Scientific Reports

https://doi.org/10.1038/s41598-026-36471-9

Article in Press

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Received: 21 October 2025

Accepted: 13 January 2026

Published online: 05 February 2026

Cite this article as: Okyere I.J., Semevor G.O., Ablordey A. *et al.* Genomic profiling of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from poultry and poultry farm workers in Accra, Ghana. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-36471-9

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Genomic Profiling of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Poultry and Poultry Farm Workers in Accra, Ghana.

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Abstract: Background: Antimicrobial resistance (AMR), driven by the extensive use of antibiotics in human and animal health, poses a significant global threat. In Ghana, the contribution of poultry farming to the high prevalence of AMR remains underexplored. This study investigates the genomic characteristics and prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in poultry and human populations. **Methods**: A total of 300 cloacal swabs from poultry and 60 stool

samples from poultry farm workers in peri-urban Accra were collected from 20 poultry farms and cultured. Bacterial isolates were identified through MALDI-TOF-MS, with ESBL production confirmed using the double disk synergy test. Whole-genome sequencing of 17 multi-drug resistant isolates selected was conducted on the MiSeq Illumina platform to characterize resistance genes, virulence genes, and sequence types. **Results**: ESBL production was detected in 84.8% (n = 123/145) in isolates from poultry and 67.5% (n = 27/40) in isolates from humans.. All isolates were resistant to cefotaxime, with significant resistance to tetracycline and sulfamethoxazole-trimethoprim also recorded. The *bla*$_{\text{CTX-M-15}}$ gene was the most prevalent ESBL gene identified, with additional genes including *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{OXA\text{-}1}$, *bla*$_{OXA\text{-}181}$, *bla*$_{TEM\text{-}1B}$, and *bla*$_{DHA\text{-}1}$ also identified. Sequence typing revealed multiple resistance-associated sequence types, notably ST10 and ST155. Plasmid replicon analysis identified IncF, Col, and IncI1 groups, many co-occurring with multiple resistance genes. Virulome profiling revealed the presence of avian pathogenic *E. coli* (APEC)-associated genes such as *iroN*, *iss*, *ompT*, and *hlyF*. **Conclusions**: This study highlights the prevalence and genomic characteristics of ESBL-producing E. coli at the human–poultry interface in Ghana, emphasizing poultry as a potential reservoir for multidrug-resistant bacteria. The findings provide actionable insights for small- to medium-scale poultry farmers, including the importance of prudent antibiotic use, enhanced hygiene, and biosecurity practices, and underscore the need for ongoing

genomic surveillance to guide interventions aimed at reducing the spread of antimicrobial resistance in Ghana.

Keywords: Antimicrobial resistance; Extended-spectrum beta-lactamase; whole-genome sequencing; Poultry farming; *Escherichia coli*; Ghana

Introduction

Antimicrobial resistance (AMR) has emerged as a significant global public health challenge, with an alarming potential to claim up to 10 million lives annually by 2050 [1]. Among the various forms of resistance, Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria, particularly *Escherichia coli*, have gained prominence due to their association with severe infections and their role in the horizontal transfer of resistance genes. ESBLs are enzymes produced by Gram-negative bacteria, particularly members of the Enterobacteriaceae, that hydrolyze extended-spectrum cephalosporins, as well as monobactams, while remaining inhibited by β-lactamase inhibitors such as clavulanic acid. The ESBL phenotype is most commonly mediated by genes belonging to the $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} families, which are frequently plasmid-encoded and often co-located with resistance determinants to other antimicrobial classes, thereby contributing to multidrug resistance [2, 3].

The increasing prevalence of ESBL-producing *E. coli* has raised significant concerns in both healthcare and agricultural sectors, particularly in low-and middle-income countries (LMICs), where the overuse of antibiotics in human

medicine and livestock farming has become widespread [4, 5]. Poultry farming in Ghana is a widely practiced agricultural activity fueled by economic incentives, inadvertently increasing the risks of antibiotic misuse and overuse in these systems [6]. The emergence of ESBL-producing *E. coli* in poultry is particularly troubling, given their association with contamination in retail chicken and their potential to facilitate the transmission of resistance genes to human populations [7, 8, 9]. The close proximity of poultry farms to human settlements, often lacking appropriate biosecurity measures, intensifies public health concerns [10]. Despite the urgency, research exploring the dynamics of AMR dissemination between poultry and humans in Ghana has been limited, and primarily focused on human health [11]. This creates a critical gap in our understanding of the complex interplay between poultry farming practices, AMR, and potential zoonotic transmission routes. While existing studies on AMR in Ghana have contributed valuable insights, they predominantly rely on phenotypic analyses and do not include poultry farm workers [5], [12], [13] , which, although useful for identifying resistance patterns, do not provide a comprehensive understanding of the underlying genetic mechanisms driving resistance. Methods such as whole-genome sequencing and multi-locus sequence typing have been rarely applied, limiting our ability to map the genetic characteristics and potential transmission pathways of ESBL-producing *E. coli* across poultry and human populations [13, 14]. This gap is concerning, considering the potential risks associated with the spread of resistant strains and their implications for public health. Moreover, the unique

environmental and agricultural factors in Ghana, such as economic pressures leading to increased, often unregulated, antibiotic use, further exacerbate the potential spread of AMR. The lack of robust biosecurity measures in poultry farms, combined with the close integration of these farms within human communities, positions them as potential reservoirs for resistant bacteria [15, 16, 17]. Addressing these gaps in knowledge is essential for informing health policy and intervention strategies aimed at curbing the threat of AMR. This study sought to conduct genomic profiling of ESBL-producing *E. coli* isolated from poultry and poultry farm workers across 20 poultry farms in Accra, Ghana. By leveraging advanced genomic techniques, the study elucidated the genetic diversity, resistance gene profiles, and dissemination of ESBL-producing *E. coli*. The findings provide insights into the dynamics of AMR in the poultry-human interface, contributing to a broader understanding of its impact on public health.

