

Diversity of Multi-Drug Resistance Genes in *Escherichia coli* Isolated from Poultry in Southern Togo

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Background and Purpose: The medical sector is facing therapeutic dead ends due to antimicrobial resistance (AMR), which leads to human death and economic losses for poultry farmers. This study aimed to investigate the diversity of resistance genes in Enterobacteriaceae on poultry farms.

Methods: Illumina sequencing was used for the cultivation of 184 poultry fecal samples, identification using the API 10S Biomerieux gallery, antibiotic susceptibility testing by disk diffusion, and DNA extraction using the Biocentric Bruker automated system.

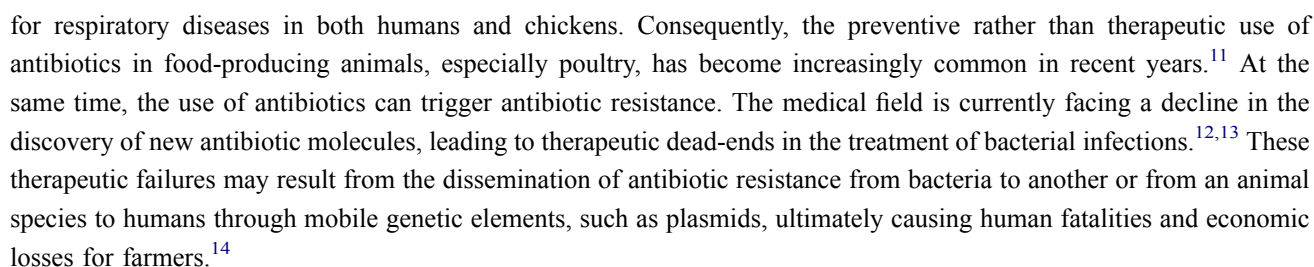
Results: Among the 164 isolates, *Escherichia coli* was the most isolated species (84.78%, n = 156), followed by *Klebsiella pneumoniae* (3.26%, n = 06), and *Salmonella* spp. (0.01%, n = 02). Among 156 *Escherichia coli* strains, 10% (n = 18) were extended-spectrum beta-lactamase (ESBL) producers. Among the 13 sequenced ESBL-producing *E. coli* strains, 53 resistance genes were identified with moderate variability and some were strain-specific. Co-localization of plasmids with virulence genes was also observed. Several *bla*TEM variants that encode beta-lactam resistance have been frequently detected. The *ompK37* and *ompK36* mutants coding for carbapenemase production were identified in one strain. One strain carried *mcr-1.1* gene.

Conclusion: Antibiotics used in poultry farming contribute to the selection of resistant and highly virulent clones. Strengthening regulations on antibiotic use in poultry farming is necessary.

Keywords: enterobacteriaceae, ESBL, plasmids, virulence, Togo

Introduction

Antibiotic resistance is the ability of a bacterium to survive exposure to a minimal therapeutic concentration of an antibiotic.¹ The emergence and spread of antibiotic resistance are attributed to the common and irrational use of antibiotics (ATB) in human and veterinary medicine.² Bacterial susceptibility to antibiotics is increasing, but the rate of antibiotic-resistant strains has been rising since the first description of a pneumococcal strain with reduced sensitivity to penicillin G in 1967.^{3,4} Notably, bacterial resistance in food-producing animals can be transmitted to humans, gene transfer contributes to spreading resistance to initially antibiotic-sensitive species.⁵ A resistance gene can be transferred from a non-pathogenic to a pathogenic bacteria. One of the most well-known resistance mechanisms is the production of extended-spectrum beta-lactamases (ESBLs).⁶⁻⁸ In Togo, poultry which is predominantly affected by respiratory and diarrhea diseases.⁹ *Escherichia coli* (*E. coli*) is a gram-negative bacterium naturally present in the intestinal flora of poultry, humans, and their environment.¹⁰ While most *E. coli* strains are harmless, some are pathogenic, and can cause urinary tract infections in humans and colibacillosis in poultry. *Klebsiella pneumoniae* is one of the bacteria responsible



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Materials And Methods

Study Area and Period

The study took place in the “maritime area” and the “Autonomous District of Greater Lomé (DAGL)” in November 2022 (Figure 1). This region was chosen because it is a real pole of concentration, with 66.06% commercial poultry farms compared to the rest of the country.¹⁵

Selection of Study Poultry Farms

This study was conducted to isolate multi-drug-resistant bacteria from fresh poultry droppings. The necessary sample size consisted of 184 fresh fecal samples collected from 153 poultry farms.¹⁷ According to National Association of Poultry Farmers of Togo (ANPAT), 305 farms were recorded in southern Togo in 2017, and this registry served as the basis for our study. Following the method outlined in the document stating that at least 30% of the farms in the target area should be sampled,¹⁸ the total number of poultry farms sampled was 153 (exceeding $305 \times 30/100 = 92$). Poultry that had been treated with antibiotics within three–seven days prior to our visit were excluded.

Sample Collection

At least one sample was collected from each poultry farm that was visited. For farms with more than two (2) poultry batches, samples were taken from two different ones each. Each fresh fecal sample consisted of a pool of five samples collected from different locations within the building. The samples were stored at a temperature between 2 °C and 4°C in a cooler and immediately

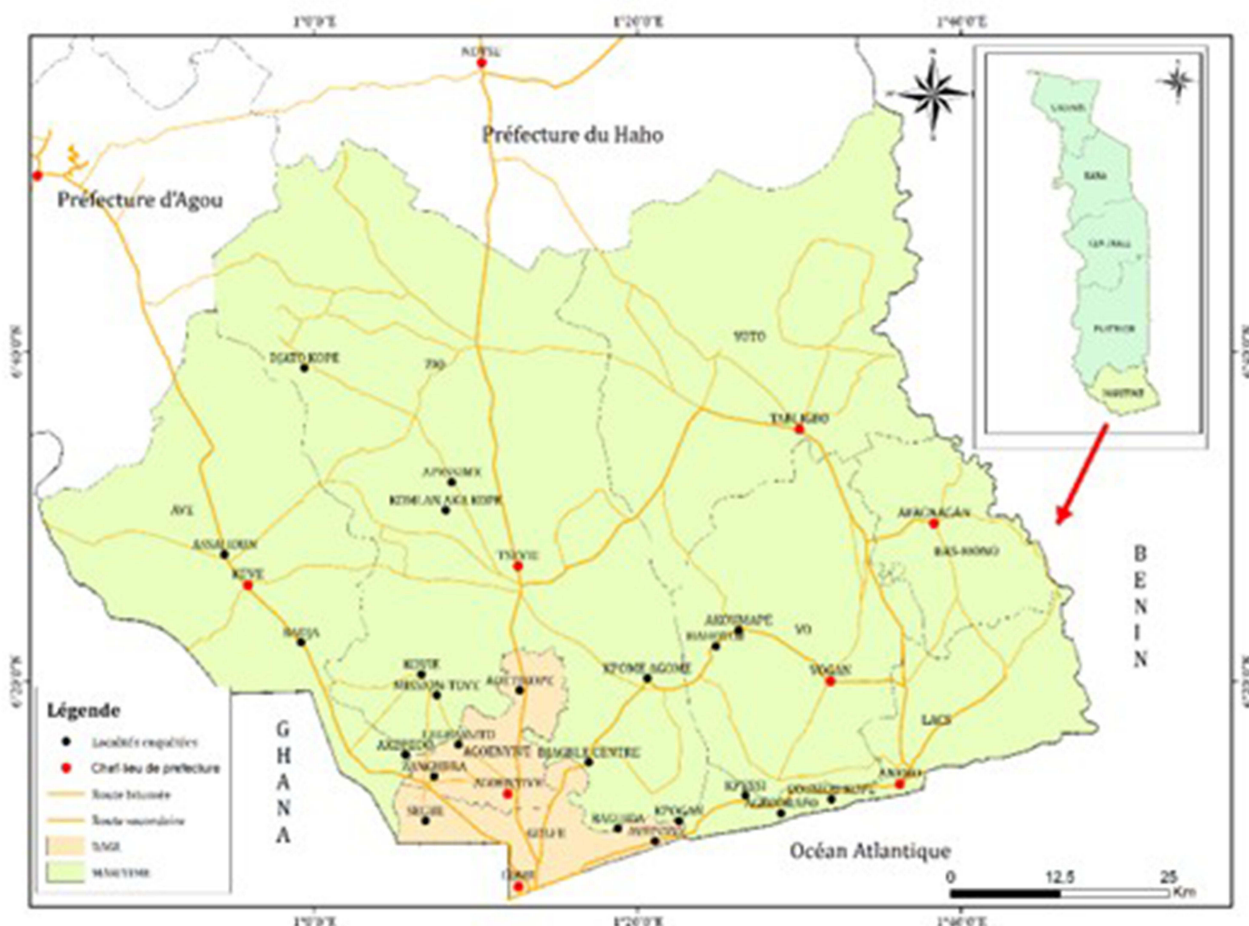


Figure 1 Epidemiological map of the “maritime region” showing the principals localities covered; Source:¹⁶

transported to the laboratory, where they were pre-enriched in Thioglycollate Broth (BT) and stored at -80°C before processing. A descriptive questionnaire was used to collect information on farm identification, hygiene practices, and the frequency of antibiotic use by poultry farmers. The Polaris GPS application was used to record geographic coordinates for map development.

