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Full Length Article

Characterization and Antibiogram of *Escherichia coli* Isolated from Indigenous Chickens in Ibadan, Nigeria

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Abstract

Escherichia coli remains a leading aetiology of bacterial disease in poultry and food-borne illness in humans, worldwide. There are reports on the changing trends in antimicrobial resistance pattern of animal pathogens with very limited information available on *E. coli* isolates from free-range chickens in Ibadan. This study was therefore designed to investigate the characteristics and antibiogram of *E. coli* from indigenous chickens in Live Bird Markets (LBMs) in Ibadan, Nigeria. In a cross-sectional survey, 100 cloacal swabs were obtained from indigenous chicken from three LBMs in Ibadan, namely Oje (n = 34), Moniya (n = 34) and Bode (n = 32). *E. coli* was isolated using standard procedures. Initial identification of *E. coli* was by morphological, biochemical, and gram staining characteristics. PCR amplification of the 16S rRNA gene of *E. coli* was done using standard primers. Sequences were analysed, deposited in the Genbank and Phylogenetic trees were constructed using MEGA 11.0 software. Antibiotic susceptibility pattern of the isolates was investigated utilizing the disc diffusion technique and interpreted according to CLSI 2020 guidelines. Data were analysed using descriptive statistics at $P < 0.05$. *E. coli* colonised 43% of the samples with a higher frequency of isolation from male chickens (46%), and those from Oje market (52.9%). There was close phylogenetic relatedness with other strains from Egypt, Portugal and Iraq. Several strains were multi-drug resistant with leading resistance to Metronidazole (100%), Doxycycline (81.40%) and Streptomycin (76.74%) and in various combinations. Isolates were mostly sensitive to Enrofloxacin (100%) and Gentamicin (88.37%). Free-range indigenous chickens in Ibadan harbor multi-drug resistant *E. coli* strains which may be a source of food contamination. Improved hygiene while handling indigenous chickens is recommended.

Keywords: Poultry infection; *Escherichia coli*; Indigenous chickens; Antibiotic resistance

Introduction

Escherichia coli continues to be the most common cause of bacterial disease in poultry, which can lead to serious health issues as well as financial losses (Alfifi *et al.* 2022). It is the causative agent of numerous poultry diseases with a wide range of clinical and pathological manifestations, such as omphalitis, peritonitis, salpingitis, septicemia and airsacculitis (Poulsen *et al.* 2017; Alfifi *et al.* 2022).

E. coli is a vital component of the typical microbiota of all warm-blooded animals, including poultry and have been known to possess varied resistance against antimicrobials (Sarba *et al.* 2019; Mandal *et al.* 2022). Generally, they have evolved to fit into many ecological niches both inside and outside of the host, such as extraintestinal or intestinal locations (Raheel *et al.* 2022).

Pathogenic *E. coli* may cause localised and/or systemic infections which may be within the gut or outside the gut, with the latter being referred to as extra-intestinal pathogenic *E. coli* (ExPEC) (Ibrahim *et al.* 2019). The pathogenic strains are defined as those that have one or more virulence factors; the serotypes O78:K80, O1:K1, and O2:K1 are the most frequently occurring isolates in poultry (Sarba *et al.* 2019).

Globally, there is considerable concern over the emergence of multi-drug resistance in *E. coli* (Sarba *et al.* 2019) with the trends in antibiotic resistance in poultry isolates developing faster than in clinical isolates from humans (Tadesse *et al.* 2012). The non-pathogenic strains are also important as reservoirs of several genes linked to antibiotic resistance that could be transferred to pathogenic strains (Blake *et al.* 2003; Parvin *et al.* 2020).

Human foodborne illness is still predominantly caused by *E. coli* and the rise of multidrug-resistant strains of the

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bacteria has led to a notable increase in morbidity and mortality in humans (Been *et al.* 2014). ExPEC strains from humans and animals have been shown to be phylogenetically and clonally related by scientific studies, with strong indications that these strains share virulence-associated genotypic or phenotypic features (Rodriguez-Siek *et al.* 2005; Moulin-Schouleur *et al.* 2007; Alfifi *et al.* 2022). The development of extended spectrum beta-lactamases (ESβL) in some *E coli* strains aggravates the challenge of drug resistance. The development of these resistance traits has been linked to the lavish use of antibiotics in poultry (Raheel *et al.* 2022).

Several studies have documented the transmission of antibiotic-resistant *E. coli* from chickens to humans (Norizuki *et al.* 2017; Ferguson-Noel *et al.* 2019; Mandal *et al.* 2022), indicating that certain pathogenic *E. coli* strains from poultry species are of public health importance (Shang *et al.* 2023).

Symptoms of food poisoning caused by *E. coli* in humans typically include vomiting, cramping in the abdomen, and in rare instances, bloody diarrhea. An infection with *E. coli* that produces Shiga toxin can occasionally result in hemolytic-uremic syndrome, which can induce kidney failure (Switaj *et al.* 2015). Many of the *E. coli* infections resolve on their own, thus treating with antibiotics may not be recommended (Parvin *et al.* 2020).

The growing trend of antibiotic resistance is primarily driven by indiscriminate and/or excessive use of antibiotics, especially in livestock (Moreno *et al.* 2008; Habib *et al.* 2021). Gene mutations or horizontal gene transfer result in the acquisition of this resistance with several drug-resistant genes typically present in multidrug-resistant (MDR) bacteria (Hughes and Andersson 2015; Habib *et al.* 2021; Ahmed *et al.* 2023; Mwafy *et al.* 2023). Typically, when antibiotics are used in humans, they are to treat specific individuals who have infections, but in food animals, they are mostly used on herd basis for growth promotion, preventive, and therapeutic purposes (Nahla *et al.* 2018). While the European Union has prohibited the use of antibiotics as growth promoters since 2006, some other countries continue to use antibiotics for this purpose (Nahla *et al.* 2018; Cige *et al.* 2023). In Nigeria, considering the relatively uncontrolled access of antibiotics to poultry farmers; they continue to use it in poultry feed or water without restrictions (Manyi-Loh *et al.* 2018; Aworh *et al.* 2019). Oxytetracycline, ciprofloxacin, neomycin, gentamicin, enrofloxacin, doxycycline, colistin, streptomycin, tylosin, nitrofurans and chloramphenicol are among the antibiotics that are widely abused (Agyare *et al.* 2019; Ahmed *et al.* 2022).