2. Materials and Methods

2.1. Study site and sampling

This cross-sectional study was conducted in peri-urban areas of Accra, Ghana, focusing on small-to-medium-scale poultry farms and their workers to determine the prevalence and genomic characteristics of ESBL-producing *E. coli*. A total of 360 samples were collected from 20 poultry farms in November 2023, comprising 300 cloacal swabs from chickens and 60 stool samples from poultry farm workers. Each farm housed approximately 900–3000 chickens,

and 15 randomly selected chickens were sampled per farm, along with stool samples from three farm workers. Cloacal swabs were obtained from live chickens using sterile swabs pre-moistened with phosphate-buffered saline (PBS) (Oxoid, Basingstoke, Hampshire, UK) and transported in a cold box to the laboratory. Stool samples were self-collected by poultry workers in sterile containers, inoculated with PBS, and transported in a cold box to the laboratory within 24 hours of collection. Participants were selected based on their willingness to participate and their involvement in poultry farming.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (CHS-Et/M.2-P 4.6/2021–2022). All methods were performed following the relevant guidelines and regulations and in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.2. Isolation and Identification

Cloacal swabs were incubated aerobically at 37 °C for 24 h, while approximately one gram of each human stool sample was inoculated into buffered peptone water (Oxoid, Basingstoke, Hampshire, UK) immediately upon arrival and incubated at 37 °C for 24 h. Immediately after incubation, a 10 µL loopful of the culture was streaked onto selective MacConkey agar plates (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 4 µg/mL cefotaxime. The inoculated plates were incubated at 37 °C for 24 hours under aerobic

conditions. Following overnight incubation, morphologically distinct colonies were observed, and up to five pink to reddish isolated colonies indicative of lactose fermentation from each sample were selected. These colonies were then sub-cultured on Nutrient agar (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 hours to obtain pure cultures. The pure cultures were initially screened using conventional biochemical tests (Triple sugar iron, Citrate utilization tests, Urease, and Sulphur Indole motility biochemical tests) and subsequently identified using the Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF, Bruker, Billerica, MA, USA) analyzer. Isolates identified as *E. coli* were retained for downstream ESBL confirmation and genomic analyses while non–*E. coli* Enterobacterales identified were excluded from further analyses. For long-term storage and future analyses, three to five representative colonies of the pure cultures of *E. coli* were preserved in skim-milk-tryptone-glucose-glycerol broth at −80 °C.

2.3. Phenotypic detection of ESBL-producing E. coli

The phenotypic detection of ESBL production was carried out utilizing the double disk synergy test, following the guidelines established by the WHO [18]. Mueller-Hinton agar plates (Oxoid, Basingstoke, Hampshire, UK) were used as the growth medium. Briefly, an inoculum preparation of a bacterial suspension equivalent to a 0.5 McFarland standard was done, and the suspension was inoculated onto the agar plates using sterile cotton swabs to ensure uniform growth. After allowing the plates to air-dry for 3-5 minutes, cefotaxime (30µg), and ceftazidime (30µg), were placed approximately 20 mm

from the centrally positioned amoxicillin/clavulanic acid (20/10µg) disk using sterile forceps. The plates were incubated for 18 hours at 37°C under aerobic conditions and then examined for synergy effects indicated by increased zones of inhibition surrounding the cephalosporin disks, which confirmed positive ESBL production.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *E. coli* isolates was conducted using the Kirby–Bauer disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. Pure colonies were selected and suspended in sterile saline, and the bacterial concentration was adjusted to a 0.5 McFarland standard using a BD PhoenixSpec™ nephelometer (Beckton Dickinson, Sparks, MD, USA). The standardized suspension was uniformly spread across Mueller-Hinton agar plates (Oxoid, Basingstoke, Hampshire, UK) using sterile cotton swabs to achieve confluent bacterial growth. Commercially available antibiotic disks (Oxoid, Basingstoke, Hampshire, UK) were carefully placed on the agar surface. The antibiotics tested included amikacin (30 µg), amoxicillin-clavulanate (20/10 µg), tetracycline (30 µg), gentamicin (10 µg), cefoxitin (30 µg), piperacillin-tazobactam (100/10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), sulfamethoxazole-trimethoprim (10 µg), meropenem (10 µg), ceftazidime (30 µg), and cefotaxime (30 µg). The plates were then incubated at 37°C under aerobic conditions for 24 hours. A quality control strain, *E. coli* ATCC 25922, was included in each batch to validate the results and ensure accuracy. The inhibition zone diameters surrounding the antibiotic

disks were measured and recorded into WHONET 2024 software for interpretation [20].

2.5. Whole Genome Sequencing and Bioinformatic Analysis

DNA extraction was performed on 17 ESBL-producing *E. coli* isolates, including 10 obtained from poultry and 7 from poultry farmworkers, selected based on their similar multidrug-resistant patterns. The isolates were cultured on nutrient agar after overnight incubation at 37°C under aerobic conditions, and DNA was extracted using the QIAamp® DNA mini kit (QIAGEN Inc. GmbH, Holden, Germany) according to the manufacturer's protocol. The quality and concentration of genomic DNA were assessed using the NanoDrop™ 2000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA). Genomic libraries were generated with the Illumina DNA prep kit, adhering to the manufacturer's guidelines for the Illumina MiSeq technology. The libraries were pooled and subsequently loaded onto the MiSeq Illumina sequencer (Illumina Inc., San Diego, CA, USA) to generate 250 bp x 2 paired-end reads for each ESBL-producing *E. coli* isolate. All raw sequences were submitted to NCBI and assigned a Bioproject accession number, PRJNA1208549.

The raw sequenced reads underwent quality assessment and trimming, using FASTQC v0.12.1 and Trimmomatic v0.36 [21]. Trimmed reads were then *de novo* assembled using the SPAdes assembler. Multi-locus sequence typing analysis was conducted on the assembled genomes using the MLST 2.0 tool

hosted by the Center for Genomic Epidemiology (CGE) (http://cge.cbs.dtu.dk/services/MLST/) according to the Achtman scheme, which identifies sequence types (STs) based on variations in seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, and recA) [22]. Isolates that could not be assigned ST through conventional MLST were further analyzed using core genome MLST (cgMLST) in EnteroBase to determine their cgSTs. *In silico* analysis was conducted using the ResFinder tool v4.6.0 [23] hosted on the CGE website at default threshold ID (90%) and minimum length (60%) values to identify acquired antimicrobial resistance genes. Virulome and plasmid analysis were also conducted using the VirulenceFinder 2.0 tool [24] and PlasmidFinder 2.1 [25] hosted on the CGE website to identify the virulence-associated genes. The phylogroups classification of the *E. coli* genomes was then performed using the in silico Clermon-Typing 1.4.1 tool [26] at default parameters before whole-genome sequences were uploaded to CSIPhylogeny to analyze the evolutionary relatedness [27] among the 17 ESBL-producing *E. coli* and identify single-nucleotide polymorphisms (SNPs), by comparing assembled sequences to the reference genome ASM584v2 (*Escherichia coli* str. K-12 substr. MG1655). The generated tree was visualized and annotated using Interactive Tree of Life, ITOL v6 [28].

