International Journal of Pathogen Research

6(4): 31-42, 2021; Article no.IJPR.67922
ISSN: 2582-3876

Molecular Detection of Extended Spectrum Beta-lactamase Resistance in *Escherichia coli* from Poultry Droppings in Karu, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author YBN designed the study, performed the statistical analysis. Author GRIP wrote the protocol and wrote the first draft of the manuscript. Authors SCT and IHN managed the analyses of the study. Author RHA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2021/v6i430169
Editor(s):
(1) Dr. Khadiga Ahmed Ismail Eltris, Ain Shams University, Egypt.
Reviewers:
(1) Mbehang Nguema Pierre Philippe, Tropical Ecology Research Institute, Gabon.
(2) Ileana Miranda Cabrera, Plant Health Centro Nacional de Sanidad Agropecuaria (CENSA), Cuba.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67922

Original Research Article

Received 23 February 2021
Accepted 30 April 2021
Published 05 May 2021

ABSTRACT

Aims: This study investigates and reports the production of extended spectrum beta-lactamase in *Escherichia coli* isolates in poultry droppings sourced from selected poultry farms in Karu, Nigeria
Study Design: Cross sectional study
Place and Duration of Study: Department of Microbiology, Nasarawa State University, Keffi, between August 2019 and February 2020.
Methodology: *Escherichia coli* was isolated from the samples using standard cultural and microbiological methods. Antibiotic susceptibility testing and minimum inhibitory concentrations were evaluated as described by the Clinical and Laboratory Standards Institute (CLSI). The detection of ESBL production in *E. coli* isolates was carried out using double disc synergy test. In addition, molecular detection of ESBL genes was carried out using Polymerase Chain Reaction (PCR) method.
Results: All (100%) samples collected had *E. coli*. Antibiotic resistances in the isolates in decreasing order were as follows: ampicillin (96.7%), streptomycin (94.4%), sulphamethoxazole /trimethoprim (87.8%), amoxicillin/ clavulanic acid (61.1%), gentamicin (52.2%), ciprofloxacin (40.0%), ceftazidime (35.6%), cefotaxime (31.1%), imipenems (22.2%), cefoxitin (13.3%). The

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commonest antibiotic resistant phenotype was AMP-SXT-S-CTX-CN (8.8%). Multiple antibiotic resistance (MAR) was observed in 92.2% (83/90) of the isolates with the common MAR indices being 0.5 (26.5%), 0.6 (19.2%), 0.4 (13.2%) and 0.9 (10.8%). Fifty nine of the eighty beta-lactam resistant isolates (73.7%) were confirmed ESBL producers. 55 of the 59 ESBL positive isolates (93.2%) carried *bla* genes as follows: bla_{SHV} (50/55, 90.9%), bla_{TEM} (31/55, 56.3%) and bla_{CTX-M} (46/55, 83.6%). Thirty six (65.5%) of the 55 isolates carried two *bla* genes (bla_{SHV} and bla_{TEM}, bla_{TEM} and bla_{CTX-M}, and bla_{CTX-M} and bla_{SHV}).

Conclusion: The *E. coli* isolates showed lower resistances to cefoxitin, imipenem, cefotaxime, ceftazidime, and ciprofloxacin and most isolates were MAR, with resistance to 5 antibiotics being the most predominant. In addition, bla_{SHV} gene was the most common ESBL gene detected in the confirmed ESBL-producing *E. coli* isolates.

Keywords: E. coli; ESBL; antibiotics; resistance; susceptible; gene; poultry.

1. INTRODUCTION

All over the world, the growth of the poultry industry as a source of food has been remarkable [1]. In Nigeria, the poultry industry is a major source of food (animal protein) to the citizens, and has a significant effect on the economy [2]. Food pathogens are responsible for a host of diseases [3-4] and these pathogens can be transmitted to humans in many ways including through contact with poultry droppings or through contact with contaminated poultry products [5].

Escherichia coli is a well-known and one of the most studied pathogens [6]. The habitat of *E. coli* is the intestinal tract of warm-blooded animals [7]. Its infection in poultry results in diseases which may or may not be responsive to treatment [8]. Pathogenic *E. coli* infections in poultry, also called colibacillosis, occur either as localized enteric colonization of intestines or as systemic infection manifesting in many ways, including as an acute fatal septicemia; and is a common disease of economic importance in poultry worldwide [8].

