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Phenotypic and Genotyping Identification of Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae Obtained from Animal Fecal Samples within Owerri Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: One of the major means for the spread or distribution of antibiotic resistance is through animal, either by consumption or as companions. Most of the antibiotic resistant bacteria investigated have been reported to exhibit multidrug resistance which can either be acquired or natural. The present study was targeted at investigating the epidemiology of extended spectrum beta-lactamase-producing Enterobacteriaceae. Using stratified randomized sample design, three hundred (n = 300) fecal samples [of cattle, and goats (n = 150 for each)] were collected from various abattoir in Owerri metropolis. The isolation, purification, and antibiotic sensitivity were

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carried out using standard procedures. The resistant genes were investigated using forward and reverse primers specific for the following genes *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$.
Results: The result showed 97.67 % growth of Enterobacteriaceae. The highest Enterobacteriaceae isolated was in cattle fecal sample (61.00 %), while 2.33 % of the total samples did not show any growth for Enterobacteriaceae. Only three Enterobacteriaceae species were identified: *Escherichia coli, Proteus vulgaris,* and *Klebsiella pneumonia*. *Escherichia coli* was the most abundant Enterobacteriaceae (42.32 %) and *P. vulgaris* was the least abundant (23.89 %). The isolates showed multiple resistances to most of the antibiotics tested. All the isolates were susceptible to Imipenem (IPM). The molecular characterization showed the presence of the three genes (*bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$) investigated. The *bla*$_{CTX}$ gene-band was the most prevalent (72.22 %) while the *bla*$_{SHV}$ gene-band was the lowest prevalent resistant gene (61.11 %).
Conclusion: It was concluded that the genes: *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ were present in Enterobacteriaceae species (*K. pneumoniae, P. vulgaris, and E. coli*) isolated from abattoir in Owerri metropolis, Imo State.

Keywords: Extended Spectrum Beta-Lactamase (ESBL), Enterobacteriaceae; antibiotic resistance, fecal sample, epidemiology.

1. INTRODUCTION

Antibiotics have been crucial in the fight against bacterial infection since the time of its discovery. Consequent to the consistent and inappropriate utilization of antibiotics, there has been an emergence of resistance among bacteria species [1]. Among these antibiotic resistant bacteria species are the Gram-negative bacteria that are resistant to multiple antibiotics and capable of producing Beta-Lactamases enzymes which have broad spectrum of activity [1]. The production of these enzymes is the most common resistant mechanism of Enterobacteriaceae to third generation cephalosporins [2]. Several researches have detected ESBL genes in bacteria from both human and animal isolates, and from products of the food chains and sewages, revealing its distribution and presence in environmental reservoirs [2].

Extended spectrum Beta-Lactamases (ESBLs) are enzymes (with extended spectrum) produced by bacteria to degrade beta-lactam antibiotics [3]. These are enzymes that confer resistance to β-lactam family of antibiotics and are produced by Gram-negative bacteria [4,5]. ESBLs consist of several hundreds of enzymes and different nomenclatures have been suggested for β-Lactamases. According to Giske et al. (as cited by Brolund et al. [3]), ESBL enzymes can be divided into three main groups: ESBL$_A$, ESBL$_M$, and ESBL$_{CARBA}$. The group ESBL$_A$ contains the CTX-M enzymes, SHV, and TEM enzymes which are horizontally transferable. The ESBL$_M$ are miscellaneous ESBLs under which AmpC acquired ESBL represent the most commonly found in this group. ESBL$_{CARBA}$ contains enzymes that confers carbapenemase activity such as KPC and metallo β-Lactamases [3,6].

Enterobacteriaceae is the major bacterial family associated to ESBL production, of which *Klebsiella pneumonia* and *Escherichia coli* are the most important (because their clinical importance as human pathogens) [3]. Enterobacteriaceae, the largest family of Gram-negative bacteria, is a rod shaped, facultative, anaerobic bacteria; majority of this species resides in the intestine of humans and animals, only a few are found in soil and water [1]. Enterobacteriaceae are clinically significant pathogens, they are the most regular gastrointestinal tract (GIT) colonizers and are responsible for several diseases such as infection in the bloodstream, central nervous system, lower respiratory tract, gastrointestinal and urinary tract [3].

ESBL have constitute a burden to health care system; it has resulted in prolonged hospital stay and increase in treatment cost. Many a times the diagnostic procedure available for detection of these isolates are slow and empirical treatment becomes necessary. The occurrence of Enterobacteriaceae species in foods of animal origin is high in many parts of the world; some studies have attributed this to the unhygienic slaughter practices, and the risk of diseases from the consumption of these food is high because of the traditional practice of consuming raw meats. The study, therefore, is design to investigate the epidemiology of ESBL producing Enterobacteriaceae obtained from animal fecal samples within Owerri metropolis.

2. METHODS

2.1 Sample Collection

Three hundred (n = 300) fecal samples [of cattle, and goats (n = 150 for each)] were collected from various abattoir in owerri metropolis. The samples were collected from the colon of recently slaughtered animals. Using a sterilized spatula, the fecal samples were collected into sterilized, properly labeled sample bottles, after which the bottles were transported to the laboratory immediately for bacteria isolation and incubation.

2.2 Bacteria Isolation

The media was prepared according to manufacturers' instruction.

