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Antimicrobial Resistance Profiles of *Escherichia coli* Bacteria from Commercial Laying Chickens in Ibadan, Nigeria

Ojja. CV[1]*; Elizabeth A. Amosun[2]
[1,2]Avian Medicine Programme, Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), Ibadan, Nigeria

Erneo B. Ochi[3]
[3]Department of Clinical Studies, School of Veterinary Medicine, University of Juba, Juba, South Sudan

Corresponding Author:-Ojja. CV[1]*

Abstract:-The investigation into the profiling of antimicrobial resistance in *E. coli* surpasses the study on the Production of laying chickens due to its potential ramifications on the poultry industry's finances. The excessive administration of drugs in poultry farming has led to the emergence of antimicrobial resistance (AMR), which ultimately renders treatment of infectious diseases ineffective and may even result in fatalities. To tackle this issue, this study aimed to eradicate and determine the prevalence of *E. coli*spp in samples from laying chickens, while also examining the rates of antimicrobial sensitivity and resistance shown by selected drugs. In order to accomplish this, a cross-sectional study design was employed, and Excel and STATA software was utilized for data analysis. The findings of this study revealed that *E. coli* were present in 95% of the 200 samples that tested positive, while the remaining 5% yielded negative results. Moreover, 287 isolates identified from the 200 swab samples with 59.6% classified as *E. coli*. Among these isolates, a significant 100% sensitivity to AM, 75% to AU, 69% to OFX, and 86% to CPX whereas *E. coli*spp was also found to be resistant to Septrin (94%), CN to 88%, and other antibiotics as well. It is noteworthy that the drug susceptibility experiment identified at least 19 patterns of multidrug resistance, which indicates the improper and excessive use of medications.

Keywords:- *Antimicrobial Resistance, Escherichia coli, Poultry, Ibadan, Nigeria.*

I. INTRODUCTION

Escherichia coli play a role in the regulation of gastrointestinal physiology (Daodu *et al.*, 2017). In instances of immunosuppression in avian species, these strains have the potential to induce secondary opportunistic infections (Abebaw et al., 2018). Investigations conducted by Kiiti et al. (2021)have identified *E. coli* as the predominant pathogen found in clinical laboratories. The overreliance on antibiotics has contributed to the emergence of antibiotic-resistant(Wang *et al.*, 2019; Zhu *et al.*, 2023). In various rural and peri-urban regions of Nigeria and other developing nations, antibiotics are readily accessible for purchase without prescriptions, resulting in misuse and compromising the efficacy of antimicrobial medications (Agyare et al., 2019; Exner *et al.*, 2017). Improper disposal of chicken waste contaminated with antibiotics can further contribute to the widespread dissemination of resistance traits within the community (Johnson et al., 2019; Joshua et al., 2018; Marshall & Levy, 2011). This investigation concentrates on antimicrobial resistance (AMR) in prevalent bacterial pathogens, particularly *E. coli* with the goal of identifying gaps in knowledge concerning the extent and prevalence of this concern. Immediate public health interventions and recommendations for farmers are imperative in addressing this issue and promoting responsible antibiotic usage to enhance poultry productivity and bolster food security. The objective of the study is to ascertain the antimicrobial resistance profiles of *E. coli* isolated from laying chickens.

II. MATERIALS AND METHODS

A. Sample Collection

200 healthy laying chickens were randomly selected from 10 farms in Ibadan, Nigeria, and sterile cotton swabs were utilized to collect cloacal swabs from each chicken.

B. Samples Preservation

Following collection, the swab samples were promptly placed in a cold box containing ice packs to maintain their integrity during transportation to the laboratory for subsequent analysis.

C. Isolation and Identification of Escherichia Coli Spp.

To isolate *Escherichia coli* strains, the cloacal fecal swabs were placed in buffered peptone water-filled bottles and incubated at 37°C for 24 hours. Subsequently, the collected swabs were streaked onto agar plates using loop sticks and incubated again at 37°C for 24hr. The resulting isolates were then sub-cultured on different agar plates to obtain pure cultures, which were subsequently used to incubate broth cultures at 37°C for an additional 24 hr. at a concentration of 100 mg/L. The growth of bacteria was recorded based on various characteristics such as size, elevation, media changes, and fermentation. Lactose fermenting gram-negative bacteria exhibited pink colonies, while non-lactose fermenting gram-negative bacteria

produced opaque, off-white colonies, and subjected to a series of Mueller-Hinton and EMB agar tests to determine their likely identity.