2.6. Data Analysis

Statistical analyses were conducted to evaluate differences in antibiotic resistance patterns between poultry and human isolates. Fisher's exact test was applied separately to each antibiotic resistance profile to determine

whether resistance frequencies varied significantly between the two sources. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using R v4.4.1 software

3. Results

3.1. Prevalence of ESBL-Producing E. coli

In this study, we assessed the prevalence of ESBL-producing *E. coli* within poultry farms and among poultry farm workers. A total of 189 third-generation cephalosporin-resistant bacterial isolates were obtained from 300 cloacal swab samples (n = 149/300) and 60 fecal samples (n = 40/60) collected from poultry workers. The isolated bacteria were predominantly *E. coli* (n = 185/189, 97.9%), with a smaller number of *Klebsiella pneumoniae* (n = 3/189, 1.6%) and *Enterobacter cloacae* (n = 1/189, 0.5%). Out of 185 *E. coli* isolates recovered, 150 were phenotypically confirmed as ESBL producers. Among poultry-derived isolates, 84.8% (n = 123/145) were confirmed as ESBL-producing *E. coli*. Among isolates recovered from poultry farm workers, 67.5% (n = 27/40) were confirmed as ESBL-producing *E. coli*. Notably, 16 out of the 20 farms exhibited at least one positive henhouse for ESBL-producing *E. coli*, while at least one human from 10 farms also tested positive for ESBL-producing *E. coli*. Farm-level analysis revealed significant variation in ESBL prevalence. At the farm level, the proportion of samples from poultry testing positive for ESBL-producing *E. coli* ranged from 40% (n = 6/15) to 60% (n =

246 9/15), while the carriage among humans ranged from 33.3% (n = 1/3) to
247 66.7% (n = 2/3) per farm (Table S2).

248 The antibiotic susceptibility testing of the 150 ESBL-producing *E. coli* isolates
249 showed widespread resistance across multiple antibiotic classes. All ESBL-
250 positive isolates from both poultry (n=123/123, 100%) and farm workers
251 (n=27/27, 100%) were resistant to cefotaxime, confirming the characteristic
252 β-lactam resistance conferred by ESBL production. Among the ESBL-producing
253 isolates, resistance to ceftazidime varied between poultry (n=118/123, 95.9%)
254 and humans (n=22/27, 81.5%). Similarly, tetracycline resistance was notable
255 in both poultry (n=121/123, 98.4%) and human (n=17/27, 63.0%) isolates.
256 Resistance to sulfamethoxazole-trimethoprim was observed in (n=112/123,
257 90.8%) poultry isolates and (n=19/27, 70.4%) of the human isolates (Table 1).

258 **Table 1.** Antimicrobial Resistance Profile of ESBL-producing *E. coli* isolates
259 from poultry (n = 123) and poultry farm workers (n = 27).

Antibiotics	**ESBL-positive isolates**		
	Poultry (n = 123)	**Humans (n = 27)**	***p*-value**
Amoxicillin/clavulanic acid (30µg)	31 (25.6%)	09 (33.3%)	0.634

Piperacillin-tazobactam (30µg)	43 (34.6%)	09 (33.3%)	1
Cefotaxime (30µg)	123 (100%)	27 (100%)	NA
Ceftazidime (30µg)	118 (95.9%)	22 (81.4%)	0.017
Cefoxitin (30µg)	43 (34.6%)	10 (37.0%)	0.828
Meropenem (10µg)	02	00 (0.0%)	1
Gentamicin (10µg)	31 (25.2%)	12 (44.4%)	0.065
Amikacin (30µg)	46 (37.2%)	10 (37.0%)	0.828
Tetracycline (30µg)	121 (98.4%)	17 (63.0%)	<0.001
Chloramphenicol (30µg)	38 (31.0%)	11 (40.7%)	0.821
Ciprofloxacin (5µg)	93 (75.6%)	16 (59.3%)	0.154
Sulfamethoxazole-trimethoprim (1.25/23.75µg)	112 (90.8%)	19 (70.4%)	0.002

NA: Statistical comparison not applicable because all isolates were 100% resistant. *The values represent the number of resistant isolates, with percentages given in parentheses.*

3.2. Distribution of acquired antimicrobial resistance genes

Genomic analysis of the 17 ESBL-producing *E. coli* isolates, selected based on their similar multidrug resistance patterns in both poultry and human sources, identified a significant diversity of antimicrobial resistance genes. Notably, a total of 32 acquired antimicrobial resistance genes were identified with *bla*$_{\text{CTX-}}$

$_{M\text{-}15}$ emerging as the most prevalent, present in all 17 isolates (Figure 1). Several other beta-lactamase genes were detected, including *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{DHA\text{-}1}$, *bla*$_{TEM\text{-}1B}$, *bla*$_{TEM\text{-}35}$, *bla*$_{OXA\text{-}1}$, and *bla*$_{OXA\text{-}181}$. The *bla*$_{OXA\text{-}181}$ gene was observed exclusively in isolates that also exhibited resistance to meropenem. Beyond beta-lactam resistance, genes conferring resistance to aminoglycoside (*aph(6)-Id, aph(3')-Ia, aph(3'')-Ib, ant(3'')-Ia*, *aadA2*), folate inhibitors (*sul1, sul2, dfrA12, dfrA14*) and quinolones (*aac(6')-Ib-cr, qnrS1*, *qepA4*) were also observed.