Isolation of Enterobacteria

E. coli and *Klebsiella* were cultured on Eosin Methylene Blue (EMB) agar. *Salmonella* was cultured on Hektoen agar after enrichment in Rappaport Vassiliadis (RV) broth. Culture purification was performed on Urinary Tract Infection (UTI) agar and identification was carried out using the API 10S BIOMERIEUX system.

Phenotypic Antibiotic Sensitivity Testing

Antibiograms were obtained using the antibiotic disk diffusion method on Muller-Hinton agar following medical procedures.¹⁹ Twenty (20) antibiotics from seven different families were tested based on their importance in human medicine (Table 1). The inhibition zone diameters were measured and interpreted as resistant, intermediate, or susceptible according to the reference diameters of CA-SFM/EUCAST.²⁰ A bacterium were considered β -lactamase-producing extended-spectrum (ESBL) if they were able to grow after 24 h of incubation at 37°C on MacConkey agar with cefotaxime (CTX) dilution. On the antibiogram, a “champagne cork pattern” was observed when third- and fourth-generation cephalosporin disks (ceftriaxone, ceftazidime, cefotaxime, and cefepime) were placed around the aminopenicillin disk (amoxicillin + clavulanic acid).

Resistance Gene Detection

To identify resistance genes, thirteen (13 of) *E. coli* ESBL strains were selected for sequencing according to the guidelines of the World Organisation for Animal Health (WOAH). According to these guidelines, at least 30% of the bacterial population is required to validate the results of Polymerase Chain Reaction (PCR) technique.²¹ DNA extraction was performed using the Biocentric Bruker automated system with the GXT NA Extraction Kit. The NGS DNA Library Prep Set (Cat No. PT004) was used for library preparation, and whole-genome sequencing was performed using

Table 1 List of Antibiotics Tested

Antibiotic Class		Generic Name	Abbreviation	Disk Load (μg)
BETA LACTAMS	Pénicillins	Ampicillin	AM/AMP	10
		Amoxicillin+acid clavulanic	AMC	20-10
		Ticarcillin	TIC	75
		Piperacillin	PIP/PRL	30
	Carbapenems	Imipenem	IPM	10
		Ertapenem	ERT/ETP	10
		Ceftriaxone	CRO	30
	Cephalosporins III and IV generation	Cefotaxime	CTX	5
		Ceftazidime	CAZ	10
		Cefoxitine	FOX	30
		Cefepime	FEP	30
AMINOSIDES PHENICOLES POLYMYXINS SULFANOMIDES QUINOLONES FLUOROQUINOLONES		Amikacin	AK	30
		Gentamicin	GM/CN	10
		Chloramphenicol/Thiamphenicol	C	30
		Colistin	CS/CT	-
		Trimethoprim/ Sulfamethoxazole	SXT/W	23,75–1,25
		Acid Nalidixic	AN	30
		Norfloxacin	NOR	5
		Ciprofloxacin	CIP	5
		Levofloxacin	LEV	5

a NovaSeq X Series 25 B Reagent Kit (300 cycles) with XLEAP-SBS chemistry from Illumina. Results were obtained in FASTQ format.²² These FASTQ data were input into the ResFinder universal genomic algorithm database (ResFinder-2.4.0), to identify various resistance genes, into VirulenceFinder to detect virulence genes, and into PlasmidFinder to detect plasmids present in each strain.^{23–25}

Data Analysis

The results obtained were entered into an Excel 2016 database and the rates were calculated. The map was modified using the ArcGIS 10.8 software.

Results

Prevalence of Enterobacteria

Among the 184 samples collected, 84.78% (n = 156) were colonized by at least one bacterial strain and 0.04% (n = 8) were colonized by two different strains. Twenty-eight samples (15.22%) were free of bacterial strains. Thus, 164 strains were isolated from 156 samples. *Escherichia coli* was the most commonly isolated species (84.78%, n = 156), followed by *Klebsiella pneumoniae* (3.26%, n = 6) and *Salmonella spp.* (0.01%; n = 2) (Figure 2). Among *Escherichia coli* species, 10% (n = 18) were ESBL producers (ESBL+). The figure below shows the distribution of ESBL-producing bacteria on poultry farms (Figure 3).

Phenotypic Antibiotic Susceptibility Testing

The prevalences of *Escherichia coli* isolates that were non-ESBL producers (BLSE-) and resistant to antibiotics were: ampicillin [78 (56.5%)]; amoxicillin + clavulanic acid [20 (14.4%)]; ticarcillin [77 (55.8%)]; piperacillin [71 (51.4%)]; imipenem/ertapenem [0 (0%)]; ceftriaxone [1 (0.7%)]; cefotaxime [0 (0%)]; ceftazidime [0 (0%)]; cefoxitin [0 (0%)]; cefepime [2 (1.4%)]; amikacin [2 (1.4%)]; gentamicin [9 (6.5%)]; chloramphenicol [12 (8.7%)]; colistin [0 (0%)]; sulfamethoxazole + trimethoprim [107 (77.5%)]; nalidixic acid [32 (23.1%)]; norfloxacin [17 (12.3%)]; ciprofloxacin [22 (15.9%)]; levofloxacin [17 (12.3%)] (Figure 4).

In *Escherichia coli* ESBL producers (ESBL+), the resistance prevalences were: [18 (100%)] against third- and fourth-generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime, cefepime); [07 (38.8%)] against amoxicillin + clavulanic acid; 0% against carbapenems (imipenem, ertapenem) and polymyxins (colistin); amikacin [01 (5.5%)]; gentamicin [05 (27.7%)]; chloramphenicol [12 (8.7%)/ 06 (33.3%)/ 01 (16.6%)/ 01 (50%)]; colistin [0 (0%)]; sulfamethoxazole + trimethoprim [15 (83.3%)]; nalidixic acid [07 (38.8%)]; norfloxacin [06 (33.3%)]; ciprofloxacin [07 (38.8%)]; levofloxacin [06 (33.3%)] (Figure 5).

In *Klebsiella pneumoniae* bacteria, the resistance prevalences were: ampicillin [06 (100%)]; amoxicillin + clavulanic acid [01 (16.6%)]; ticarcillin [06 (100%)]; piperacillin [06 (100%)]; imipenem/ertapenem [0 (0%)]; ceftriaxone [0 (0%)];

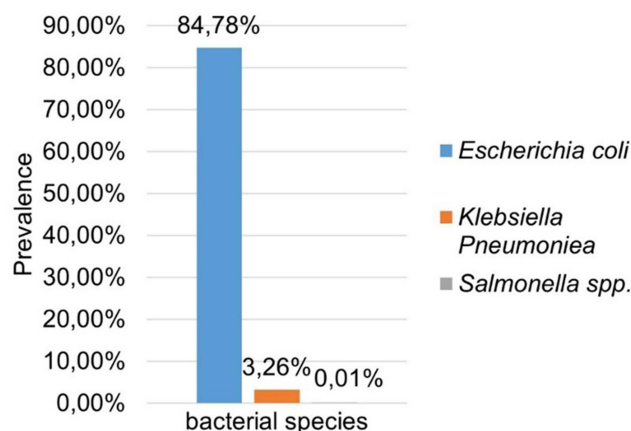


Figure 2 Prevalence of targeted enterobacteria.