Poultry contributes significantly to the economy of Nigeria with the indigenous breeds of chicken being more important to the rural economy as they require less input to survive. The use of native breeds of chicken in rural poultry farming remains a common practice in many developing and underdeveloped countries throughout the world (Padhi 2016). These birds are usually raised on free-range, while mixing with other domestic and wild animals and are allowed to shelter within or near human habitation. Although the indigenous chickens have lower productivity compared to exotic breeds (Mahoro *et al.* 2017; Cyuzuzo *et al.* 2023); their role in dissemination of pathogen, while being raised, handled or prepared for consumption needs investigation.

Notable aggregation points for indigenous and exotic chickens in Nigeria are the Live Bird Markets (LBMs). They form a critical point for the exchange of pathogens during aggregation and handling of the birds for sale. The market designs usually ensure remarkable contact between the chickens, marketers, and consumers. Considering that it has been established that zoonotic infections can spread to people either directly through contact with chicken litter or indirectly through infected poultry products, this presents a certain degree of risk (Chen and Jiang 2014). On the other hand, no report is available on the molecular characteristics and antibiotic resistance pattern of *E. coli* isolates from indigenous birds in Ibadan. This study was therefore designed to investigate the cloacal carriage of *E. coli* in indigenous chickens at LBMs in Ibadan, Nigeria. Further, their molecular characteristics and antibiogram were also determined. The findings may provide useful information on the epidemiology of *E. coli* in indigenous chickens in Ibadan, Nigeria.

Materials and Methods

Study area

The study was conducted in Ibadan, Oyo State, Nigeria which is known as a major hub for poultry activities in the south-western part of Nigeria (Owoade *et al.* 2004; Adene and Oguntade 2006). Oyo State has a coordinate of 7.377°N and 3.947°E (Pulsipher and Pulsipher 2014).

Study design

This study was a cross-sectional investigation covering the three most prominent Live Bird Markets (Oje, Moniya and Bode) within Ibadan. The investigation was conducted between February and August 2023. Free-range indigenous chickens aggregated at the markets were randomly selected and sampled. The estimated age and sex of the birds were also noted.

Sample size determination

Thrusfield's formula was used for estimating the sample size. With a 95% confidence interval, 5% absolute precision required and a 50% expected prevalence (Thrusfield 2005). N is the sample size, Z is the statistic for an interval level of confidence at a 95% constant value (1.96), D is the required absolute accuracy, and (5% = *0.05*) *P* is the anticipated

prevalence level of the subject.

$$N = z^2 p (1-p)$$

$$N = \frac{1.96^2 Pexp (1-Pexp)}{d2}$$

$$N = \frac{3.84 \times 0.05 \times (0.886)}{0.0025}$$

$$N = 69.46$$

Sample size calculated n = 69.46
Using 10% non-response, sample size N = 100

A total of 100 indigenous chickens were sampled in the three Live Bird Markets comprising Oje (n = 34), Moniya (n = 34) and Bode (n = 32).

Sample collection

Faecal samples were collected directly from the cloaca of the indigenous chickens using sterile swabs. The sterile swabs with labels were transported to the University of Ibadan's Department of Veterinary Medicine's Aquatic Animal and Wildlife Medicine Laboratory.

Bacteria isolation

The faecal samples were enriched in 10 mL of buffered peptone water, and incubated at 37°C for 24 h on nutritient agar plates (HiMEDIA®, USA). The dominant colonies were repeatedly sub-cultured on MacConkey (MAC) and Trypticase Soy Agar (TSA) for the purpose of determining colonial shape and purity (Aynadis and Aweke 2021).

Bacterial colonies on MAC with typical traits and colour of lactose fermenter were cultivated on Blood Agar and Eosin Methylene Blue. The morphological characteristics of bacteria colonies, such as their color, size, and form were assessed using TSA. The bacteria isolates were identified by colonial morphological characteristics, gram staining, and biochemical tests such as methyl red, the Vogues Proskauer test, the Triple Sugar Iron agar slant culture, oxidase, catalase, motility, Simon citrate, indole, glucose (gas), hydrogen sulfide, nitrate reduction, lysine, ornithine and urease, and sugar fermentation (Oliveira *et al.* 2014; Aynadis and Aweke 2021). The bacteria were identified by Global Infectious Diseases and Epidemiology Network Online (GIDEON) microbiology database and Systemic Bacteriology Bergey's Manual (2012). The outcomes of the biochemical tests were input into the software for confirmation and differential diagnosis. *E. coli* pure cultures were re-cultured in Tryptic soy broth. The bacteria were maintained in bijou bottles kept at a temperature of 20°C in 50% glycerol supplemented with TSB.

Molecular identification and characterisation

Isolates already identified as *E. coli* by morphological methods were further investigated with molecular techniques. Conventional Polymerase Chain Reaction (PCR) was used to identify the species from the 43 samples (E1-E43) using standard primers. Bacterial gDNA was extracted using DNA isolation kit (ZR Tissue & Insect DNA MiniPrep™, ZYMO Research, U.S.A.) according to manufacturer's instructions. The amplification of the 16S rRNA gene of *E. coli* was done using the standard primers (Forward primer (ECO-1) 5'-GACCTCGGTTTAGTTCACAGA -3' and reverse primer 5'- CACACGCTGACGCTGACCA-3') (Hassan *et al.* 2014) sourced from African Biosciences Limited, Ibadan. 1 μL template DNA, 12.5 μL One Taq Quick-Load 2X Master Mix (1.8 m*M* $MgCl_2$,, 22 m*M* NH_4Cl, 22 m_M KCl, 20 m*M* Tris-HCl, 0.2 m*M* dNTPs, 0.06% IGEPAL® CA-630, 5% glycerol, 0.05% Tween® 20, Xylene Cyanol FF, Tartrazine, 25 units/mL One Taq DNA Polymerase), 0.2 μM of each primer and 10.5 μL of nuclease free water were used in the 25 μL reaction. Amplification was done on a C 1000 Touch (Bio-Rad, Foster City) PCR machine. After initial denaturation at 95°C for 3 min, a 30-cycle amplification protocol was followed, including 94°C for 45 s, 58°C for 45 s and 72°C for 60 s and a final extension step of 72°C for 3 min and held at 4°C. The PCR products of 585 bp were analyzed by electrophoresis in 1.5% agarose dyed with Ethidium Bromide and examined by transillumination on Kodak Gel Logic 200 Imaging System.