MaConkey agar: 49.53g of MacConkey agar was weighed and poured into a conical flask containing 1000ml of distilled water and 1g of Cefoxitin antibiotics. The solution in the conical flask was properly sealed with cotton and aluminum foil, autoclaved at 121 ^{o}C for 15 minutes, allowed to cool at 45 ^{o}C and aseptically poured into petri dishes and allowed to gel. The samples were cultured by dipping a sterile swab stick into the samples and evenly robbing the swab stick against the medium in the petri dish. The petri dish is then put into the incubator and left for 24 - 48 hours. Bacteria isolates sensitive to Cefoxitin will not survive while positive growth will be found on plates containing bacteria isolates resistant to the antibiotics Cefoxitin. This will help in the isolation of bacteria with potential extended spectrum antibiotic resistance activity.

Inoculation and incubation: Using sterile wire loop, distinct colonies were collected and inoculated onto the nutrient agar gel. The inoculated plates were incubated at 37 ^{o}C for 24 hrs in an incubator. The inoculation was carried under a flame inoculating cabinet. The isolates were preserved in aseptic condition for further analysis.

Nutrient agar: 35g of Nutrient agar was weighed and poured into a conical flask containing 1000ml of distilled water and swirled to obtain a homogenous mixture. The solution in the conical flask was properly sealed with cotton and aluminum foil, autoclaved at 121 ^{o}C for 15minutes, allowed to cool at 45 ^{o}C and aseptically poured into petri dishes and allowed to gel. The bacteria isolates were sub-cultured on nutrient agar in order to provide additional nutrients for their growth, in other to obtain pure colonies of Enterobacteriaceae.

Inoculation and incubation: using sterile wire loop, distinct colonies were collected and inoculated onto the nutrient agar gel. The inoculated plates were incubated at 37 ^{o}C for 24 hrs in an incubator. The inoculation was carried under a flame inoculating cabinet. The isolates were preserved in aseptic condition for further analysis.

2.3 Gram Staining

A suspension of each isolate was prepared and smeared on a clean grease free slide and air dried for a couple of minutes. Crystal Violet was poured on the slide and kept for about 30 seconds to 1 minute and rinsed with water. Iodine was poured on the dry slide and immediately washed away with alcohol. Safranin was added for about 1 minute and washed with water. After proper air drying, it was observed under the microscope.

2.4 Antimicrobial Susceptibility Testing

All the isolates from the study were subjected to antimicrobial susceptibility test using the "Kirby-Bauer" disc diffusion method following the recommendation of CLSI [7]. Sterile petri-dishes that were used to plate Mueller Hinton agar based on the manufacturer's instruction. The culture was left overnight in an incubator for growth, bacteria colonies from the culture was collected using sterile wire loop and transferred into sterile water to create a suspension. The suspension was brought to McFarland standard. Using sterile swabs standardize inoculums were collected and inoculated on the surfaces of the Mueller Hinton agar plates. It is pertinent to note that to remove excess fluid the swabs were rotated (applying little pressure) on the side wall of the test tube containing the suspensions.

The antibiotics employed for the susceptibility testing include Ceftazidime 30µg, Cefotaxime 30µg, Cefpime 30µg, Imipenem 30µg, Gentamicin 30µg, Ofloxacin 5µg, chloramphenicol 20µg, Streptomicin 20µg, Augmentin 30µg, Trimethropin 10µg, Septrin 30µg, Pefloxacin 10µg, and Amoxicilin 30µg (oxoid, UK). The plates were incubated at 37 ^{o}C for 24 hours and a transparent metre rule was used to measure the zone of inhibition caused by the antibiotics (to the nearest millimetre).

2.5 Phenotypic Detection of ESBL by Double-Disk Synergy Test (DDST) Method

The Double-Disk Synergy Test method was used for the phenotypic detection of ESBL in accordance with CLSI [7]. Several plates of Mueller Hinton ager were prepared and 30µg disc of Ceftazidime, Cefotaxime, and Cefepime, were placed 50mm centre to centre from the amoxicillin clavolanic acid disc (10µg).

Following the standard specified by CLSI [7] inoculums were inoculated into plates of Mueller Hinton and incubated at 37 ^{o}C overnight. Enhanced zones of inhibition between any of the beta-lactam discs and the centre disc were recorded as ESBL producers according to the CLSI [7] criteria.

2.6 DNA Extraction

A 4:1 ratio of genomic lysing buffer and the liquid sample was prepared, mixed thoroughly by vortexing and allowed to stand for 7.5 minutes at room temperature. After that, the mixture was separated by centrifuging for 5 minutes at 10,000 rpm. It was then transferred to a Zymo-Spin™ IICR Column using a collection tube and centrifuged for one minute at 10,000 rpm. The flow-through was discarded and the Zymo-Spin™ IICR Column was transferred into a new collection tube. Then 100 µl of DNA Pre-Wash Buffer was introduced into the column which was then centrifuged for one minute at 10,000 rpm. The g-DNA Wash Buffer (250 µl) was then added to the spin column and centrifuged for another one minute at 10,000. A sterile microcentrifuge tube was used to collect the mixture before 65 µl of DNA Elution Buffer was added and incubated for 3.5 minutes at room temperature. It was centrifuged at top speed for 30 seconds to elute the DNA; the eluted DNA was stored at -20 ºC for future use.

