Multiple Antimicrobial Resistance of Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* from Small-Scaled Poultry Farms and Retail Chicken

Agnes Kilonzo-Nthenge^{1,2}, Samuel Nahashon², Siqin Liu², Ravneet Sandhu² and Kourtney Daniels²

1. Department of Human Sciences, Tennessee State University, 3500 John A. Merritt Boulevard, Nashville, Tennessee 37209

2. Department of Agricultural and Environmental Sciences, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, Tennessee 37209, USA

Abstract: Antibiotics used for agricultural purpose has contributed to the increased prevalence of antibiotic-resistant bacteria. The goal of this study was to investigate the prevalence and antimicrobial resistance of ESBL-producing *E. coli* in small-scaled poultry farms and retail chicken. The cultured *E. coli* isolates were subjected to phenotypic tests, susceptibility tests, and the polymerase chain reaction for detection of *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} genes. From 120 samples each of chicken feces, retail chicken, soil and chicken feed, ESBL-producing *E. coli* isolates were detected in 75.9%, 63.6%, 39.2%, and 13.3% of the samples, respectively. Minimum inhibitory concentration (MICs) values indicated that ESBL-producing *E. coli* were resistance to ampicillin (MIC $\geq$ 32 μ g/mL), gentamicin (MIC $\geq$ 16 μ g/mL), cefotaxime (MIC $\geq$ 4 μ g/mL) and ceftriaxone (MIC $\geq$ 4 μ g/mL), respectively. The total resistance for imipenem was also observed at 1.0% (MIC $\geq$ 4 μ g/mL) and none of the isolates were resistant to ceftazidime (MIC $\geq$ 16 μ g/mL). ESBL-producing *E. coli* from chicken feces and retail chicken carried *bla*_{SHV} gene at a rate of 6.8% and 5.7%, respectively and *bla*_{CTX-M} gene was also revealed at 2.9% in retail chicken. Moreover, ESBL-producing *E. coli* isolated from soil harbored *bla*_{SHV} and *bla*_{CTX-M} genes at 5%. None of the feed samples yielded ESBLs genes. Twenty three resistance patterns were observed for multi-resistant ESBL-producing *E. coli*. This study highlights the prevalence of multi-antimicrobial resistant ESBL-producing *E. coli* in small-scaled poultry farms and retail chicken, hence the need to review poultry management practices to minimize the occurrence.

Key words: *E. coli*, β -lactamases, poultry farms, retail chicken.

1. Introduction

Several findings suggest that the misuse of antimicrobials in animals' production results to antimicrobial resistant bacteria that could find their path into the environment [1]. *Escherichia coli* found in the gastrointestinal tract of human and animals are harmless, however that causes a number of significant illnesses [2]. *E. coli*, a zoonotic and a widespread *Enterobacteriaceae* is linked to animal and human infections [3] and also reflected as an indicator of fecal contamination in food. *E. coli* resides in soil,

water, farm animals, and food products [4] and is known to produce extended spectrum β -lactamases (ESBLs) [5] which exhibit effective hydrolyzation of β -lactam antibiotics. Beta-lactam resistance is caused by ESBL genes that are encoded by plasmid [6]. According to Habeeb et al. [7], the resistance usually depends on expression of *bla* genes belonging to the inter alia *bla*_{TEM}, SHV *bla*_{SHV} and *bla*_{CTX-M} genes family. Management for *E. coli* infections has been progressively complicated by the emergence of resistance to most first-line antimicrobial agents such as cephalosporins [8]. Cephalosporins, particularly third and fourth-generation are essential antimicrobial drugs in human and animal medicine [9]. The rising of *Enterobacteriaceae* resistance to cephalosporins over

Corresponding author: Agnes Kilonzo-Nthenge, Ph.D., associate professor, research fields: food microbiology and food safety.

the years is indebted to the spreading of ESBLs [10].

Evidence exists that antimicrobial drugs' use in the livestock plays a significant role in the contamination of food with ESBL-producing bacteria [11]. According to Bergenholtz et al. [12], the recovery of ESBL-producing bacteria from foods and animals has been reported and this has caused major and significant attention to the food industry [13]. In view of the accumulative concerns of ESBL-producing *E. coli* and the potential risk to the public health, it is important to recognize the potential reservoirs of ESBL-producing *E. coli* in agricultural settings. ESBL-producing *E. coli* may be used to trace the spread of antibiotic resistant bacteria [14] in the environment and food chain. Thus, this study focused on the search for ESBL-producing *E. coli* and confirmation thereof through the PCR amplification of the *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} genes in small-scaled poultryfarms' environment and retail chicken. Additionally, due to the rise of *Enterobacteriaceae* ESBL-positive strains exhibiting multidrug-resistance, an additional objective of our study was to evaluate the sensitivity of ESBL-producing *E. coli* to a panel of antibiotics.