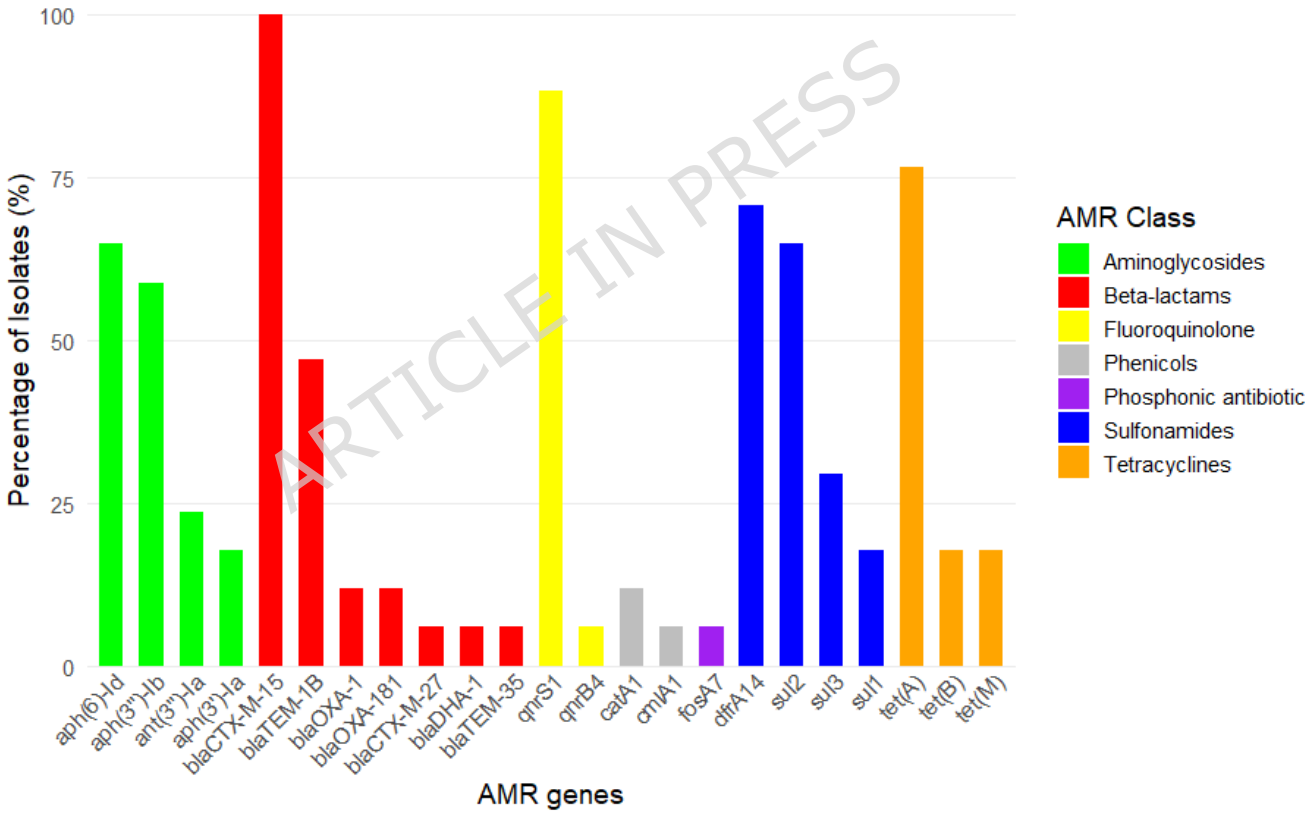


Figure 1. Antimicrobial resistance gene distribution.

3.3 Distribution of Beta-lactam resistance genes by source

The patterns of beta-lactam resistance genes in the ESBL-producing *E. coli* isolates from human and poultry sources reveal interesting trends. The gene $bla_{CTX\text{-}M\text{-}15}$ was the most commonly identified resistance gene in both groups, with a higher prevalence in poultry samples (n = 10) compared to human isolates (n = 7). In addition to $bla_{\text{CTX-M-15}}$, the gene $bla_{TEM\text{-}1B}$ was frequently detected, again showing greater occurrence in poultry (n = 6) than in humans (n = 2). Notably, other beta-lactam resistance genes such as $bla_{OXA\text{-}1}$, $bla_{OXA\text{-}181}$, $bla_{CTX\text{-}M\text{-}27}$, and $bla_{TEM\text{-}35}$ were unique to poultry samples, whilst $bla_{DHA\text{-}1}$ (n = 1), was identified only in one isolate from the human samples. (Figure 2).

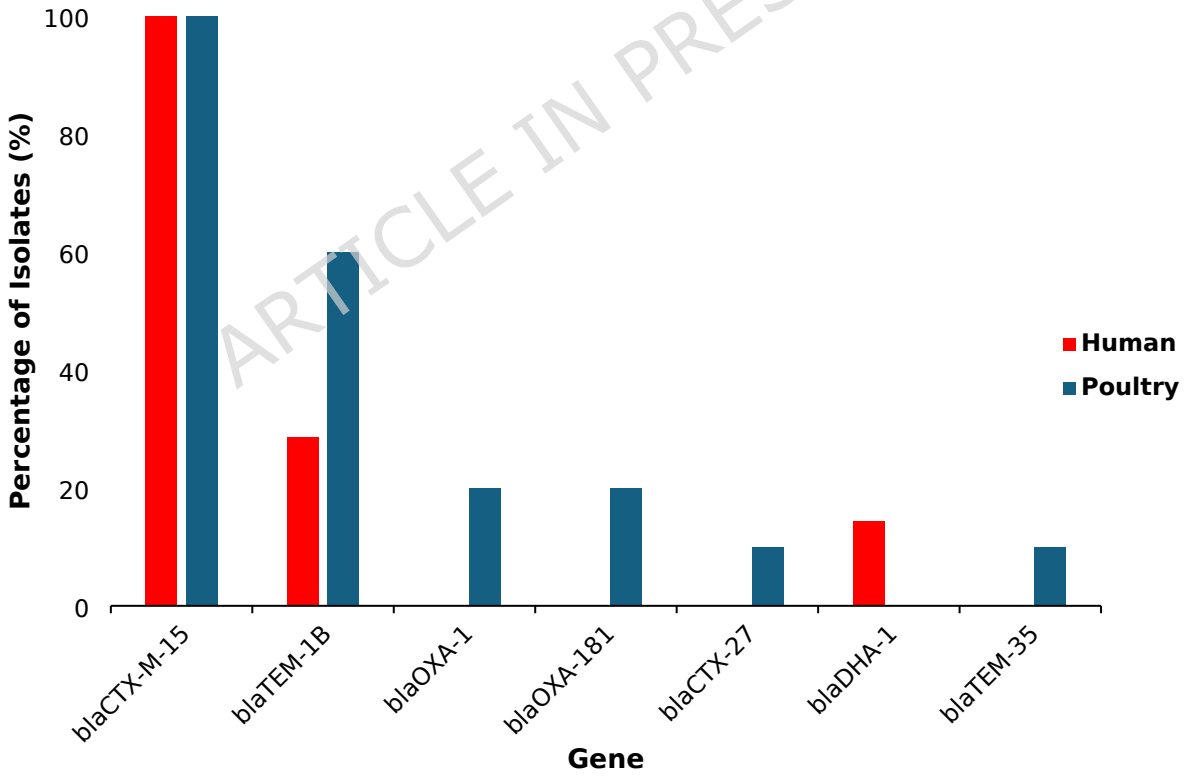


Figure 2. Distribution of Beta-lactam resistance genes by source.