2.7 PCR Protocol

A 12.5 µl of Taq Quick-Load 2X Master Mix with standard buffer (New England Biolabs Inc.); 0.5 µl each of forward and reverse primers; 8.5µl of Nuclease free water and 3µl of DNA template was used to prepare 25µl reaction volume of the PCR cocktail. The reaction was gently mixed and transferred to an Eppendorf nexus gradient Mastercycler (Germany).

2.8 Statistical Analysis

The data acquired from the research were analyzed and represented in charts and tables. The mean and standard error values were obtained. The formulae applied in the study include:

$$Mean\ X = \frac{\sum X}{n}, standard\ error\ SE = \frac{\sigma}{\sqrt{n}}$$

Where, x = number of occurrences, n = total number of samples, σ = standard deviation

3. RESULTS AND DISCUSSION

3.1 Isolation and Cultivation of Enterobacteriaceae from Animal Fecal Samples Collected from Livestock Farms

The total number of fecal samples collected from cattle and goats were 300. After cultivation for Enterobacteriaceae species, growth was observed in 293 plates. It was observed that as were no growth on 7 (2.33 %) plates. Samples collected from cattle showed higher number of Enterobacteriaceae species [183 (61.00 %)]. Samples collected from goats were 36.67% (n = 110) of the samples size (Fig. 1). The cultivation and isolation of Enterobacteriaceae species from the fecal samples showed higher prevalence of Enterobacteriaceae from cattle fecal samples compared to that of goats (Fig. 1). But on a general note, Enterobacteriaceae species were isolated from the two animal fecal samples. This lends support to Smet et al. [8], that Enterobacteriaceae can be observed in companion animals and increasingly in livestock. In contrast to this, Ayitso and Onyango [9], reported the presence of Enterobacteriaceae species in the gut of termites. According to Olowe et al. [2], Enterobacteriaceae bacteria are not only detected in human clinical samples but can also be isolated from animals and products of the food chain. Hall et al. [10], have shown a relationship between Enterobacteriaceae species isolated from human samples and those isolated from retail chicken meat.

A total of three Enterobacteriaceae species were identified (*Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae*). This finding is in line with Nguema et al. [4], which identified *E. coli*, *K. pneumoniae*, and *P. vulgaris* in the fecal samples of fruit bats; alongside other Enterobacteriaceae. Abayneh et al. [11], identified *E. coli* from cattle

meat samples collected from retail shops in Jimma town. It was observed that *Escherichia coli* was the most abundant organism observed in both of the sample animals (cattle 79, goat 45). *Proteus vulgaris* showed the lowest abundance in both sample animals (cattle 43, goats 27). The result from the isolation of Enterobacteriaceae species from animal fecal samples showed that of all the three species identified, *E. coli* had the highest abundance [124 (42.32 %)], *K. pneumoniae* was the second most abundant species observed [99 (33.79 %)], while *P. vulgaris* showed the lowest abundance level [70 (23.89 %)] (Fig. 2). Nguema et al. [4], also recorded *E. coli* (39.93 %) as the most abundant Enterobacteriaceae species identified from their study and *K. pneumoniae* (17.24 %) as the second abundant species recorded; lending support to the current findings. According to Brolund [3], *E. coli* and *K. pneumoniae* are common Enterobacteriaceae associated with antibiotic resistivity and are of clinical importance because they are responsible for numerous human infections. It is hypothesized that food-producing animals have become infection sources or reservoir for several bacteria particularly *E. coli* and *K. pneumoniae* [2].

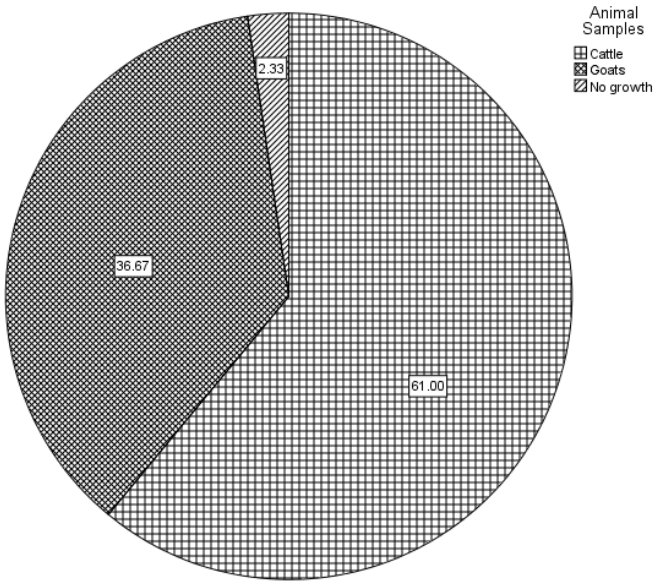

Fig. 1. Percentage of Enterobacteriaceae species isolated from cattle and goats' fecal samples