2. Materials and Methods

2.1 Sampling of Materials

Environmental samples tested in this study included chicken feces, soil, and feed from six poultry farms (two farms per county) in Davidson, Williamson, and Cheatham counties in Tennessee, USA. These samples were acquired at private properties and consent was granted by the collaborating poultry farms. In the current study, small-scaled poultry farms raising the commercial chicken layer variety Single Comb White Leghorn were defined as those rearing 20 to 250 birds and vended their products to farmer's markets, roadside stands, or had direct delivery to their clients. In addition, retail chicken was purchased from 6 chain supermarkets in Davidson County. To keep confidentiality, the farms and stores were

identified by ID numbers, sample type, date and source of collection.

2.2 Isolation of Escherichia coli

Conventional poultry farms were sampled for chicken semi-fresh droppings, feed, and soil within the farm vicinity. All samples were collected over a 10 month period (February 2016—November 2016). For each sample type, five sub-samples were collected and pooled to make one composite sample (i.e., 5 samples per composite). Two samples for each environmental sample from all six farms were collected once in a month and analyzed for *E. coli*. At the same time, retail chicken was purchased from six different grocery stores. In total, 480 samples (feces = 120; soil = 120, feed = 120, retail chicken = 120) were analyzed. The background information of drugs used on farms was unknown; farmers were reactant to display farm records. Samples were processed in the laboratory within 24 h of collection or were stored overnight at 4°C until processed the next day. Briefly, 25 grams of each sample were suspended in 225 mL LB (Luria-Bertani) broth (Becton, Dickinson and Company, USA) and homogenized (Stomacher 400 circulator, Seward, London, UK) at 230 rpm for 2 minutes. The homogenates were incubated at 37 °C for 20 h. Subsequently, 10 µL were streaked on MacConkey agar (MAC; BD, Franklin Lakes, NJ) plates and sub cultured onto Eosin Methylene Blue (EMB; BD, Franklin Lakes, NJ) agar plates at 37 °C for 24 h. Colonies that exhibited a dark blue color with characteristic metallic sheen were selected and identified as *E. coli* by oxidase test and the API 20E (bioMérieux, Hazelwood, MO).

2.3 Isolation of ESBL-Producing Escherichia coli

E. coli isolates identified at 98% and above confidence level by API 20E test were tested for ESBL production. To screen for ESBL-producing *E. coli*, isolates were cultured in nutrient broth (BD, Franklin Lakes, NJ) for 20 h at 37 °C. Subsequently,

10 µL of grown cultures were streaked on CHROMID ESBL selective chromogenic media (bioMérieux, Hazelwood, MO) and incubated for 18-20 h at 37 °C. Presence of ESBL production was confirmed by combination disc method for ESBL detection. For this purpose, antibiotic susceptibility disks (BBL, BD) containing cefotaxime (30 µg) and ceftazidime (30 µg) with or without clavulanic acid (10 µg) were used. Briefly, overnight LB broth cultures were adjusted to a 0.5 McFarland opacity standard (BD, Franklin Lakes, NJ) and evenly streaked onto Mueller-Hinton agar (MHA) (Remel, Lenexa, KS) plates to form a lawn culture. After 10 min at ambient temperatures, third generation cephalosporins with and without clavulanic acid were placed onto inoculated plates and incubated at 37 °C for 18 h. A difference in the zone of inhibition of ≥ 5 mm for cefotaxime and ceftazidime with and without clavulanic acid was considered to indicate ESBL producing *E. coli*. The *E. coli* ATCC 25922 was used as the reference strain.

