# Prevalence and Molecular Characterization of Antibiotic-Resistant *Escherichia coli* O157:H7 Isolated from Human and Poultry Faeces in the Federal Capital Territory, Nigeria

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## ABSTRACT

This study accessed the prevalence and molecular characteristics of antibiotic-resistant *Escherichia coli* O157:H7 in humans and poultry within the Federal Capital Territory (FCT), Nigeria. A total prevalence of 9% was observed in humans, with 7.5% in Internally Displaced Persons (IDP) camps and 10.5% in hospitals, while poultry showed a prevalence of 0.8%. The isolates exhibited multiple antibiotic resistance, with 100% resistance to oxytetracycline in both human and poultry samples. Extended-Spectrum Beta-Lactamase (ESBL) enzymes were detected, and virulent genes including *rfbE*, *eaeA*, *stx1*, and *stx2* were identified. Phylogenetic analysis revealed close genetic relationships between human and poultry isolates, suggesting possible cross-transmission and zoonotic potential. These findings highlight the urgent need for judicious antibiotic use, regular surveillance, and molecular monitoring to guide antimicrobial therapy and prevent outbreaks. The study underscores the importance of adopting a One Health approach to address antimicrobial resistance and safeguard public health.

**Keywords**: Escherichia coli O157:H7, Antibiotic resistance, Oxytetracycline, Zoonosis and Extended-Spectrum Beta-Lactamase (ESBL)

## INTRODUCTION

*Escherichia coli* (*E. coli*) *i*s a Gram-negative enteric bacterium and a significant zoonotic foodborne pathogen worldwide (Hassan *et al.*, 2021; Abebe, *et al.*, 2020). It is grouped based on virulence factors and infection types: intestinal pathotypes [Entereopathogenic *E. coli* (EPEC), Entereotoxigenic *E. coli* (ETEC), Entereohaemorrhagic *E. coli* (EHEC) like O157:H7, Entereoadhesive *E. coli* (EAEC), Entereoinvasive *E. coli* (EIEC), Diffuseadherent *E. coli* DAEC] and extra intestinal pathotypes [Uropathogenic *E. coli* (UPEC), Meningitis-associated *E. coli* MNEC, Avian Pathogenic *E. coli* APEC] (Riley, 2020). Shiga-toxin producing *E. coli* (STEC) O157:H7 is responsible for intestinal and extra-intestinal disease syndromes in humans and animals, causing numerous outbreaks globally (Naidoo and Zishir, 2025).

*E. coli* O157:H7 and non-O157 STEC can cause hemorrhagic colitis HC, hemolytic uremic syndrome HUS, thrombotic thrombocytopenic purpura TTP, and death. Isolation has been reported worldwide, but transmission pathways remain unclear (Meng *et al*., 2012). Globally, STEC causes about 2.8 million acute illnesses annually, with thousands of HUS cases and deaths (Joseph *et al.,* 2020). In the US, *E. coli* O157:H7 was first recognized in 1982, with 390 outbreaks between 2003–2012 leading to thousands of illnesses and deaths. Similar outbreaks have been reported worldwide, including Germany and Africa, where O157:H7 contributes significantly to disease burden (Ison, 2016). The first African case was reported in 1990 in South Africa, with notable prevalence in Nigeria (Azubuike *et al.*, 2018). Shiga toxins (Stx1 and Stx2), especially Stx2, are implicated in severe disease and HUS (Joseph *et al*., 2020. Symptoms include abdominal pain, watery diarrhea, and progression to bloody diarrhea. These toxins bind to eukaryotic cell receptors, causing tissue damage (Cohen, 2022).

Antimicrobial resistance (AMR) is a major global threat, responsible for millions of deaths annually and projected to rise annually (Tang *et al.*, 2023). Multiple antibiotic resistance (MAR) in pathogens like *Salmonella, Proteus*, and *E. coli* O157:H7 has emerged due to widespread antimicrobial use in food animals (Grudlewska-Buda *et al*., 2023; Gambushe *et al*., 2022). Transmission from beef and poultry to humans is well-documented, with recent reports in Nigeria (Sanni, 2024). Commonly used antibiotics in poultry include tylosin, doxycycline, streptomycin, chloramphenicol, oxytetracycline, ciprofloxacin, neomycin, nitrofurantoin, gentamicin, and colistin, facilitating resistant strain spread (Fadipe and Hölzle, 2025).

## MATERIALS AND METHODS

### Study Area

The research was conducted in the Federal Capital Territory (FCT), Nigeria, which covers about 8,000 km² and consists of six Area Councils of Abaji, Kwali, Gwagwalada, Kuje, Abuja Municipal Area Council AMAC and Bwari (Madu, 2023; Ibilewa 2018). The FCT is mainly inhabited by the Gbagyi people, alongside other ethnic groups such as Hausa, Fulani, Yoruba, and Igbo, each engaged in farming, trade, politics, or civil service (Bawa, 2025). Agriculture is the backbone of the economy, with crops like maize, millet, yams, and beans widely grown (Ayuba *et al.*, 2025). Poultry farming is significant, with 117 commercial farms reported in 2025, many establishing digital presence (Aminullah *et al.*, 2025). There are five Internally Displaced Persons (IDP) camps in the FCT, located in poor sanitary conditions, lacking amenities, and prone to water-borne and infectious diseases (Ekoh *et al*., 2023).