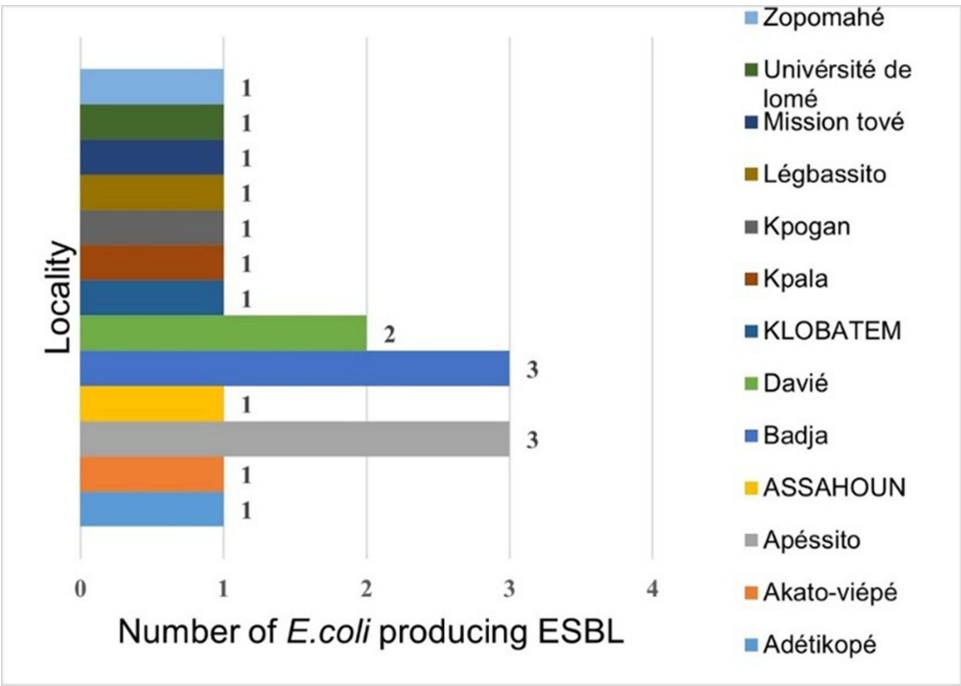


Figure 3 Distribution of *Escherichia coli* ESBL-producing by locality.

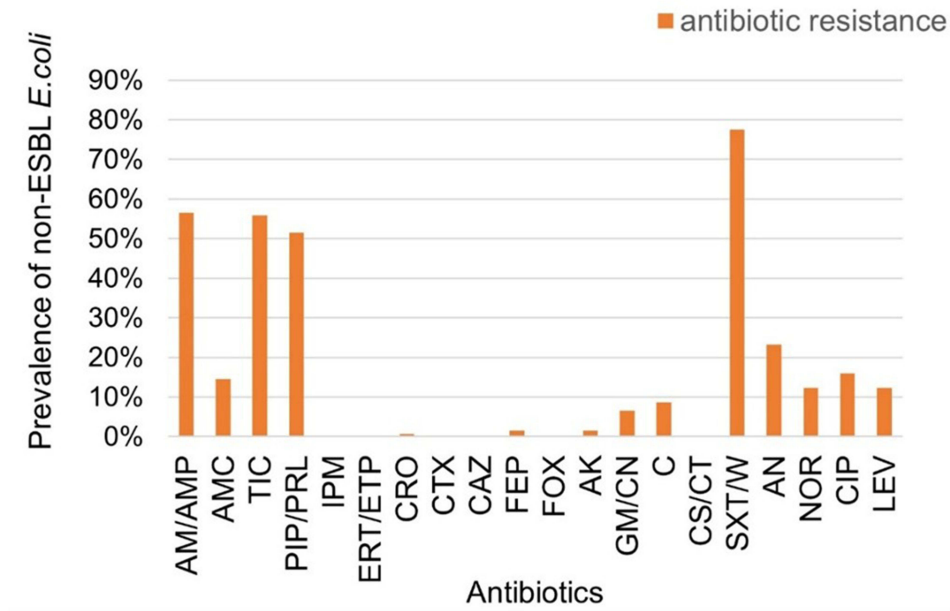


Figure 4 Antibiotic resistance profiles of non-ESBL-producing *Escherichia coli*.

cefotaxime [0 (0%)]; ceftazidime [0 (0%)]; cefoxitin [0 (0%)]; cefepime [0 (0%)]; amikacin [0 (0%)]; gentamicin [01 (16.6%)]; chloramphenicol [01 (16.6%)]; colistin [0 (0%)]; sulfamethoxazole + trimethoprim [02 (33.3%)]; nalidixic acid [0 (0%)]; norfloxacin [01 (16.6%)]; ciprofloxacin [01 (16.6%)]; levofloxacin [0 (0%)] (Figure 6).

Of the two *Salmonella spp.* isolates, only one was resistant to ampicillin, amoxicillin + clavulanic acid, ticarcillin, piperacillin, gentamicin, chloramphenicol, sulfamethoxazole + trimethoprim, and nalidixic acid.

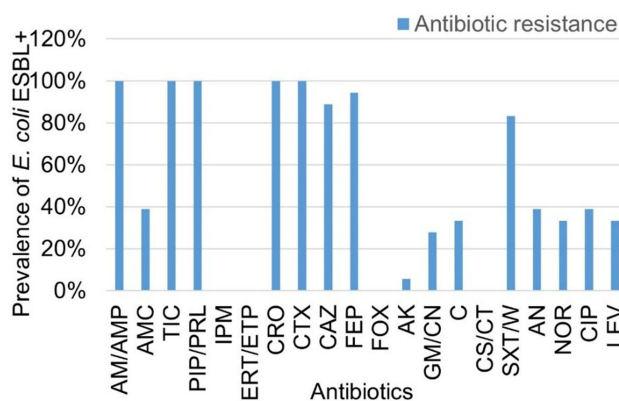


Figure 5 Antibiotic resistance profile of ESBL-producing *Escherichia coli*.

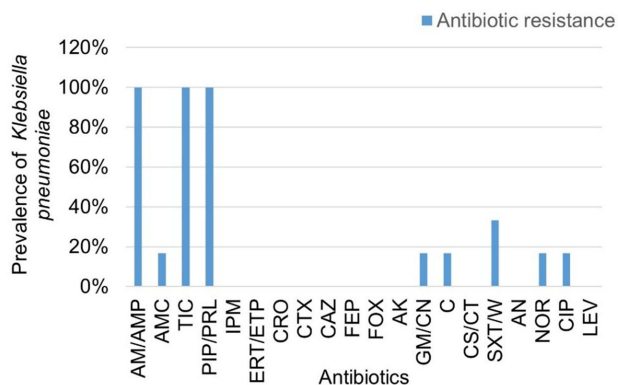


Figure 6 Antibiotic resistance profile of *Klebsiella pneumoniae*.

The prevalence of resistance to different antibiotic families is summarized in Tables 2–4. Overall, it appears from the tables above that enterobacteria developed resistance to sulphonamides at 78%, followed by beta-lactams at 57%, and quinolones at 23%.

Table 2 Prevalence of Resistance to Penicillins

Beta-Lactams				
Penicillins				
	Ampicillin	Amoxicillin + Clavulanic Acid	Ticarcillin	Piperacillin
<i>Escherichia coli</i> ESBL - (N=138)	78 (56.52%)	20 (14.49%)	77 (55.80%)	71 (51.45%)
<i>Escherichia coli</i> ESBL + (N=18)	18 (100%)	07 (38.89%)	18 (100%)	18 (100%)
<i>Klebsiella pneumoniae</i> (N=06)	06 (100%)	01 (16.66%)	06 (100%)	06 (100%)
<i>Salmonella</i> spp. (N=02)	01 (50%)	0 (0%)	01 (50%)	01 (50%)

Table 3 Prevalence of Resistance to Carbapenems and Cephalosporins

Beta-Lactams						
	Carbapenems		3rd- and 4th-Generation Cephalosporins			
	Imipenem	Ertapenem	Ceftriaxone	Cefotaxime	Ceftazidime	Cefoxitine
<i>Escherichia coli</i> ESBL - (N=138)	0 (0%)	0 (0%)	01 (0.72%)	0 (0%)	0 (0%)	0 (0%)
<i>Escherichia coli</i> ESBL + (N=18)	0 (0%)	0 (0%)	18 (100%)	18 (100%)	16 (88.89%)	0 (0%)
<i>Klebsiella pneumoniae</i> (N=06)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Salmonella</i> spp.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 4 Prevalence of Resistance to Aminoglycosides, Phenicol, Polymyxins, Sulfonamides, and Quinolones