Antimicrobials, including beta-lactams, have been used extensively in poultry feeds at sub-therapeutic concentrations for growth promotion, disease prevention and treatment [9-11]. Classes of antimicrobials used in agriculture include penicillins, cephalosporins, fluoroquinolones, sulfonamides, amino glycosides, and tetracyclines [12]. The use of antibiotics is considered as a great factor in the emergence, selection and dissemination antibiotic-resistant organisms, particularly extended-spectrum beta-lactamase (ESBL) producing ones [13]. The ESBLs are plasmid-mediated beta-lactamases capable of hydrolyzing broad-spectrum cephalosporins, penicillins and monobactam, but not cephamycins or carbapenems; and are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam [14]. Many ESBLs have been described in different pathogens, including *E. coli*; the commonest are CTX-M, SHV, TEM, and OXA types, being the most prevalent and disseminated across various epidemiological niches [15]. The trend of antimicrobial resistance among *E. coli* in food animals and birds such as chickens is a cause of concern, especially due to the possibility and potential for the transfer of these pathogens to the human population [16].

In Nigeria, poultry farmers used one or more antibiotics for their birds [11]. Beta-lactam drugs are commonly used, and some have been abused and/or misused in many occasions [17]. Hence, ESBL resistance in poultry is of great significance as it places great burden economically and on healthcare systems. This study focuses on the detection of ESBL genes in *E. coli* isolates from poultry droppings from farms in Karu, Nigeria.

2. MATERIALS AND METHODS

2.1 Bacteria Isolates

Three poultry farms, designated A, B and C, were randomly selected from Karu Local Government Area of Nasarawa West Senatorial zone, in North Central Nigeria. Farm A is a commercial poultry farm located in Gunduma, a district under Karu Local Government, having a hatchery, processing plant and over 4000 chickens. Farm B is a medium sized poultry farm in Karshi, a different vicinity of Karu and housing about 600 to1,000 chickens aged from 50 to 60 days as at the time of sampling. Farm C is a large scale poultry farm having a large population of chickens, and situated at Keffi Shanu, in Karu Local Government Area.

A total of 90 chicken droppings (30 from each of the three poultry farms) were randomly collected over a four-month period. Each dropping was picked using a sterile spoon as described by Opere et al. [18] with some modifications. The droppings were scooped and dropped into a sterile stool container before being transported to the Microbiology Laboratory at the Nasarawa State University, Keffi, for same-day analysis or stored in a refrigerator (Model PRN 1313 HCA, BEKO, Germany) at 5°C for latter-day analysis.

Presumptive *E. coli* was isolated from the poultry droppings as follows: 1.0 g of poultry dropping was inoculated into 9 ml of nutrient broth (NB: Oxoid Ltd., UK) and incubated in an incubator (Quincy Lab Inc. Model12-140E, USA) at 37ºC for 24 h. A loopful of the 24- h broth was streaked on MacConkey agar (MCA: Oxoid Ltd., UK) plate and incubated at 37ºC for 24 h. Pinkish colonies from the 24-hour MCA plates were further streaked on Eosine Methylene Blue agar (EMB: Oxoid Ltd., UK) plates and incubated at 37ºC for 24 h. Colonies with a greenish-metallic sheen appearance were selected as presumptive *E. coli* [19].

Identification of *E. coli* was done by morphological, cultural and biochemical characteristics using Gram staining, Motility Test and biochemical tests (Indole, Methyl Red-Voges-Proskauer, Citrate, Nitrate Reduction Test, Urease Test, H_2S production Test, etc) as described in the Bacteriological Analytical Manual [20] and Cheesbrough [19]. The API20E system (Analytical Profile Index) (BioMerieux™, USA), a commercial kit designed for the identification of Enterobacteriaceae and other non-fastidious Gram negative bacteria, was used to confirm the suspected isolates as described in the manufacturer's manual. Colonies with a characteristic pink color on MCA, which grew with a greenish-metallic sheen on EMB agar, Gram-negative, rods, indole positive, citrate negative, methyl-red positive, Voges-Proskauer negative, urease negative, nitrate reduction positive, and a positive motility test indicated *E. coli.* The bacterium was stored in the refrigerator on nutrient agar (Oxoid Ltd, UK) slants and reactivated by sub-culturing on MCA for use in further research.