### Study Design

A cross-sectional epidemiological study design was adopted to investigate the prevalence and molecular characteristics of antibiotic-resistant *Escherichia coli* O157:H7 in human stool and poultry fecal samples within the Federal Capital Territory (FCT), Nigeria. A multi-stage sampling technique was employed. Three area councils (AMAC, Kwali, Gwagwalada) were selected by simple random sampling, and one major hospital from each council was purposively chosen: National Hospital (AMAC), University of Abuja Teaching Hospital (Gwagwalada), and General Hospital Kwali (Kwali). Stool samples of diarrhea patients were purposively collected. Two IDP camps (Kuchingoro and Karamajiji) were purposively selected, mainly occupied by children under 10 years, and stool samples from children and few adults were collected.

Commercial Production Poultry (CPP) farms with 2,500–10,000 birds in the three councils (totaling 58 farms) were listed, and three farms from each council were selected by simple random sampling. Faecal samples of birds in battery cages were purposively collected.

### Sample Size Estimation

Sample size determined as described by Thrusfield (2018): Prevalence by Robert *et al*.2002.

The calculated sample sizes of 383 for poultry and human were each increased by 5% to increase precision and rounded to 400, resulting in 400 human stool samples and 400 poultry fecal samples. In total, 800 samples were analyzed.

### Faecal Sample Collections from Poultry Farms

Faecal samples were collected from three commercial production poultry farms in AMAC, Gwagwalada, and Kwali (total 400 samples) over four weeks, following Siwela *et al.* (2007), and transported in ice packs to Namiroch Research Laboratory, Kwali.

### Stool Sample Collections from Hospitals and IDP Camps

A total of 400 stool samples were purposively collected from three hospitals (AMAC (70), Gwagwalada (70), Kwali (60)) and two IDP camps (100 each from Kuchingoro and Karamajiji), with assistance from laboratory scientists and health workers, following Adeleke and Omafuvbe (2011). Samples were transported daily in ice packs at -4 to -8°C to Namiroch Research Laboratory for storage and analysis.

## Laboratory Analysis

### Sample Preparation

All samples were refrigerated before analysis, serially diluted, and plated on agar media as described by Diana *et al*. (2017). Media Preparation and Sterilization Media were prepared according to manufacturers' instructions and sterilized by autoclaving or hot air oven.

The Nutrient Broth (Fluka, USA) was prepared and sterilized at 121°C for 15 minutes, refrigerated. *Escherichia coli* (EC) Broth (Oxoid, UK) was prepared as per APHA guidelines, sterilized at 121°C for 15 minutes. Nutrient Agar (Fluka, USA) was also prepared, autoclaved, cooled in slants, and refrigerated. Eosin Methylene Blue Agar (DIFCO, USA) was prepared, sterilized, cooled, dispensed into Petri dishes, incubated at 37°C for sterility check. And Cefixime–Tellurite Sorbitol MacConkey Agar (HIMEDIA, UK) was prepared with supplement, sterilized, cooled, dispensed into Petri dishes, incubated at 37°C for sterility check.

### Bacterial Culture and Identification

### Bacterial Isolation

Samples were inoculated into EC broth, streaked onto EMB agar, and incubated. *E. coli* colonies were identified by metallic sheen and confirmed through biochemical tests (Indole, Methyl red, Voges-Proskauer, Citrate, Catalase, Oxidase, Motility, Gelatin liquefaction, Triple sugar iron, Urease, $H_2S$ production, Sugar fermentation). Presumptive isolates were further confirmed on CT-SMAC agar for *E. coli* O157:H7 (Alu *et al.*, 2021)

Biochemical characterization of the isolated colonies was performed using the following tests: Indole Test confirmed indole production from tryptophan with a dark pink layer. Methyl Red Test confirmed acid production with a cherry-red color. Voges-Proskauer Test indicated positive results with pink to burgundy color. Citrate Utilization Test confirmed citrate use with a color change to blue. Catalase Test confirmed catalase enzyme production with bubble formation. Oxidase Test confirmed oxidase activity with a dark blue, maroon, or black color change. Motility Test confirmed motility with diffuse growth radiating from the inoculation line. Gelatin Liquefaction Test confirmed gelatinase production with liquefaction of gelatin. Triple Sugar Iron (TSI) Test confirmed glucose fermentation with $CO_2$ gas production, lactose and sucrose fermentation with a yellow slant, and absence of $H_2S$ production with no blackening. Gram Staining Technique classified bacteria as Gram-positive (blue/purple) or Gram-negative (pink/red).

### Identification and Characterization of *E. coli* O157:H7

Presumptive *E. coli* isolates were confirmed on Sorbitol MacConkey Agar with cefixime-tellurite supplement (CT-SMAC), where O157 appeared as colorless colonies while non-O157 strains fermented sorbitol and appeared pink. Antimicrobial susceptibility was tested using Kirby-Bauer disk diffusion with ten commonly used antibiotics from different classes, and results interpreted by CLSI guidelines to classify isolates as sensitive, intermediate, or resistant. Multiple Antibiotic Resistance (MAR) strains were identified, and ESBL production confirmed by double-disc diffusion test (DDDT) with ceftazidime and ceftazidime plus amoxicillin-clavulanic acid discs. Molecular confirmation was performed using Multiplex PCR assay targeting rfbE, eaeA, stx1, stx2, blaTEM, blaSHV, and blaCTXM genes, with DNA extracted by Phenol-Chloroform Method and analyzed by Agarose Gel Electrophoresis (AGE).