2.4 Minimum Inhibitory Concentrations (MIC) for ESBL-Producing Escherichia coli

MICs for ESBL-producing *E. coli* (n = 105) against selected cephalosporins, carbapenem, and aminoglycoside were determined by E-test (gradient methods) and agar dilution (reference method) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. E-test strips were purchased from Sigma-Aldrich (St. Louis, MO, USA). MICs were documented by determining where the ellipse edge intersected with the strip (MIC reading scale in µg/mL). Briefly, selected *E. coli* colonies from an overnight agar plates were emulsified in 0.85% NaCl to achieve a turbidity equivalent to a 0.5 McFarland standard. The suspensions were swabbed on a MHA plates and allowed to dry for 15 minutes. E-test strips were then applied to the agar surface with sterile forceps and incubated at 37 °C for 18-20 h. The resistance was interpreted as S (Susceptible), I (Intermediate) or R (Resistant) by comparing the

breakpoint values of each antibiotic with the CLSI recommended criteria [15]. *K. pneumonia* ATCC 700603 and *E. coli* ATCC 25922 were used as controls strains.

2.5 Detection and PCR Confirmation of b-Lactamase Genes in ESBL-Producing Escherichia coli

Presumptive ESBL-producing *E. coli* was confirmed through amplification of the *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} genes using PCR assays. In total 105 ESBL-producing *E. coli* isolates were randomly selected and utilized for *bla* gene detection by PCR. The isolates were cultivated overnight in Tryptic Soy Broth (Difco Dickinson and Company, Sparks, MD) to $> 5 \times 10^6$ cells and 1 mL of the overnight culture was centrifuged for 3 min at $13,000 \times g$ at 4 °C. DNA was isolated using the Pure link Genomic DNA Mini Kit (Life Technologies, Grand Island, New York) and respective concentrations were measured using a NanoDrop 2000 (Thermo Scientific, Pittsburgh, PA). Template DNA was diluted to a final working concentration of 25 ng/µL and its integrity was confirmed using agarose gel electrophoresis. Primer sequences, their melting temperature and expected size of amplicons of the *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} genes first reported by Feizabadi et al. [16] and Mahrouki et al. [17] are presented in Table 1. Using a PCR CORE Kit (Sigma, St. Louis, MO), PCR reactions were carried out in a final volume of 25 µL consisting of 125 ng DNA template, 2.5 µL 10X PCR buffer containing 10 mM Tris-HCl (PH 8.3), 3 mM MgCl₂, 400 µM dNTPs, 2.5 U Taq DNA polymerase, 0.5 µM each forward and reverse primer and deionized H₂O. Amplification was carried out in an Eppendorf Mastercycler nexus GSX1 thermal cycler (Fisher Scientific, Fair Lawn, NJ, USA) following thermal cycling profile adapted from Seyedjavadi et al. [18] for each *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} primers as follows: Initial denaturation at 94 °C for 5 minutes, 35 cycles of amplification at 94 °C for 45 seconds, 54 °C for 30 seconds, and 72 °C for 1 minute; initial

Table 1 Oligonucleotide primers used to amplify the *bla* genes of the ESBL-producing *E. coli* isolated from small-scaled poultry farms.

Gene target	Nucleotide sequence	TM ^a	Product size (bp)	Reference
<i>bla</i> _{CTX-M}	5-ACGCTGTTGTTAGGAAGTG-3	58	857	[16]
	5-TTGAGGCTGGGTGAAGT-3	57		
<i>bla</i> _{TEM}	5-TCGGGGAAATGTGCGCG-3	62	972	[17]
	5-TGCTTAATCAGTGAGGCACC-3	60		
<i>bla</i> _{SHV}	5-GGGTTATTCTTATTTGTCGC-3	56	615	[16]
	5-TTAGCGTTGCCAGTGCTC-3	60		

^a Melting temperature.

denaturation at 94 °C for 5 minutes, 30 cycles of amplification at 94 °C for 45 seconds, 56 °C for 1 minute, and 72 °C for 1 minute; Initial denaturation at 94 °C for 5 minutes, 36 cycles of amplification at 94 °C for 1 minute, 58 °C for 30 seconds, and 72 °C for 1 minute, respectively. Each of these reactions was followed by a final extension at 72 °C for 10 minutes. Samples were held at 4 °C until further use. Negative control reactions were prepared for each primer to identify contamination from reactions with DNA that was not targeted. After PCR amplification of the genes, PCR products were analyzed by electrophoresis in a 1.2% agarose gel (Fisher Scientific, Fair Lawn, NJ, USA) stained with 50 µg ethidium bromide. The PCR products were separated in a 1 × TBE buffer in a Fisher Biotech gel electrophoresis system (Fisher Scientific, Fair Lawn, NJ, USA) at 100 volts for 2 hours. *K. pneumonia* ATCC 7000603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