2.2 Antibiotic Susceptibility Testing

The antibiotic susceptibility test for *E. coli* isolates from poultry droppings was carried out using the Kirby-Bauer disc diffusion method as modified by the Clinical and Laboratory.

Standards Institute (CLSI) [21]. Briefly, 5 colonies of *E. coli* isolates were inoculated into 5 ml of Mueller-Hinton broth (MHB: Oxoid Ltd, UK) and incubated at 37ºC for 24 h after which the 24-h MHB was standardized to the turbidity equivalent to 0.5 McFarland Standard. The 0.5 McFarland Standard was prepared as follows: 99.5 ml of 1% ($\frac{v}{v}$) H_2SO_4 + 0.5 ml of 1.172% ($\frac{w}{v}$) $BaCl_2.2H_2O$. A sterile cotton swab stick was dipped into the standardized *E. coli* suspension and streaked on Mueller-Hinton Agar (MHA: Oxoid Ltd, UK) plates. Antibiotics discs (Oxoid Ltd, UK) were gently placed on the MHA plates using a pair of sterile forceps and the plates were allowed to incubate at room temperature for 1 h before re-incubating at 37ºC for 17 h. After incubation, the diameters of the zones of inhibition were measured to the nearest millimetre (mm) using a ruler and the result of the susceptibility test was interpreted using susceptibility breakpoint earlier described by CLSI [21].

2.2.1 Extended spectrum β-Lactamase production test

The phenotypic confirmatory test for ESBL production by isolates resistant to cefotaxime and ceftazidime was carried out using Double-Disc Synergy Test (DDST) method earlier described by Giriyapur et al. [22]. Briefly, 10^5 cfu/ml bacterial suspension was streaked on sterile Mueller-Hinton agar plates and amoxicillinclavulanic acid (30 µg) disc was placed at the centre of the plate. Cefotaxime (30 µg) and ceftazidime (30 µg) discs were then placed 15 mm (edge-to-edge) from the disc at the centre. Enhancement of zone of inhibition in the area between the amoxicillin-clavulanic acid disc and any one of the β-lactam discs compared with the zone of inhibition on the far side of the drug disc was interpreted as indicative of the presence of an ESBL in the tested strain.

2.2.2 Molecular detection of extended spectrum β-Lactamase genes

Isolates that were confirmed ESBL producers were screened to detect the presence of some ESBL resistance genes namely: bla_{SHV}, bla_{TEM} and bla_{CTX-M}.

2.3 DNA Extraction

The DNA extraction was performed by the boiling method as described previously [23]. Following purification on MacConkey agar, bacterial DNA was isolated from a 24-h culture in Luria-Bertani broth (LB: Oxoid Ltd, UK) prepared according to the manufacturers' protocol. The bacterial cells were harvested by centrifugation at 3200 rpm in a microcentrifuge (Model 5417R, Lab-line Instrument Inc USA) for 2 min at room temperature and the supernatant was discarded. The harvested cells were re-suspended in 1 ml of sterile normal saline and the microcentrifuge tubes were placed in the vortex for 5 sec. Centrifugation was carried out at 3200 rpm for 1 min and the supernatant was discarded. 0.5 ml of sterile normal saline was added to the pellets and the tubes were mixed on a vortex mixer (Lab-line Instrument Inc, USA) for 5 sec after which they were heated in the block heater at 90°C for 10 min. immediately after heating, rapid cooling was done by transferring the tubes into the freezer for 10 min. Cell debris was removed after centrifugation was done at 3200 rpm for 1 min and 300 µl of the supernatant was transferred into a sterile 2 ml Eppendorf tube as DNA and stored at -10°C until use.

Estimation of the concentration, purity and yield of the DNA sample was accessed using the absorbance method (the measurement of absorbance) with the spectrophotometer (Nanodrop 1000, InqabaBiotec, South Africa). For DNA concentration, absorbance readings were performed at 260 nm (A260) and the readings were observed to be within the instrument's linear range (0.1 – 1.0). DNA purity was estimated by calculating the A260/A280 ratio and this was done by the spectrophotometer's computer software (where A260/A280 ratio ranges from 1.7 – 1.9).

2.3.1 DNA amplification of extended spectrum β-Lactamase genes

Simplex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes being assessed in the isolates. The presence of *blaCTX-M, blaSHV and blaTEM* genes were tested for using previously published primer sets and conditions. The primer sequences and expected amplicon sizes for each gene are listed in Table 2.