Bioinformatic analysis

A non-redundant NCBI GenBank database was used for the 16S rRNA gene sequence analysis of the identified seven sequences utilizing BLAST to identify closely similar bacterial 16S rRNA gene sequences. Another ten high-quality 16S rRNA gene sequences were selected from the NCBI GenBank database and subjected to multiple sequence alignment using the Clustal W program (Anifowose *et al.* 2023). The phylogenetic tree was constructed according to the maximum likelihood method using MEGA 11.0 software. The 16S rRNA gene sequence of *E. coli* isolates were submitted to the NCBI database.

Antimicrobial susceptibility testing

The susceptibility of pure isolates of *E. coli* to antibiotics was examined. The agar-disc diffusion method was performed in accordance with CLSI Mo2 criteria and evaluated for their sensitivity and resistance to antibiotics (CLSI 2020). The susceptibility was evaluated against 13 antimicrobials from the following eight classes: Penicillin [amoxicillin-clavulanic acid (AC; 30 μg); ampicillin (AP; 10 μg); amoxicillin (AM; 25 μg)], Cephalosporin (third-generation) [ceftriaxone (CEF; 30 μg)], Tetracycline [doxycycline (D; 30 μg), tetracycline (T; 30 μg)], Chloramphenicol (CH; 30 μg), Aminoglycoside [gentamicin (G; 10 μg) and streptomycin (S; 10 μg)], Metronidazole (M; 5 μg), Fluoroquinolone [ciprofloxacin (CIP; 5 μg), enrofloxacin (ENR; 5 μg)] and Furaltadone (F; 30 μg) using the Kirby-Bauer disc diffusion technique (CLSI 2020; Hartantyo *et al.* 2020).

Preparation of antibiotic disc

The disc was constructed in accordance with the CLSI Mo2 disc diffusion guide. Briefly, six 6-mm sterilized Whatmann filter paper discs impregnated with the known antimicrobial concentrations stated above were inserted on a 100-mm plate. Each isolate on sterile Mueller-Hinton agar plate was made and appropriately labeled with an indelible marker.

Preparation of inoculums

E. coli was grown overnight on Nutrient agar, and pure cultures were harvested using a sterile loop. The colonies were suspended in sterile normal saline that had been blended to a consistent turbidity. Saline or additional bacteria were added in order to get the *E. coli* suspension's density to McFarland 0.5. After that, Muller-Hinton agar was streaked with the suspended colonies.

Inoculation of agar plates

Sterile pointed forceps were used to pick one antibiotic disc (for each bacterial isolate) and applied to the Mueller-Hinton agar plates. This allowed the discs to effectively touch the channel. The plates were then incubated for a further 18 h at 37°C (CLSI 2020). For the purpose of identifying which antibiotics, the isolate was sensitive to or resistant to, the visible zone of growth inhibition encircling various discs was assessed in terms of millimeters. The antibiogram was used to establish the resistance pattern for each isolate (Akond *et al.* 2009).

Measurement of zones and interpretation of susceptibility

A meter ruler in millimeters was used to measure the diameters of the inhibitory zones. Each isolate's sensitivity or resistance were determined using the resistance breakpoints in the "Performance Standards for Antimicrobial Susceptibility Testing" developed by Clinical and Laboratory Standards Institute (CLSI 2020). *E. coli* strains were categorized as susceptible, resistant to one or two antimicrobial categories or multidrug-resistant (MDR).

Data presentation and analysis

Data were entered into an MS Excel spreadsheet, and the Statistical Package for Social Sciences (SPSS) was used to summarize the data using descriptive statistics. Sex and source of sample were used to categorize prevalence and computed for *E. coli* using Chi-square analysis, the relationships between the investigated factors and the result variables were examined. The odds ratio was used to compare the strength of the relationship, and the difference was considered significant when the *P*-value was less than *0.05*. The antimicrobial resistance (AMR) percentages were calculated and categorized into susceptible, intermediate, and resistant classes.

Results

Prevalence of *E. coli* in indigenous free-range chicken from three markets in Ibadan

Based on colony morphology, Gram staining and biochemical tests; the prevalence of *E. coli* was 43% (Table 1; Fig. 1). The isolation rate was higher in males 46% than in females 37.8%. In addition, based on source of the samples, the prevalence increased in the order of Moniya (26.5%), Bode (50.0%) to Oje (52.9%) markets (Table 1).

Molecular identification and characterisation

The amplification of the 16S rRNA gene of *E. coli* in all of the 43 samples provided a confirmation for the morphological identification. Seven of the samples with high quality amplicons were succesfully sequenced and labelled OyNG 1-7. The sequences were deposited to the GenBank and accession number were assigned (Table 2).

Evaluation of phylogeny of isolates of *E. coli* from indigenous chickens in Ibadan

To determine the evolutionary history and relationships among or within the *E. Coli* isolates, phylogenetic trees were constructed to evaluate the relatedness between the seven strains of *E. coli* (Oyo 1-7) from indigenous chickens in Ibadan and strains from other parts of the world (Fig. 2). Three of the isolate (2,4 and 7) clustered closely while the others were dispersed and clustered with strains from other parts of the world including Egypt, Portugal and Iraq. This reveals that *E coli* colonising indigenous chicken in Ibadan were by diverse population. The evolutionary history of the isolates showed they were likely from different origins or sources.

