

DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCTION IN *Escherichia coli* ISOLATED FROM CATTLE FEACES IN OWO METROPOLIS

ABSTRACTS

Escherichia coli which synthesize extended spectrum beta-lactamases (ESBL) have been implicated in severe human diseases. There is substantial evidence that cattle feces have a role in developing and spread of multidrug-resistant pathogens, raising public health concerns. The study is aimed at detecting ESBL producing *E. coli* associated with cattle feces within Owo metropolis. Freshly passed fecal samples were taken aseptically from 9 apparently healthy cattle that were about to be slaughtered and placed in correctly labeled sterile capped universal bottles with sterile spatula. Using standardized method, *Escherichia coli* was isolated using Eosin Methylene Blue Agar. The antibiotic susceptibility of the isolates was evaluated using the Kirby Bauer disc diffusion method, and the expression of ESBL was detected using the double disc synergy test. According to the findings, 25 (55.6%) of the 45 *E. coli* isolates showed probable production of ESBL during screening and 11 (44.0%) were confirmed to be ESBL producers. The isolates' antibiotic resistance pattern revealed that they were most resistant to gentamicin (81.8%) and least resistant to imipenem (9.1%). As a result, imipenem was the most effective antibiotic against ESBL producing *E. coli* isolates. Antibiotypes of ESBL-producing *E. coli* isolates also showed that 9 (81.8%) of ESBL-positive *E. coli* isolates were multidrug-resistant. This studies confirmed that *E. coli* isolated from bovine feces had high prevalence of antibiotic resistance and revealed that ESBL-producing bacteria are present in cattle and subsequent consumers. This necessitates the implementation of mitigating strategies to limit the transfer of antibiotic-resistant bacteria from animals to humans.

Keywords: ESBL, *E. coli*, antibiotic resistance, cattle, faeces.

INTRODUCTION

Resistance to antibiotic is a major concern in public health, it accounts for almost a million human deaths annually (European Commission, 2017; Jasovský *et al.*, 2016). Factors responsible for resistance to antibiotics have been documented such as indiscriminate use of antimicrobials in disease management (both in human and animals), it is most times abused in the form of misuse or overuse in these settings (Holmes *et al.*, 2016). Domestic animals and pets are prospective reservoirs of antibiotics resistant microorganisms according to Schmid *et al.* (2013). Microorganisms in faeces generally consists of the Family Enterobacteriaceae which are notorious for production of extended spectrum beta-lactamase that account for their resistance to these rather effective antibiotics (dos Santos *et al.*, 2013).

A major source of animal protein is cattle and in combination with milk and milk products derived from it is gaining momentum to become the most consumed animal throughout the globe

(Alexandratos and Bruinsma, 2012). Moreover, the massive production of faecal matter by these animals makes them a good source of manure/biofertilizer (Smith and Williams, 2016). All these, points to the overwhelming significance of cattle rearing in food production and as reservoir or vehicle for transmitting Enterobacteriaceae Extended spectrum beta-lactamases (E-ESBLs) as a result, constituting a major danger to global public health (Robinson *et al.*, 2016).

Saravanan *et al.* (2018) described extended spectrum beta-lactamases (ESBLs) as enzymes that are capable of hydrolysing different beta-lactam antibiotics, hence mediating the resistance to penicillins, third and fourth generation cephalosporins. It has been reported that plasmid-borne genes are the precursors of extended spectrum beta-lactamases and are freely transferred between different species according to Paterson and Bonomo (2005) and Schmid *et al.* (2013). These resistance genes are dispersed by the food chain and through contact between man and animals during handling (Schmid *et al.*, 2013; Dahms *et al.*, 2015). Earlier studies however have reported that the Cefotaximase munich (CTX-M) beta-lactamases are by far the most often detected extended spectrum beta-lactamases in animals.

The occurrence of Enterobacteriaceae extended spectrum beta-lactamases (E-ESBL) in many habitats including as benign commensal organisms in man and livestock as well as contaminants in various environments has been observed throughout the world. Albeit, in recent years animal production has constituted the main source of genuine concern for public health because these animals are potential reservoirs and also vehicle for dispersal of E-ESBL since they are directly linked with the food chain (Madec *et al.*, 2017).

Escherichia coli species are the most commonly isolated ESBL producing organism. This organism is frequently implicated in several infections in humans including urinary tract infections (UTI), pneumonia and sepsis as reported by Abraham *et al.* (2012). *E. coli* producing ESBL has been documented in animal medicine as etiological agent causing mastitis in dairy cattle (Brinas *et al.*, 2003; Haftu *et al.*, 2012). However, there is dearth of information on the occurrence or prevalence of ESBL-producing bacteria (particularly *E. coli*) in livestock showing their subsistence in apparently healthy cattle ready for slaughter in Nigeria. Therefore, this study was aimed at determining the production of extended spectrum beta-lactamase in *E. coli* isolated from cattle faeces in Owo metropolis.