	Aminosides		Phenicols	Polymyxins	Sulfonamides
	Amikacin	Gentamicin	Chloramphenicol/ Thiamphenicol	Colistin	Sulfaméthoxazole/ Triméthoprim
<i>Escherichia coli</i> ESBL - (N=138)	02 (1.45%)	09 (6.52%)	12 (8.70%)	0 (0%)	107 (77.54%)
<i>Escherichia coli</i> ESBL + (N=18)	01 (5.56%)	05 (27.78%)	06 (33.33%)	0 (0%)	15 (83.33%)
<i>Klebsiella pneumoniae</i> (N=6)	0 (0%)	01 (16.66%)	01 (16.66%)	0 (0%)	02 (33.33%)
<i>Salmonella</i> spp. (N=02)	0 (0%)	01 (50%)	01 (50%)	0 (0%)	01 (50%)
	Quinolones			Fluoroquinolones	
	Nalidixic acid		Norfloxacin	Ciprofloxacin	Levofloxacin
<i>Escherichia coli</i> ESBL - (N=138)	32 (23.19%)		17 (12.32%)	22 (15.94%)	17 (12.32%)
<i>Escherichia coli</i> ESBL + (N=18)	07 (38.89%)		06 (33.33%)	07 (38.89%)	06 (33.33%)
<i>Klebsiella pneumoniae</i> (N=6)	0 (0%)		01 (16.66%)	01 (16.66%)	0 (0%)
<i>Salmonella</i> spp. (N=02)	01 (50%)		0 (0%)	0 (0%)	0 (0%)

Distribution of Phenotypic Resistance Levels Among Isolates

Table 5 shows that 37 (26.8%) non-ESBL-producing *E. coli* isolates were multidrug-resistant. Additionally, 4 (66.66%) *Klebsiella pneumoniae* isolates had developed resistance to a one class of antibiotics.

Analysis of the Different Genetic Determinants of Bacterial Resistance

The figure above shows the proportion of isolates according to the different genetic determinants associated with resistance among the 13 *Escherichia coli* ESBL-producing strains. It is evident from this figure that 100% of the sequenced strains acquired antibiotic resistance genes against antibiotics, all of which were associated with the presence of at least one plasmid and one virulence gene. Among the 13 strains, 92.3% (n=12) had multiple chromosomal mutations, conferring resistance to several antibiotic molecules. Acquired resistance genes against disinfectants were detected in 46.15% (n=6) of the strains (Figure 7).

Serotyping revealed that all 13 sequenced strains carried H and O serotype genes, marked by the presence of the corresponding antigens, except for one strain that presented a KL128 capsular locus, which was non-typeable and lacked an H type (Table 6).

Table 5 Prevalence of Multidrug-Resistant Enterobacteria

Resistance Level	<i>Escherichia coli</i> (ESBL-)		<i>Klebsiella pneumoniae</i>	
	Count	Proportion	Count	Proportion
Susceptible	18	13.04%	0	00%
Resistant to 1 ATB class	31	22.46%	4	66.66%
Resistant to 2 ATB class	52	37.68%	2	33.33%
Resistant to 3 ATB class (MDR)	23	16.66%	0	00%
Resistant to 4 ATB class (XDR)	9	6.52%	0	00%
Resistant to 5 ATB class (XDR)	4	2.17%	0	00%
Resistant to 6 ATB class (PDR)	1	0.72%	0	00%

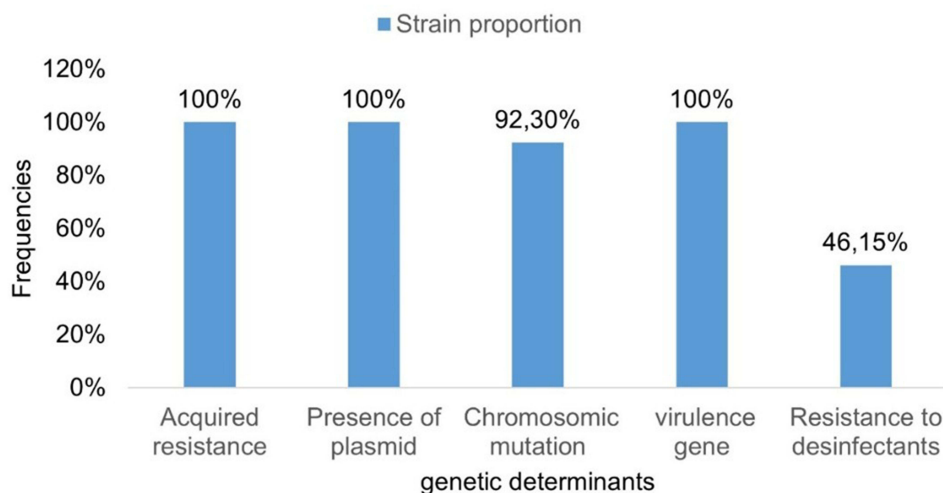


Figure 7 Genotypic determinants in 13 ESBL-producing *E. coli*.

Distribution of Resistance Genes Among Different Sequenced Strains

Figure 8 illustrates the frequency of occurrence of the 53 identified resistance genes among the 13 sequenced multidrug-resistant *Escherichia coli* strains. Genes such as *bla*_{CTX-M-55}, *sul2*, *tet(A)*, *floR*, *aph (6)*, *aph(3'')*, *gyrA*, *gyrB*, *gyrC*, *parC*, *parE*, *pmrA*, *pmrB*, *folp*, *23S*, *16-rrsB*, *16-rrsC*, *16-rrsH*, *ampC-promoter*, and *rpoB* were highly prevalent, appearing in more than 84.6% of the strains (Table 7). Other genes were less widespread and were found in only two to three samples (15–23%). One *mcr-1.1* gene was detected in each strain. Of the 53 genes, 56.60% were associated with acquired resistance to various antibiotics under selective pressure from various antibiotics. Additionally, 35.85% of the genes (*gyrA*, *gyrB*, *gyrC*, *parC*, *parE*, *pmrA*, *pmrB*, *folp*, *23S*, *16-rrsB*, *16-rrsC*, *16-rrsH*, *ampC-promoter*, and *rpoB*) were resistant to ciprofloxacin, nalidixic acid, fluoroquinolones, and other unknown antibiotics (Table 8).

The Plasmid Profile of Sequenced ESBL-Producing *E. coli* Strains

Each of the 13 ESBL-producing *Escherichia coli* strains carried at least one plasmid, with some harboring multiple plasmids. Seventeen unique plasmids were identified in this study. The following table (Table 9) provides a summary of

Table 6 Summary of Serotype Diversity in 13 *E. coli* Strains

Serotypes and Variants	Strain Count
H; O182	1
H11; O38	1
H19;O134;O46;O8	1
H19;O61	3
H32	1
H32;O149	1
H4; O44;O17;O77	1
H4;O8	1
H40; H53	1
H5; O110	1
Locus KL182; O12	1
Total strains	13