The reactions were carried out in 20 µl reaction volume made up of 10 µl of Mastermix (Inqaba Biotec, South Africa), 0.32 µl of primers (0.16 µl each of forward and reverse primers), 3 µl of DNA and 6.68 µl of nuclease-free water. The primer concentration stood at 0.2 M [24]. The reaction tubes were placed in the holes of the thermocycler (Model TC-312, Techne, England) and the door of the machine was closed.

Conditions for amplification of all the genes during the reactions were set as 3 min of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 40 sec, initial extension at 72°C for 50 sec, final extension at 72°C for 3 min and a hold at 4°C infinitely.

2.3.2 Agarose gel electrophoresis

Exactly 7 µl of the amplified DNA was transferred into the wells of a 1.5% Agarose gel by stabbing the wells using a micropipette and this was done carefully to ensure that each well had only one sample. Each gel had one well which contained a DNA ladder (1500 bp, Inqaba Biotec, South Africa) in order to estimate the size of the DNA amplicons. Electrophoresis was run at 125 volts for 20 min, after which the gels were viewed using ultra-violet trans-illuminator (Vilberb Lourmat TFX-35-M serial no NoV02 8104, France).

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Resistance Profile

Resistance to the antibiotics tested was observed in 83 (92.2%) of the 90 isolates. The prevalences of antimicrobial resistance profile of the *E. coli* isolates from poultry droppings are shown in Table 1. Resistance to beta-lactams was in decreasing order: ampicillin (96.7%), amoxicillin/clavulanic acid (61.1%), ceftazidime (35.6%), cefotaxime (31.1%), imipenem (22.2%) and cefoxitin (13.3%). Resistance to non-beta-lactams decreased in the order: streptomycin (94.4%), sulphamethoxazole / trimethoprim (87.8%), gentamicin (52.2%) and ciprofloxacin (40.0%).

3.2 Antimicrobial Resistance Phenotypes

The distribution of the antibiotic-resistant isolates into the observed phenotypes is shown in Table 3. The commonest phenotypes were the AMP-SXT-S-CTX-CN having 7 isolates (8.8%), AMP-SXT-S-CIP-AMC, and AMP-SXT-S-FOX-CAZ-CIP-CTX-CN-AMC-IPM combinations, all having 4 isolates (5.0 %). The commonest ESBL phenotype was: AMP,SXT,S,FOX,CAZ,CIP,CTX,CN,AMC,IPM (3.6%)

Table 1. Antimicrobial resistance profile of *Escherichia coli* isolates from poultry droppings from selected poultry farms in Karu, Nasarawa State, Nigeria

Antibiotics	Disc Content (µg)	No. (%) resistance in *E. coli* (n=90)
Ampicillin (AMP)	10	87 (96.7)
Gentamicin (CN)	30	47 (52.2)
Amoxicillin/Clavulanic acid (AMC)	30	55 (61.1)
Sulphamethoxazole/Trimethoprim (SXT)	25	79 (87.8)
Cefotaxime (CTX)	30	28 (31.1)
Streptomycin (S)	10	85 (94.4)
Ceftazidime (CAZ)	30	32 (35.6)
Ciprofloxacin (CIP)	5	36 (40.0)
Cefoxitin (FOX)	30	12 (13.3)
Imipenem (IPM)	10	20 (22.2)

3.3 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in 83 (92.2%) of the 90 isolates. Two isolates (2.22%) had a MAR index of < 0.20 and this suggests that most of the isolates originated from an environment where abuse of antibiotics was regular [12]. The commonest indices were 0.5 (26.5%), 0.6 (19.2%), 0.4 (13.2%) and 0.3 (10.8%).

3.4 Phenotypic Confirmation of Extended-spectrum Beta-lactamase Production

Fifty nine (73.7%) of the 80 beta-lactam resistant isolates tested showed enhanced zones of clearing towards the amoxicillin-clavulanic acid disc when examined by DDST method. Hence, most of isolates resistant to beta-lactam antibiotics were ESBL positive.

3.5 Molecular Detection of Extended Spectrum Beta-lactamase Genes

Fifty five of the fifty nine ESBL positive *E. coli* isolates (55, 93.2%) carried the *bla* genes as follows: bla_{SHV} (50; 90.9%), bla_{CTX-M} (46 83.6%) and bla_{TEM} (31, 56.3%). Thirty six (65.4%) of the 55 isolates carried two *bla* genes (either of the combinations: (bla_{SHV} and bla_{CTX-M}, bla_{TEM} and bla_{CTX-M} and bla_{TEM} and bla_{SHV}).