### PCR Amplification Reactions

The amplification reaction mixture consisted of 2µl of extracted template DNA mixed with primers for blaTEM, blaSHV, blaCTX-M1, CTX-M2, CTX-M9, rfbE, eaeA, stx1, stx2, probes, and nuclease-free water. The PCR cycling conditions included pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, 30 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 5 minutes.

**Gel Electrophoresis and Visualization under UV Lights through Transilluminator**

PCR products were analyzed in 1.5% agarose gel with ethidium bromide, run at 100 volts for 60 minutes, and visualized under a UV transilluminator. Bands corresponding to expected sizes confirmed the presence of rfbE, eaeA, stx1, stx2, blaCTX-M1,2,9, blaSHV, and blaTEM genes in *E. coli* O157:H7 isolates.

**Sequencing and Phylogeny of *E. coli* O157:H7-ESBL Positive Isolates from Human and Poultry in FCT**

Amplified PCR fragments were purified using Nucleospin Gel Extraction Kit and sequenced with Inqaba Biotech tools. Results were analyzed using BLAST, and phylogenetic trees were constructed to show relatedness of human and poultry isolates.

**Data Analysis**

Data analysis were performed using Excel Spreadsheet 2016. Chi-square tests, relative risks (RR), and odd ratios (OR) were calculated, and results presented in tables and charts.

## RESULTS

The prevalence of *E. coli* across the five selected sites was 179 (44.8%), while *E. coli* O157:H7 was 36 (9%). In IDP camps, Kuchingoro recorded 20 (20%) for *E. coli* and 5 (5%) for *E. coli* O157:H7, while Karamajiji had 37 (37%) and 10 (10%) respectively. In hospitals, Gwagwalada showed 51 (72.9%) and 11 (15.7%), Kwali 24 (36.9%) and 4 (6.2%), and AMAC 47 (72.3%) and 6 (9.2%) for *E. coli* and *E. coli* O157:H7 respectively.

Table 1: Prevalence of *E. coli* and *E. coli* O157:H7 in Humans in FCT

| **Location** | **No. of Samples** | ***E. coli*** | ***E. coli* O157:H7** |
|---|---|---|---|
| Kuchingoro IDP | 100 | 20 (20%) | 5 (5%) |
| Karamajiji IDP | 100 | 37 (37%) | 10 (10%) |
| **IDP Prevalence** | **200** | **57 (28.5%)** | **15 (7.5%)** |
| National Hospital | 65 | 47 (72.3%) | 6 (9.2%) |
| UATH Gwagwalada | 70 | 51 (72.9%) | 11 (5.7%) |
| Kwali Gen. Hospital | 65 | 24 (36.9%) | 4 (6.2%) |
| **Hospital Prevalence** | **200** | **122 (61 %)** | **21 (10.5%)** |
| **Overall Prevalence** | **400** | **179 (44.8%)** | **36 (9%)** |

**$\chi^2$ = 27.6, p= 0.0014, P< 0.05, OR =13, RR= 12.4**

Overall, across the three area councils, the prevalence of *E. coli* in poultry in the FCT was 20 (5%), while *E. coli* O157:H7 had a prevalence of 3 (0.8%). The prevalence in AMAC was 4 (3%) for *E. coli* and 0 for *E. coli* O157:H7; in Gwagwalada 8 (5.9%) for *E. coli* and 2 (1.5%) for *E. coli* O157: H7; in Kwali 8 (6.2%) for *E. coli* and 1 (0.8%) for *E. coli* O157:H7 respectively (Table 2).

Table 2: Prevalence of *E. coli* and *E. coli* O157:H7 in Poultry in FCT

| **Location** | **No of Samples** | ***E. coli*** | ***E. coli* O157:H7** |
|---|---|---|---|
| AMAC | 135 | 4 (3%) | 0 (0.0 %) |

| | | | |
|---|---|---|---|
| Gwagwalada | 135 | 8 (5.9%) | 2 (1.5%) |
| Kwali | 130 | 8 (6.2%) | 1 (0.8%) |
| **Total** | **400** | **20 (5%)** | **3 (0.8%)** |

*Escherichia coli* O157:H7 in humans showed 100% resistance to Oxytetracycline, 71.8% to Amoxicillin-clavulanate, 28.2% to Erythromycin, Nitrofurantoin, and Sulphamethoxazole-Trimethoprim, and 25.6% to Ciprofloxacin. The lowest resistance was observed for Gentamicin (0.3%), Ceftazidime (2.6%), Meropenem (2.6%), and Colistin (2.6%). The multiple antibiotic resistance index (MARI) was calculated at 0.6 (Figure 1).