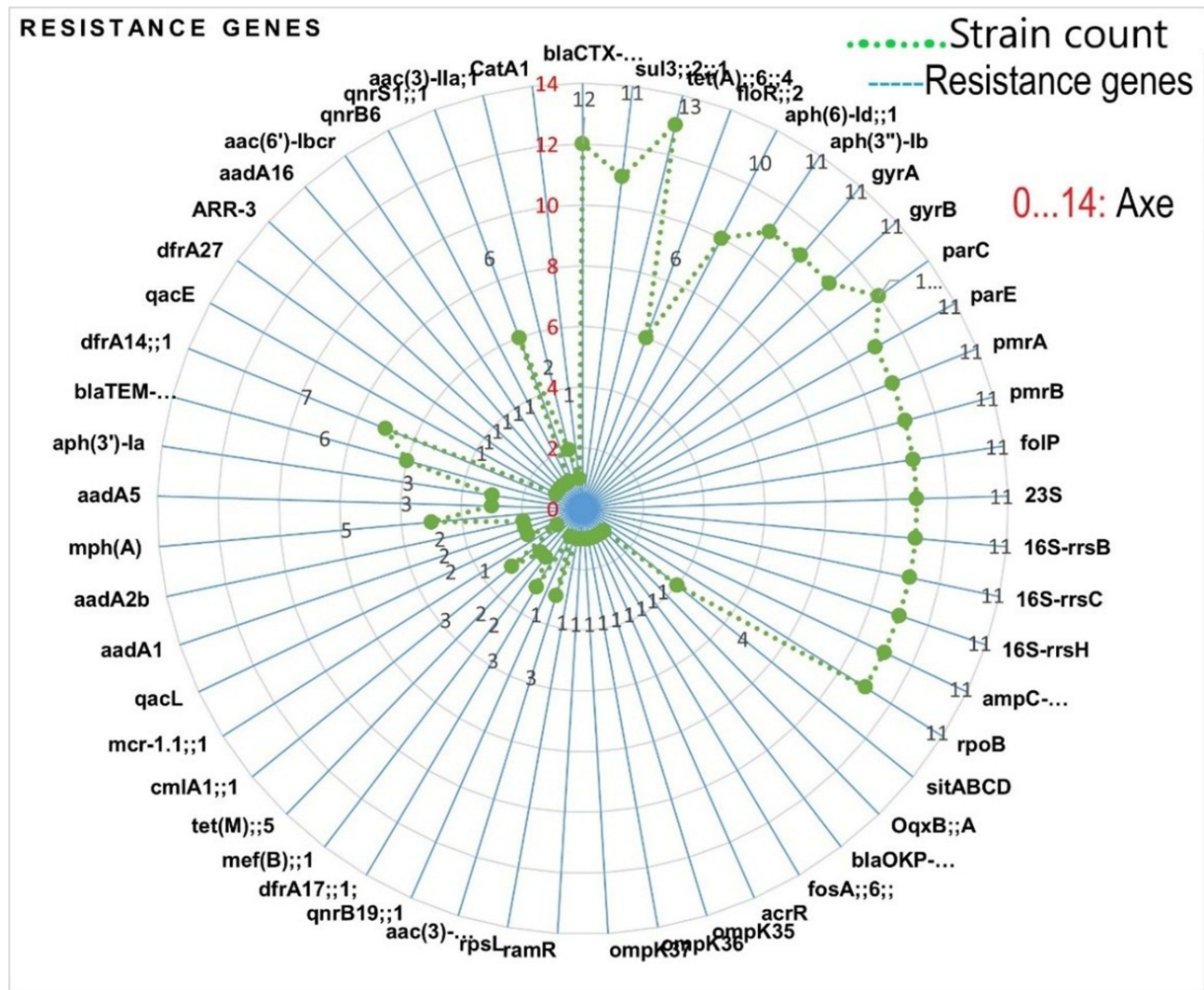


Figure 8 Frequency scale of different resistance genes in 13 E.coli ESBL strains.

the plasmid diversity and their frequencies among the sequenced strains. The most frequently identified plasmid across multiple strains was *IncFIB(pHCM2)* (38.46% of strains), followed by *p0111*, *ColpVC*, and *IncHI1B(pNDM-CIT)* (each detected in 30.76% of strains). Several other plasmids were only detected once.

Table 7 Frequencies of Different Resistance Genes in 13 ESBL-Producing E. coli Strains

Genes	Strain Count	Proportion
<i>blactx-M-55;1;15;27</i>	12	92,30%
<i>sul3;2;1</i>	11	84,61%
<i>tet(A);6;4</i>	13	100%
<i>flor;2</i>	6	46,15%
<i>aph(6)-Id;1</i>	10	76,92%
<i>aph(3'')-Ib</i>	11	84,61%
<i>gyrA</i>	11	84,61%
<i>gyrB</i>	11	84,61%

(Continued)

Table 7 (Continued).

Genes	Strain Count	Proportion
<i>parc</i>	12	92,30%
<i>pare</i>	11	84,61%
<i>pmra</i>	11	84,61%
<i>pmrb</i>	11	84,61%
<i>folp</i>	11	84,61%
<i>23S</i>	11	84,61%
<i>16S-rrsB</i>	11	84,61%
<i>16S-rrsC</i>	11	84,61%
<i>16S-rrsH</i>	11	84,61%
<i>ampc-promotor</i>	11	84,61%
<i>rpoB</i>	11	84,61%
<i>sitabcd</i>	4	30,76%
<i>OqxB₂A</i>	1	7,60%
<i>blaKp-B-1₂;;1₂</i>	1	7,60%
<i>fosa₂6₂</i>	1	7,60%
<i>acrr</i>	1	7,60%
<i>ompK35</i>	1	7,60%
<i>ompK36</i>	1	7,60%
<i>ompK37</i>	1	7,60%
<i>ramR</i>	1	7,60%
<i>rpsL</i>	1	7,60%
<i>aac(3)-11d₂1₂</i>	3	23,07%
<i>qnrB19₂1₂</i>	1	7,60%
<i>dfrA17₂1₂</i>	3	23,07%
<i>meff(B)₂1₂</i>	2	15,20%
<i>tet(M)₂5</i>	2	15,20%
<i>cmlA1₂1₂</i>	3	23,07%
<i>mcr-1.1₂1₂</i>	1	7,60%
<i>qacL</i>	2	15,20%
<i>aadA1</i>	2	15,20%
<i>aadA2b</i>	2	15,20%
<i>mph(A)</i>	5	38,46%
<i>aadA5</i>	3	23,07%
<i>aph(3')-Ia</i>	3	23,07%
<i>blaTEM-1B₂1₂</i>	6	46,15%
<i>dfrA14₂1₂</i>	7	53,84%
<i>qacE</i>	1	7,60%
<i>dfrA27</i>	1	7,60%
<i>ARR-3</i>	1	7,60%
<i>aadA16</i>	1	7,60%
<i>aac(6')-Ibcr</i>	1	7,60%
<i>qnrB6</i>	1	7,60%
<i>qnrS1₂1₂</i>	6	46,15%
<i>aac(3)-IIa₂1₂</i>	2	15,20%
<i>CatA1</i>	1	7,60%

Virulence Genes Associated with Resistance

Several virulence genes with varying distribution have been identified. Each strain carried at least one virulence gene, and the most frequently detected virulence genes included *fimH*, *yeh*, *gad*, *terC*, *csaA*, *nlpl*, and *hlyE*, which were present in all the 13 strains. Notably, the resistance gene *blaCTX-M-55* was identified in one strain and associated with virulence (Figure 9).

Table 8 Classification of Genes According to Resistance Types and Their Genotypic Expression

Resistance Types and Corresponding Genes	Genes Count	Proportion
Acquired Resistances	30	56,60%
Amoxicillin, ampicillin, ticarcillin, piperacillin, cephalothin	1	
<i>blaTEM-1B;;I</i>	1	
Apramycin, tobramycin, netilmicin, dibekacin, gentamicin, sisomicin	3	
<i>aac(3)-IIId;;I</i> ;	1	
<i>aac(3)-IIa;;I</i>	1	
<i>aac(6')-Ibcr</i>	1	
Azithromycin, erythromycin	1	
<i>mef(B);;I</i>	1	
Benzylkonium chloride, ethidium bromide, cetylpyridinium chloride, chlorhexidine	3	
<i>qacE</i>	1	
<i>qacL</i>	1	
<i>sitABCD</i>	1	
Betalactams	1	
<i>blaOKP-B-I;;I;;</i>	1	
Chloramphenicol	2	
<i>CatA I</i>	1	
<i>cmlA I;;I</i>	1	
Ciprofloxacin	3	
<i>qnrB19;;I</i>	1	
<i>qnrB6</i>	1	
<i>QnrS I;;I</i>	1	
Ciprofloxacin, acid nalcidic, trimethoprim, chloramphenicol	1	
<i>OqxB;;A</i>	1	
Acquired Resistances	30	56,60%
Colistin	1	
<i>mcr-1.1;;I</i>	1	
Erythromycin, spiramycin, telithromycin, azithromycin	1	
<i>mph(A)</i>	1	
Fosfomycin	1	
<i>fosA;;6;;</i>	1	
Neomycin, lividomycin, kanamycin, ribostamycin, paromomycin	1	
<i>aph(3')-Ia</i>	1	
Rifampicin	1	
<i>ARR-3</i>	1	
Streptomycin	2	
<i>aph(3'')-Ib</i>	1	
<i>aph(6)-Id;;I</i>	1	
Streptomycin, spectinomycin	4	
<i>aadA I</i>	1	
<i>aadA2b</i>	1	
<i>aadA5</i>	1	
<i>aadA I 6</i>	1	
Tétracyclin	1	
<i>tet(M);;5</i>	1	
Trimethoprim	3	
<i>dfrA I 4;;I</i>	1	
<i>dfrA I 7;;I</i> ;	1	
<i>dfrA27</i>	1	

(Continued)

Table 8 (Continued).