3.6 Discussion

Generally, there has been an increase in the number of infections due to ESBL *E. coli*, and African countries are not exempted [25]. Also, bacteria in food-producing animals are well spread in the food chain (14). In many African countries, the desire to make huge returns in animal production has led to extensive and heavy use of antibiotics [26]. Interestingly, reports have shown that the heavy usage of antibiotics is a risk factor for the acquisition of ESBL-producing organisms and this has resulted in the increase in resistance of many common antibiotics, such as, gentamicin, ampicillin, tetracycline, and cephalosporins (third generation) [27].

From this study, it was observed that *E. coli* isolates were more resistant to Amoxicillin/Clavulanic Acid, Streptomycin, Ampicillin, and Sulphamethoxazole /Trimethoprim, but less resistant to Imipenem, Cefotaxime, and Ceftazidime. This finding is similar to a study conducted by Chishimba et al. [26] and Seni et al. [28]. The high susceptibility to imipenem and cefotaxime, supports their use as last β-lactam antibiotic of choice when treating infections caused by *E. coli*. Moawad et al. [29] (2018) observed high resistance to antibiotics such as Penicillin (98.2%), Erythromycin (96.4%) and Rifampicin (96.4%). Antimicrobial resistance rates for Imipenem, in the study location was below 15%, being in a similar range with those of *E. coli* from poultry as reported in Abuja (6.3%) by Aworh et al. [30], Keffi (12%) by Tama et al. [24], Ecuador (0%) by Vinueza-Burgos et al. [31] and in Anambra (0%) by Carissa et al. [32]. This observation differs from one by Chika et al. [33] who reported a high percentage of resistance to imipenem (51%) from poultry samples in Abakaliki.

The occurrence of MAR isolates observed in this study (as calculated in Table 4) suggests that almost all the bacteria exhibited multiple antibiotic resistance rates. A significant number of *E. coli* isolates in this study had a MAR index >0.2. Also, it is noteworthy that MAR of 0.5 had a high percentage of isolates in the study location. MAR index values greater than 0.2 suggests that the isolates were gotten from high-risk sources. The resistance of isolates to these antibiotics may be due to antibiotic misuses, poor dosing regimen or prolonged therapy of infection [34]. These high MAR index isolates, all from poultry droppings points to the fact that antibiotic resistance in humans does not only come from hospitals, but also from food-producing animals.

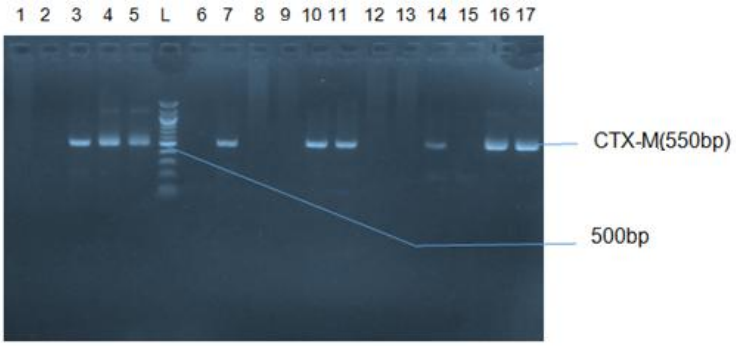

Plate 1. Agarose gel electrophoresis of the amplified CTX-M gene; Lanes 3-5, 7, 10, 11, 14, 16 and 17 represent the CTX-M bands at 550bp while Lane L represents the 100bp molecular ladder

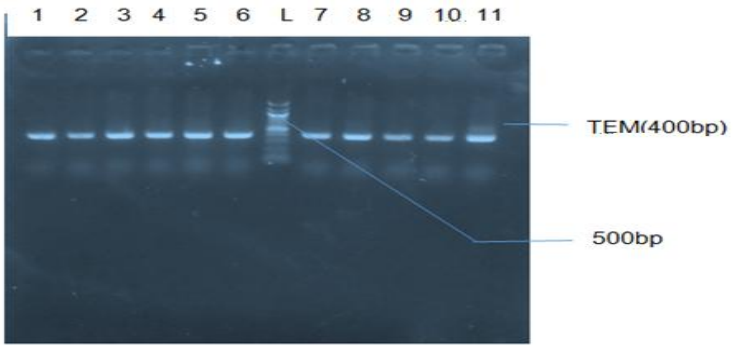

Plate 2. Agarose gel electrophoresis of the amplified TEM gene; Lanes 1-11 represent the TEM bands at 400bp while Lane L represents the 100bp molecular ladder