2.6 ESBL-Producing *Escherichia coli* Antibiotic Susceptibility Test

Antibiotic susceptibility testing of ESBL-producing *E. coli* isolates (n = 105) was done by Kirby Bauer disk diffusion method and according to the CLSI [15]. Briefly, overnight LB cultures for ESBL-producing *E. coli* isolates were adjusted to 0.5 McFarland standards and spread evenly on MHA plates and incubated at 37 °C for 24 h; commercially available antibiotic disks (St. Louis, MO, USA) were used for antimicrobial susceptibility testing. The panel of 12 different antimicrobial drugs included: amikacin (30 µg),

ciprofloxacin (5 µg), erythromycin, (15 µg), chloramphenicol (30 µg), kanamycin (30 µg), streptomycin (10 µg), novobiocin (30 µg), nalidixic acid (30 µg), tetracycline (30 µg), vancomycin (30 µg), colistin (10 µg), and amoxicillin (30 µg). *E. coli* ATCC 25922 was used as positive reference strain in all tests.

2.7 Statistical Analyses

Differences in mean prevalence and antimicrobial resistance of the ESBL-producing *E. coli* from poultry feces, retail chicken, soil and feed were determined by a one-way analysis of variance using SPSS software for Windows, version 12 (SPSS, IBM, Armonk, NY) and the chi-square test, respectively. Results for all analyses were considered significant at $p < 0.05$.

3. Results and Discussion

3.1 *Escherichia coli* Isolates

Biochemically confirmed *E. coli* was detected in 87.1% (418 out of 480) of various samples as listed in Table 2. In this work, chicken feces were highly (96.7%) contaminated and this demonstrates that poultry feces are reservoirs *E. coli* [19]. Likewise, 91.7% of the retail chicken samples were also contaminated with *E. coli*, however not statistically different from chicken feces ($p > 0.05$). There is a continuous defecation of *E. coli* by birds with the probability of contaminating the environment and entering the food chain. Through excretion, *E. coli* can potentially be transferred to agricultural products by avian manure to humans [20]. The practice of spreading contaminated chicken litter as a soil amendment

Table 2 Incidence of antimicrobial resistant *E. coli* from small poultry farms and retail chicken.

Sample type	No. of samples	Incidence of <i>E. coli</i> (%), 95% CI	Incidence of ESBL-producing <i>E. coli</i> (%), 95% CI
Chicken feces	120	116 (96.7) ^a	88 (75.9) ^a
Retail chicken	120	110 (91.7) ^{ab}	70 (63.6) ^a
Soil	120	102 (85) ^b	40 (39.2) ^b
Feed	120	90 (75) ^c	12 (13.3) ^c
Total No. of samples	480	418	210

may also result to *E. coli* entering water that is used for drinking and irrigation of crops. Our results are in agreement with an earlier finding that meats are a common source of *E. coli* [21]. The contamination of poultry meats arising from slaughter process is often primarily of fecal origin, due to intestinal leakage of fowls. Additionally, poultry meat may also be adulterated during processing, and transportation to the retail stores [22]. Transfer of bacteria from poultry products to human population may occur through the consumption or handling of contaminated meats [23]. Therefore, consumers should follow good hygienic practices during cooking and meal preparation, especially when raw chicken is an ingredient.

Soils within the vicinity of the poultry farms displayed *E. coli* contamination at a rate of 85%. Trawińska et al. [24] assessed *E. coli* contamination of the environment surrounding the poultry farms and established that the highest count was in the soil samples. Previous reports have demonstrated that poultry manure used for field application or rinse water during cleaning can be a source of entrance of bacteria into the soil environment [25]. Other avenues would include air transmission or spread by poultry house workers from contaminated floors to the outdoors. Our study also revealed 75% prevalence of *E. coli* in the poultry feed. This highlights the need for sanitary handling of poultry feed during processing, transpiration, and storage; since animal feed is at the begging of the food safety chain. Bacteria can readily diffuse to food animals via tainted feed and potentially contaminate animal carcasses intended for human consumption [26].

