

## THE DISTRIBUTION OF EXTENDED-SPECTRUM BETA-LACTAMASE GENES IN FOMITES, HEALTHCARE WORKERS, AND PATIENTS FROM TWO HOSPITALS IN LAGOS STATE, NIGERIA

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Antibiotics resistance is a rapidly emerging issue through the misuse of antibiotics to treat human and animal-related infections. The use of beta-lactams has increased considerably since its discovery so also resistant genes leading to Extended-Spectrum Beta-Lactamases (ESBL) mediated by the presence of bla<sub>CTX-M</sub>, bla<sub>TEM</sub>, and bla<sub>SHV</sub> genes present in most Gram-negative bacteria. This study aimed to detect the widespread distribution of ESBL genes from fomites, healthcare workers, and patients suffering from urinary tract infection in two hospitals in Lagos state, Nigeria. A total of 150 swab samples were collected from fomites, health care workers, and catheters of patients suffering from urinary tract infection (UTI). Antibiotics susceptibility test was performed by Kirby-Bauer technique according to CLSI guidelines. Organisms that tested positive phenotypically for ESBL were subjected to PCR for molecular analysis. ESBL prevalence rate of 21.8% and a carbapenemase-resistance rate of 16.7% were recorded. The ESBL producing isolates showed the highest resistance to ceftriaxone (82.4%) and the least resistance to tigecycline (5.9%). The existence of bla<sub>CTX-M</sub> and bla<sub>TEM</sub> was detected in 76.5% and 17.6% of the isolates respectively, while bla<sub>SHV</sub> encoding gene was not detected in this study. The distribution of bla genes detected in this study is of great concern which necessitates strict control measures in the usage of antibiotics especially the third-generation cephalosporin. In summary, the presence and distribution of ESBL encoding genes within two hospitals in Lagos were tested and the highest occurrence was recorded in bla<sub>CTX-M</sub> gene reducing and limiting the available treatment option for infections.

### INTRODUCTION

The number of resistant microbial strains, geographic areas affected by drug resistance, and the extent of resistance in clinical isolates continues to escalate therefore contributing to one of the most serious jeopardies to global public health in the 21<sup>st</sup> century (Lewis and Allen, 2001; Fair and Tor, 2014; Amann *et al.*, 2019). The spread of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-producing *Enterobacteriaceae* has been reported globally in increasing numbers (Haller *et al.*, 2018). Extended-Spectrum Beta-Lactamases (ESBLs) are transmissible  $\beta$ -lactamases capable of hydrolysing third-generation cephalosporin and aztreonam but are inhibited by clavulanic acid, tazobactam, or sulbactam (Rawat and Nair, 2010; Shaikh *et al.*, 2015).  $\beta$ -lactamases may be chromosomally encoded or plasmid-mediated encoding genes that can be exchanged between bacteria in a process of vertical gene transfer (from parents to offspring) or horizontal gene transfer (from a donor to a recipient that is not its offspring (Rawat and Nair, 2010; Shaikh *et al.*, 2015). These beta-lactamases

have been found worldwide in many different species of the *Enterobacteriaceae* family and other Gram-negative bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp (Lewis and Allen, 2001). The major agents of the propagation of these strains are misuse and over usage of antibiotics in humans, animals, the circulation of antibiotic residues, and ESBL in the environment (Huijbers *et al.*, 2014; Hou *et al.*, 2015; Gundran *et al.*, 2019).

Curiously, ESBLs are most commonly detected in *Klebsiella pneumoniae* (Podschun and Ullmann, 1998). This organism is a cause of significant community-and hospital-acquired infections (Paterson *et al.*, 2003). Multidrug-resistant clinical isolates have important clinical consequences in community and hospital settings (Li and Webster, 2018). This organism is a cause of significant community-and hospital-acquired infections (Paterson *et al.*, 2003). Multidrug-resistant clinical isolates have important clinical consequences in community and hospital settings (Li and Webster, 2018). They have evolved as a global concern, exacerbated by under-reporting in some regions

of Africa and the world at large (Essack *et al.*, 2017). The tendency of these isolates to concurrently be resistant to other groups of antibiotics significantly limits the selection of antibiotics for the treatment of infections. The development of resistance to third-generation cephalosporin is attributed to the production of beta-lactamases including Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemase.