3.4. Virulence factors Distribution in Poultry and Human ESBL-producing E. coli Isolates

A virulome analysis conducted using the Virulence Finder tool from CGE identified 25 unique virulence-associated genes (VAGs) across the 17 ESBL-producing *E. coli* isolates. Among these, the most prevalent VAGs were *fimH*, present in 88% of the isolates (n = 15/17), followed closely by *terC* at 82% (n = 14/17), *lpfA* at 59% (n = 10/17), and *gad* at 41% (n = 7/17). The identified virulence factors were categorized based on their functional roles, including adhesion molecules, toxin production, iron acquisition systems, immune evasion, and acid resistance (Figure 3).

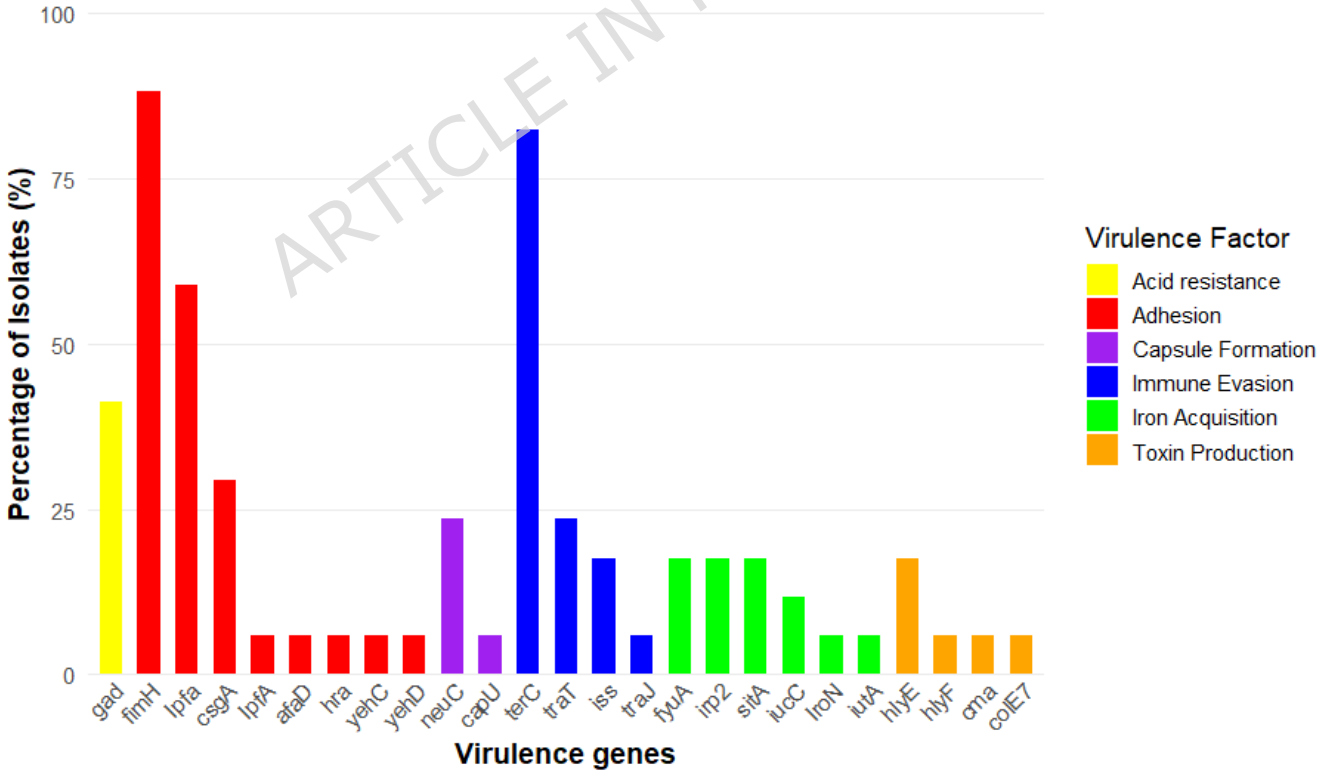
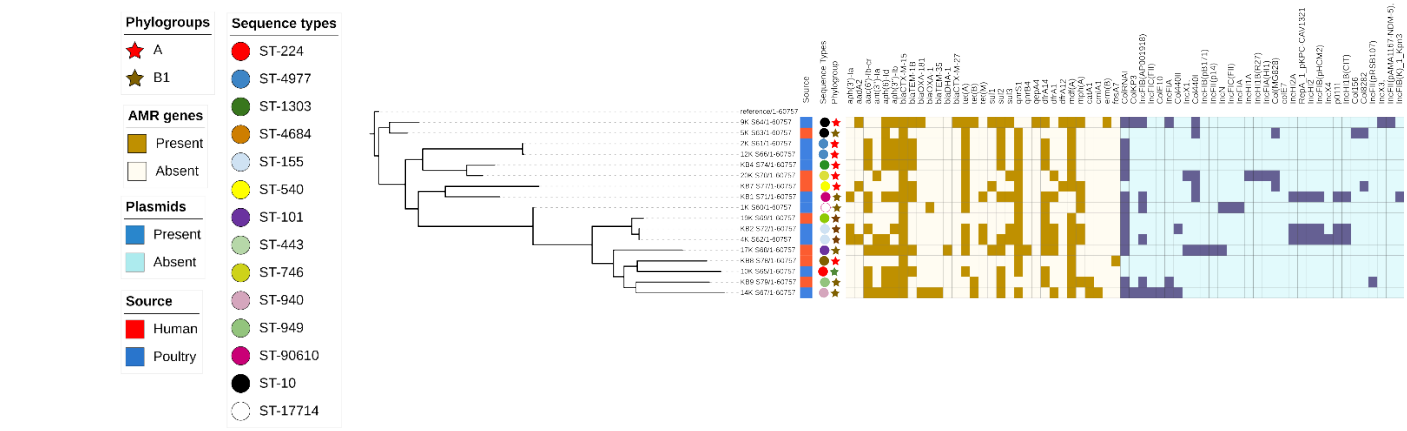


Figure 3. Distribution of Virulence genes in the ESBL-producing *E. coli* isolates.