Resistance Types and Corresponding Genes	Genes Count	Proportion
Chromosomics mutations	19	35,85%
Ciprofloxacin, acid nalcidic	1	
<i>gyrA</i>	1	
Fluoroquinolone	2	
<i>acrR</i>	1	
<i>ompK35</i>	1	
Antibiotics unknown	16	30,19%
<i>16S-rrsB</i>	1	
<i>16S-rrsC</i>	1	
<i>16S-rrsH</i>	1	
23S	1	
ampC-promotor	1	
<i>folP</i>	1	
<i>gyrB</i>	1	
<i>ompK36</i>	1	
<i>ompK37</i>	1	
<i>parC</i>	1	
<i>parE</i>	1	
<i>pmrA</i>	1	
<i>pmrB</i>	1	
<i>ramR</i>	1	
<i>rpoB</i>	1	
<i>rpsL</i>	1	
Resistance type unknown	4	7,55%
Ampicillin, cefepime, cefotaxime, ceftazidime	1	
<i>blaCTX-M-55;;1;;15;27</i>	1	
Chloramphenicol	1	
<i>floR;;2</i>	1	
Sulfamethoxazole	1	
<i>sul3;;2;;1</i>	1	
Tetracyclin	1	
<i>tet(A);;6;;4</i>	1	
Total	53	100,00%

Table 9 List of Plasmids Detected Among 13 ESBL-Producing *E. coli* Strains

Plasmids	Strain Count	Frequencies
<i>IncB/O/K/Z</i>	2	15.38%
<i>Col(pHAD28)</i>	2	15.38%
<i>Col(BS512)</i>	1	7.69%
<i>IncFII(pHN7A8)</i>	2	15.38%
<i>IncX4</i>	1	7.69%
<i>IncFIB(AP001918)</i>	3	23.07%
<i>Col440I</i>	1	7.69%
<i>IncQI</i>	2	15.38%
<i>IncHII B(pNDM-CIT)</i>	4	30.76%

(Continued)

Table 9 (Continued).

Plasmids	Strain Count	Frequencies
<i>IncX1</i>	2	15.38%
<i>pO111</i>	4	30.76%
<i>ColpVC</i>	4	30.76%
<i>IncFIB(pHCM2)</i>	5	38.46%
<i>IncFIB(K)</i>	2	15.38%
<i>IncHI2/IncHI2A</i>	1	7.69%
<i>IncFIA(HII)</i>	1	7.68%
<i>IncFII(pCoo)</i>	1	7.69%

Discussion

Phenotypic Tests

Prevalence of Isolated Enterobacteria

Most *Escherichia coli* are enterobacteria considered to be normal or commensal hosts in the gastrointestinal tract of humans and most warm-blooded animals.²⁶ Thus, the 15.22% of our composite samples that were negative for any bacterial colony suggest an excess of antibiotics leading to dysbiosis or intestinal imbalance in the subjects included in this study.^{27–29} The work in 2018 showed that excess antibiotics can also lead to imbalance and immunosuppression, especially in young animals, making them more susceptible to secondary infections.³⁰ On the other hand, some bacteria were not cultured, likely due to a loss of viability during storage at -80°C . Freezing techniques, including cooling kinetics, can induce bacterial stress, thus affecting their survival.³¹ *Klebsiella spp.* are responsible for several diseases, including respiratory issues in humans and poultry; therefore, their low prevalence in this study justifies the absence of disease reported by the majority of poultry farmers during sample collection.

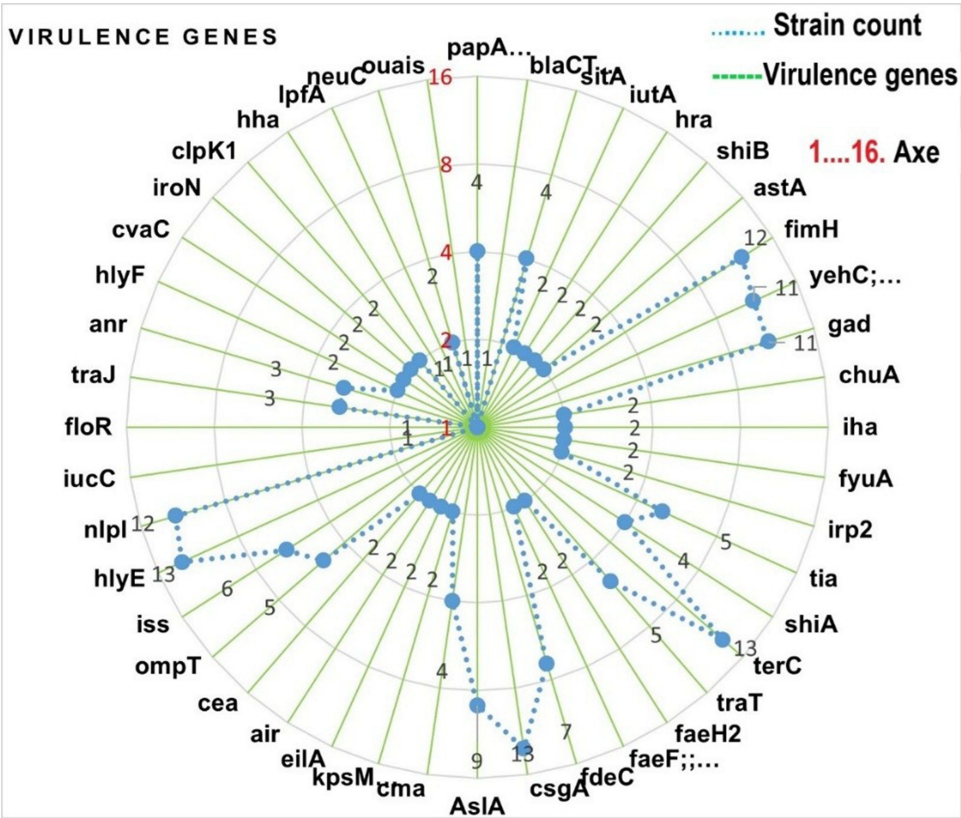


Figure 9 Frequency scale of the distribution of virulence genes.

Regarding *Salmonella*, recent reports indicate concerning trends; these are associated with foodborne illnesses of avian origin in countries of the West African subregion, particularly Togo, Ghana, Benin, and Côte d'Ivoire.³² In Togo, an increase in cases of salmonellosis has been observed, mainly related to ineffective farming and hygiene practices. Salmonellosis is one of the most recurrent diseases, causing significant losses in poultry farms in Togo, according to unpublished local data. However, its low isolation rate could be due to the common use of antibiotics for prevention, as noted in this and previous ones.³³ Indeed, *Salmonella* is very sensitive, even in contact with older antibiotic molecules. One study isolated a high rate of *Salmonella* during the rainy season; thus, our sampling season could have affected our isolation rate.³⁴ Infections caused by *Salmonella* constitute a serious global epidemiological and economic problem in the context of food safety and public health. In 2014, the European community (EU) reported a total of 88,715 cases of salmonellosis in humans, with 34.4% requiring hospitalization.³⁵ Regional initiatives, such as promoting good hygiene practices and the responsible use of antibiotics in poultry farming, could help strengthen food safety.

Susceptibility of Non-ESBL-Producing *E. coli* (ESBL-)

The results showed varied antibiotic resistance profiles among the ESBL-producing *Escherichia coli* (ESBL-) strains. Resistance was particularly high for beta-lactam antibiotics such as ampicillin (56.5%), ticarcillin (55.8%), piperacillin (51.4%), and sulfamethoxazole+ trimethoprim (77.5%), likely reflecting the widespread use of these antibiotics in poultry farming, promoting the selection of resistant strains. In 2020, a study in poultry showed similar trends, with resistance prevalence in *E. coli* ranging from 55.17% to 100% against penicillin and sulfonamide antibiotics.³³ There is a similarity with the results in 2021, who found 80% of *E. coli* isolates resistant to sulfonamides in poultry in Côte d'Ivoire.³⁶ Indeed, their good tolerance and bactericidal properties make these molecules the most widely used for treating bacterial infections and even for prevention in poultry farming, thereby exerting selective pressure on bacteria.^{37,38}

In contrast, complete absence of resistance was observed for antibiotics such as imipenem/ertapenem (0%), cefotaxime (0%), ceftazidime (0%), and colistin (0%), likely due to regulations that restricted access to these antibiotics in the veterinary market. Piperacillin is an antibiotic that is not therapeutically used in poultry. However, 51.45% of *E. coli* strains were resistant to its composition containing piperazine, a molecule commonly used as an antiparasitic in poultry and swine farms, according to unpublished 2020 surveys in Togo.