3.2 Prevalence of ESBL-Producing *Escherichia coli*

Understanding the frequency of ESBL-positive bacteria is quite significant, particularly that infections caused by these bacteria are more challenging to treat in humans. This study demonstrated a high prevalence of ESBL-producing *E. coli* (75.9%) in chicken feces from small-scale poultry farms (Table 2). In contrast to our results, ESBL-producing *E. coli* has been previously reported in broiler fecal samples at a lower rate of 42.1% [27]. The abundance and the movement of antibiotic resistance bacteria in agricultural soils may be enhanced by the application of animal manures that contain antibiotic resistant bacteria [28]. It is important to uphold environmental hygiene principles, notably with reverence to ensuring that crops do not come into contact with antimicrobial resistant pathogens carried in raw manure that is used as a fertilizer. The uptake of these antimicrobial resistant bacteria by vegetables that are consumed raw can cause imminent risk to the health of the consumer.

ESBL-producing *E. coli* frequency among retail chicken was 63.6% in our study. This is in agreement with a study in Portugal [29] where ESBL-producing *E. coli* was isolated in 60% from chicken carcasses at the retail level. In contrast to these results, ESBL-positive *E. coli* was notably lower compared to previous studies by Egea et al. [30] and Cohen et al. [31], which reported 93.3% and 94% contamination of raw poultry meat, respectively. It is a concern that ESBL producing *E. coli* is found in retail chicken, this bacteria hydrolyses several antibiotics, including third-generation cephalosporin [32] which is critical in managing infections.

In total, 39.2% of all soil samples from poultry farms turned out to be positive for ESBL-producing *E. coli*. However, the isolation rate was lower as compared to 77.0% observed in a previous study [33]. The prevalence of ESBL-producing *E. coli* in animal farms could also affect public health through environmental pollution [34]. According to Laube et al. [35], ESBL-producing *E. coli* has been detected on the land adjacent to animal farms. Soil is reported to harbor a diversity of microorganisms that are considered potential reservoirs for antibiotic resistance [36]. Overall, 13.3% ESBL-producing *E. coli* was isolated from poultry feed, significantly ($p < 0.05$) lower as compared to bacteria found in chicken feces, retail chicken, and soil. In some farms, antimicrobials are routinely added to animal feeds, thus repetitively exposing bacterial populations to sub therapeutic doses; ideal for the emergence and spread of antimicrobial resistance [37]. Therefore, effort must be made towards withdrawing the use of essential antimicrobial drugs in agriculture and strictly practice the therapeutic use under the oversight of veterinarians [38].

3.3 MICs for ESBL-Producing *Escherichia coli*

The MICs for ESBL-producing *E. coli* from various sources are detailed in Table 3. Our analysis showed ESBL-producing *E. coli* resistance to cephalosporins (cefotaxime cefoxitin, ceftriaxone), carbapenem (imipenem), aminoglycoside (gentamicin), and penicillin (ampicillin). The MIC determination revealed that 12.4% and 3.9% of the isolates had MIC values of ≥ 4 $\mu\text{g/mL}$ to both cefotaxime and ceftriaxone; thus confirming resistance to third generation cephalosporin (Table 3). It was also observed that 57.1% isolates were resistant to ampicillin (MIC ≥ 32 $\mu\text{g/mL}$) and 51.4% were resistant to gentamicin (MIC ≥ 16 $\mu\text{g/mL}$). The lowest rates of resistance in ESBL-producing isolates were observed for ceftazidime 0% (MIC ≥ 16 $\mu\text{g/mL}$) and imipenem 1.0% (MIC ≥ 4 $\mu\text{g/mL}$). Although few

ESBL-producing *E. coli* isolates were resistant to imipenem, it is still a concern since this antimicrobial agent is commonly used in human medicine [39]. According to Samaha-Kfoury and Araj [40], the treatment of ESBL-producing bacteria is limited to carbapenems such as imipenem. Moreover, our findings exhibited resistance among *E. coli* isolates to penicillin which is used routinely in the treatment of urinary tract infection [18]. More disturbing, ESBL-producing *E. coli* has been documented to be resistant to clinically essential β -lactam antibiotics including third and fourth-generation cephalosporins and penicillins [41]. Carcasses are often contaminated with resistant *E. coli* [42] at slaughter and probably, ESBL-producing *E. coli* and may be transmitted to humans through the consumption of contaminated chicken. Of note, ESBL-producing *E. coli* isolates were resistant to ampicillin and cefotaxime. The resistance to these antibiotics may be largely linked with the frequently use of ampicillin and cefotaxime in local human and animal clinical practices [43]. Our study provides evidence that poultry farms and retail chicken should be reflected as an important reservoir of ESBL-producing *E. coli*.