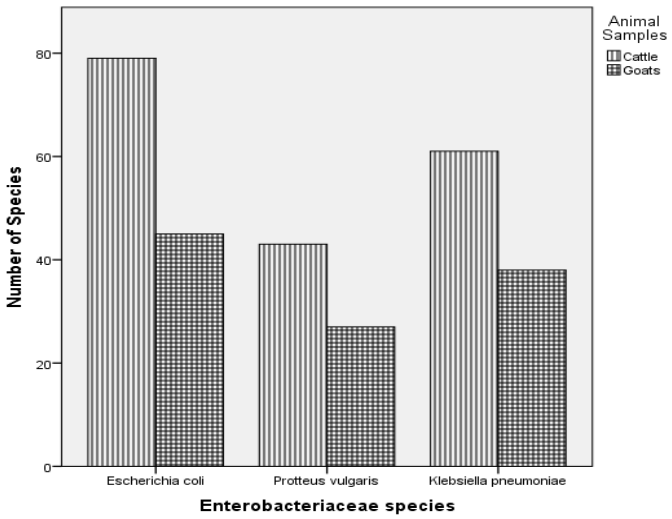

Fig. 2. Number of Enterobacteriaceae species (*E. coli*, *P. vulgaris* and *K. pneumoniae*) isolated from cattle and goats' fecal samples

The distribution of the Enterobacteriaceae according to the locations the sample were collected from was represented in Table 1. It was observed that isolates from the animal fecal samples collected from Obinze abattoir had the largest Enterobacteriaceae isolated n = 99 (which was 33.79% of the total isolate), this was followed by samples from Uborumi which showed n = 78 Enterobacteriaceae isolated (26.62% of the total isolates) [12]. Samples from Relief market showed n = 60Enterobacteriaceae isolated (20.48% of the total isolates) while samples from Somachi showed the lowest number of Enterobacteriaceae isolate (n = 56; 19.11%) (Table 1).

3.2 Antibiotic Resistivity of the Isolates through Antibiotic Susceptibility Pattern

To ascertain that the species isolated were capable of producing ESBL, the isolates were subjected to antibiogram using multiple beta-lactam antibiotic discs. The percentage of resistivity was calculated and summarized in Table 2 and Fig, 3. The following antibiotics were used in the study: Ceftazidime (CAZ), Cefotaxime (CTX), Imipenem(IPM), Gentamicin (CN), Ofloxacin (OFX), chloramphenicol (CH), Streptomicin (S), Augmentin (AUG), Trimethropin (SXT).Septrin (SP), Pefloxacin (PEF), Cefepine (CEF), Ciprofloxacin (CPX), Cefepime (CFP).

The result showed resistance of Enterobacteriaceae species isolated to most of the antibiotic discs. The Enterobaceriaceae investigated showed resistance to all the antibiotic discs under study except for Imipenem (IPM). For *E. coli*, a total of 42.1 % resistivity was recorded. The highest percentage resistivity was 4.8%, to Augmentin antibiotics, which accounted for 13 of the *E. coli* isolates. *E. coli* was susceptible to Gentamicin and Imipenem having a 1.1% and 0.7% resistivity towards Imipenem and Gentamicin respectively. *Klebsiella pneumoniae* showed a total resistance of 33.4%. The highest resistivity recorded in *K. pneumoniae* samples was 3.7% against Cefepime antibiotics. *Proteus vulgaris* showed the lowest overall resistivity percentage of 23.6 %. Its highest resistance was recorded against ciproflaxicinn antibiotics (3.7%) (Table 2). The graph (Fig. 3) and Table 3, further explains the antibiotic profile in terms of species isolated. It can be seen that *E. coli* and *K. pneumoniae* showed equal percentage resistance to CFP and CH (3.69% and 3.69% respectively). *K. pneumoniae* was the isolate with the highest resistance to Streptomicin (3.32%), Gentamicin (1.48%) and the lowest to Imipenem (IPM) (0.37%). *E. coli* was the most resistant isolate to CPX (4.43%), OFX (4.43%), AM (4.06%), SP (4.06 %), PEF (3.32%), SXT (3.32%), and CAZ (2.95 %).

3.3 Phenotypic Identification of ESBL Producing Isolates

The isolates that showed resistance to multiple antibiotics were further subjected to ESBL detection test using the DDST method. The isolates that showed resistivity (zone of inhibition as seen in Plate 2) were analyzed and the result represented in Fig. 4. It can be seen that *E. coli* showed the highest resistivity to Cefotaxime and Cefepime (19.67 ± 3.67 mm and 18.67 ± 1.67 mm respectively). *K. pneumoniae* showed highest resistivity to Ceftazidime and Amoxicillin (17.00 ± 3.51 mm and 12.33 ± 1.45 mm respectively). *P. vulgaris* showed the lowest resistivity to all the antibiotics except for Cefepime. The zone of inhibition observed for Cefepime (18.00 ± 2.31 mm) in *P. vulgaris* isolate was higher than the zone observed in *K. pneumoniae* (16.00 ± 3.00 mm). The zones measured for Cefotaxime, Ceftazidime, and Amoxicillin in *P. vulgaris* isolates were 15.67 ± 2.19 mm, 6.33 ± 6.00 mm, and 7.00 ± 6.54 mm (Fig. 4).