Antibiogram of *E. coli* isolates

The antibiotics susceptibility profile of the *E. coli* isolates (n = 43) to the 13 tested antibiotics is shown in (Table 2). There was very high resistance (76.74-100%) to three of the antibiotics tested; specifically, to the Aminoglycoside (Streptomycin) (76.74%), Tetracycline (Doxycycline) (81.40%) and Metronidazole (100%), but with intermediate susceptibility to Phenicol (Chloramphenicol) (51.16%) and Penicillin (Amoxicillin 25 μg) (60.47%) while it was highly susceptible to Aminoglycoside (Gentamicin) (88.37%) and Fluoroquinolones (Enrofloxacin) (100%) (Table 3). The diameter of the zone of inhibition was widest to Ciprofloxacin, Gentamycin and Enrofloxacin (Fig. 3).

Antimicrobial resistance pattern (AMR)

Most of the *E. coli* organism strains were observed to show resistance to at least two antimicrobial agents. There was the

Table 1: Prevalence of *E. coli* isolated from cloaca of indigenous free-range chicken from three Live-Bird Markets in Ibadan

No.	Sample source (live bird markets)	Sex	Distribution of samples collected	Percentage of *E. coli* isolates and prevalence based on sex	Prevalence based on location
1	Oje	Male	22	13 (59.1%)	52.9%
		Female	12	5 (41.7%)	
2	Moniya	Male	20	6 (30.0%)	26.5%
		Female	14	3 (21.4%)	
3	Bode	Male	21	10 (47.6%)	50.0%
		Female	11	6 (54.5%)	
	Total		100	43	
	Overall prevalence			43%	

Table 2: Sample Identification and Accession numbers of the sequences of *E. coli* submitted to the NCBI GenBank

S. No.	Sample ID	Accession No	Host
1	OyNG1	OR226372.1	Indigenous Chicken
2	OyNG2	OR226392.1	Indigenous Chicken
3	OyNG3	OR226394.1	Indigenous Chicken
4	OyNG4	OR226395.1	Indigenous Chicken
5	OyNG5	OR226396.1	Indigenous Chicken
6	OyNG6	OR226397.1	Indigenous Chicken
7	OyNG7	OR226399.1	Indigenous Chicken

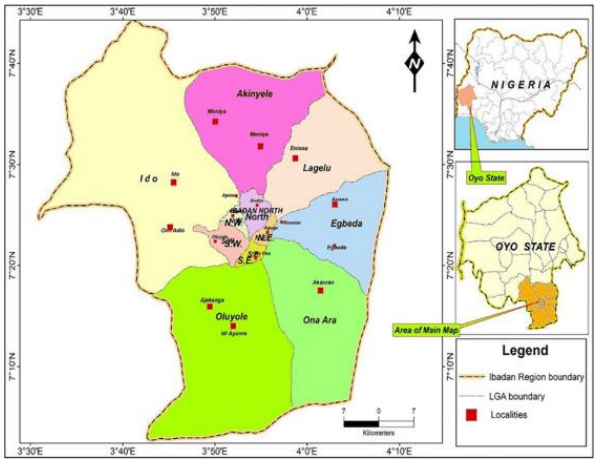

Fig. 1: Maps of the Ibadan, Oyo State, Nigeria

highest (100%) resistance phenotype observed to Streptomycin-Metronidazole antibiotics. Antibiotic resistance was lowest Enrofloxacin, Gentamicin, Ampicillin, Ciprofloxacin and Ceftriaxone (Table 3).

Many of the *E. coli* organism strains were observed to show resistance to a range of two to six classes of antibiotics. The highest (100%) multi-drug resistance phenotype observed in Streptomycin-Metronidazole antibiotics. Antibiotic resistance was lowest to Gentamicin, Ampicillin, Ciprofloxacin and Ceftriaxone (Table 4).

Discussion

This investigation provides information on the cloacal carriage of *E. coli* by indigenous chickens that were aggregated at live bird markets in Ibadan, Nigeria. We report the colonisation rate, phenotypic and molecular characteristics, and antimicrobial susceptibility patterns of the isolated strains. In this study, the colonisation rate of *E. coli* was 43%. Previous research has indicated that this bacterium is present in indigenous chickens from different places at varying frequencies. Mwambete and Stephen (2015) reported a 50% prevalence in Tanzania's Dar es Salaam while Manishimwe *et al.* (2017) reported 82% in Kigali city, Rwanda and Cige *et al.* (2023) reported 19.5% in Somalia. In Nigeria, Kolapo *et al.* (2022) reported a prevalence of 16.7% in a specific search for *E. coli* O157:H7. Prevalence of *E. coli* reported in poultry has been demonstrated to vary depending on several factors including source/type of sample collected, host susceptibility, environment, season, and identification methods used. Akond *et al.* (2009) reported differences in prevalence of *E. coli* based on source/type of sample collected in poultry with isolates from feces of bird being highest followed by isolates from the intestine while isolates from egg surfaces were the lowest. In the present study, *E. coli* isolates were from cloacal swabs. Also, the 16.7% prevalence reported by Kolapo *et al.* (2022) was specifically for *E. coli* O157:H7 and basic morphological method of *E. coli* identification was used. In the present study, *E. coli* was identified by morphological method and further confirmed by molecular technique. In this instance, all *E. coli* strains in the samples were identified, hence the higher prevalence.