Susceptibility of ESBL-Producing *E. coli*

One of the most important mechanisms of antibiotic resistance in Enterobacteriaceae is the production of enzymes that hydrolyze penicillin, cephalosporins, and monobactams. These enzymes are called extended-spectrum beta-lactamases (ESBLs).³⁹ Publications have reported that the mechanism, first diagnosed in 2008 in *K. pneumoniae* and *E. coli*, involves the production of a metallo-beta-lactamase enzyme; conferring resistance to many beta-lactam antibiotics, including carbapenems and amino-penicillins (combination of clavulanic acid with ampicillin, amoxicillin, ticarcillin, or piperacillin).^{40,41} These last families were discovered to maintain the effectiveness of beta-lactam antibiotics.³⁷ Although susceptibility to the combination of amoxicillin and clavulanic acid (AMC) has been observed, studies conducted in Spain and France have shown that more than 70% of *E. coli* and 50% of *Klebsiella* isolates were susceptible to AMC in vitro but clinically resistant. This suggests an uncertain prognosis when AMC are used to treat infections caused by ESBL-producing bacteria.^{42,43} Furthermore, Figure 5 shows an extension of resistance to fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones, thus justifying the overuse of antibiotics which has led to the expansion of the enzymatic spectrum.⁴⁴ This poses a public health problem. Fortunately, no carbapenemase-producing strains have been identified, indicating a positive public health outcome. Carbapenems are considered last-resort drugs and are mainly used to treat infections caused by bacteria that are resistant to other antibiotics. Indeed, substances in this group are particularly important because of their broad antibacterial activity against both aerobic and anaerobic bacteria as well as gram-positive and gram-negative bacteria.^{45,46}

Susceptibility of *Klebsiella pneumoniae*

All *Klebsiella pneumoniae* isolates were resistant to amoxicillin (AM), ampicillin (AMP), ticarcillin (TIC), and piperacillin (PIP). The trends are almost similar to those observed in 2020.³³ Although the *Klebsiella* genus has intrinsic

resistance to AM, AMP, TIC, and PIP,^{20,47} this represents a significant risk to animal and public health due to its ability to cause opportunistic infections, spread resistance genes via plasmids and mobile genetic elements to other pathogenic bacteria, thereby amplifying the resistance problem within the bacterial community and reducing therapeutic options.⁴⁸

An opportunistic infection caused by these bacteria, whether in animals or humans, necessitates the use of more potent antibiotics such as third-generation cephalosporins, carbapenems, polymyxins, or fluoroquinolones, which must be used with caution to avoid selecting multidrug-resistant strains. If these bacteria are resistant to poultry, they can be transmitted to humans through the food chain or through contact with feces in fields and water sources. It is essential to raise awareness regarding the strict application of hygiene measures at the individual level to protect the health of poultry farmers and consumers.

No colistin resistance (0%) was observed in this study. Colistin resistance limits treatment options for severe infections, including those caused by human pathogens (*Escherichia coli* and *Klebsiella pneumoniae*). Resistant bacteria present in poultry can contaminate the meat and other food products, thereby increasing the risk of infection for consumers and poultry farmers. The use of colistin in poultry farming for preventive purposes or growth promotion can lead to the selection of resistant bacteria. Recently, some publications in 2023 reported the spread of strains carrying plasmid-mediated colistin resistance genes (*mcr-1* to *mcr-4*) across all continents, with the vast majority being found in food-producing animals.⁴⁹ In Côte d'Ivoire, a 2021 study isolated 26% of *Escherichia coli* strains resistant to colistin.³⁶ In China, the *mcr-1* plasmid gene has been detected in *E. coli* isolates from both animals and humans, suggesting a probable zoonotic transmission from animals to humans.⁵⁰ However, in Togo, the use of colistin whether in its pure form, combined with vitamins, or in association with other compounds in poultry farming is becoming increasingly common, showing positive results in animals with pathological symptoms. In human medicine, colistin has long been excluded from treatment protocols because of its toxicity, particularly nephrotoxicity. However, since the global spread of resistance to last-generation cephalosporins and carbapenems, along with the discovery of the first colistin resistance mechanism (*mcr-1* gene) in China in pig and chicken meat sold for human consumption transferable to humans,⁵¹ colistin has re-emerged as a prescribed antibiotic for the treatment of severe human infections caused by bacteria resistant to all other therapeutic options, according to a scientific alert from the National Agency for Food Safety.⁵²

Susceptibility of *Salmonella* Spp

The low number of *Salmonella* isolates (two) among the 184 samples in this study is a major limitation that prevents any statistical extrapolation. A single resistant strain out of two was not sufficient to determine a clear trend in the studied population, as sample size is crucial in microbiology and epidemiology to ensure the reliability and validity of results.⁵³ Further studies on similar populations, using alternative sampling techniques, are necessary to obtain more *Salmonella* spp. isolates and provide statistically valid conclusions.

Distribution of Phenotypic Resistance Levels Among Isolates

The analysis in Table 5 reveals marked differences in resistance levels among the isolated bacterial species. Most of *E. coli* isolates exhibited significant multidrug resistance. Only 13.04% of the isolates were non-resistant, indicating a low proportion of sensitive bacteria. Thirty-one isolates (22.46%) were resistant to a single class of antibiotic. The highest proportion (37.68%) consisted of isolates resistant to two antibiotic classes, followed by a notable proportion (16.66%) of multidrug-resistant (MDR) isolates, and 9.41% classified as extensively drug-resistant (XDR) or pandrug-resistant (PDR). Compared with *E. coli*, a significant proportion of *K. pneumoniae* isolates were resistant to only one antibiotic class (66.66%), indicating an initial response to selective pressure. Isolates resistant to the two antibiotic classes represented 33.33% of the isolates, but no multidrug resistance (MDR, XDR, or PDR) was detected. The absence of extreme resistance cases (XDR and PDR) is noteworthy and may reflect lower exposure to a diverse range of antibiotics.

Escherichia coli shows more advanced resistance patterns, with cases of MDR, XDR, and PDR, whereas *Klebsiella pneumoniae* remains limited to resistance against one or two antibiotic families. This difference could be attributed to species-specific resistance mechanisms^{41,54} as well as environmental^{55,56} or behavioral factors in poultry farming.^{57,58} The high prevalence of resistance to one or more antibiotic classes in both species poses a challenge for the management

of animal and human infections. MDR and extremely resistant *E. coli* isolates (XDR and PDR) can limit the therapeutic options and increase bacterial virulence in human infections. For *K. pneumoniae*, although extreme resistance is absent, the rapid progression of resistance to one or two classes of antibiotics necessitates proactive monitoring.

Overall, these data highlight concerns regarding antimicrobial resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolated from poultry farms in Togo. These results indicate a major issue of antibiotic resistance, which can be attributed to the systematic and excessive use of antibiotics in poultry farming, often without veterinary prescriptions or medical supervision, combined with poor hygiene and biosecurity measures that facilitate the spread of resistant bacteria within poultry farms and potentially to humans. Furthermore, they demonstrated that human medicine practitioners are not the only practitioners responsible for selecting resistant bacteria; veterinarians and poultry farmers also play a role.⁵⁹

According to the FAO, an estimated 700,000 people die each year from antimicrobial-resistant (AMR) infections, and an incalculable count of sick animals fail to respond to treatments.⁶⁰ If no action is taken, a pandemic caused by multidrug-resistant bacteria could lead to massive human losses by 2050 and severe economic damage, with an estimated 3.8% decline in global GDP.⁶¹

Genotypic Tests

Analysis of Different Genetic Determinants of Bacterial Resistance

The high prevalence of plasmids in all (100%) multidrug-resistant strains is of particular concern, as these plasmids can facilitate the spread of resistance to multiple antibiotic families within initially susceptible bacterial populations.⁶² The presence of virulence genes indicates a high pathogenic potential of these strains, increasing their ability to cause severe infections in humans upon contamination. The 92.3% rate of chromosomal mutations suggests that most isolates have undergone DNA modifications in response to environmental pressures, particularly antibiotic treatment, thereby enhancing their adaptability. This resistance mechanism, though less easily transmissible than plasmid-mediated resistance, contributes to the emergence of multidrug-resistant strains, as bacterial adaptation is closely linked to mutation occurrence.⁶³ In addition to other resistance mechanisms, a combination with disinfectant resistance was observed in 46.15% of the isolates. A study showed that antimicrobial-resistant *E. coli* isolates tended to harbor more diverse combinations of disinfectant-resistance genes than susceptible isolates.⁶⁴ This relatively low rate may be explained by lower selective pressure, as disinfectants are less frequently used in poultry farming than antibiotics. However, such resistance remains concerning as it compromises the effectiveness of aseptic protocols and infection prevention in healthcare settings. Regarding strain variability, the results in Table 6 for the serotypes indicated that 23.07% ($n=3$) of the strains exhibited very similar characteristics.