3.5. Phylogenetic analysis of ESBL-producing E. coli isolates

The phylogenetic analysis of the ESBL-producing *E. coli* isolates among the strains derived from human and poultry sources (Figure 4) classified the isolates into two major phylogroups, A and B1, with B1 predominantly associated with poultry-derived strains, while A was more evenly distributed between human and poultry isolates. Twelve sequence types (STs) were identified, including ST-224, ST-746, and ST-10, with the identification of two novel sequence types. Two isolates that could not be assigned STs through conventional MLST were further analyzed using cgMLST in EnteroBase, which identified them as cgST 17714 (1K S60) and cgST 90610 (KB1 S70). Thirty different plasmids were observed among the 17 ESBL-producing *E. coli* isolates sequenced from poultry and human sources. The most prevalent plasmid replicons belonged to Col (*ColRNAI*, *Col440I*, *ColKP3*, *ColE10*, *Col(MG828)*, *Col440I*, *Col156*, *Col8282, Col(MG828), Col8282)* and Inc (*IncY, IncFIB(AP001918), IncFIC(FII), IncFIA, IncX1, IncFIB(pB171), IncFII(p14), IncN*, *IncHI1A*, *IncHI1B(R27), IncFIA(HI1), IncHI2A*, *IncHI2*, *IncHI1B(CIT), IncX4, IncX3, IncFII(pAMA1167-NDM-5), IncFII(pRSB107), IncFIB(K)_1_Kpn3)* groups. The prevalent plasmid types were *ColRNAI* (n = 13/17), *IncY* (n = 7/17), *IncFIB (AP001918),* and *Col440I* (n = 6/17 each), as shown (figure 4). *ColRNAI* was the most prevalent in poultry (n = 9/17), whilst *Col440I* and *ColRNAI* (n = 4/17, each) were prevalent in the isolates from poultry farm workers.



323

324 **Figure 4.** A phylogenetic tree generated based on single-nucleotide
325 polymorphisms of the core genes of 17 ESBL-producing *E. coli* isolates,
326 showing resistance genes and plasmids against sequence types with reference
327 genome *E. coli* str. K-12 substr. MG1655 as an outgroup.

328 **4. Discussion**

329 Studies worldwide have shown increasing concerns regarding AMR impacting
330 both human and animal health [29, 30]. This study highlights the genomic
331 diversity of ESBL-producing *E. coli* from poultry and poultry farm workers in
332 Accra, Ghana.

333 The detection rates of ESBL-producing *E. coli* observed suggest potential
334 differences in exposure risks, biosecurity measures, or antibiotic use practices
335 [31], which may contribute to variations in colonization patterns between
336 poultry and farm workers. These ESBL-producing *E. coli* isolates showed
337 significant resistance to multiple antibiotics across different classes such as

tetracycline, ciprofloxacin, and sulfamethoxazole-trimethoprim, highlighting their multidrug-resistant nature and a widespread presence of antimicrobial-resistant ESBL-producing *E. coli* in both animal and human populations on farms. The findings align with previous studies that have similarly linked high rates of ESBL production in *E. coli* to the development of multidrug resistance in both poultry and human sources [32, 33, 34], reinforcing the notion that farming practices may be instrumental in shaping the resistance profiles of these pathogens. Notably, the observed resistance pattern was most evident against penicillin, tetracycline, aminoglycosides, and sulfonamides which are antimicrobials frequently employed for therapeutic purposes in both human medicine and the poultry industry in Ghana [5, 35]. The high resistance rates observed in poultry may reflect antibiotic exposure driven by agricultural practices such as the use of antibiotics for growth promotion and disease prevention. This trend raises questions about the efficacy of current antimicrobial stewardship practices, highlighting the urgent need for revised strategies to mitigate the selection and spread of resistance.

Whole genome sequencing revealed $bla_{CTX\text{-}M\text{-}15}$ as the most occurring ESBL gene in all the isolates. The predominance of $bla_{CTX\text{-}M\text{-}15}$ among the ESBL-producing *E. coli* isolates in this study aligns with its widespread distribution in both clinical and agricultural settings [14, 36]. CTX-M-15 has been frequently implicated in human infections and has been reported in various geographic regions, particularly in LMICs where the burden of antimicrobial resistance is high [37, 38, 39]. Its presence in Ghanaian hospital settings

361 further supports this trend, as previous studies have consistently identified
362 *bla*$_{CTX\text{-}M\text{-}15}$ as the dominant ESBL gene in clinical isolates [40]. A study reported
363 that all ESBL-producing *E. coli* isolates carried a *bla*$_{CTX\text{-}M}$ gene, with *bla*$_{CTX\text{-}M\text{-}15}$
364 detected in 98% of cases in 2016 at a hospital in Ghana [39]. Similarly, a more
365 recent study on ESBL-producing *E. coli* and *Klebsiella pneumoniae* isolates
366 from pediatric patients found that *bla*$_{CTX\text{-}M}$ was the most prevalent ESBL gene,
367 detected in 92.4% of isolates in a hospital in Ghana [41]. This trend is not
368 unique to Ghana but is also in other LMICs, where a high prevalence of the
369 *bla*$_{CTX\text{-}M}$ gene has been reported. In Senegal, *bla*$_{CTX\text{-}M\text{-}15}$ has been reported to
370 be the predominant ESBL gene detected in ESBL-producing *E. coli* isolated
371 from broiler chickens sold in open wet markets [42]. Similarly, prevalence
372 rates of 83-88% have been reported for the detection of *bla*$_{CTX\text{-}M\text{-}15}$ within
373 hospitals in Ethiopia [43, 44]. The detection of other beta-lactamase genes
374 such as *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{TEM\text{-}1B}$, *bla*$_{TEM\text{-}35}$, and *bla*$_{OXA\text{-}1}$, in this study, further
375 underscores the genetic diversity of resistance within these isolates and
376 highlights their functional significance in conferring broad-spectrum β-lactam
377 resistance. Although *bla*$_{OXA\text{-}1}$ and bla$_{\text{OXA-181}}$ were identified in a few isolates,
378 their presence warrants attention. *bla*$_{OXA\text{-}1}$ is often associated with resistance
379 to amoxicillin-clavulanate, compromising the effectiveness of β-lactamase
380 inhibitor combinations in treating infections caused by these resistant strains
381 [45, 46]. The bla$_{OXA\text{-}181}$ gene, identified only in isolates that were meropenem
382 resistant, suggests a potential role in conferring meropenem resistance in
383 these specific strains. bla$_{OXA\text{-}181}$ is a carbapenemase-producing gene and has