3.4 Occurrence of *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} Genes

This study revealed that ESBL-producing *E. coli* from small-scale poultry farms and retail chicken harbored *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} genes. A representation of PCR amplification for the genes is shown in Fig. 1. About 6.8% (3 of 44) of ESBL-producing *E. coli* isolates from chicken feces and 5.7% (2 of 35) from retail chicken carried the *bla*_{SHV} gene. On the other hand, gene *bla*_{CTX-M} was detected at 2.9% (1 of 35) bacteria isolates from retail chicken. The *bla*_{SHV} and *bla*_{CTX-M} were both detected in soils at 5% (1 of 20) of sample isolates. *E. coli* strains producing CTX-M enzymes have been reported to be the most frequent ESBL-positive *Enterobacteriaceae* detected in humans [44]. None of the isolates showed the presence of any combination

Table 3 ESBL-producing *Escherichia coli* resistance to cephalosporins, carbapenem, and aminoglycoside.

Antimicrobial agent	MIC interpretive criteria (µg/mL)			Antibiotic MIC µg/mL)	Resistance CF isolates (%)			Resistance RC isolates (%)			Resistance SL isolates (%)			Resistance FD isolates (%)			Total resistance (%)		
	S	I	R		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Cephalosporins	≤ 1	2	≥ 4	0.002-32	34.3	4.8	2.9	23.8	2.9	6.7	18.1	-	1.0	3.8	-	1.9	80	7.6	12.4
Cefotaxime	≤ 8	16	≥ 32	0.016-256	22.9	11.4	7.6	29.5	-	3.8	7.6	8.6	2.9	1.9	2.9	1.0	61.9	22.9	15.2
Cefoxitin	≤ 1	2	≥ 4	0.016-256	31.4	9.5	1.0	21.9	11.4	-	15.2	1.9	1.9	1.9	2.9	1.0	70.4	25.7	3.9
Ceftriaxone	≤ 4	8	≥ 16	0.016-256	28.6	13.3	-	29.5	3.8	-	17.1	1.9	-	2.9	2.9	-	78.1	21.9	-
Ceftazidime	≤ 1	2	≥ 4	0.002-32	38.1	2.9	1.0	33.3	-	-	19.0	-	-	5.7	-	-	96.2	2.9	1.0
Carbapenem	≤ 4	8	≥ 16	0.19-96	8.6	9.5	23.8	11.4	8.6	13.3	2.9	1.9	14.3	4.8	1.0	-	27.6	21	51.4
Imipenem	≤ 8	16	≥ 32	0.016-256	7.6	5.7	28.6	5.7	6.7	21.0	11.4	1.9	5.7	2.9	1.0	1.9	27.6	15.2	57.1
Aminoglycoside																			
Gentamicin																			
Penicillin																			
Ampicillin																			

Based on testing of 105 isolates in duplicate on two separate occasions: Chicken feces (CF); retail chicken (RC); soil (SL); feed (FD); minimum inhibitory concentration (MIC); susceptibility (S); Intermediate (I); and Resistance (R).

Fig. 1 PCR amplification products generated by primer *bla*_{SHV} (A), *bla*_{CTX-M} (B) and *bla*_{TEM} (C) from *E. coli* in small-scaled poultry farms (Lanes 1-7, 1-5 and 1-2, respectively) suspected of being positive for ESBL-producing *E. coli*. Arrows point to the 625, 857, and 972 base pair representing the *bla*_{SHV}, *bla*_{CTX} and *bla*_{TEM} gene and M is corresponding weights of the molecular marker (100 kb DNA ladder). Lane N is negative control *E. coli* 11775 and Lane P is positive control ESBL-producing *E. coli*. Lane B is a blank and BP depicts size of DNA fragment in base pairs.

of the genes in a single ESBL-producing *E. coli* isolate. According to Sebastian et al. [45], ESBL genes including *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} are common among in ESBL-producing *E. coli*. The presence of these genes in *E. coli* is worrisome; particularly because they cause resistance to carbapenems that are used for the management of infections suspected to be initiated by multidrug-resistant bacteria. Foods of animal origin are therefore, potential reservoirs for ESBL-producing *Enterobacteriaceae* and their encoding genes positions a critical health risk for consumers [46]. As such, efforts should be concentrated on Best Management Practices (BMPs) and hygienic practices during the course of meat harvesting and processing, to minimize the spread of antimicrobial resistance in the food chain.