D. Antibiotic Susceptibility Testing of Escherichia Coli Spp

Assessment of the antimicrobialresistance/susceptibility patterns in the *Escherichia coli* isolates was conducted using the disc diffusion technique as recommended by the Clinical and Laboratory Standards Institute(CLSI, 2008). This technique involved the use of various commonly employed antibiotics, including Septrin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Sparfloxacin (10 µg), Amoxicillin (30 µg), Augmentin (10 µg), Gentamycin (30 µg), Pefloxacin (30 µg), Streptomycin (30 µg), and Trarivid (10 µg). Sterile forceps were employed to place the antibiotic discs on sterile solidified Muller-Hinton agar, and the seeded agar plates were subsequently incubated at 37°C for 24 hours. Following incubation, the plates were further incubated aerobically at 37°C for another 24 hours, and the diameters of the respective zones of inhibition were measured and interpreted according to the guidelines set forth by the Clinical and Laboratory Standards Institute.

E. Study Design

A cross-sectional descriptive study was conducted in Ibadan, Nigeria, with the aim of collecting fecal samples from apparently healthy chickens in 10 poultry farms/LGAs over the course of one month. It is important to note that any chicken displaying symptoms or signs of illness was excluded from the study.

F. Data Analysis

The collected data, including the sample checklist and laboratory results, were entered into Microsoft Excel 2010 and subsequently analyzed using STATA 17.0 software. Multiple linear regression analysis was employed to compare data from different sample categories, with statistical significance set at $p<0.05$. The results were presented in the form of tables and graphs, displaying frequencies and percentages.

III. RESULTS

A. Isolation and Identification of Escherichia Coli Spp.

Out of 200 bacteria isolates, 95%*E. coli* strains tested positive (*Fig.* 1).