primarily been identified in enterobacteriaceae in hospitals in Ghana [44]. Although carbapenems are not used in poultry production, the presence of this gene in poultry-associated isolates may reflect horizontal transfer of carbapenemase genes within the farm environment. Detecting these clinically significant resistance genes in poultry highlights the need for close monitoring to control their spread into the broader community. While studies from Africa, including this study, indicate a high predominance of $bla_{CTX\text{-}M\text{-}15}$, research conducted in Europe reveals markedly lower rates of this gene in poultry isolates [47, 48]. This disparity emphasizes the regional variations in the prevalence of ESBL genes, which are likely influenced by differing antibiotic usage patterns, agricultural practices, and the robustness of monitoring frameworks [47].

A range of resistance determinants to non-beta-lactam antibiotics was also identified. These included genes conferring resistance to quinolones, aminoglycosides, tetracyclines, phenicols, and folate pathway inhibitors. These findings are consistent with those reported in similar studies [12, 49, 50]. The presence of such genes underscores a multidrug-resistant profile among the *E. coli* isolates from both poultry and human sources. This occurrence of resistance determinants to multiple antibiotic classes poses a significant challenge to existing antimicrobial therapies, as it greatly reduces the pool of effective antibiotics. The diversity and prevalence of these resistance genes highlight the adaptability and resilience of the ESBL-producing *E. coli* isolates. This emphasizes the urgent need for enhanced

regional surveillance and strategic antimicrobial stewardship measures to combat the spread of antimicrobial resistance effectively.

The genomic analysis further elucidated the genetic diversity present among the ESBL-producing *E. coli* isolates, revealing 12 STs that were grouped into two principal commensal phylogenetic groups: B1 and A. Phylogroup B1 was predominantly composed of isolates from poultry, suggesting host adaptation or persistence within the poultry environment. In contrast, phylogroup A included isolates from both poultry and farm workers, indicating potential genetic overlap and exchange across hosts. Within these, only ST10 emerged as a shared sequence type between poultry and human sources, both of which were recovered from the same farm. This co-occurrence points to a possible overlap between hosts within a shared environment, suggesting possible zoonotic potential. Conversely, ST155 and ST4977, found exclusively in poultry isolates, may indicate a possible adaptation to avian hosts. The association of ST10 and ST155 with high virulence and resistance-associated gene counts raises concerns about their pathogenic potential, as their adaptability and prevalence within microbial communities could facilitate the transfer of critical genetic material, complicating efforts to control the spread of antimicrobial resistance. Previous studies have established that these STs are prevalent in both poultry and human populations, with frequent identification in clinical settings [13, 51, 52]. These STs harbor a range of virulence factors, such as adhesion factors (*fimH*), iron acquisition systems (*fyuA*, *iroN*, *irp2*), serum resistance genes (*iss*), and hemolysins (*hlyF*), which

are commonly found in extraintestinal pathogenic *E. coli* strains [10, 53, 54]. This is consistent with our study, where ST10 and ST155 were predominantly identified with these virulence genes (fig S1). The overlap between these STs in agricultural and healthcare environments points to the risk of cross-species transmission and emphasizes the necessity for monitoring these strains.

Virulome analysis of the ESBL-producing *E. coli* isolates revealed a complex landscape of virulence factors linked to the pathogen's adaptability and survival in both host environments. The high occurrence of *fimH* and *lpfA* observed in this study highlights their critical roles in *E. coli* adhesion to host cells. *FimH,* a key virulence factor mainly associated with Uropathogenic *E. coli* encodes type 1 fimbriae [55] while *lpfA,* typically linked to Enterotoxigenic *E. coli* encodes long polar fimbriae [56]. Both facilitate bacterial attachment to epithelial surfaces, promoting colonization in poultry and humans [57, 58]. Together, these findings highlight the significant role of *fimH* and *lpfA* in enhancing the ability of the ESBL-producing *E. coli* isolates to adhere to host tissues, a key factor in their adaptability, persistence, and pathogenic potential across diverse environments. Notably, none of the isolates carried the set of virulence determinants defining diarrheagenic *E. coli* (DEC) pathotypes, suggesting that classical DEC lineages were absent in this population [59], [60], [61]. In contrast, several isolates harbored combinations of avian pathogenic *E. coli* (APEC)-associated genes such as *iutA* (aerobactin receptor gene), *traT* (outer membrane protein complement resistance), *iss* (serum survival protein), *hlyE; hlyF* (haemolysins), *ompT* (outer membrane

453 protease), and *iroN* (salmochelin siderophore receptor) [62], [63], [64]. These
454 findings indicate that while diarrheagenic lineages were not represented, the
455 isolates exhibited virulence characteristics of APEC-like strains. This overlap of
456 virulence determinants in both poultry and human isolates highlights their
457 zoonotic relevance and raises concerns about the potential for extraintestinal
458 pathogenic infections, particularly in agricultural settings where frequent
459 human–animal contact occurs.

460 The widespread presence of *terC* among 82% of the isolates suggests that
461 this gene plays a significant role in environmental stress resistance [65, 66],
462 its contribution to the survival of *E. coli* in harsh conditions cannot be
463 overlooked. This suggests a potential evolutionary advantage, especially in
464 agricultural settings where selective pressures, such as antimicrobial use, are
465 common. Additional insights were gained from the identification of the *gad*
466 gene in 41% of the isolates, indicative of an enhanced resilience to acidic
467 environments, an essential trait for pathogens colonizing the gastrointestinal
468 tract at low pH [67, 68].