MATERIALS AND METHODS

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Sample Collection

Between February and March 2021, freshly passed cattle fecal samples were aseptically collected at the Central Abattoir, Owo in Ondo State. The samples were taken with the aid of sterile spatula into well labelled sterile sample bottles from apparently healthy cattle that were ready for slaughtering, and transported under controlled temperature to the Microbiology laboratory of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic Owo (RUGIPO) for immediate analyses.

Isolation and Identification of *Escherichia coli*

The cattle fecal samples were serially diluted and plated out on MacConkey Agar (MAC) and Eosin Methylene Blue Agar (EMB) using pour plate technique. The plates were incubated at 35-37°C for 24-48 hours. Distinct colonies were sub-cultured on freshly prepared EMB Agar plates and repeated streaking was done to obtain pure culture. Identification of isolates was carried out using their colonial morphology, microscopic and standard biochemical tests.

Screening for potential Extended Spectrum Beta-Lactamase producing *Escherichia coli*

All the *E. coli* isolates were assessed for the production of extended spectrum beta-lactamase. Mueller-Hinton agar plates were inoculated with homogeneous inoculum of the test isolates and separate antibiotic discs that contain ceftazidime (30 µg) and cefotaxime (30 µg) respectively were positioned aseptically on the agar surface at a distance of 30 mm apart. The plates were placed on workbench for about half-hour to allow the antibiotics to diffuse into the agar, then they were incubated at 37 °C for 24 hrs. Thereafter, meter rule was used to measure the zones of inhibition. A reduced susceptibility/resistance to either of the two antibiotics used for the screening was taken as suspected case of extended spectrum beta-lactamase production (CLSI, 2018).

Recognition of Extended Spectrum Beta-Lactamase producing *E. coli*

An 18 hrs old culture of isolates that shows resistance to one or more of the third generation cephalosporins were inoculated into test tubes which contain sterile normal saline, then the turbidity of the content was adjusted to 0.5 MacFarland standard. The bacterial suspension was inoculated on prepared Mueller Hinton agar plates by uniformly swabbing the entire surface of the agar plates. The double disc synergy test was done using discs of augmentin alongside the discs of ceftazidime (30 mg) and cefotaxime (30 mg); which were placed at 20 mm equidistance from augmentin disc. Then, the plates were incubated for 24 hrs at 37 °C, the plates were

observed and the organisms was considered to be producing extended spectrum beta-lactamase when the zone of inhibition is ≤ 5 mm in diameter of either cefotaxime or ceftazidime in combination with augmentin against its zone when tested alone (CLSI, 2018). ESBL-positive *Escherichia coli* ATCC 25922 strain were used as control.

Antibacterial screening of the ESBL-producing *Escherichia coli*

The ESBL producing *Escherichia coli* was subjected to susceptibility test against nine antibiotics using the disc diffusion method (CLSI, 2018). The antibiotics used include the following classes of antibiotics: beta-lactam combination agent (amoxicillin/clavulanate 20/10 μ g), cephem (cefotaxime 30 μ g, ceftazidime 30 μ g, cefixime 5 μ g), carbapenem (imipenem 10 μ g), aminoglycosides (gentamicin 10 μ g), fluoroquinolone (ciprofloxacin 5 μ g, ofloxacin 5 μ g), and nitrofurans (nitrofurantoin 300 μ g). The discs were positioned on freshly prepared Mueller Hinton agar plates which had been inoculated with the test isolate and incubated for 24 hrs at 35 $^{\circ}$ C. Then, the zones of inhibition were measured with meter rule, recorded (in mm) and interpreted based on CLSI guidelines (CLSI, 2018). However, those isolates that show intermediate resistance to the antibiotics were designated resistant while those that show resistance to minimum of three classes of antibiotics were referred to as multidrug resistant (Magiorakos *et al.*, 2012). The positive control was a strain of *E. coli* ATCC25922.

RESULTS

All the isolates were Gram-negative rods. They were motility, catalase, indole, methyl red, glucose, lactose positive but were citrate, maltose, Voges-Proskauer negative (Table 1). The preliminary screening for ESBL production by *E. coli* isolated from cattle feces is shown in Table 2 where it was revealed that 25 (55.6%) of the *E. coli* were suspected to be ESBL producers since they showed resistance to cefotaxime. Furthermore, the confirmatory screening for ESBL production by *E. coli* isolated from cattle feces indicated that 11 (44.0%) out of the 25 suspected ESBL positive *E. coli* isolates from cattle feces were confirmed to be ESBL positive (Table 3).