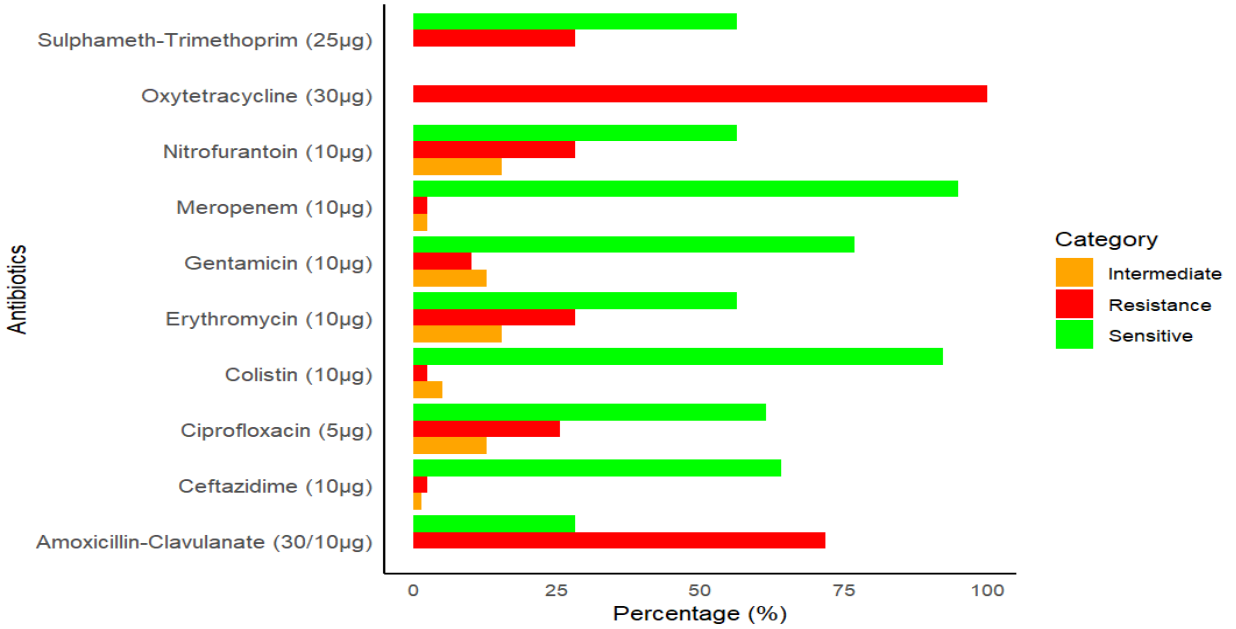


Figure 1: Antimicrobial Susceptibility Profile of *E. coli* 0157:H7 Isolates in Human (FCT)

*E. coli* O157:H7 isolates from poultry showed complete (100%) resistance to oxytetracycline, sulphamethoxazole-trimethoprim, nitrofurantoin, erythromycin, gentamicin, and colistin. Ciprofloxacin resistance was 90%, while amoxicillin-clavulanate, ceftazidime, and meropenem recorded 60%, 30%, and 10% resistance respectively. The multiple antibiotic resistance index (MARI) was notably high at 0.9 (Figure 2).

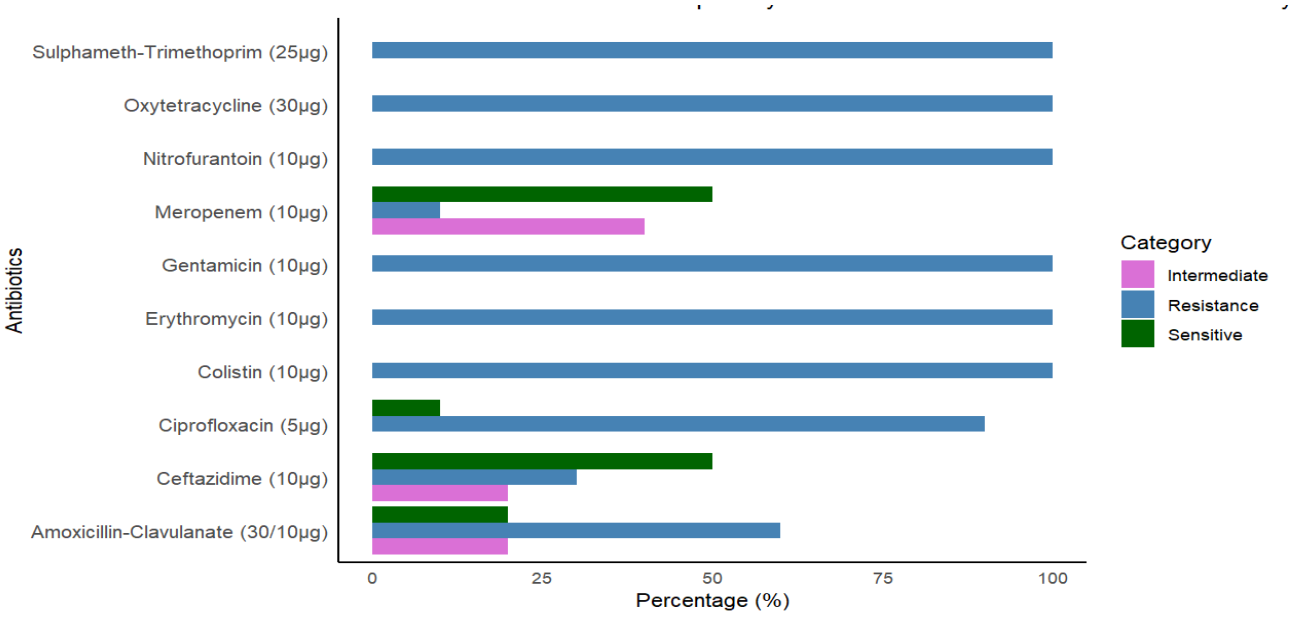


Figure 2: Antimicrobial Susceptibility Profile of *E. coli* 0157:H7 Isolates in Poultry

