence of antimicrobial resistance in the environment. (Amosun et al, 2012)

In conclusion, this study established that guinea fowls and quails' meats are safe and wholesome for human consumption in that antimicrobial agents have not been abused in guinea-fowls and quails. Their meats will be free from antimicrobial residues and possibility of transmission of antimicrobial resistant genes from animals to humans through ingestion or contact may not likely occur.

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