3.5 Antimicrobial Susceptibility of ESBL-Producing *Escherichia coli*

Our findings demonstrate that 50.2% (210 of 418) of the ESBL-producing *E. coli* from poultry farms and retail chicken were resistant to a panel of antibiotics (Table 4). Differences in proportions of ESBL-producing *E. coli* resistant to these antibiotics were high and statistically significant ($p < 0.05$). The occurrence of ESBL producing *E. coli* is a reason for alarm; their multi drug resistance has immensely increased worldwide and reported as one of the most common reasons of morbidity and mortality associated with hospital-acquired infections [47].

Our results established that ESBL positive *E. coli* isolates from feces demonstrated absolute resistance against erythromycin (100%; 88 of 88) and streptomycin (100%; 88 of 88), which was significantly

higher than other antibiotics ($p < 0.05$). ESBL positive *E. coli* resistance to streptomycin is widespread among many enteric bacteria [48] and has become a concern to public health. Among the ESBL-producing isolates from feces, high resistance was also observed for tetracycline (88.6%; 78 of 88), followed by kanamycin (86.4%; 76 of 88), and nalidixic acid (68.2%; 60 of 88); moderate resistance rates were observed for chloramphenicol (63.6%; 56 of 88), ampicillin (40.9%; 36 of 88); and relatively low resistance rates were displayed for novobiocin (31.8%; 28 of 88), vancomycin (29.5%; 26 of 88), colistin and ciprofloxacin (11.4%; 10 of 88), and amikacin (4.5%; 4 of 88). Among the ESBL-producing *E. coli* from retail chicken, highest resistance rates were observed for erythromycin (100%; 70 of 70), followed by streptomycin (88.6%; 62 of 70), tetracycline (82.9%; 58 of 70), and nalidixic acid (71.4%; 50 of 70); moderate resistance rates were observed for kanamycin (51.4%; 36 of 70) and relatively lower resistance rates ($p < 0.05$) were shown for chloramphenicol, ampicillin, colistin, ciprofloxacin,

and amikacin (Table 4). None of the isolates from feed showed resistance to chloramphenicol, novobiocin, colistin, ciprofloxacin, and amikacin. ESBL-producing isolates from the soil were not resistant to colistin, ciprofloxacin, and amikacin. Generally, ESBL-producing *E. coli* from soil and feed generally showed lower resistance as compared to the isolates from feces and retail chicken. If not controlled, diffusion of multidrug resistance from poultry environment to the human community can be a major public health challenge [49]. The rest of the resistance rates among the ESBL-producing *E. coli* isolates are represented in Table 4.

Several phenotypes among antibiotic-resistant ESBL-producing *E. coli* isolates are presented in Table 5. Twenty three resistance patterns were observed and the predominant resistant pattern was ERY-STR (30.5%; 32 of 105), followed by ERY-STR-KAN-NAL (10.5%, 11 of 105), ERY (7.6%, 8 of 105), and ERY-STR-KAN-TET-VAN-AMC (6.7%, 7 of 105). The least (0.95%; 1 of 105) resistance patterns were

Table 4 Percentage of ESBL-producing *E. coli* resistant to select antibiotics.

Antibiotic	Source of <i>E. coli</i>			
	Feces	Chicken	Soil	Feed
			(%)	
CHL	63.6 ^c	23.1 ^d	18.2 ^b	0.0 ^e
ERY	100 ^a	100 ^a	81.8 ^a	46.2 ^a
STR	100 ^a	88.6 ^b	86.4 ^a	23.1 ^{bc}
TET	88.6 ^b	82.9 ^b	80.2 ^a	30.8 ^b
NAL	68.2 ^c	71.4 ^b	4.5 ^c	7.7 ^d
KAN	86.4 ^b	51.4 ^c	77.3 ^a	15.4 ^c
VAN	29.5 ^e	28.2 ^d	9.1 ^c	7.7 ^d
NOV	31.8 ^{de}	21.0 ^d	4.5 ^c	0.0 ^e
COL	11.4 ^f	4.7 ^e	0.0 ^d	0.0 ^e
AMC	40.9 ^d	20.5 ^d	22.7 ^b	15.3 ^c
CIP	11.4 ^f	5.1 ^e	0.0 ^d	0.0 ^e
AMK	4.5 ^g	2.6 ^e	0.0 ^d	0.0 ^e
N	88	70	40	12

Antibiotics: chloramphenicol (CHL), erythromycin (ERY), streptomycin (STR), tetracycline (TET), nalidixic acid (NAL), kanamycin (KAN), vancomycin (VAN), novobiocin (NOV), colistin (COL), amoxicillin (AMC), ciprofloxacin (CIP), amikacin (AMK).

