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Research Article

Antimicrobial Susceptibility Profiling and Detection of Cefotaxime-resistant *Escherichia coli* from Commercial-laying Hens, Indigenous Ducks and Chickens in Ibadan, Nigeria

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ABSTRACT

Cefotaxime is a critically important antimicrobial agent for the treatment of infections in humans and animals. The upsurge in the incidence of cefotaxime-resistant *Escherichia coli* from animal sources is of global public health importance. Avian pathogenic *Escherichia coli* is a Gram negative zoonotic bacterial pathogen. Infections by *Escherichia coli* usually occur following consumption of foods and water contaminated with faeces. The development of antimicrobial resistance in *Escherichia coli* is a concern worldwide. This study evaluated the prevalence, antimicrobial susceptibility and cefotaxime resistant *Escherichia coli* in commercial laying hens, indigenous ducks and chickens in Ibadan, Nigeria. Cefotaxime resistant *Escherichia coli* isolates from the cloacae of these poultry sources were tested for antimicrobial agents. The overall isolation rate of cefotaxime resistant *Escherichia coli* was 6.5% (6/93), 3.2% (3/93) and 10.0% (20/200) from indigenous ducks, indigenous chickens and commercial laying hens respectively. Cefotaxime resistant *Escherichia coli* isolates were 89.7%, 86.2%, 65.5%, 55.2%, 37.9%, 27.6%, 20.7% and 20.7% resistant to sulphamethoxazole, tetracycline, ceftazidime, amoxicillin -clavulanic acid, chloramphenicol, ciprofloxacin, gentamicin and ceftriaxone respectively. Whereas, 75.9%, 68.9%, 62.1%, 51.7%, 41.4%, 27.6%,10.3% and 10.3% susceptible to ceftriaxone, ciprofloxacin, gentamicin, chloramphenicol, amoxicillin-clavulanic acid, ceftazidime, sulphamethoxazone and tetracycline respectively. Multidrug resistant (MDR) was observed in 89.7% (26/29) of the isolates which exhibited 2 (in indigenous ducks), 3 (in indigenous chickens) and 13 (in commercial laying hens) different MDR patterns to 7 antimicrobial classes of drug. Higher isolation rate of cefotaxime resistant *Escherichia coli* and remarkable numbers of the isolates from commercial laying hens showed multidrug resistant than that of indigenous ducks and chickens. Misused of drugs was predicted in commercial laying hens. This study showed that the indigenous ducks and chickens harbour multidrug resistant *Escherichia coli* and may contribute to environmental contamination through faecal shedding.

Keywords: *Antimicrobial susceptibility, Escherichia coli, Commercial Laying Hens, Indigenous ducks, Indigenous chickens, Ibadan, Nigeria*

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INTRODUCTION

Poultry is one of the most widespread food industries worldwide and chicken is the most commonly farmed species with over 90 billion tons of chicken meat produced yearly worldwide (FAO, 2017). Nigeria as a developing country, many families depend on poultry production as a means of income and livelihood due to the increased consumption of poultry products (Akintunde *et al*, 2015). Poultry refers to all birds of economic usefulness to man including chickens, duck, quail, guinea fowl, pigeon, pheasant and ostrich. The advancement of the poultry industry is interrupted by several constraints, of which a major one is disease outbreaks causing about 30% mortality of chickens every year (Afolabi *et al*., 2013).

Microbial food safety is an increasing public health concern worldwide. Viral and bacterial pathogens of poultry

are a concern on both local and international scale because they represent a burden to human health and economy (Hossain et al ., 2015). Diseases that can be transmitted to bird flock through drinking water may originate from contaminated faeces and secretions of sick birds, or by utilization of water already contaminated by pathogenic organisms (Linden, 2015). Pathogenic microorganisms in the food chain are transmitted to humans through a variety of foods including beef, poultry, and eggs (Baran and Gulmez, 2010). Bacterial contamination of food can occur at all stages of production so it can affect the quality of poultry products (Herman *et al*., 2003). Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning (Muldler, 1999). *Escherichia coli* (*E. coli*) is considered as a member of the normal microbiota of all warm-blooded animals including poultry (Kaper *et al*., 2004). *E. Coli* causes diseases in the gut (diarrhoea), and other body regions such as the kidney (haemolytic uremic syndrome) as well as the brain (meningitis). Infections by *E. Coli* usually occur following consumption of foods and water contaminated with faeces as well as contact of the bacterium with cut body surfaces. However, in the debilitated or in immune suppressed hosts, or when gastro-intestinal barriers are violated, even normal "non-pathogenic" strain of *E. Coli* can cause infection to poultry, humans and animals. Moreover, there are certain *E. Coli* strains designated as avian pathogenic *E. Coli*, spread into various internal organs and cause colibacillosis characterized by systemic fatal disease (Nakazato *et al*., 2009).