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The ESBL positive isolates from Kuchingoro IDP (AMAC) were 2 (40.0%); Karamajiji IDP (AMAC), 2 (20%); National Hospital (AMAC), 0; UATH (Gwagwalada) 1 (16.7%); and General Hospital (Kwali), **1 (25%) (Figure 3).**

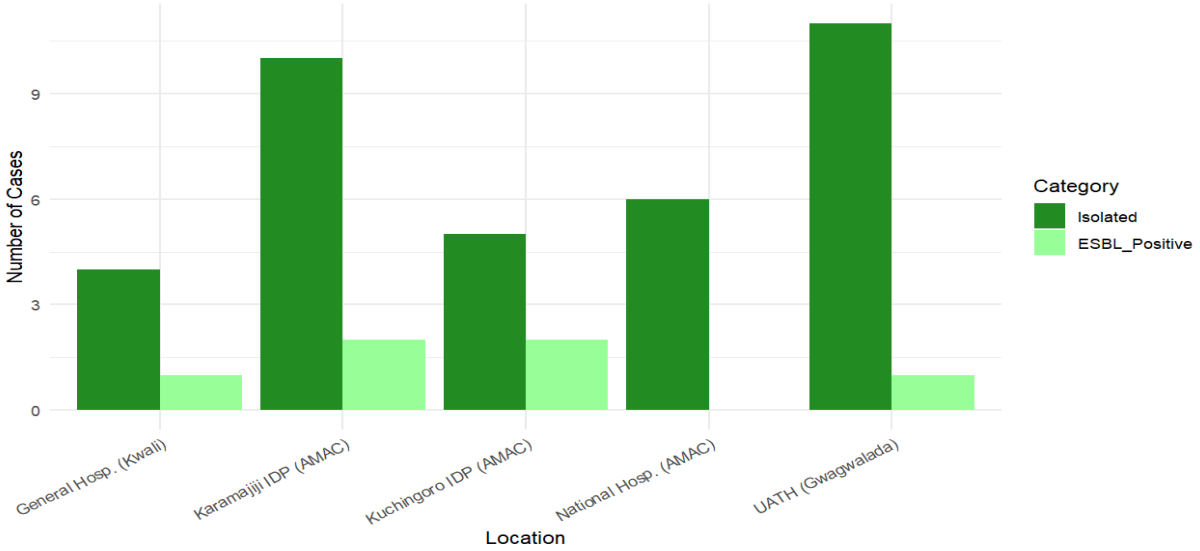


Figure 3: E. coli O157:H7 ESBL Production and Distribution in Humans in FCT

There was no ESBL positive *E. coli* O157:H7 isolated from AMAC farms, 0; Gwagwalada was 1 (100%); and Kwali farms 1 (50%) (Figure 4).

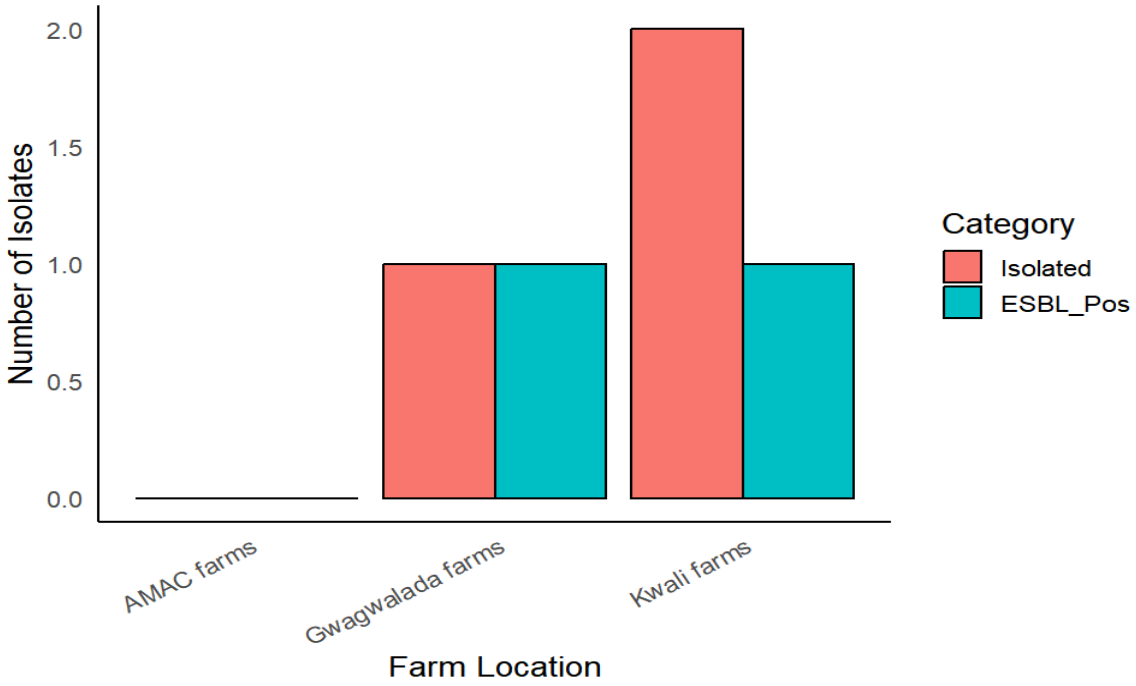


Figure 4: *E. coli* 0157:H7 ESBL Production in Poultry (FCT)