We observed a higher frequency of isolation from male chickens (46%) compared to females (37.8%). The reason for this sex influence in prevalence is yet to be elucidated but factor as age and varied sources of origination of the birds, human factors such as hygiene and environment may be associated with this finding. We also isolated *E. coli* in varying frequencies across the sampling locations. It increased in the order of Moniya (26.5%), Bode (50.0%) to Oje (52.9%) markets. The isolation of *E. coli* in varying frequencies across the sampling locations in Ibadan had earlier been reported with a similar pattern (Kolapo *et al.* 2022). However, this pattern is subject to human factors including personal hygiene of traders, handling of birds and

Table 3: Antimicrobial susceptibility for *E. coli* isolated from the cloaca of indigenous chickens from Ibadan (n = 43)

Antimicrobial Class	Antimicrobial agent	Disc potency (μg)	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Aminoglycosides	Streptomycin	10	5 (11.63)	5 (11.63)	33 (76.74)
	Gentamicin	10	38(88.37)	0 (00)	5 (11.63)
Cephalosporin (3rd generation)	Ceftriaxone	30	21 (48.84)	12(27.91)	10 (23.26)
Penicillins	Ampicillin	10	19 (44.19)	15 (34.88)	9 (20.93)
	Amoxicillin	25	0 (00)	26 (60.47)	17 (39.53)
	Amoxicillin cloxacillin	30	0 (00)	20 (46.51)	23 (53.49)
Fluoroquinolones	Enrofloxacin	5	43 (100.00)	0 (00)	0 (00)
	Ciprofloxacin	5	28 (65.11)	7 (16.28)	8 (18.60)
Tetracyclines	Doxycycline	30	0 (00)	8 (18.60)	35 (81.40)
	Tetracycline	30	0 (00)	13 (30.23)	30 (69.77)
Phenicols	Chloramphenicol	30	0 (00)	22 (51.16)	21 (48.83)
Nitroimidazole	Metronidazole	5	0 (00)	0 (00)	43 (100.00)
Nitrofuran	Furaltadone	30	0 (00)	17 (39.53)	26 (60.47)

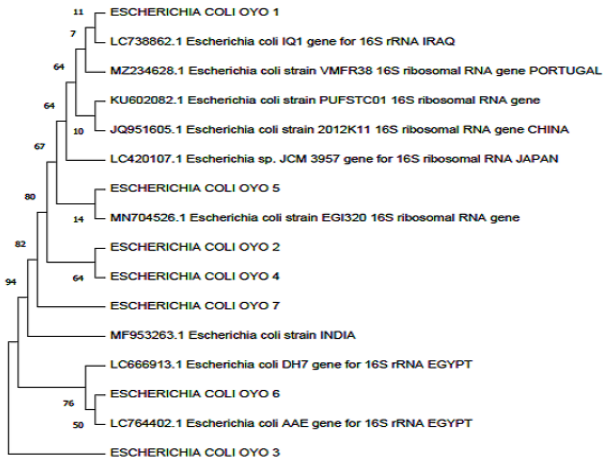

Fig. 2: Phylogenetic tree showing relationship between *E. coli* from indigenous free-range Chickens in Ibadan, Nigeria and other strains reported elsewhere

other trade considerations as inter market transport of birds. Anecdotal evidence has shown that some traders do display the same birds for sale at different markets on different days of the week, especially when sales appear to be more favorable at one market than the other. Also, the variation in prevalence between locations was mostly attributed to chance as the chickens were usually bought from farmers in different locations and mixed at the markets. This pattern is not limited to Ibadan; it has also been reported in other countries. Langata *et al.* (2019) also observed a similar pattern where they isolated *E. coli* from birds in varying rates from Kawangware (80%), Mathare (60%) and Kibera (48%) in Kenya.

All the samples previously identified by morphological method were confirmed as *E. coli* by molecular technique. Genomic sequencing and nucleotide BLAST analysis of the 16S rRNA gene was used to characterise the strains. The NCBI BLAST results indicated 99 to 100% homology to other *E. coli* sequences from other countries including Egypt, Portugal and Iraq. The phylogenetic tree revealed that three isolates (2, 4 and 7) were closely related to each other while the others clustered closely with strains from other parts of the world. This infers that the ancestry, and likely the source of the *E coli* strains colonizing indigenous chickens in Ibadan are diverse. The nucleotide sequences from our investigation that were deposited at the NCBI Genbank should aid further research and better understanding of the epidemiology of *E. coli* in indigenous chicken in Africa.

E. coli showed very high resistance to three of the antibiotics tested; specifically, to the Aminoglycoside (Streptomycin) (76.74%), Tetracycline (Doxycycline) (81.40%) and Metronidazole (100%), but with intermediate susceptibility to phenicol (Chloramphenicol) (51.16%) and Penicillin (Amoxicillin 25 μg) (60.47%), while it was highly susceptible to Aminoglycoside (Gentamicin) (88.37%) and Fluoroquinolones (Enrofloxacin) (100%).

In Nigeria, the use of antibiotics for growth promotion in addition to prevention and treatment of diseases is still a regular practice (Aworh *et al.* 2019). The widespread use of antibiotics in humans, animals, and crops remains the main cause of the emergence, selection, and spread of antibiotic-resistant bacteria (Habib *et al.* 2021). In our investigation using 13 different antibiotics, the pattern of susceptibility of *E. coli* isolates from indigenous chickens were noted. The isolates showed varying levels of antibiotic resistance with some isolates displaying multiple antibiotics resistance to different antibiotic combinations. These findings are in line with earlier reports of growing resistance to antibiotics (Apun *et al.* 2008; Akond *et al.* 2009). The persistent abuse of antibiotics, especially the tetracycline class is a growing concern (Omotosho *et al.* 2023). Kang *et al.* (2005) revealed that the tetracycline-resistant *E. coli* isolates from chicken were extremely resistant. Tetracycline resistance was discovered in 78.1% of the cases, according to Mandal *et al.* (2022). This is less than the findings of Sarker *et al.* (2019), who indicated that tetracycline resistance was present in all *E. coli* isolates from broiler chickens. Tetracycline-resistant bacteria are plasmid-based, and a broad range of genetic factors contribute to the continuous acquisition of resistance genes through conjugation or transformation (Miles *et al.* 2006).