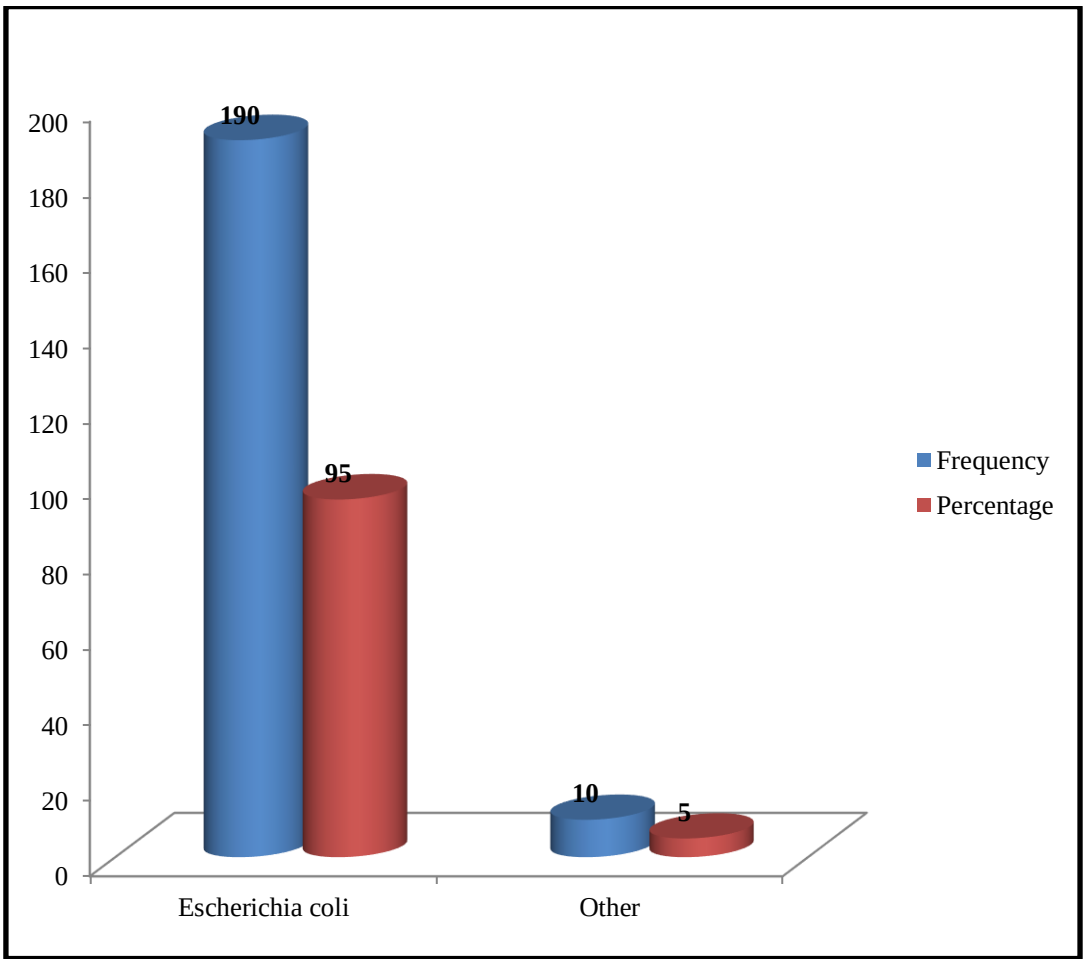

Fig. 1: Isolation and Identification of Escherichia Coli Isolated in Ibadan, Nigeria.
Note:More Positive E. Coli Strains found at the Rate of 95%

B. Prevalence of E. Coli Bacteria

The frequency of *E. coli* bacteria during isolation. 121 was the frequency obtained from *E. coli*(*Fig.* 2) (P<0.05). At least 287 of bacteria isolates were obtained of which 171 or 59.6% belongs to *E. coli*(P<0.05) (*Fig.* 3).

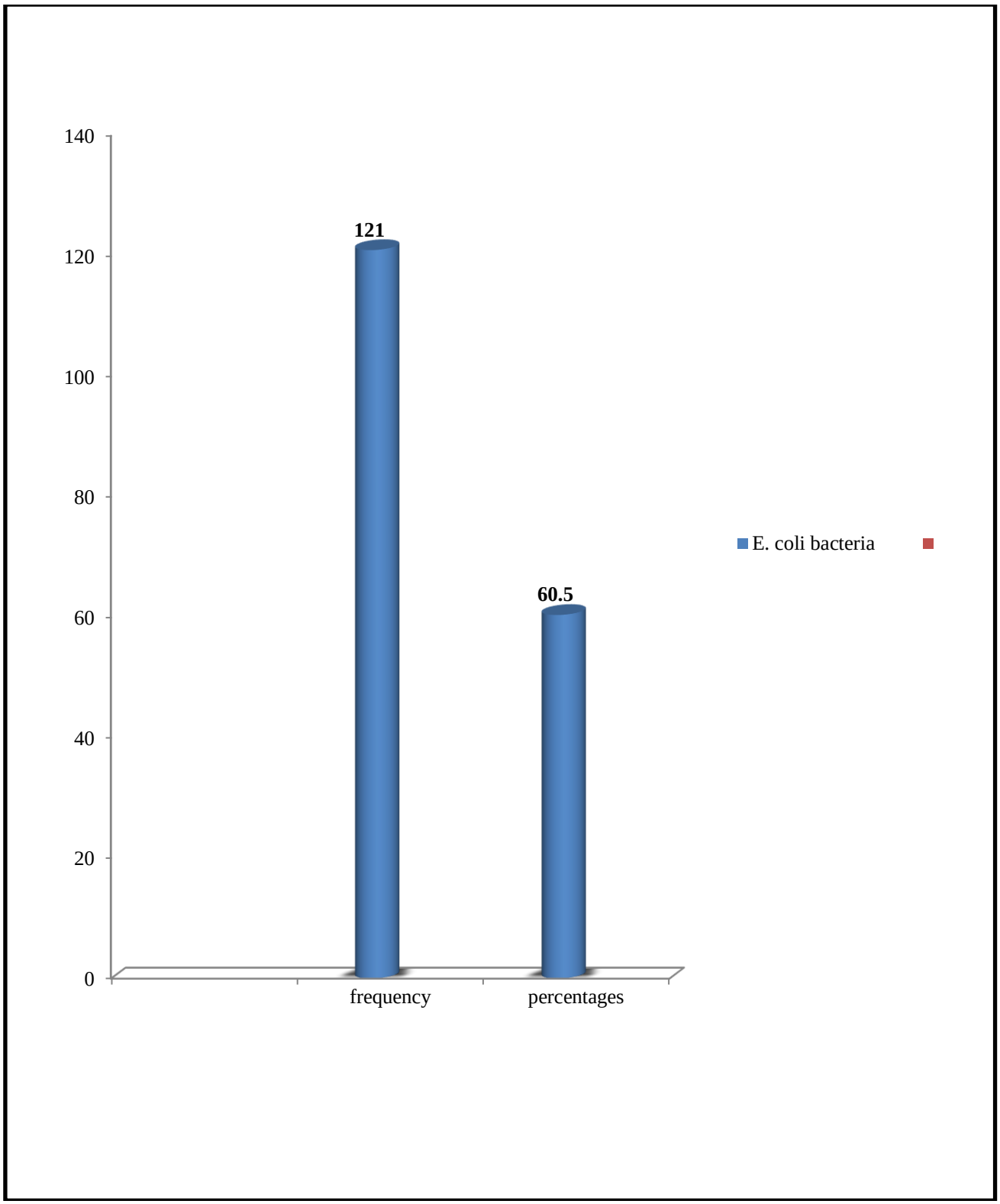

Fig. 2: Frequency of E. Coli Bacteria during Isolation. 121 or 60.5% was the Frequency Obtained from E. Coli