Table 1. Distribution of Enterobacteriaceae species and their prevalence within the positive sample

Location (Abattoir)	Total	E.c	%E.c	K.p	%K.p	P.v	%P.v	Total % for each site
Obinze	99	38	13.0	37	12.6	24	8.2	33.79
Relief Market	60	30	10.2	20	6.8	10	3.4	20.48
Uborumi	78	32	10.9	29	9.9	17	5.8	26.62
Somachi	56	24	8.2	13	4.4	19	6.2	19.11
Total	293	124	42.3	99	33.7	70	23.6	

Legend: E.c – Escherichia coli, %E.c – Frequency of Escherichia coli, K.p – Klebsiellapneumonia, %K.p – Frequency of Klebsiella pneumonia, P.v – Proteus vulgaris, %P.v – Frequency of Proteus vulgaris

Table 2. Antibiotic resistance profile of the *Enterobacteriaceae* isolates to multiple antibiotics

Antibiotics	*E. coli*	%*E.c*	*K. pneumoniae*	%K.p	*P. vulgaris*	%P.v	% Resistivity
Imipenem IPM	3	1.1	1	0.4	1	0.4	1.9
Ofloxacin (OFX)	12	4.4	8	3.0	5	1.8	9.2
Ceftazidime (CAZ)	8	3.0	6	2.2	4	1.5	6.7
Cefotaxime (CTX)	7	2.6	7	2.6	4	1.5	6.7
Augmentin (local disc) AM	13	4.8	8	3.0	3	1.1	8.9
Trimethoprim (SXT)	9	3.3	8	3.0	5	1.8	8.1
Chloramphenecol (CH)	10	3.7	10	3.7	5	1.8	9.2
Septrin (SP)	11	4.1	7	2.6	5	1.8	8.5
Ciprofloxacin (CPX)	12	4.4	7	2.6	10	3.7	10.7
Gentamicin (CN)	2	0.7	4	1.5	3	1.1	3.3
Pefloxacin (PEF)	9	3.3	8	3.0	6	2.2	8.5
Streptomicin (S)	8	3.0	9	3.3	5	1.8	8.1
Cefepime (CFP)	10	3.7	10	3.7	8	3.0	10.4
Total	114	42.1	93	34.3	64	23.6	

Legend: E.c – number of Escherichia coliisolates, %E.c – Percentage of Escherichia coli, K.p – number ofKlebsiellapneumoniae isolates, %K.p – Percentage of Klebsiellapneumoniae, P.v –number ofProteus vulgaris isolates, %P.v – Percentage of Proteus vulgaris

Table 3. Antibiotics resistance profile of the isolates to various antibiotics tested

Antibiotics	Number of Antibiotics	Number of Isolates	Percentage
AM, CPX, OFX,PEF	4	10	3.69
AM, CTX, S, SXT	4	18	6.64
CFP, CH, CPX, PEF	4	12	4.43
CAZ, CFP, CPX, CTX	4	18	6.64
AM, CAZ, CN, SP, PEF	5	5	1.85
AM, CFT, CTX, S, SXT	5	4	1.48
CAZ, CFP, CTX, SP, SXT	5	6	2.21
CAZ, CH, CTX, S, SXT	5	20	7.38
AM, CFP, CH, CPX, S, SP	6	42	15.50
AM, CPX, CTX, CN,OFX, S, SXT	7	19	7.01
CFP, CPX, CTX, OFX, S, SP, SXT	7	15	5.54
CH, CFP, CN, CPX, PEF, S, SP, SXT,	8	19	7.01
CAZ, CFP, CH, CTX, OFX,PEF,S, SXT	8	10	3.69
CAZ, CH, CN, CPX, CTX, OFX, PEF, SXT	8	13	4.80
AM, CFP,CH, CPX, PEF, S, SP, SXT, IPM	9	29	10.70
AM, CFP, CH, CN, CPX, CTX,OFX, PEF, S, SP, SXT	11	16	5.90
AM, CAZ, CFP, CH, CN, CPX, CTX, OFX, PEF, S, SP, SXT	12	15	5.54

3.4 Molecular Characterization of ESBL-Producing Enterobacteriaceae Isolates

To identify the ESBL-producing Enterobacteriaceae, the isolates were investigated for multiple Beta-lactam antibiotics (CTX, SHV and TEM). The results from the PCR and gel electrophoresis are represented in Table 4. It was observed that the genes (CTX, SHV and TEM) under investigation were identified in most of the bacteria isolates. CTX was the most prevalent in the samples [n =13 (72.22 %) positive results were identified from the isolates]. CTX bands were observed at 500 and 600 bp. TEM resistant gene was the second most prevalent observed from the samples [n = 12 (66.67 %) positive results were identified from the isolates]. The TEM bands were 1000 bp of the DNA ladder. The SHV resistant gene was the least prevalent; 11 (61.11 %) positive results were observed from the isolates. The SHV bands were identified at 300 bp of the DNA ladder (Appendix II).