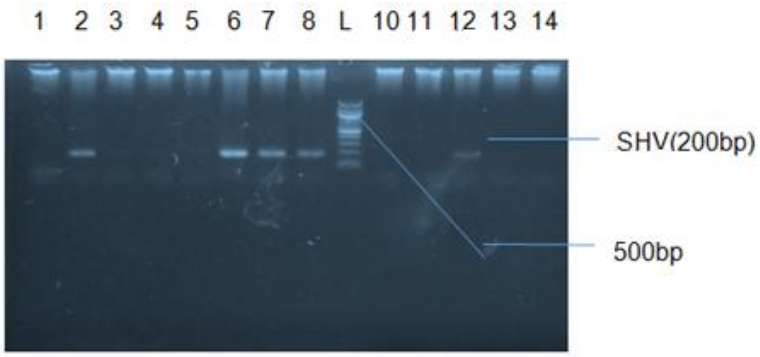

Plate 3. Agarose gel electrophoresis of the amplified SHV gene; Lanes 2, 6-8, 12 represent the SHV bands at 200bp while Lane L represents the 100bp molecular ladder

Table 2. Primers and their sequences

Target Gene	Primer Name	Sequence (5' – 3')	Product Size (bp)	Reference
bla_{SHV}	bla_{SHV}-F	TCAGCGAAAAACACCTTG	472	(40)
	bla_{SHV}-R	TCCCGCAGATAAATCACC		
bla_{CTX-M}	bla_{CTX-M} F	CGCTTTGCGATGTGCAG	550	(40)
	bla_{CTX-M} R	ACCGCGATATCGTTGGT		
bla_{TEM}	bla_{TEM}-F	CTTCCTGTTTTTGCTCACC	636	(40)
	bla_{TEM}-R	AGCAATAAACCAGCCAGC		

Table 3. Antimicrobial resistance phenotypes of *Escherichia coli* isolated from poultry droppings from selected poultry farms in Karu, Nasarawa State, Nigeria

Antibiotic Resistance Phenotypes	Frequency (%) (n=80)
ESBL Phenotypes	
AMP,CTX,AMC	1(1.2)
AMP,CTX,CAZ	1(1.2)
AMP,FOX,CAZ,CTX	2(2.5)
SXT,FOX,S,CAZ,CTX	2(2.5)
AMP,FOX,S,CAZ,CTX	1(1.2)
AMP,SXT,FOX,S,CAZ,CTX	2(2.5)
AMP,SXT,S,CAZ,CIP,CTX	1(1.2)
AMP,SXT,CAZ,CTX,CIP,AMC	1(1.2)
AMP,SXT,S,CAZ,CTX,AMC	2(2.5)
AMP,SXT,FOX,S,CAZ,AMC	1(1.2)
AMP,SXT,FOX,S,CIP,CTX,IPM	1(1.2)
AMP,SXT,S,FOX,CAZ,CTX,IPM	2(2.5)
AMP,SXT,S,FOX,CAZ,CTX,CN,AMC	1(1.2)
AMP,SXT,S,FOX,CAZ,CIP,CTX,AMC	2(2.5)
AMP,SXT,S,FOX,CAZ,CIP,CTX,AMC,IPM	2(2.5)
AMP,SXT,S,FOX,CAZ,CIP,CTX,CN,AMC,IPM	3(3.6)
Non-ESBL Phenotypes	
CIP	1(1.2)