Table 1: Gram Reaction and Biochemical Characterization of the isolates from the Cattle faeces

S/N	Shape	Gram	MOT	CAT	CIT	IND	MR	VP	GLU	LAC	MAL	Identity
1-45	Rod	-	+	+	-	+	+	-	+	+	-	<i>E. coli</i>

KEY: S/N = Serial number, + = Positive, - = Negative, MOT = Motility, CAT = Catalase, CIT = Citrate, IND = Indole, MR = Methyl red, VP = Voges-proskauer, GLU = Glucose, LAC = Lactose, MAL = Maltose

Table 2: Preliminary screening for ESBL production by *E. coli* isolated from Cattle faeces

Antibiotics used	Total number of <i>E. coli</i> Isolated	No. (%) resistance	No. (%) Susceptibility
Cefotaxime	45	25 (55.6)	20 (44.4)
Ceftazidime	45	22 (48.9)	23 (51.1)

KEY: No. = Number, % = percentage

Table 3: Confirmatory screening for ESBL production by *E. coli* isolated from Cattle faeces

No. of <i>E. coli</i> Screened	No. of ESBL + (%) <i>E. coli</i>	No. of ESBL – (%) <i>E. coli</i>
25	11 (44.0)	14 (56.0)

Table 4 showed the level of resistances and susceptibilities exhibited by the isolated ESBL-producing *E. coli* from the Cattle faeces to the test antibiotics. The ESBL-producing *E. coli* isolates from the cattle faeces were highly resistant to gentamicin (81.8%) and augmentin (72.7%) but were highly susceptible to imipenem (90.9%), ofloxacin (72.7%), nitrofurantoin (72.7%) and ciprofloxacin (81.8%).

The Antibiotypes of the ESBL-producing *E. coli* showed that nine (81.8%) of the isolates that produced ESBL and showed resistance to antibiotics were multidrug resistance. In addition, two *E. coli* were resistant to a combination of three different antibiotics (NIT-AUG-CXM), one to four and five different antibiotics respectively (NIT-AUG-CXM-GEN and NIT-AUG-CXM-GEN-CPR) (Table 5).

Table 4: Antibiotic susceptibility pattern of ESBL-producing *E. coli* isolated from cattle faeces

S/N	Antibiotics	Total No. of ESBL+ <i>E. coli</i>	No. (%) resistivity	No. (%) susceptibility
1	Nitrofurantoin	11	3 (27.3)	8 (72.7)
2	Augmentin	11	8 (72.7)	3 (27.3)
3	Cefixime	11	5 (45.5)	6 (54.5)
4	Cefotaxime	11	7 (63.6)	4 (36.4)
5	Ofloxacin	11	3 (27.3)	8 (72.7)
6	Ceftazidime	11	8 (72.7)	3 (27.3)
7	Imipenem	11	1 (9.1)	10 (90.9)
8	Gentamicin	11	9 (81.8)	2 (18.2)
9	Ciprofloxacin	11	2 (18.2)	9 (81.8)

KEY: S/N = Serial number, No. = Number, ESBL+ = ESBL positive

Table 5: Antibiotypes of the ESBL-producing *E. coli* Isolated from cattle faeces

Antibiotypes	Class of Antibiotics	No. (%) ESBL+ <i>E. coli</i>
CTX	1	1 (9.1)
IMP	1	1 (9.1)
AUG-CTX	2	2 (18.2)
GEN-CPR	2	0 (13.6)
NIT- AUG -CXM	3	2 (18.2)
NIT- AUG -CTX	3	1 (9.1)
NIT-CTX-GEN	3	1 (9.1)
NIT-CXM-GEN	3	1 (9.1)
NIT- AUG -CXM-GEN	4	1 (9.1)
NIT-IMP-GEN-CPR	4	0 (0.0)
NIT- AUG -CXM-GEN-CPR	5	1 (9.1)
TOTAL		11

KEY: NIT = Nitrofurantoin (300 µg), AUG = Augmentin (30 µg), CTX = Cefotaxime (30 µg), CXM = Cefixime (30 µg), IMP = Imipenem (10 µg), GEN = Gentamicin (10 µg), CPR = Ciprofloxacin (5 µg).