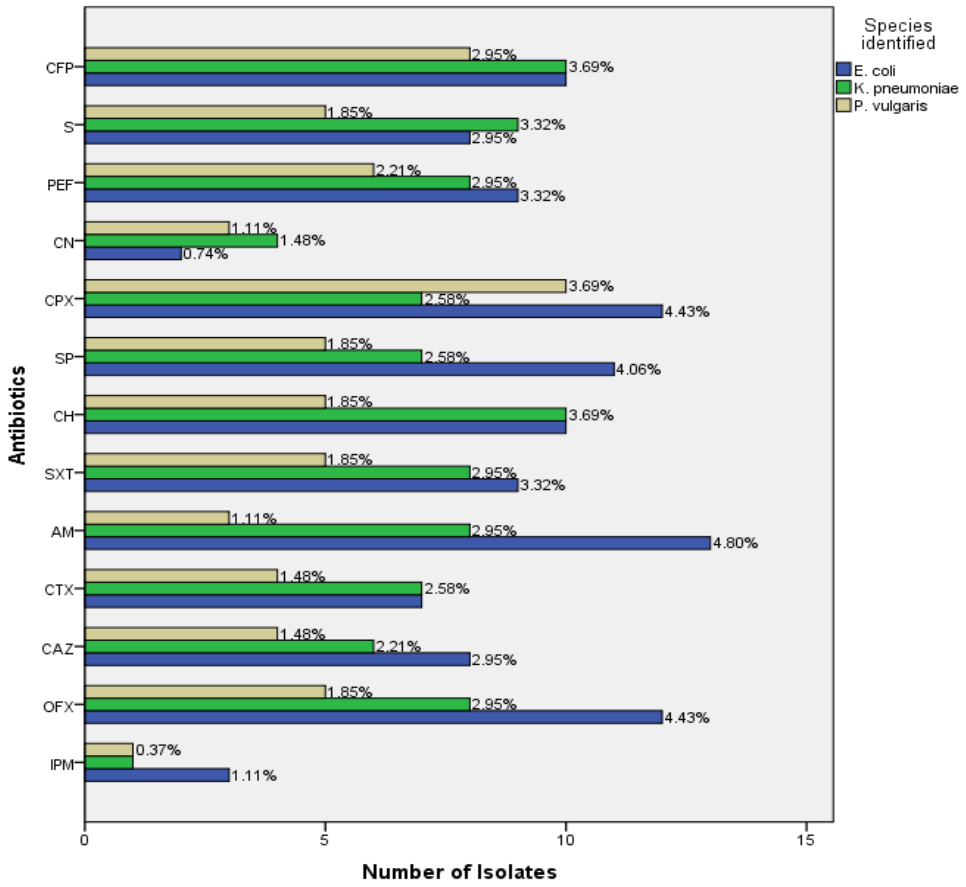

Fig. 3. Antibiotic susceptibility pattern of Enterobacteriaceae species to multiple antibiotics treatment

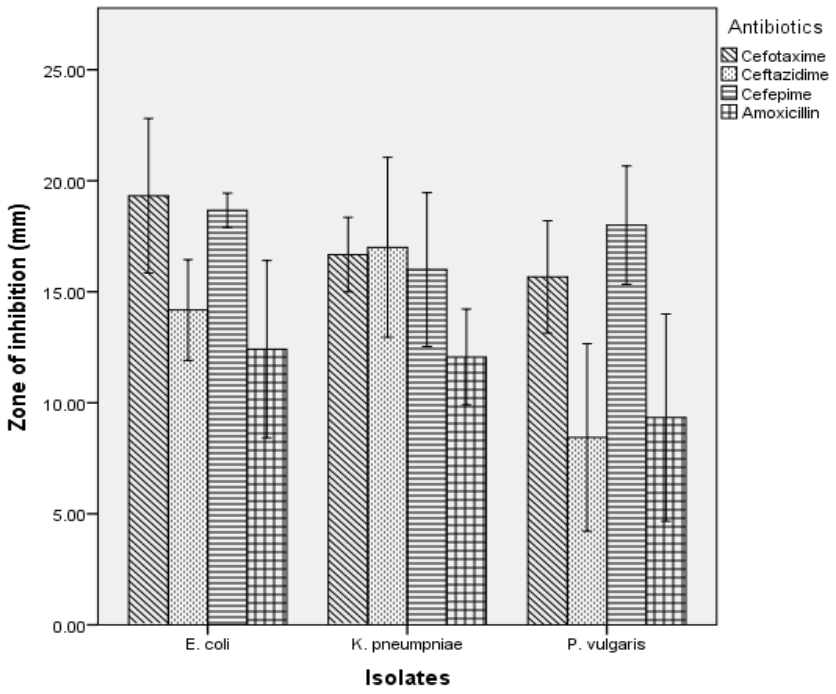

Fig. 4. Zone of inhibition of isolates for the phenotypic identification of ESBL producer

Table 4. ESBL band detection for the isolates' DNA

S/N	Samples	ESBL genes		
		CTX	SHV	TEM
1	UCF47	+	+	+
2	UCF79	-	-	-
3	UCF43	-	-	-
4	UCF65	-	-	-
5	UCF26	+	+	+
6	UCF77	+	+	+
7	UCF75	+	+	+
8	UCF112	+	-	-
9	UCF135	+	+	+
10	UCF99	-	-	+
11	UCF71	+	+	+
12	UCF143	+	+	+
13	UCF26	+	+	+
14	UCF125	+	+	+
15	UCF75	-	-	-
16	UCF73	+	+	+
17	UCF139	+	+	+
18	UCF68	+	-	-
	Total	13	11	12
	Percentage (%)	72.22	61.11	66.67