Diseases associated with *E. Coli* in poultry are manifested by yolk sac infection, omphalitis, respiratory tract infection, septicaemia, polyserositis, enteritis, cellulitis and salpingitis (Lutful, 2010). Cefotaxime is a third -generation cephalosporin that are used in the management and treatment of Gram-negative and Gram-positive organisms because of it's potentcy and broad-spectrum antimicrobial activity (Plosker *et al*., 1998). Cefotaxime inhibits peptidoglycan cross-linkage by crossing the bacterial cell wall, avoiding inactivation by β-lactamases, and binding to and inactivating critical penicillin-binding proteins (Chaudhry *et al*., 2019) Cefotaxime has considerably antimicrobial activity, good penetration into vascular tissue (Duceac *et al*.,2020). Cefotaxime and ceftizoxime provide essentially the same antimicrobial (Chalhoub *et al*., 2015). The development of bacterial resistance has affected all steps of the cefotaxime mechanism of action, including production of β-lactamases, alterations in penicillin-binding proteins, and modification of the cell wall (McPherson *et al*., 2018). Food-producing animals are among the most important reservoirs of antimicrobial resistance genes due partly to the frequent and indiscriminate use of antimicrobial agents as feed additives for prophylaxis and in the treatment of gastrointestinal illness in animals thus facilitating the shading of resistance genes and pathogenic foodborne bacteria into the environment. However, the use of antimicrobials improves the health and production of our poultry because of the benefits of antimicrobials, there is an obvious overdependence on their use in poultry as prophylactic supplements or growth-promoting agents in feed, which has contributed to antimicrobial resistance, resulting in ineffective treatment of infectious diseases and also contributing to adverse health outcomes such as treatment failure, prolonged illness, and mortality.

Antimicrobial resistance associated with inappropriate use of antimicrobial drugs in humans and animals has been the major factor for the emergence and spread of drug-resistance traits among pathogenic and commensal bacteria. The development of multi-drug resistance in *E. Coli* is one of major concern worldwide (Von Baum, 2005). In Nigeria, there is unrestricted access to antimicrobials, high level of fake and substandard drugs, antimicrobial misuse in animals, inadequate laboratory diagnosis and antimicrobial susceptibility testing as the basis for prescription by veterinary doctors. Moreover, unhygienic practices in processing, marketing and serving of animal-derived food, poor personal hygiene, as well as close contact between animals and humans are common. All these factors contribute to the development and spread of antimicrobial resistance in bacteria. The present study aimed to evaluate antimicrobial susceptibility profiling and cefotaxime- resistant of *Escherichia coli* isolated from commercial laying hens, indigenous ducks and chickens in Ibadan, Nigeria.

Table 1.
Sample Collection in Local Government Areas (LGA) within Study Location

S/no	Local Government Area	Sample Location	Commercial Laying Hens	Ducks	Indigenous Chickens	Samples Collected
1	Akinyele	Shasha	0	31	31	62
2	Akinyele	Elyon farm	20	0	0	20
3	Ibadan South-East	Molete	0	31	31	62
4	Ibadan South-East	Bode	0	31	31	62
5	Ibadan South-East.	Ayo's farm	20	0	0	20
6	Egbeda	BL Goshe	20	0	0	20
7	Ibadan North	Olu Oluwa	20	0	0	20
8	Ibadan North – East	Freedom farm	20	0	0	20
9	Lagelu	His Grace farm	20	0	0	20
10	Ona-Ara	James's farm	20	0	0	20
11	Ibadan North-West	Kay's farm	20	0	0	20
12	Ido	Twins farm	20	0	0	20
13	Oluyole	Sun Boy's farm	20	0	0	20
Total			200	93	93	386

MATERIALS AND METHODS

Samples collection: A total of 386 faecal samples were collected using sterile swabs directly from the cloacal of commercial laying hens (200), indigenous ducks (93) and indigenous chickens (93). The samples were properly labelled and transported to the laboratory in ice packs for microbiological analysis.