Furthermore, the diversity of serotypes, which is almost common among all bacteria studied, suggests that these bacteria share similar characteristics. This similarity may indicate a common origin or clone spreading across the study area, which could support their role in the epidemic transmission chain.⁶⁵

However, even if these strains were mistakenly classified within the same clone due to their shared characteristics, the *H* and *O* serotypes identified in all strains confirm that they indeed belong to the same clone but exhibit variant expressions over time (*H*: 4, 5, 11, 19, 33, 40, 53; and *O*: 8, 12, 17, 38, 44, 48, 61, 77, 110, 134, 148, 182).⁶² The only strain identified with a *KL128* locus instead of the *H* antigen, and which could not be matched to any other strain in the sequencing data base, may indicate the emergence of a subclone. In 1994, a researcher described several *Staphylococcus aureus* bacteria originating from the same parental clone that evolved into two distinct subclones or lineages over time and under selective pressure.⁶⁶

Distribution of Resistance Genes Among Different Sequenced Strains

Analysis of antibiotic resistance gene frequencies among the 13 multidrug-resistant bacterial strains revealed a nearly uniform distribution of resistance genes. Certain genes, such as *blaCTX-M-55* and *sul2*, were detected in almost all samples ($\geq 90\%$), suggesting a high prevalence of resistance to β -lactams and sulphonamides in these bacterial populations. This trend is consistent with numerous studies showing the global dissemination of extended-spectrum beta-lactamases (ESBLs) and sulfonamide resistance genes, which may lead to a pandemic in both clinical and environmental contexts.^{67–69} Other genes, such as *aph(3'')-Ib* and *parC*, are also very common ($\geq 75\%$), indicating a significant selective

pressure favoring resistance to aminoglycosides and fluoroquinolones. In contrast, some genes are present in a small number of bacteria, indicating a rare distribution due to recent acquisition and are therefore not yet widespread in the bacterial population. This variability may be due to genetic differences between bacterial strains, environmental factors, or specific selective pressures (eg, exposure to antibiotics and horizontal gene transfer).^{70–72}

The presence of some genes at lower frequencies suggests acquisition via plasmids, increasing the risk of the emergence of new resistance combinations through co-localization phenomena.⁷³ A notable element of this study is the detection of the *mcr-1.1* gene in one strain (7.7%). This gene is particularly concerning as it confers resistance to colistin, a last-resort antibiotic used to treat infections caused by multidrug-resistant bacteria.⁷⁴ In some cases, resistance to colistin can lead to the production of carbapenemases, limiting treatment options for severe infections, including those caused by human pathogens. The presence of the mutant *ompK36* and *ompK37* genes in one strain is also an alarming signal, as they encode resistance to carbapenems. Therefore, it is important to regulate its use in livestock farming.

Plasmid Profiles Associated with Resistance

The results showed a high diversity of plasmids in the studied *E. coli* ESBL strains. The presence of plasmid types *IncF*, *IncH*, *IncX*, and *IncH1B(pNDM-CIT)* in several strains (38.46% of strains) is particularly concerning as these plasmids are frequently associated with antibiotic resistance and virulence genes. Indeed, the roles of *IncF* and *IncH* plasmids are well known in the transport and dissemination of extended-spectrum β -lactamase (ESBL) genes, such as *blaCTX-M* and *blaTEM*.⁷⁵ The presence of the *BlaOKP-B* gene in an *E. coli* strain could lead to dissemination via a plasmid, as this gene codes for β -lactamases that confer resistance to β -lactams in *Klebsiella oxytoca*. Furthermore, the presence of plasmids *IncH1B(pNDM-CIT)* and *IncFII(pCoo)* alerts us to the rapid dissemination potential in the case of the emergence of a gene encoding carbapenemases in these bacteria. These plasmids can only harbor genes encoding carbapenemases (*blaNDM*, *blaKPC*, which were not identified in this study), and they confer increased resistance to carbapenems and other β -lactams.⁷⁶ Moreover, the presence of plasmids *p0111* and *ColpVC* in some strains could increase the prevalence of pathogenic strains in livestock and the community. These plasmids, in association with virulence genes, may play a role in virulence by carrying toxic genes and factors that promote colonization and persistence in the host against antibiotics.⁷⁷ The heterogeneous presence of plasmids across the same *E. coli* clone highlights the strong selective pressure depending on the level of antibiotic use in the farms, but with a low potential for vertical gene transfer of resistance.⁷⁸ Indeed, the maintenance of a multi-resistant plasmid in a bacterial population through vertical transmission makes it more difficult to eradicate resistance.⁷⁹ However, the identification of plasmids associated with multi-resistance and virulence calls for strengthened biosecurity measures and a rational use of antibiotics to limit selective pressure and their dissemination through the environment.

Virulence Genes Associated with Resistance

The results showed highly virulent *E. coli* ESBL strains characterized by the combined presence of virulence and antibiotic resistance genes. The high prevalence of *fimH*, *csgA*, and *papA* indicates an increased ability for adhesion and biofilm formation, facilitating the persistence of the bacteria in both the host and the environment.⁸⁰ The simultaneous presence of *blaCTX-M-55* and virulence genes reflects a dual threat, as this co-localization makes the strain highly pathogenic and complicates treatment with β -lactams. The presence of these strains in a clinical setting could make managing infections more challenging, particularly urinary tract infections, sepsis, and hospital-acquired infections in humans.^{81–83} Other genes such as *fyuA*, *irp2*, *chuA*, *iutA*, and *astA* play a role in iron acquisition, immune evasion, and cytotoxicity. Enhanced epidemiological surveillance and controlled antibiotic use in livestock is necessary to contain highly pathogenic bacteria.

Conclusion

Considering the emergence and spread of multidrug-resistant bacteria worldwide, monitoring antibiotic resistance has become a major priority for public health preservation. In conclusion, the prevalence of multidrug-resistant enterobacteria was assessed, and resistance genes, virulence genes, and plasmids associated with this multidrug resistance were identified. It was found that 84.78% of fecal samples were colonized by at least one enterobacterium, with

a predominance of *Escherichia coli* (84.78%), followed by *Klebsiella pneumoniae* (3.26%), and *Salmonella spp.* (0.01%). The majority of the isolates were resistant to sulfamides (78%) and penicillins (57%), followed by quinolones and fluoroquinolones (23%). Additionally, 10% of *E. coli* isolates were extended-spectrum beta-lactamase (ESBL) producers. Excluding the ESBL-producing isolates, 26.07% of *E. coli* isolates were extremely resistant (XDR and PDR). Sequencing of *E. coli* ESBL strains revealed moderate variability in resistance genes, providing therapeutic options for cases of severe infections in a hospital setting. However, the colocalization of plasmids carrying both virulence and resistance genes, along with the frequent detection of variants of the gene *blaTEM* conferring resistance to beta-lactams, highlights the high risk of resistance dissemination. Moreover, although rare, the presence of the gene *mcr-1.1*, which is not expressed in the phenotypic test but is involved in resistance to polymyxins, is a warning signal, as it could spread rapidly via the mobile plasmids identified in this study (*IncX4* and *IncHI2*). This study demonstrated the genetic diversity of bacterial populations in the context of prophylactic antibiotic use in poultry farming. These results emphasize the need for both individual and collective action to improve farming practices and strengthen biosecurity measures to limit the spread of multidrug-resistant bacteria. Further studies, including sequencing enterobacteria from poultry farmers, will help measure the level of colonization of these strains in farmers. Sequencing of plasmids associated with the *mcr-1.1* gene and the mutant *ompK36* and *ompK37* genes will help assess their potential for dissemination and any associated co-resistance. Research into alternatives to antibiotic use in poultry farming and the antibiotic sensitivity testing of these ESBL bacteria to new classes of antibiotics, such as Vabomere and Cefiderocol (Cephalosporin), should be considered as future research avenues for preserving antibiotic efficacy in a “One Health” context.

Data Sharing Statement

Data supporting the findings of this study are available from the corresponding author upon request.

Ethical Approval

This study was conducted in accordance with the ethical guidelines of the Bioethics Committee for Health Research at the University of Lome, Togo. The research protocol was approved by the committee (approval N° 012/2024/CBRS, dated May 16, 2024). All procedures adhered to the committee recommendations regarding confidentiality and the appropriate use of data, ensuring the protection of participants and ethical management of the information collected during the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest related to this manuscript.

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