+ presence of band; - absence of band

The Table 4 revealed that majority of the isolates possessed the CTX, SHV and TEM genes; as it was observed that 11 isolates (UCF47, UCF26, UCF77, UCF75, UCF135, UCF71, UCF143, UCF26, UCF125, UCF73, UCF139) contained the CTX, SHV and TEM bands; 2 isolates UCF112 and UCF68 contained bands only for CTX; isolate UCF99 was positive for TEM only; while the other 4 isolates (UCF79, UCF43, UCF65, UCF75) were negative for the three ESBL genes (CTX, SHV and TEM) (Table 4). According to Giske et al. [13], ESBL are categorized broadly into three; $ESBL_A$, $ESBL_M$, and $ESBL_{CARBA}$. The $ESBL_A$ group contains the CTX-M, SHV and TEM [13]. Amongst these, CTX-M was the first discovered and most common ESBL class [6]. In line with this finding, Olowe et al. [2], observed high levels of bla_{CTX-M} (44.7 %) compared to bla_{TEM} (42.1 %) in fecal samples of cattle and pigs. Unlike this research finding, bla_{SHV-1} was not detected in any of the isolates [2]. The findings suggested that bla_{CTX-M} was more common among the ESBL genes in these isolates; this conforms to the findings of Villa et al. [14] investigation on ESBLs conferring transferrable resistance to new lactam agents in Enterobacteriaceae. Also, to the findings of Olowe et al. [2] investigation on the phenotypic and molecular characterization of extended-spectrum beta-lactamase producing *E. coli* from animal fecal samples in Ado Ekiti. Several other researchers have identified the $ESBL_A$ class genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV-1}) *in* Enterobacteriaceae isolates [14,15].

4. CONCLUSION

ESBL-producing Enterobacteriaceae frequently displays multidrug-resistant phenotypes thereby limiting therapeutic options; this has resulted to increased morbidity and mortality of patients. The occurrence of Enterobacteriaceae species in foods of animal origin is high in many parts of Nigeria. Some studies have attributed this to the unhygienic slaughter practices; and the risk of diseases from consuming this food is high due to the traditional consumption practices (partially cooked meal, eating some food raw, unhygienic eating habits etc.). The study reported the presence of Enterobacteriaceae in the fecal samples of the studied livestock animals (cattle and goat). These Enterobacteriaceae isolates were identified as *E. coli*, *P. vulgaris*, and *K. pneumoniae*. *E. coli* showed the highest occurrence, followed by *K. pneumoniae*, while *P. vulgaris* showed the least occurrence rate. All the Enterobacteriaceae species isolated were resistant to multiple antibiotics (Pefloxacin, Septrin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Streptomycin, Ceftazidime, Cefotaxime, Augmentin, Cefepime, Gentamicin, Amoxicillin); only Imipenem was effective against

P. vulgaris. Area of technical uncertainty was also reported for Imipenem effect on *E. coli* and *K. pneumoniae*. The molecular characterization of the resistant genes in the isolates showed the presence of *bla*$_{CTX}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ ESBLs. The CTX genes was the most common ESBL observed while the TEM genes were the second most common. Most of the Enterobacteriaceae isolates contained the three ESBLs investigated (*bla*$_{CTX}$, *bla*$_{TEM}$, and *bla*$_{SHV}$); 11.11 % of the isolates contained either one of the ESBLs, and 22.22 % of the isolates tested negative for ESBL genes. Although there is no specific source of origin of ESBL producing Enterobacteriaceae, but various research studies have suggested food animals, companion animals, the environment and humans. It is therefore, paramount for proper sensitization on the dangers of ESBL-producing Enterobacteriaceae to human health. Proper hygiene and ethical livestock farming should be practiced to reduce the occurrence of resistant Enterobacteriaceae in food animals.

5. RECOMMENDATION

Based on the results obtained from the study, the following recommendations are provided:

- There is need for proper hygiene practices in livestock farming, slaughterhouses, food preparation, and the environment at large. Ethical farm practices should also be implemented; the use of antibiotics for animal growth promotion should be avoided, as they contribute to the increase in bacteria resistivity to antibiotics.
- There is urgent need for investigating and implementing the appropriate policies directed to reduce the prevalence of ESBLs.
- More investigations are required, on alternative therapies (including combinational therapy with herbs, natural compounds) for treating sick animals; in order to reduce the incidence of antibiotic resistance among ESBL-producing bacteria.
- The route of transmission of these ESBL-producing Enterobacteriaceae should be well understood, so as to control the spread of these bacteria.
- There is need for rational antibiotic use in the farm sector to minimize the selective pressure for resistant bacteria. Novel antibiotics are also needed.
- The research was on cattle and goats; there is need for further investigation on other food animal such as poultry and poultry produce. Also, other areas within Nigeria should be investigated to provide adequate data on the epidemiology of ESBL-producing Enterobacteriaceae in Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX I

Plate 1. Enterobacteriaceae growth on MacConkey agar

Plate 2. Detection of ESBL production based on double disc synergy test (DDST) method. From the figure, a positive ESBL result is indicated when the zone of inhibition around any of the cephalosporin disc is augmented in the direction of the Amoxicilin Acid disc placed in the center plate