The agarose gel electrophoresis (1.5%) confirms the successful amplification of virulence and antibiotic resistance genes in *Escherichia coli* O157:H7 isolates from human and poultry sources. Human isolates H1, H2, and H3 consistently show the presence of *rfbE* and *eaeA*, with additional resistance genes such as CTX-M1, CTX-M2, CTX-M9, and TEM variably detected, indicating pathogenic and multidrug-resistant profiles. Poultry isolates P4 and P5 also exhibit *rfbE* and *stx2*, with P4 showing a broader resistance pattern including CTX-M variants and SH, suggesting a highly virulent strain. The gel image validates the multiplex PCR assay and highlights the genetic diversity and resistance potential of *E. coli* O157:H7 strains circulating in both human and poultry populations.

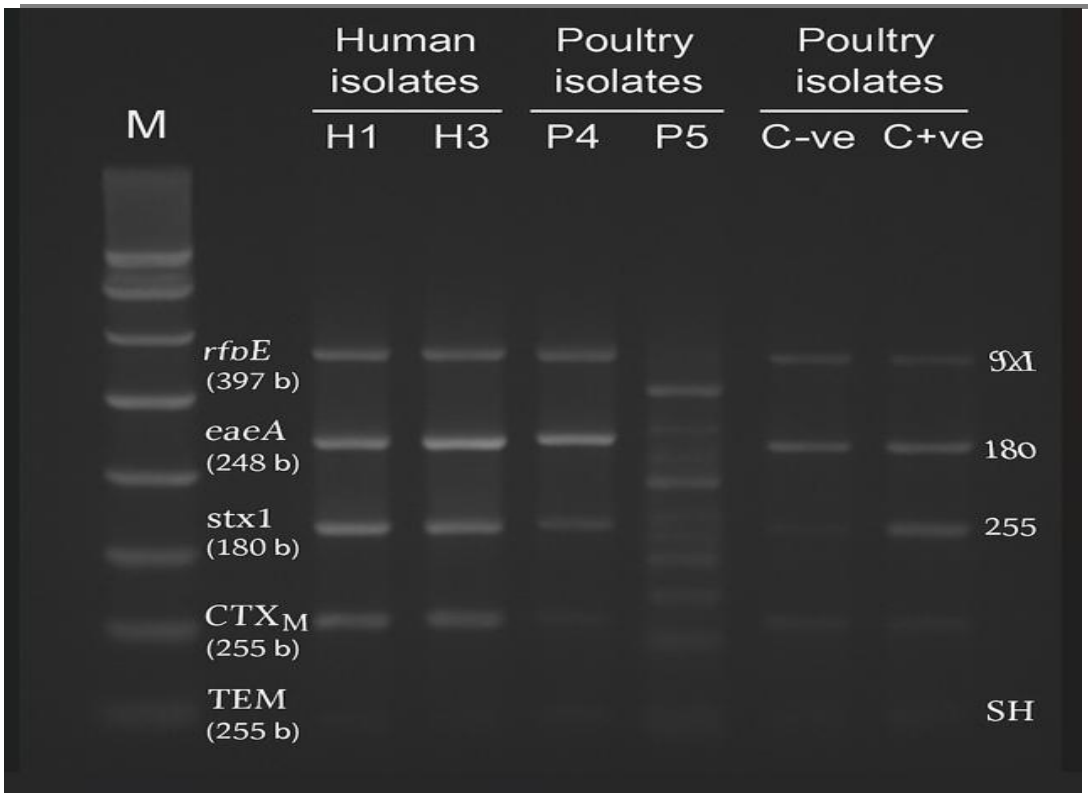


**Plate 1: Agarose Gel Electrophoresis (1.5%) of Multiplex PCR Products**

The phylogenetic tree analysis of *E. coli* O157:H7 ESBL-positive isolates in the FCT showed that the evolutionary relationships were rooted using 16S rRNA genes and core genome SNPs, with *E. coli* O55:H7 serving as the reference strain. The numbered nodes represent points of shared ancestry, from which lineages diverged. Genetic distances between isolates, measured in SNPs, ranged from a few to several hundred, reflecting a largely homogeneous population with occasional gene acquisitions and chromosomal rearrangements driven by prophages and plasmids. The evolutionary history indicates a gradual transition from sorbitol-positive (SOR+) ancestors to the typical sorbitol-negative (SOR–), beta-glucuronidase-negative progenies, highlighting the stepwise emergence of pathogenic lineages.