469 The plasmid replicons in this study indicate the potential of these isolates to
470 acquire new genetic material and transfer AMR genes among similar species
471 and other pathogens via plasmid-mediated resistance gene transmission. The
472 frequent use of antibiotics in poultry farming exerts selective pressure,
473 fostering the persistence of resistance genes within poultry-associated
474 bacteria, which can be transferred to human pathogens [14, 69, 70, 71, 72].
475 Notably, resistance genes, such as $bla_{OXA-181}$, *qnrS1*, *tet(A),* and *sul2* were

detected together with the *ColKP3* plasmid replicon (Table S3), suggesting a robust capacity for conferring resistance to third-generation cephalosporins, tetracyclines, and fluoroquinolones, further complicating treatment options. Another notable plasmid group identified in this study is the Inc plasmid replicons with 19 different types observed as shown in figure 4. This group of plasmids is often identified in Enterobacteriaceae isolated from humans and animals [73, 74, 75, 76], where they serve as carriers of genes that code for resistance against beta-lactam antibiotics and other resistance genes that contribute to multidrug resistance mostly in *E. coli* [73, 74, 75, 76, 77, 78]. This aligns with the findings of this study, as these plasmids were observed to co-occur with resistance genes such as $bla_{TEM\text{-}1B}$, *qnrS1*, *dfrA1, tet(A)*, *ter(C)* and *aph(3'')-Ib* (Table S3), conferring resistance to beta-lactams, trimethoprim, tetracyclines, fluoroquinolones, and aminoglycosides, further emphasizing their role in the dissemination of MDR traits.

Given the proximity of poultry farms to human communities in peri-urban Ghana, the spread of AMR from animals to humans poses a public health risk, particularly in settings with limited biosecurity measures, as it facilitates the exchange of resistant bacteria between humans and animals and increases the risk of treatment failures in clinical settings. These findings call for targeted interventions, such as regulated antibiotic usage and enhanced biosecurity practices in poultry farms, to mitigate the risk of AMR spread.

Antimicrobial susceptibility testing in this study was performed using the disc diffusion method, which provides qualitative resistance profiles. Factors such

as agar thickness and potential AmpC β-lactamase overproduction may influence resistance interpretation. Future studies incorporating quantitative methods, such as broth microdilution for minimum inhibitory concentration determination, would allow more precise assessment of resistance levels. While this study provides valuable insights into AMR at the human–poultry interface in Ghana, several limitations should be considered. First, the human sample size was relatively small, which may limit the generalizability of the findings. Future studies should consider increasing the human sample size and incorporating more diverse populations. Additionally, environmental samples were not included, which could have provided further context on potential reservoirs of resistant strains. Also, expanding the WGS analysis to include a larger and more phenotypically diverse set of isolates would further enhance the robustness and representativeness of the genomic findings. In this study, only 17 ESBL-producing *E. coli* isolates selected based on similar multidrug-resistant patterns were subjected to WGS; therefore, the genomic results reflect this highly resistant subset and do not fully capture the genetic diversity of the total ESBL-producing *E. coli* population recovered. Also, including antibiotic usage data could have strengthened the conclusions by linking resistance patterns to specific antimicrobial exposures. Future research should incorporate longitudinal sampling, which would provide a more comprehensive understanding of the selective pressures driving AMR and allow for assessment of transmission dynamics in poultry farming systems.

5. Conclusions

This study provides insights into the prevalence and genomic characteristics of ESBL-producing *E. coli* at the human-poultry interface in Ghana, highlighting the significant role of poultry as a potential reservoir for antimicrobial-resistant bacteria. A high detection of ESBL production in *E. coli* isolates from both poultry and farm workers was observed, underscoring the public health risks associated with antimicrobial resistance at the human–poultry interface in peri-urban Ghana. We report the co-occurrence of the $bla_{CTX\text{-}M\text{-}15}$ gene in all sequenced isolates, often with non-beta-lactamase resistance genes such as *tetA*, *sul2*, and *qnrS1.* This suggests an increasing spread of multidrug-resistant *E. coli* bacteria within the Ghanaian animal farming landscape. Our data further revealed that ST10 and ST155 were the two sequence types that predominantly carried a broad range of virulence factors typically associated with both avian and human infections among the sequenced isolates. Additionally, the co-occurrence of resistance determinants and plasmids suggests a significant potential for horizontal gene transfer, facilitating the spread of multidrug resistance between bacteria. While the genomic analysis was based on a limited set of isolates, the findings remain highly relevant for small- to medium-scale poultry farmers. These farming systems often operate with fewer biosecurity safeguards and limited veterinary oversight, making them particularly vulnerable to resistant bacteria. By providing evidence of shared MDR *E. coli* patterns between poultry and humans, this study offers actionable knowledge that can inform farmer-level practices, such as prudent

antibiotic use, enhanced hygiene, and farmworker training, thereby supporting both poultry health and farmer well-being. These findings underscore the urgency of implementing stricter antibiotic stewardship and biosecurity measures to mitigate the spread of antibiotic resistance. Continuous genomic surveillance is essential to monitor emerging resistance patterns and inform targeted interventions that address the growing threat of AMR within agricultural and healthcare systems.

Author Contributions: Conceptualization, ESD, SJ, AA, IJO; methodology, IJO, ESD, GOS, AA, SJ; software, IJO.; validation, IJO, GOS, ESD, SJ, AA; formal analysis, IJO.; investigation, IJO, GOS, ESD, SJ, AA.; resources, ESD.; data curation, IJO, GOS; writing—original draft preparation, IJO, GOS, ESD, SJ, AA; writing—review and editing, IJO, ESD, SJ, AA; visualization, IJO, GOS; supervision, ESD, SJ, AA; project administration, ESD, SJ, AA; funding acquisition, ESD. All authors have read and agreed to the published version of the manuscript."

Funding: This work was funded by the National Institutes of Health, USA, through the "Application of Data Science to Build Research Capacity in Zoonoses and Food-Borne Infections in West Africa (DS-ZOOFOOD) Training Programme" hosted at the Department of Medical Microbiology, University of Ghana Medical School (Grant Number: UE5TW012566). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (CHS-Et/M.2-P 4.6/2021–2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Clinical trial number: Not applicable.

Data Availability Statement: The raw sequencing data are available at the National Center for Biotechnology Information Sequence Read Archive under the BioProject ID PRJNA1208549. All other raw data supporting the reported results can be made available upon request from the corresponding author.

Acknowledgments: We are grateful to the poultry farm owners, workers, and the laboratory and technical staff of the Department of Medical Microbiology, University of Ghana Medical School.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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