APPENDIX II

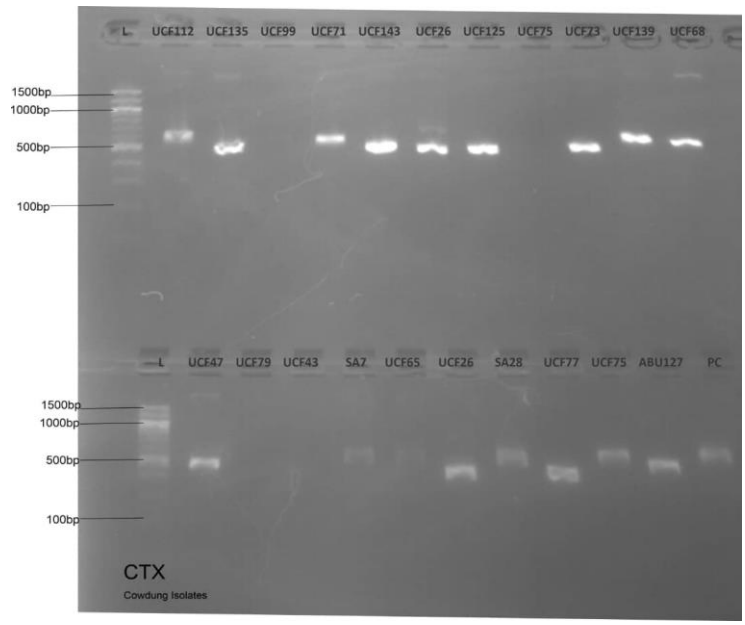

Plate 3. PCR amplification product for *bla*$_{CTX-M}$ gene from cattle and goat fecal sample. The *bla*$_{CTX-M}$ was observed between 500 bp and 600 bp. This might be due to DNA addition or deletion increasing or decreasing (respectively) the molecular weight of the gene isolated. Some of the isolates such as UCF99 and UCF75 did not contain the *bla*$_{CTX-M}$ as such, there was no band observed in their pit

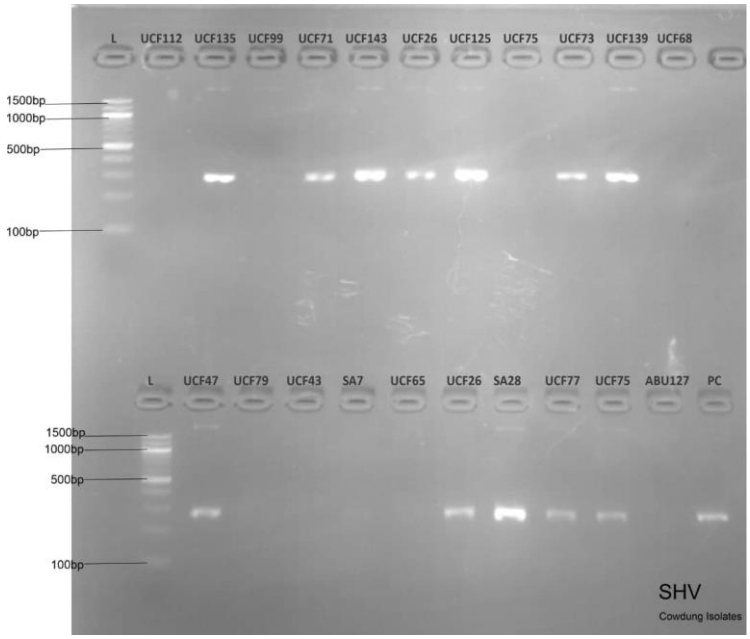

Plate 4. PCR amplification product for *bla*$_{SHV}$ gene from cattle and goat fecal sample. Majority of the isolate showed the absence of the *bla*$_{SHV}$ gene as seen in pit UCF112, UCF135, UCF75, and UCF68 in the cattle fecal sample. For the goat fecal sample, the gene *bla*$_{SHV}$ was only observed in few isolates such as UCF47, UCF26, UCF28, UCF77, and UCF75. The band for *bla*$_{SHV}$ was observed at around 300 bp for all the isolates with positive result. This indicates that there haven't been mutations in the gene band for *bla*$_{SHV}$

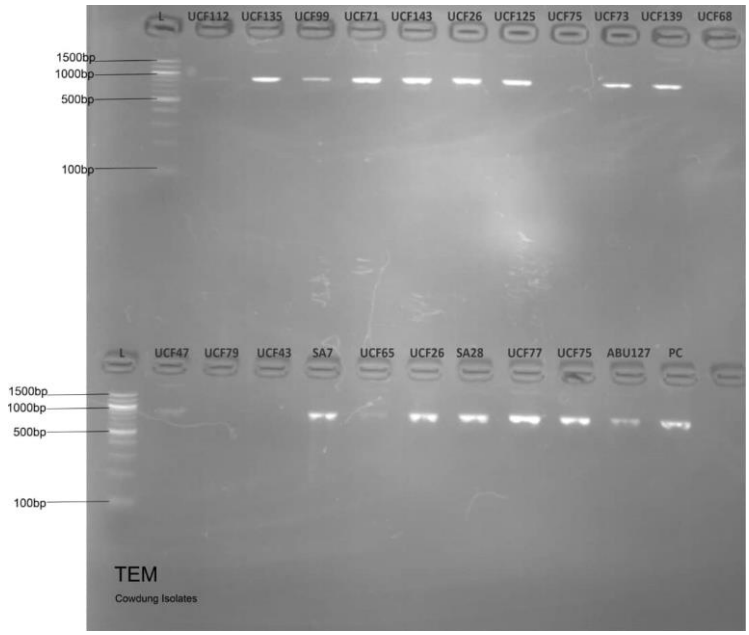

Plate 5. PCR amplification product for *bla*$_{TEM}$ gene from cattle and goat fecal sample: The *bla*$_{TEM}$ gene was common in most of the isolates both for cattle and goat fecal sample. It was absent in only isolates UCF112 and UCF75 cattle fecal sample. Isolates from goat fecal sample showed negative result for *bla*$_{TEM}$ gene in UCF47, UCF75, and UCF43 isolates. For cattle fecal samples the *bla*$_{TEM}$ gene was observed at 1000 bp while for goat fecal sample, it was observed at 1000 bp also. This indicated the absence of mutation the gene

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