The prolonged, extensive, and widespread administration of antibiotics to chickens and other food producing animals mounts selection pressure on microbes

Table 4: Multi-drug resistance pattern of *E. coli*

Antimicrobial agent	*E. coli* (n = 43)	
	Resistance	
	Frequency	Percentage
STP	33	(76.74%)
GEN	5	(11.63%)
CEF	10	(23.26%)
AMP	9	(20.93%)
AMO	17	(39.53%)
AMC	23	(53.49%)
CIP	8	(18.60%)
DOX	35	(81.40%)
TET	30	(69.77%)
CHL	21	(48.83%)
MET	43	(100%)
FUR	26	(60.47%)
STP-GEN	38	(44.19%)
DOX-TET	65	(75.58%)
CHL-MET	64	(74.42%)
CHL-FUR	47	(54.65%)
MET-FUR	69	(80.23%)
CHL-MET-FUR	90	(69.77%)
STP-GEN-CEF	48	(37.20%)
AMP-AMO-AMC	49	(37.98%)
STP-GEN-CEF-CIP	56	(32.56%)
AMP-AMO-AMC-CIP	57	(33.14%)
STP-GEN-DOX-TET	103	(59.88%)
STP-AMO-CIP-DOX	93	(54.10%)
CIP-CHL-MET-FUR	98	(56.98%)
STP-AMO-CIP-DOX-MET	136	(63.25%)
GEN-AMP-CIP-TET-FUR	78	(36.28%)
STP-AMC-TET-CHL-MET-FUR	176	(68.22%)

STP – Streptomycin, GEN – Gentamicin, AMP – Ampicillin, AMO – Amoxicillin, AMC – Amoxicillin clavulanic acid, DOX – Doxycycline, TET – Tetracycline, CIP – Ciprofloxacin, CHL – Chloramphenicol, MET – Metronidazole, FUR – Furaltadone, CEF – Ceftriaxone

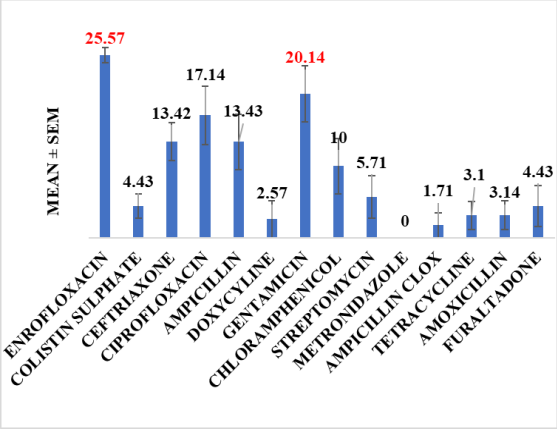

Fig. 3: Diameter of inhibition zone (Mean ± SEM) of Antimicrobial tested against *Escherichia coli* isolates

which drive development of resistance (Taye *et al.* 2013; Omotosho *et al.* 2016). The obtained isolates displayed various patterns of antibiotic resistance with a high proportion displaying resistance to a range of one to four classes of antibiotics. Single antibiotic resistance was found in all strains to Metronidazole (100%). Divalent antibiotic resistance was found to Metronidazole and Furaltadone (80.23%) while there was trivalent antibiotic resistance to Chloramphenicol, Metronidazole and Furaltadone (69.77%). Tetravalent resistance was observed in 59.88% of the *E. coli* isolates to Streptomycin, Gentamycin, Doxycycline and Tetracycline.

In another study with similar findings by Rahman *et al.* (2020), observed that all isolates of *E. coli* were resistant in varying degrees to 8 different antimicrobial agents. Most of the isolates were sensitive to Ciprofloxacin (82.6%), Gentamicin (78.2%), Azithromycin (60.8%) and were resistant to Amoxicillin (91.4%), Erythromycin (73.9%) and Doxycycline (47.8%). Our observation of widespread antibiotic resistance in *E. coli* corroborates several other reports of growing resistance in this population of organisms (Azad *et al.* 2019). The finding of multidrug resistant *E. coli* isolates also strengthen the earlier observations of Talukder *et al.* (2006) and Akond *et al.* (2009). However, from our findings, ciprofloxacin, enrofloxacin and gentamicin are still effective antibiotics for controlling *E. coli* infection as they were highly effective.

Conclusion

Indigenous chickens in live-bird markets in Ibadan are potent carriers of multidrug resistant strains of *E. coli* with diverse ancestry. There is leading resistance to streptomycin, doxycycline and metronidazole while the isolates were most susceptible to ciprofloxacin, gentamycin and enrofloxacin. An improved stewardship of the use of these antibiotics is needed to stop the growing trend of antibiotic resistance. The findings of this study may provide insight into the level of threat these pathogens pose to public health. Extra prevention strategies should be adopted when handling indigenous chickens at the live-bird markets or during processing for food.

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Author Contributions

Gbanamou W: Conceptualization, Methodology, Sample collections, Laboratory investigation, Data curation, Writing-Review and Editing, Funding Acquisitions; Omotosho OO: Conceptualization, Methodology, Writing-Original Draft, Writing-Review and Editing; Anifowose OR: Laboratory investigation, Writing-Original Draft, Writing-Review and Editing; Olukole SG: Conceptualization, Methodology, Project Administration, Writing-Original Draft.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethics Approval

Ethical approval for the study was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan with ethical approval number UI-ACUREC/046-0623/02.

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References

Adene DF, AE Oguntade (2006). Poultry sector review: Nigeria. *In: Food and Agriculture Organization of the United Nations*, pp:1–95. Available at https://www.fao.org/publications/card/en/c/97f1a303-d6e1-544f-9dc4-ecd0f5e47752/ accessed on April 19, 2024

Agyare C, VE Etsiapa, CN Zumbi, FB Osei (2019). Antibiotic use in poultry production and its effects on bacterial resistance. *In: Antimicrobial Resistance - A Global Threat*, pp:1–20. IntechOpen London

Ahmed HAA, AAA El-Tawab, FI El-Hofy, WMM Hassan, ME El-Khayat (2023). Surveillance of multi drug resistant bacteria isolated from virally infected broilers. *Intl J Vet Sci* 12:199–205

Ahmed MH, EM Riad, OM Diab, HA Mansour, MKH El-Mossalami (2022). Antibiotic residues in locally marketed fresh and frozen livers in Cairo and Giza, Egypt. *Intl J Vet Sci* 11:37–42

Akond A, SMR Hassan, S Alam, M Shirin (2009). Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *Amer J Environ Sci* 5:47–52