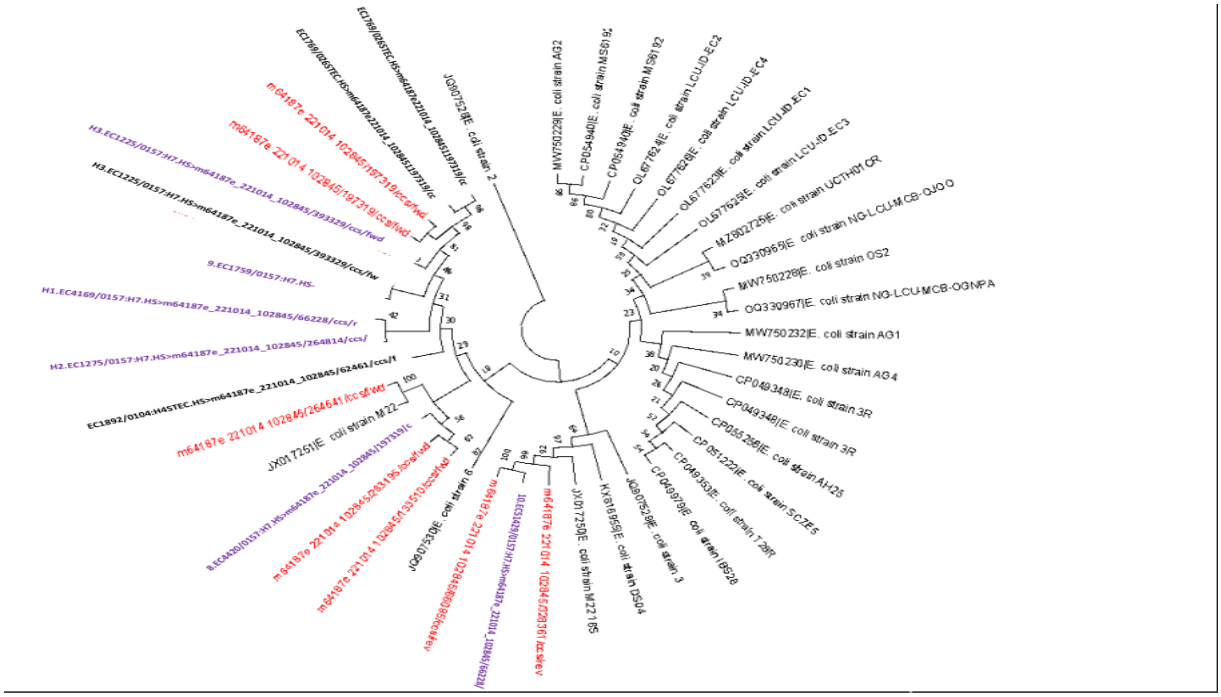

Figure 5: Human Phylogenetic Tree of *E. coli* O157:H7 identified from the Study (in purple) relative to the reference *E. coli* O157/other non-O157 Serotypes (in red) and *E. coli* strains (in black) from the GenBank

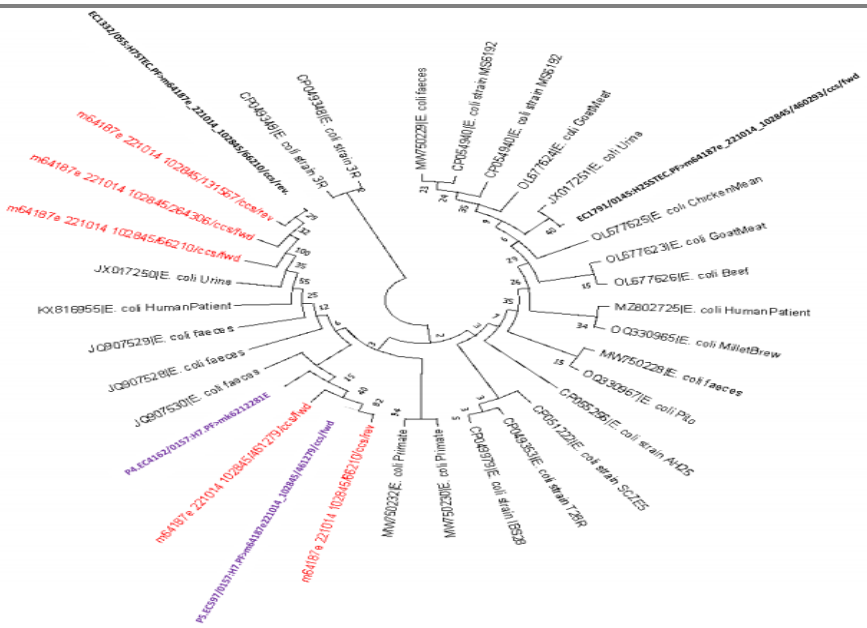


Figure 6: Poultry Phylogenetic Tree of *E. coli* O157 identified from the Study (in purple) relative to the reference *E. coli* O157/other non-O157 Serotypes (in red) and *E. coli* strains (in black) from the GenBank