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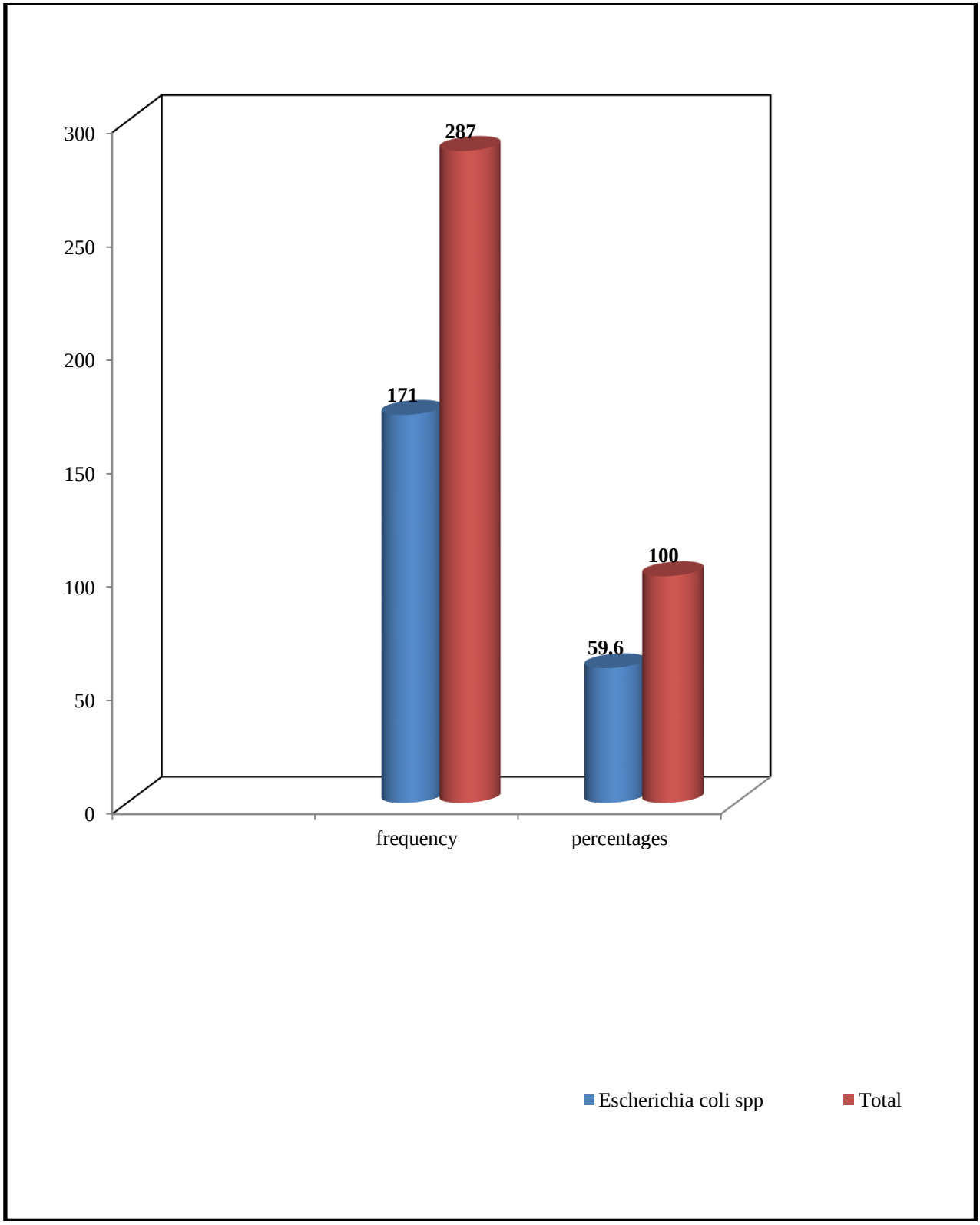

Fig. 3: Prevalence of E. coliIsolates in Ibadan, Nigeria.Note:287 Isolates Recorded, of which (n= 171 or 59.6%) belong to E. coli Isolates (P<0.05).