Alfifi A, JP Christensen, YMG Hounmanou, M Sandberg, A Dalsgaard (2022). Characterization of *Escherichia coli* and other bacteria isolated from condemned broilers at a Danish abattoir. *Front Microbiol* 13:1020586

Anifowose OR, OO Akinniyi, OG Banwo, GA Oladosu (2023). Occurrence and characterization of *Enterococcus faecalis* from infected farmed african catfish in Ogun State, Nigeria. *Intl J Oceanogr Aquac* 7:252

Apun K, YL Chong, MT Abdullah, V Micky (2008). Antimicrobial susceptibilities of *Escherichia coli* isolates from food animals and wildlife animals in Sarawak, East Malaysia. *Asian J Anim Vet Adv* 3:409–416

Aworh MK, J Kwaga, E Okolocha, N Mba, S Thakur (2019). Prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria. *PLoS One* 14:e0225379

Aynadis T, E Aweke (2021). Isolation, Identification and antimicrobial susceptibility profile of *E. coli* (O157:H7) from fish in lake Hawassa, Southern Ethopia. *Life Sci J* 17:64–72

Azad MARA, MM Rahman, R Amin, MIA Begum, R Fries, A Husna, AS Khairalla, ATM Badruzzaman, MEE Zowalaty, KN Lampang, HM Ashour, HM Hafez (2019). Susceptibility and multidrug resistance patterns of *Escherichia coli* isolated from cloacal swabs of live broiler chickens in Bangladesh. *Pathogens* 8:118

Been MD, VF Lanza, M de Toro, J Scharringa, W Dohmen, Y Du, J Hu, Y Lei, N Li, A Tooming-Klunderud, DJJ Heederik, AC Fluit, MJM Bonten, RJL Willems, F de la Cruz, W van Schaik (2014). Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. *PLoS Genet* 10:e1004776

Blake DP, K Hillman, DR Fenlon, JC Low (2003). Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. *J Appl Microbiol* 95:428–436

Bergey's Manual of Systematic Bacteriology (2012). The Actinobacteria, Vol 5. Springer, New York, USA

Chen Z, X Jiang (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A review. *Agriculture* 4:1–29

Cige H, O Omotosho, A Ibrahim (2023). Evaluation of antibiotic resistance pattern of *Escherichia Coli* and *Salmonella Species* isolated from cloaca of indigenous chickens in live-bird markets in Marodi Jeh Region, Somalia. *J Appl Vet Sci* 8:44–54

CLSI (2020). Performance Standards for Antimicrobial Susceptibility Testing. *In: The Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards*, 30th edn. Available at https://clsi.org/media/3481/m100ed30_sample.pdf. (Accessed: 21 April 2024

Cyuzuzo E, EA Amosun, M Byukusenge, V Musanayire (2023). Antimicrobial resistance profiling of *Escherichia coli* isolated from chickens in Northern Province of Rwanda. *Afr J Biomed Res* 26:151–154

Eltai NO, EA Abdfarag, H Al-Romaihi, E Wehedy, MH Mahmoud, OK Alawad, MM Al-Hajri, AA Al Thani, HM Yassine (2018). Antibiotic resistance profile of commensal *Escherichia coli* isolated from broiler chickens in Qatar. *J Food Prot* 81:302–307

Ferguson-Noel N, NK Amour, AH Noormohammadi, M El-Gazzar, JM Bradbury (2019). Mycoplasma. *Diseases of Poultry*, 14th edn, Vol. 1, pp:1–25. Dinev I (Ed.). Wiley-Blackwell, Hobokon, New Jersey, USA

Habib A, F Afroz, M Rahman, D Begum, M Kamruzzaman, A Haque (2021). Molecular characterization and antimicrobial resistance of *Escherichia coli* isolated from retail chicken meat. *South Asian J Biol Res* 4:38–47

Hartantyo SHP, ML Chau, TH Koh, M Yap, T Yi, DYH Cao, RA Gutiérrez, LC Ng (2020). Foodborne *Klebsiella pneumoniae*: Virulence potential, antibiotic resistance, and risks to food safety. *J Food Prot* 83:1096–1103

Hassan J, MS Parvej, MB Rahman, MSR Khan, MT Rahman, T Kamal, KNH Nazir (2014). Prevalence and characterization of *Escherichia coli* from rectal swab of apparently healthy cattle in Mymensingh, Bangladesh. *Microb Health* 3:12–14

Hughes D, DI Andersson (2015). Evolutionary consequences of drug resistance: Shared principles across diverse targets and organisms. *Nat Rev Genet* 16:459–471

Ibrahim RA, TL Cryer, SQ Lafi, EA Basha, L Good, YH 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

Kang HY, YS Jeong, JY Oh, SH Tae, DC Moon, WK Lee, YC Lee, SY Seol, DT Cho, JC Lee (2005). Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J Antimicrob Chemother* 55:639–644

Kolapo A, E Amosun, O Olatoye, F Adeoye, O Oladele (2022). *Escherichia coli* O157:H7 and *Salmonella species* isolated from ducks and indigenous chickens in live-bird markets in Ibadan, Oyo State, Nigeria. *Vet Ital* 58:369–378

Langata LM, JM Maingi, HA Musonye, J Kiiru, AK Nyamache (2019). Antimicrobial resistance genes in *Salmonella* and *Escherichia coli* isolates from chicken droppings in Nairobi, Kenya. *BMC Res Notes* 12:22

Mahoro J, TK Muasya, F Mbuza, R Habimana, AK Kahi (2017). Characterization of indigenous chicken production systems in Rwanda. *Poult Sci* 96:4245–4252

Mandal AK, S Talukder, MM Hasan, ST Tasmim, MS Parvin, MY Ali, MT Islam (2022). Epidemiology and antimicrobial resistance of *Escherichia coli* in broiler chickens, farmworkers, and farm sewage in Bangladesh. *Vet Med Sci* 8:187–199