Isolation and identification of *Escherichia coli*: Each sample was pre-enriched by inoculating a faecal swab in to 9ml of sterile tryptone soy broth (TSB) in universal bottles and incubated at 370C for 18-24hr. A loopful of the pre-enrichment culture was inoculated on MacConkey agar and incubated at 370C for 18-24hr. Rose pink colonies from MacConkey agar were subculture on Eosin Methylene Blue agar (EMB) and incubated at 370C for 24hr. Rose pink colonies on MacConkey agar plates (Putative *E. Coli*) that have showed greenish metallic sheen colonies on Eosin Methylene Blue agar were selected for biochemical identification tests. The isolates were identified using colonial morphology and microscopy after Gram staining of greenish metallic sheen colonies from EMB agar. Gram negative rods isolates that were oxidase negative and catalase positive were selected for biochemical test. Biochemical test such as indole test, urease test and substrate utilization tests were used for the identification of *Escherichia coli*.

Isolation and identification cefotaxime -resistant *Escherichia coli*: Each colony of identified *Escherichia coli* was streaked on MacConkey agar supplemented with ampicillin at 100mg/L and incubated at 370C for 24hr. Ampicillin- resistant *E. Coli* was streaked on MacConkey agar supplemented with cefotaxime at 1mg/L and incubated at 370C for 24 hr. Cefotaxime -resistant *E. Coli* was selected, purified and preserved on nutrient agar slope for further investigation.

Antimicrobial Susceptibility Testing of cefotaxime -resistant *Escherichia coli*: All cefotaxime resistant *Escherichia coli* were tested for antimicrobial susceptibility using the Kirby-Bauer method. This test was performed using eight different antibiotic single discs. Discs with different antibiotics were placed firmly on inoculated Mueller-Hinton agar plates and incubated at 37ºC for 24 hours. The diameter of zones of inhibition around each disc was measure and interpreted according to the recommendation of Clinical and Laboratory Standard Institute (CLSI, 2013). The antibiotics used and their concentration were: Amoxicillin-clavulanic acid (30µg), ceftazidime (30µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (120µg), sulphamethoxazole (25µg), tetracycline (30µg).

RESULTS

Prevalence of *Escherichia coli* from commercial laying hens, indigenous ducks and chickens: Out of 200 commercial laying hens, 93 indigenous ducks and 93 indigenous chickens examined, *Escherichia coli* was isolated from 122 (61.0%) ,38(40.9%) and 47(50.5%) cloacal swabs of commercial laying hens, indigenous ducks and chickens respectively (Table 2.).

Cefotaxime – resistant *Escherichia coli* from commercial laying hens, indigenous ducks and chickens: Twenty-nine (7.5%) of 386 samples yielded cefotaxime -resistant isolates as follows: Indigenous ducks 6(6.5%), indigenous chickens 3(3.2%), commercial laying hens 20(10.0%). (Table 2). The rate of detection of cefotaxime resistant *Escherichia coli* higher in commercial laying birds

Antimicrobial susceptibility testing of *Escherichia coli* from commercial laying hens, indigenous ducks and chickens: The results of susceptibility testing showed that there was variation in susceptibility of cefotaxime – resistant *Escherichia coli* isolates to the antimicrobials used. Cefotaxime -resistant *Escherichia coli* isolates revealed susceptibility 75.9%, 64.9%, 62.1%, 51.7%, 41.4%, 27.6%, 10.3% and 10.3% to ceftriaxone, ciprofloxacin, gentamicin, chloramphenicol, amoxicillin/ clavulanic acid, ceftazidime, sulphamethoxazole and tetracycline respectively. Cefotaxime – resistant *Escherichia coli* were resistant to one to seven of the antimicrobials tested. Resistant 89.7%, 86.2%, 65.5%, 55.2%, 37.9%, 27.6%, 20.7%, and 20.7% was observed to tetracycline, sulphamethoxazole, ceftazidime, amoxicillin/clavulanic acid chloramphenicol, ciprofloxacin, gentamicin and ceftriaxone respectively (Table 3). Among the cefotaxime resistant *Escherichia coli* 89.7% were multi drug resistant (MDR) and exhibited 16 different MDR patterns to 7 antimicrobial classes (Table 4.). Cefotaxime MDR *Escherichia coli* to as few as three and as many as7 antimicrobial classes.

Table 2:
Prevalence and cefotaxime -resistant *Escherichia coli* isolated from commercial laying hens, ducks and indigenous chickens in Ibadan, Nigeria.