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C. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility patterns of the isolates represented both the sensitivity and resistance pattern of the study. 171 (59.6%) were *Escherichia coli* isolates, which showed 100% sensitivity to AM,75% to AU, 69 % OFX, and 86 to CPX. Whereas *E. coli* found to be resistant to Septrin (94%), CN (88%), and other antibiotics (Table 1).19 antimicrobial resistance patterns(Table 2).

Table 1: Antimicrobial Susceptibility Testing of *E. coli Strains*

Antibiotics	*Escherichia colispp*	
	Sensitive n (%)	Resistant n (%)
AM	171 (100)	0 (0)
AU	128 (75)	43 (25)
OFX	118 (69)	53 (31)
SP	10 (6)	161 (94)
CPX	147 (86)	24 (14)
PEF	125 (73)	46 (27)
CH	100 (58)	71 (42)
SXT	50 (29)	121 (71)
S	48 (28)	123 (72)
CN	21(12)	150 (88)

AM=amoxicillin; AU=Augmentin; OFX=Tarivid; SP=sparfloxacin; CN=gentamicin; CPX=ciprofloxacin; PEF=pefloxacin; CH=chloramphenicol; SXT=Septrin; S=streptomycin

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Table 2: Antimicrobial resistance patterns of *E. coli*strains

Antibiotic patterns	*E. coli strains*	*Types of patterns*
OFX	2	Mono resistance
SP	1	Mono resistance
AM	1	Mono resistance
SXT	1	Mono resistance
OFX-CPX	1	Double resistance
OFX-AM	1	Double resistance
OFX-SXT	1	Double resistance
OFX-S-CH	1	Triple resistance
OFX-S-SXT	1	Triple resistance
OFX-S-CH-AU	1	Quadruple resistance
S-CH-CPX-AM-CN	1	Quintuple resistance
S-CH-AM-CN-PEF-SXT	7	Sextuple resistance
Total	**19**	

AM=amoxicillin; AU=Augmentin; OFX=Tarivid; SP=sparfloxacin; CN=gentamicin; CPX=ciprofloxacin; PEF=pefloxacin; CH=chloramphenicol; SXT=Septrin; S=streptomycin

IV. DISCUSSION

In the present investigation, the detection and categorization of *E. coli*strainsyielded a prevalence rate of 95% with statistical significance at P<0.05. This study aligns with the preceding research conducted by (Ibrahim et al., 2019), wherein it was explicitly affirmed that the emergence and dissemination of ESBL-E associated with poultry, particularly chickens, are of specific concern. Moreover, Abdallah et al.(2022), explicitly indicated that *E. coli strain* is a prevalent enteric pathogen, with certain strains capable of causing both human and animal ailments.The overall occurrence of *Escherichia coli* strains was found to be more frequent compared to other bacterial species, with a prevalence rate of 13.6% in Ibadan, Nigeria, which is consistent with the earlier investigation conducted by Abebaw et al. (2018). A majority of previous studies have demonstrated that the shedding frequency of *Escherichia coli* strains from chicken feces exhibits substantial variation based on the timing of sample collection, the age of the chickens, and the dietary regimen provided to them (Exner et al., 2017). Unfortunately, poultry farms have played a role in the contamination of the environment with antibiotic-resistant bacteria, primarily belonging to the *Escherichia coli* strains, which are transmitted to chickens, livestock, and humans through direct contact or consumption of contaminated food products (Moawad et al., 2018; Nhung et al., 2017). The prevalence of *Escherichia coli* poses a significant challenge for commercial poultry breeders worldwide, as elucidated by (Rahman et al., 2020), leading to severe illness among chickens, substantial economic losses, and complications in disease transmission. Insufficient adherence to recommended antimicrobial treatment regimens or the improper stockpiling of medication for future use in chickens can culminate in inappropriate usage, thereby fostering the development of resistance in bacterial strains (Barton, 2000).

V. CONCLUSION

The results of this study indicate a significant prevalence of *Escherichia coli* strains in cloacal fecal samples, and the excessive use of tetracycline antibiotics as growth promoters in chickens has contributed to the emergence of antimicrobial resistance.

- Ethical Approval: The author seekeda letter of ethical approval for data collection from University of Ibadan.
- Conflict of Interest: Throughout the course of this study, the author did not encounter any conflicts of interest.
- Funding: The African Union Commission, specifically through the PAULESI program in Ibadan, Nigeria, provided full funding for this research project.

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