Antibiotic Resistance Phenotypes	Frequency (%) (n=80)
AMP	1(1.2)
AMP,CTX	3(3.6)
AMP,IPM	2(2.5)
AMP,CTX,AMC	1(1.2)
AMP,FOX,CTX	2(2.5)
AMP,FOX,S	1(1.2)
AMP,S,AMC	2(2.5)
AMP,SXT,S	1(1.2)
FOX,S,CAZ	1(1.2)
AMP,SXT,S,IPM	1(1.2)
AMP,SXT,S,CTX	2(2.5)
AMP,SXT,S,AMC	3(3.6)
AMP,FOX,S,CAZ	2(2.5)
AMP,SXT,S,CIP,AMC	4(5.0)
AMP,SXT,S,CTX,CN	7(8.8)
AMP,SXT,FOX,S,CTX	2(2.5)
AMP,FOX,S,CAZ,CTX	1(1.2)
AMP,SXT,FOX,S,CAZ,CIP	2(2.5)
AMP,SXT,S,CIP,CTX,CN	3(3.6)
AMP,SXT,CAZ,CTX,CIP,AMC	1(1.2)
AMP,SXT,S,CIP,CN,AMC	2(2.5)
AMP,SXT,FOX,S,CAZ,AMC	2(2.5)
AMP,SXT,S,CAZ,CIP,CTX,AMC	2(2.5)
AMP,SXT,FOX,S,CIP,CTX,IPM	2(2.5)
AMP,SXT,S,FOX,CAZ,CTX,CN,AMC	1(1.2)
AMP,SXT,S,FOX,CAZ,CIP,CTX,AMC	1(1.2)
AMP,SXT,S,FOX,CAZ,CIP,CTX,AMC,IPM	1(1.2)
AMP,SXT,S,FOX,CAZ,CIP,CTX,CN,AMC,IPM	1(1.2)

Amp: Ampicillin, CN: Gentamicin, AMC: Amoxycillin/Clavulanic Acid, SXT: Sulphamethoxazole/Trimethoprim, CTX: Cefotaxime, S: Streptomycin, CAZ: Ceftazidime, CIP: Ciprofloxacin, FOX: Cefoxitin, IPM: Imipenem

Table 4. Multiple antibiotic resistance (MAR) index of resistant *Escherichia coli* isolated from poultry droppings from selected poultry farms in Karu, Nasarawa State, Nigeria

No of antibiotics isolate resistant to (a)	No. of antibiotics tested (b)	MAR Index $(\frac{a}{b})$	No. (%) MAR isolates (n=90)
10	10	1.0	4(4.8)
9	10	0.9	3(3.6)
8	10	0.8	5(6.0)
7	10	0.7	8(9.6)
6	10	0.6	16(19.2)
5	10	0.5	22(26.5)
4	10	0.4	11(13.2)
3	10	0.3	9(10.8)
2	10	0.2	5(6.0)

**MAR isolates are those with resistance to at least two antibiotics [35]*

The occurrence of ESBL producers in *E. coli* isolates jointly resistant to ceftazidime and cefotaxime observed in this study was higher than 46.9% reported by Tama et al. [24] in Keffi, but was lower than 85.7% reported by Abayneh et al. [36] in, Ethiopia, and 70% reported by Shin et al. [37] in South Korea. This study showed that bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ ESBL genes were expressed in all the study locations. An interesting observation in this study was the percentage occurrence of the bla_{SHV} gene in the confirmed *E. coli* isolates which was higher than the bla_{TEM} and $bla_{CTX\text{-}M}$ genes. This finding is in disagreement with some reports that show $bla_{CTX\text{-}M}$ as the commonest of ESBL genes, over others such as bla_{TEM} and bla_{SHV} as reported by Olowe et al. [38], Aliasadi et al. [39], and Chishimba et al. [26]. The presence of ESBL genes observed in this study suggests that the genes are possibly responsible for the production of ESBL enzymes that is resistant to most β-lactam antibiotics. The coexistence of different β-lactamase genes within the same isolates in this study was observed.

Results from this study and similar studies around Nigeria have shown how widespread the incidence of ESBL is nationwide. This is cause for worry because poultry meat and other products from poultry serve as food for humans. The possibility of the transmission of ESBL *E. coli*, which are also multidrug resistant and known to be responsible for community infections, being transmitted via poultry droppings calls for better strategy to reduce contamination of poultry products.

4. CONCLUSION

The *E. coli* isolates from poultry droppings from selected poultry farms in Karu were more susceptible to imipenem, cefoxitin and cefotaxime. This implies that the antibiotics are useful in the treatment of infection caused by *E. coli.* Also, most *E. coli* isolates jointly resistant to cefotaxime and ceftazidime were confirmed ESBL producers. In addition, the most ESBL gene detected was bla_{SHV} than bla_{TEM} and $bla_{CTX\text{-}M}$.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Consent was obtained from the various poultry farm managers before sample collection. Samples were collected only with the farmer's consent and willingness to participate in the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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