Poultry species	Sample size	Number (%) of *Escherichia coli*	Number (%) of Ampicillin Resistant *E.coli*	Number (%) of Cefotaxime Resistant *E.coli*
Indigenous ducks	93	38 (40.9)	14 (15.1)	6 (6.5)
Indigenous chickens	93	47 (50.5)	22 (23.7)	3 (3.2)
Commercial laying hens	200	122 (61.0)	52 (26.0)	20 (10.0)
Total	386	207 (53.6)	88 (22.8)	29 (7.5)

Table 4:
Multi-drug Resistance Pattern Cefotaxime Resistant *Escherichia coli*

Antibiotics	**Number of Cefotaxime- resistant *E. Coli* isolates [n (%)]**			**Resistant pattern**
	Commercial laying hens n=20	Indigenous ducks n=6	Indigenous chickens n= 3	Resistant patterns
TET	0(0.0)	1(16.7%)	0 (0.0)	Mono resistance
SXT-AMC	1(5.0%)	0(0.0)	0(0.0)	Double resistance
CAZ- TET	1(5.0%)	0(0.0)	0(0.0)	Double resistance
TET-SXT-CH	1(5.0%)	3(50.0%)	1(33.3%)	Triple resistance
CAZ-SXT-AMC	1(5.0%)	0(0.0)	0(0.0)	Triple resistance
CAZ-SXT-CH	1(5.0%)	0 (0.0)	0(0.0)	Triple resistance
CAZ-TET-SXT	0 (0.0)	0(0.0)	1(33.3%)	Triple resistance
CAZ-TET-SXT-CH	0 (0.0)	2(33.3%)	0(0.0)	Quadruple resistance
TET-SXT-CIP-CH	0(0.0)	0(0.0)	1(33.3%)	Quadruple resistance
CAZ-TET-SXT-CN	1(5.0%)	0(0.0)	0 (0.0)	Quadruple resistance
CAZ-SXT-TET-AMC	1(5.0%)	0(0.0)	0(0.0)	Quadruple resistance
CAZ-CRO-TET-AMC	3(15.0%)	0(0.0)	0(0.0)	Quadruple resistance
CAZ-CN-TET-AMC	1(5.0%)	0(0.0)	0(0.0)	Quadruple resistance
CAZ-CRO-SXT-TET-AMC	1(5.0%)	0(0.0)	0(0.0)	Quintuple resistance
CAZ-CN-SXT-TET-AMC	1(5.0%)	0(0.0)	0(0.0)	Quintuple resistance
CAZ-SXT-CIP-TET-AMC	3(25.0%)	0(0.0)	0(0.0)	Quintuple resistance
CAZ-SXT-CIP-TET-AMC-C	1(5.0%)	0(0.0)	0(0.0)	Sextuple resistance
CAZ-CN-SXT-CIP-TET-AMC	2(10.0%)	0(0.0)	0(0.0)	Sextuple resistance
CAZ-CN-SXT-CIP-TET-AMC-C	1(5.0%)	0(0.0)	0(0.0)	Septuple resistance

Key: CAZ– Ceftazidime; CRO – Ceftriaxone ; CN– Gentamicin; SXT– Sulphamethoxazole; TET- Tetracycline; C- Chloramphenicol AMC- Amoxicillin/Clavulanic acid; CIP - Ciprofloxacin

Table 3.
Frequency of antibiotics susceptibility for Cefotaxime -Resistant *Escherichia coli.*

Antimicrobial class	**Antimicrobial agent**	**Disk Potency**	Number of Isolates, T=29		
			Sensitive [n (%)]	**Intermediate [n (%)]**	**Resistance [n (%**
Beta- Lactams	Amoxicillin/Clavulanic acid	30µg	12 (41.4)	01(03.4)	16 (55.2)
Cephalosporins (3rd Generartion)	Ceftazidime	30µg	08 (27.6)	02 (06.9)	19 (65.5)
	Ceftriaxone	30µg	22 (75.9)	01 (03.4)	06(20.7)
Phenicol	Chloramphenicol	30ug	15 (51.7)	01 (03.4)	11(37.9)
Aminoglycoside	Gentamicin	12µg	18 (62.1)	05 (17.2)	06 (20.7)
Fluoroquinolone	Ciprofloxacin	30µg	20 (69.0)	01 (03.4)	08 (38.7)
Sulphonamides	Sulfamethoxazole	25µg	03 (10.3)	01 (03.4)	25 (86.2)
Tetracycline	Tetracycline	30µg	03 (10.3)	00 (00)	26 (89.7)