Discussion

Antibiotic resistance has continued to constitute serious problems not only in human medicine but also in animal husbandry, livestock management, and veterinary medicine (Witte, 1998; Lawson, 2008). The results of this study indicating the presence of ESBL positive (44.0%) *E. coli* is in agreement with the earlier reports of Adator *et al.* (2020) in Canada, Montso *et al.*

(2019) in South Africa, Ugwu *et al.* (2018) and Ogefere *et al.* (2017) in Nigeria, Bhoomika *et al.* (2016) in India and Peternel *et al.* (2014) in Austria each of whom respectively reported the presence of ESBL producing *E. coli* (22.5 %, 66.3 %, and 100.0 % and 16.3 %, 12.0 %, 10.99 % and 20 % respectively) in their samples. However, the 44.0% ESBL producing *E. coli* observed in this study is much lower than 66.3% and 100.0% but much higher than 12.0%, 16.3%, 22.5%, 10.99% and 20% respectively reported by same authors. The reason for this disparity could be due the number of isolates studied and the level of animal exposure to antibiotics. In addition, the findings from this present study is in disagreement with the outcome of a study in South-West Nigeria which reported an nonexistence of ESBL production in organisms isolated from faeces of cattle ready for slaughter at various abattoirs (Reist *et al.*, 2013; Inwezerua *et al.*, 2014).

The high prevalence of ESBL+ *E. coli* in cattle faeces in this study was not a surprise because *E. coli* is a normal flora of intestinal tract of warm blooded animals including ruminants (Said *et al.*, 2015).

The prevalence of ESBL observed in this study is of public health importance as it point toward a great health risk for the consumers in the area of study. The contamination of meat meant for public consumption raises a potential risk infection among the consumers as well as the handlers of these meats with ESBL producing *E. coli* particularly when these meats are eaten without adequate cooking. It is important to note that most of the internal organs particularly the intestines are used for making peppersoups and barbequed meats popularly called suya in the studied area both of which undergo minimal heat processing which are incapable of neutralizing the microbial contaminants on the meat. On the other hand, the type of resistance caused by ESBLs is usually enables the organism to be resistant to other forms of antibiotics, and this in effect makes antibiotic therapy ineffective (Reist *et al.*, 2013). Deaths due to ESBL-producing *E. coli* infections are reported to be three-times greater than those caused by *E. coli* that are incapable of producing ESBLs (Ismail and Haydar, 2016; Melzera and Petersen, 2007).

The phenomenon of antibiotic resistance is presently a very grave challenge which is attracting a great deal of efforts from many researchers all over the world due to its effect on hospitals and communities (Shaikh *et al.*, 2015). The major finding in the present study is the presence of antibiotics resistant commensal *E. coli* in cattle faeces to commonly used antibiotics such as CTX (63.6%), CAZ (72.7%), NIT (27.3%), OFL (27.3%), CPR (18.2%), GEN (90.9%), CXM (45.5%), AUG (81.8%) and IMP (9.1%). This observation reiterates the finding in other studies

that have reported antibiotic resistance among bacteria especially *E. coli* isolated from cattle and other animals is increasing at an alarming rate (Aibinu *et al.*, 2004; Kozak *et al.*, 2009; Ajayi *et al.*, 2011; Ogunleye *et al.*, 2008). Furthermore, resistance to antibiotics particularly CAZ, GEN and AUG were very high. The most obvious reason that could be responsible for the high frequency of antibiotic resistance to the antibiotics is their heavy use for various purposes among the cattle sampled. Furthermore, it has been reported by earlier researchers that ESBLs mediate resistance to the third generation cephalosporins (Paterson and Bonomo, 2005; Ogbolu *et al.*, 2011; Snow *et al.*, 2012). The susceptibility pattern of the ESBL producing *E. coli* to antibiotics used in this study was harmonious with the fact that ceftazidime and cefuroxime (both cephalosporins) are known to have low *in vitro* effectiveness against Gram negative organisms. The result indicated alarming multidrug-resistance frequency of 9 (81.8%) out of 11 ESBL⁺ *E. coli* to at least two or more of the tested antibiotics. This observation is in correlation with reports of earlier researchers who obtained similar results of various levels of multi-drug resistance in bacteria isolated from food sources particularly in *Escherichia coli* isolated from beef and other meat products (S'aenz *et al.*, 2001; Umolu *et al.*, 2006). This could constitute public health concern because the cattle faeces can act as reservoir of resistant strains which could be transmitted to humans through food chain.

The present result showed that the ESBL⁺ *E. coli* are from high risk contaminated sources with frequent use of antibiotics in the cattle production. In Nigeria, there are no strict laws on grazing and it is commonplace to find cattle in streets, farm lands and river banks being led by a herdsman. They may therefore be potential reservoirs of ESBL-producing *Escherichia coli* and other organisms when they defecate in such open grazing places.

Conclusion

The high prevalence of antibiotics resistance, ESBL producing and multidrug resistance (MDR) strains of *E. coli* in this study is an indication that cattle faeces can serve as a reservoir for the development and dissemination of clinically significant antibiotics resistance among bacterial pathogens and into the community. Hence, there is need to stop open grazing by enacting laws against it and by providing ranches. Also, cautious efforts should be made to limit the misuse of antibiotics in animal husbandry in order to reduce the emergence of antibiotics resistant organisms.

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