^{a,b,c,d,e,f} Means within columns with no common superscript differ ($p < 0.05$).

Table 5 Antimicrobial resistance of ESBL-producing *E. coli* from poultry farms and retail chicken.

**Multiple Antimicrobial Resistance of Extended Spectrum Beta-Lactamase-Producing
Escherichia coli from Small-Scaled Poultry Farms and Retail Chicken**

No. of antimicrobials	Most frequent patterns (% of isolates)	No. of bacteria resistance (%)	Source
1	ERY	8 (7.6)	FM (5), RC (3)
	STR	2 (1.9)	FM (1), RC (1)
	TET	1 (0.95)	FM (1)
2	ERY-STR	32 (30.5)	FM (26), RC (6)
	NAL-TET	1 (0.95)	RC (1)
	ERY-TET	2 (1.9)	FM (2), RC (0)
3	ERY-TET-VAN	1 (0.95)	FM (1)
	ERY-CHL-STR-NAL	6 (5.7)	FM (4), RC (2)
	ERY-STR-KAN-NAL	11 (10.5)	FM (8), RC (3)
4	ERY-STR-TET-VAN	5 (4.8)	FM (4), RC (1)
	ERY-TET-VAN-AMC	1 (0.95)	RC (1)
	ERY-CHL-STR-NOV	1 (0.95)	RC (1)
6	CIP-ERY-STR-TET-COL-AMC	1 (0.95)	FM (1)
	AMK-ERY-STR-NOV-TET-VAN	5 (4.8)	FM (4), RC (1)
	ERY-STR-KAN-TET-VAN-AMC	7 (6.7)	FM (7)
7	ERY-CHL-STR-KAN-TET-VAN	1 (0.95)	FM (1)
	ERY-STR-KAN-TET-VAN-NOV	5 (4.8)	FM (3), RC (2)
	CIP-ERY-STR-TET-NAL-VAN	4 (3.8)	RC (4)
8	AMK-ERY-CHL-NAL-TET-VAN-AMC	3 (2.8)	FM (1), RC (2)
9	CIP-ERY-CHL-STR-NOV-TET-VAN-AMC	4 (3.8)	FM (2), RC (2)
	AMK-ERY-CHL-KAN-STR-TET-VAN-AMC	2 (1.9)	FM (2)
11	ERY-CHL-STR-NOV-NAL-TET-VAN-COL-AMC	1 (0.95)	FM (1)
11	AMK-CIP-ERY-STR-CHL-KAN-NAL-TET-VAN-COL-AMC	1 (0.95)	FM (1)

Chloramphenicol (CHL), erythromycin (ERY), streptomycin (STR), tetracycline (TET), nalidixic acid (NAL), kanamycin (KAN), vancomycin (VAN), novobiocin (NOV), colistin (COL), amoxicillin (AMC), ciprofloxacin (CIP), amikacin (AMK).

Source: Farm (FM), retail chicken (RC).

AMK-CIP-ERY-STR-CHL-KAN-NAL-TET-VAN-COL, ERY-CHL-STR-NOV-NAL-TET-VAN-COL-AMC, among others. An isolate was considered to be multidrug resistant (MDR) when it displayed resistance to three or more antibiotics [50]. Resistance of ESBL-producing *E. coli* to erythromycin and tetracycline in this study is a concern; these drugs are approved for treatment of poultry infections [51]. In addition, our result displayed ESBL-producing *E. coli* resistance to ciprofloxacin and tetracycline, which are commonly used in clinical settings. According to Le et al. [52], contamination of food with antibiotic resistant bacteria is a route to spread ESBL-producing bacteria in communities.

4. Conclusion

In conclusion, our findings indicate that poultry farms and retail chicken are potential reservoirs of multi-drug resistant ESBL-producing *E. coli*. The spread of ESBL-producing *E. coli* in food animals

represents a challenge in food safety and must be reflected as a public health concern, since the transmission to humans is possible. The data from this study provide contextual information for future studies of ESBL-producing *E. coli* trends in small-scaled poultry farms and retail meats. Therefore, further molecular studies are warranted to determine the genetic profile of ESBL-producing *E. coli* from poultry farms and poultry meats.

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