## DISCUSSION

This study investigated the prevalence of antibiotic-resistant *Escherichia coli* O157:H7 in humans and poultry in FCT, Nigeria. The study found a prevalence of 9% in humans (7.5% in IDP camps; 10.5% in hospitals) and 0.8% in poultry. The IDP camps had poor sanitary conditions, which may have contributed to the high prevalence. The study showed multiple antibiotic resistance (MAR) in *E. coli* O157:H7 from both humans and poultry. In humans, the highest resistance was to oxytetracycline (100%), amoxicillin-clavulanate (71.8%), and erythromycin (28.2%). In poultry, *E. coli* O157:H7 showed 100% resistance to oxytetracycline, sulphamethoxazole-trimethoprim, nitrofurantoin, erythromycin, gentamycin, and colistin. The study also detected Extended-Spectrum Beta-Lactamase (ESBL) enzymes in *E. coli* O157:H7, which confer resistance to different antibiotics. The Multiplex PCR assay amplified the target virulent and resistant genes, and Agarose Gel Electrophoresis (AGE) separated the genes based on molecular weights.

The study identified the virulent genes rfbE (100%), eaeA (100%), stx1 (33% human; 100% poultry), and stx2 genes (33% human; 50% poultry). The phylogenetic tree constructed from the identified *E. coli* O157:H7 showed a marked close phylogenetic relationship with other *E. coli* strains, especially the Shiga toxin-producing non-O157:H- *E. coli* (STEC) group. The study suggests possible cross-transmission and zoonosis of *E. coli* O157:H7 infections from poultry to human and vice versa. The findings highlight the need for regular surveillance and monitoring to guide antimicrobial therapy.

## CONCLUSION

The study reported a prevalence of 7.5% in IDP camps, 10.5% in hospitals, and 0.8% in poultry in FCT, providing data that will guide policy makers in making decisions on the prevalence of *E. coli* O157:H7 during outbreaks and the choice of antibiotics. *E. coli* O157:H7 is pathogenic even at very low doses (10–100 CFU) and poses serious public health risks, especially in children under 5 years, as it can cause hemorrhagic colitis, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and death. The study showed a MAR and 100% resistance to Oxytetracycline in both humans and poultry, emphasizing the need for clinicians and veterinary doctors to avoid Oxytetracycline and other tetracycline drugs due to 0% susceptibility, as their use may lead to treatment failures and further outbreaks. The specificity of AMC combo in the Kirby-Baur Double Disk Synergy Test (DDST) was equivocal, with a MAR Index of 0.6 and resistance rates of 72% in humans and 60% in poultry, suggesting AMC use may produce false negatives. The study detected and separated the *eaeA* virulent gene, which may serve as a candidate for developing new antibiotics, as

conventional ones are increasingly compromised by microbial lactamase enzymes. Targeting *eaeA* could help solve MAR. The Multiplex PCR assay detected *E. coli* O157:H7, its Shiga toxin genes (*stx1* and *stx2*), and non-O157 STEC strains, highlighting their public health importance. MPCR offered advantages such as confirming detection without reliance on costly *rfbE* primers, using Shiga toxin profiles as genetic markers, differentiating *stx1* and *stx2* to aid HUS management, and serving as a clue for STEC detection. Finally, the phylogenetic tree analysis of *E. coli* O157:H7 in humans and poultry, and its relatedness to GenBank reference serotypes, will help trace ancestral origins, guide antibiotic choices during outbreaks, and support new drug development by linking susceptibility patterns to evolutionary lineages.

## RECOMMENDATION

1. The FCT Health Management Board (HMB), through the FCT Ministry, should curb the prevalence of microorganisms of public health importance by utilizing the data provided in this study.

2. Antibiotics in the tetracycline class should not be used in humans or animals to avoid treatment failures and unnecessary costs. Instead, carbapenems (e.g., meropenem) should be considered for effective therapy.

3. The use of AMC in the Double Disk Synergy Test (DDST) for detecting ESBL should be discouraged, as it may yield false results. More sensitive antibiotics should be employed to improve diagnostic accuracy.

4. Future pharmaceutical molecular research should focus on developing antibiotics that target the *eaeA* gene, thereby preventing bacterial attachment to host cells and reducing virulence.

5. Phylogenetic tree construction and sequencing of detected serotypes and strains should be incorporated into outbreak screening to trace ancestral relationships and guide cost-effective, non-resistant antibiotic treatments in humans and animals.

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### Conflicts Of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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### ETHICAL APPROVAL

The study protocol was approved by the Ethical Clearance Committee (ECC) of the FCT Health Department. Informed consent was obtained from poultry farm owners, hospital authorities, IDP camp managers, and participants, with the study's purpose and scope clearly explained before consent.

### Data Availability

The data supporting this research are available from the corresponding author(s) upon reasonable request.

**Author's Contributions**

The author conceived and designed the study, collected and processed the samples, and performed the laboratory experiments including culture, biochemical characterization, antibiotic susceptibility testing, multiplex PCR assays, and phylogenetic analysis. The author analyzed and interpreted the data, prepared the tables, figures, and charts, and wrote the manuscript. Guidance and supervision were provided by the project supervisors, while technical assistance was received from laboratory technologists. All contributions were made under the author's direct coordination, and the final manuscript was reviewed and approved by the supervisors.

**Provenance And Peer Review**

Not commissioned; externally peer reviewed.

**Disclaimer**

The views and opinions expressed in this research are solely those of the authors and do not necessarily reflect the official policy or position of the University of Abuja or any affiliated institution.

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