DISCUSSION

Cefotaxime is a broad-spectrum antimicrobial agent with high potency and good penetration into vascular tissue (Duceac *et al*.,2020). The development of bacterial resistance has affected all steps of the cefotaxime mechanism of action, including production of β-lactamases, alterations in penicillin-binding proteins, and modification of the cell wall (Gustaferro and Steckelberg, 1991). *Escherichia coli* harbouring resistance determinant originating in the poultry industry are therefore of great epidemiological interest because they can serve as reservoirs of resistance genes that can be transferred to human pathogens (WHO, 2017). A relationship between resistant strains of *Escherichia coli* from poultry and those found in human has been suggested in several studies (Kluytmans *et al*., 2013; Moser *et al*., 2018). In this study the prevalence of *Escherichia coli* from commercial laying hens 122(61.0%), indigenous chickens 47(50.5%), indigenous ducks 38(40.9%). Cefotaxime-resistant *E. Coli* isolates were identified in 29 samples (7.5%) from 3 different birds' species from areas included in the study. Cefotaxime resistant *Escherichia coli* was isolated from commercial laying hens 20 (10.0%), ducks 6(6.5%), indigenous chickens 3(3.2%). Commercial laying hens have the highest rate of detection of cefotaxime- resistant *Escherichia coli*, probably due to the reason that antimicrobials have been used in the birds to ensure high levels of production, disease prevention and growth promotion. All the 29 isolates were resistant to cefotaxime and ampicillin (Table 2). The prevalence in the present study was higher than 13.4% prevalence that was

reported from chickens' cloacal samples examined in eastern Ethiopia (Mude *et al*., 2017). It was also higher compared with 5.7% from local chickens in Zaria (Ejeh *et al*., 2017) and 6.7% in strayed chickens from Calabar (Nfongeh *et al*., 2018). The variations observed in the prevalence of *Escherichia coli* might be due to different in sampling method, study location, time and season of the year that the sampling were carried out. Cefotaxime-resistant *Escherichia coli* isolates were identified in 65 birds (15.7%) from cloacal swabs from carcasses of Dutch wild birds, (Veldman *et al*., 2013). Also, cefotaxime resistant *Escherichia coli* were confirmed in 362 flocks (94.3%) from 112 farms (97.4%) in Ecuador (Vinueza-Burgos *et al*., 2019), these findings were higher than our findings in this present study. Ayeni *et al*., (2 015) reported 5% cefotaxime- resistant *Escherichia coli* from poultry in Ibadan which was lower than the finding in this present study, also Duru *et al*., (2013) observed 22% in a study carried out in Owerri South-east Nigeria. The findings in this study however, agree with the fact that the prevalence of cefotaxime-resistant *Escherichia coli* varies from region to region. The detection of cefotaxime-resistant *Escherichia coli* in this study is of public health importance as commercial laying hens may serve as reservoirs of these resistant organisms which could be transferred to humans (Geser *et al*., 2012) and also a means whereby human pathogens acquire these resistance genes (Akinlabi *et al*., 2008). The presence of cefotaxime resistant *Escherichia coli* in indigenous ducks and chickens may be important in the dissemination of cefotaxime resistant *Escherichia coli* through their faecal shedding and this may contribute to the contamination of the studied areas.

In this study 55.2% resistance to amoxicillin-clavulanic acid and 65.5% to ceftazidime were observed which is lower than the findings by Duru *et al* (2013) who reported 90% resistance to amoxicillin-clavulanic acid and 100% resistance to ceftazidime. Ejikeugwu *et al*., (2013) reported a 91.7% resistance rate of cefotaxime- resistant *Escherichia coli* to ceftazidime which is higher than the finding in this study. Gundogan *et al*., (2013) reported an 8.9% resistance rate to ceftazidime. Cefotaxime resistant *Escherichia coli* were susceptible to ceftriaxone, ciprofloxacin, gentamicin and chloramphenicol. The resistance rate to gentamicin (20.7%) is lower than the 79.6% reported by Kibret *et al*., (2011). High antimicrobial resistant rates and multi-resistance patterns could be related to the intensive use of antimicrobials in poultry production, which in some cases are not only used as therapeutics but also as prophylactics and growth promotors (Chalhoub *et al*., 2015; Bar-Oz *et al*.,2003).

In conclusion, this study detected cefotaxime resistant *Escherichia coli* in commercial laying hens, indigenous ducks and chickens in Ibadan, Nigeria at a prevalence of 7.5%. Therefore, commercial laying hens, indigenous ducks and chickens are a potential source of spread of multidrug resistant bacteria with consequent spread of resistance genes to other bacteria in human and animal populations.

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