Manishimwe R, M Buhire, A Uyisunze, JB Turikumwenayo, M Tukei (2017). Characterization of antibiotic resistant *Escherichia coli* in different poultry systems of eastern Province and Kigali City in Rwanda. *Rev Méd Vét Des Pays Tropic* 70:13–19

Manyi-Loh C, S Mamphweli, E Meyer, A Okoh (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules* 23:795

Miles TD, W McLaughlin, PD Brown (2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Vet Res* 2:7

Moreno A, H Bello, D Guggiana, M Domínguez, G González (2008). Extended-spectrum *β*-lactamases belonging to CTX-M group produced by *Escherichia coli* strains isolated from companion animals treated with enrofloxacin. *Vet Microbiol* 129:203–208

Moulin-Schouleur M, M Répérant, S Laurent, A Brée, S Mignon-Grasteau, P Germon, D Rasschaert, C Schouler (2007). Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: Link between phylogenetic relationships and common virulence patterns. *J Clin Microbiol* 45:3366–3376

Mwafy A, DY Youssef, MM Mohamed (2023). Antibacterial activity of zinc oxide nanoparticles against some multidrug-resistant strains of *Escherichia coli* and *Staphylococcus aureus*. *Intl J Vet Sci* 12:284–289

Mwambete KD, WS Stephen (2015). Antimicrobial resistance profiles of bacteria isolated from chicken droppings in Dar es Salaam. *Intl J Pharm Pharm Sci* 7:268–271

Norizuki C, JI Wachino, M Suzuki, K Kawamura, N Nagano, K Kimura, Y Arakawa (2017). Specific blaCTX-M-8/IncI1 plasmid transfer among genetically diverse *Escherichia coli* isolates between humans and chickens. *Antimicrob Agents Chemother* 61:10-1128

Oliveira RV, PG Peixoto, DC Ribeiro, MC Araujo, CTBD Santos, C Hayashi, MM Pedreira, A Pelli (2014). *Klebsiella pneumoniae* as a main cause of infection in nishikigoi *Cyprinus carpio* (carp) by inadequate handling. *Braz J Vet Pathol* 7:86–88

Omotosho O, S Ukatu, M Okandeji, O Okunlade, A Adeola, B Emikpe (2023). Antimicrobial resistance profile of aerobic nasal microflora of nigerian indigenous pigs. *Alexandr J Vet Sci* 76:30–37

Omotosho O, S Olaogun, A Odukaye (2016). Occurrence of skin abscess and sensitivity pattern of associated bacterial organisms in pigs on ifelodun farm settlement, Ogun State, Nigeria. *Alexandr J Vet Sci* 51:10–16

Owoade AA, DO Oluwayelu, OA Fagbohun, W Ammerlaan, MN Mulders, CP Muller (2004). Serologic evidence of chicken infectious anemia in commercial chicken flocks in southwest Nigeria. *Avian Dis* 48:202–205

Padhi MK (2016). Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. *Scientifica* 2016:2604685

Parvin MS, S Talukder, MY Ali, EH Chowdhury, MT Rahman, MT Islam (2020). Antimicrobial resistance pattern of *Escherichia coli* isolated from frozen chicken meat in Bangladesh. *Pathogens* 9:420

Poulsen LL, I Thøfner, M Bisgaard, JP Christensen, RH Olsen, H Christensen (2017). Longitudinal study of transmission of *Escherichia coli* from broiler breeders to broilers. *Vet Microbiol* 207:13–18

Pulsipher M, A Pulsipher (2014). World regional geography atlas. In: *The New Global Order*, 6th edn. Tomar N (Ed.). WH Freeman and Co, New York, USA

Raheel I, A Orabi, A Erfan, MA Raslan, SHAE Wahab, EAA Mohamed (2022). Intestinal tract of broiler chickens as a reservoir of potentially pathogenic curli producing ES*β*L *Escherichia coli. Intl J Vet Sci* 11:498–503

Rahman MM, A Husna, HA Elshabrawy, J Alam, NY Runa, ATM Badruzzaman, NA Banu, M Al Mamun, B Paul, S Das, MM Rahman, ATM Mahbub-E-Elahi, AS Khairalla, HM Ashour (2020). Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. *Sci Rep* 10:107

Rodriguez-Siek KE, CW Giddings, C Doetkott, TJ Johnson, MK Fakhr, LK Nolan (2005). Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology* 151:2097–2110

Sarba EJ, KA Kelbesa, MD Bayu, EZ Gebremedhin, BM Borena, A Teshale (2019). Identification and antimicrobial susceptibility profile of *Escherichia coli* isolated from backyard chicken in and around ambo, Central Ethiopia. *BMC Vet Res* 15:85

Sarker MS, MS Mannan, MY Ali, M Bayzid, A Ahad, ZB Bupasha (2019). Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. *J Adv Vet Anim Res* 6:272–277

Shang K, JH Kim, JY Park, YR Choi, SW Kim, SY Cha, HK Jang, B Wei, M Kang (2023). Comparative Studies of Antimicrobial Resistance in *Escherichia coli*, *Salmonella*, and *Campylobacter* isolates from broiler chickens with and without use of enrofloxacin. *Foods* 12:2239

Switaj TL, KJ Winter, SR Christensen (2015). Diagnosis and management of foodborne illness. *Amer Fam Phys* 92:358–365

Tadesse DA, S Zhao, E Tong, S Ayers, A Singh, MJ Bartholomew, PF McDermott (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerg Infect Dis* 18:741–749

Talukder KA, Z Islam, DK Dutta, MA Islam, BK Khajanchi, IJ Azmi, MS Iqbal, MA Hossain, ASG Faruque, GB Nair, DA Sack (2006). Antibiotic resistance and genetic diversity of *Shigella sonnei* isolated from patients with diarrhoea between 1999 and 2003 in Bangladesh. *J Med Microbiol* 55:1257–1263

Taye M, F Zewdu, D Ayalew (2013). Characterizing the climate system of Western Amhara, Ethiopia: A GIS Approach. *Amer J Res Commun* 1:319–355

Thrusfield MV (2005). V*eterinary Epidemiology*, 3rd edn, pp: 228–246. Blackwell science Ltd., London