The most significant beta-lactamase genes are variants of CTX-M, SHV, and TEM which have broadened the substrate specificity against ceftazidime, cefotaxime, and ceftriaxone (Oteo *et al.*, 2006). This study was carried out to investigate the presence of ESBL resistant genes in clinical isolates and to check the frequency of third-generation cephalosporin resistance and distribution of key genetic determinants in Lagos state, Nigeria.

## MATERIALS AND METHODS

### Study Design

This is a cross-sectional study involving two public hospitals in Lagos State namely Lagos University Teaching Hospital (LUTH) and Randle general hospital, Surulere, Lagos. The isolates were collected from intensive care unit centres in both hospitals.

### Samples Collection

Fomite samples were collected from intensive care unit door handles, patients' beds, waste bin, and thermometers at random using sterile swab sticks which were placed in plain bottles containing the transport medium (peptone water). Hand swabs were collected from health care workers' using sterile swab sticks moistened with sterile normal saline for the organisms to adhere more. The patient's swab samples were collected from the catheter tips of patients suffering from urinary tract infection.

### Bacterial Identification and Characterization

The specimens were inoculated on MacConkey agar (Oxoid) and incubated for 24 hours aerobically at 37 °C. Distinct colonies' appearance and characteristics of the isolates on MacConkey agar were noted and the pure isolates were then subjected to Gram staining using standard

procedures to identify 78 non-duplicate Gram-negative rods. All suspected isolates of *Enterobacteriaceae* were confirmed by Microbact Gram-negative identification systems (Oxoid, United Kingdom) according to the manufacturer's instructions.

### Antibiotics Susceptibility Testing

Antibiotics sensitivity was determined using the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1978) on Mueller-Hinton Agar using commercially available single antibiotics paper disks (Oxoid, UK). The antibiotics used were amoxicillin-clavulanate (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), aztreonam (30 µg), colistin (10 µg), tetracycline (30 µg), tigecycline (15 µg), meropenem (10 µg), and imipenem (10 µg).

A discrete colony of the isolate was picked using a sterile swab stick and was suspended into sterile saline water to the density of 0.5 MacFarland turbidity standard according to the Clinical Laboratory Standards Institute (CLSI) 2017 guideline. Excess saline water was drained from the swab stick to prevent over inoculation of the organism on Mueller-Hinton agar. Drained swab sticks were used to inoculate the standardized organisms to MacFarland standard to dry Mueller Hinton agar plates in such a way that ensures uniform growth of the organism across the plates. The antibiotic discs were placed on the plates within 15 minutes to prevent the organism from growing before the placement of discs. The plates were incubated aerobically for 18 hours at 37 °C. The zone of inhibition surrounding each disk was measured with a ruler and interpreted as either resistant or sensitive according to CLSI 2017 standard.

### Extended-Spectrum Beta-Lactamase Test

The double synergy test (DDST) method described by CLSI (2017) was employed. A standardized inoculum of the bacteria was inoculated using sterile swab sticks on Mueller-Hinton Agar with turbidity adjusted to 0.5 MacFarland standard (Oxoid, UK). Amoxicillin/Clavulanic acid (30 µg) disc was placed at the centre of the inoculated Mueller-Hinton Agar. Ceftazidime (30 µg) and cefotaxime

(30 µg) (Oxoid UK) were placed 25 mm centre-to-centre away from the amoxicillin/clavulanic acid disc. The plates were incubated aerobically at 37 °C for 18 hours. After incubation, ESBL production was detected by the measurement of the zone of inhibition of either or both ceftazidime and cefotaxime discs towards amoxicillin/clavulanic acid discs.

### DNA Extraction (By Boiling)

The phenotypically positive isolates were cultured on Nutrient Agar and incubated at 37 °C for 24 hours. This was done to provide freshly cultured bacteria for accurate results and was then put in a 1.5 ml micro-centrifuge tube containing 1000 µl of sterile water. The isolates in the micro-centrifuge tubes were homogenized using a vortex mixer and centrifuged at 10,000 revolutions per minute for 5 mins. The supernatants were removed and the pellet was re-suspended in 200 µl of sterile water, vortexed, and re-centrifuged for another 5 mins. The supernatant in each tube was discarded and the pellet was collected and boiled at 100 °C for 10 mins. The micro-centrifuge tubes were cooled in ice for 10 mins and centrifuged at

10,000 rpm for about 5 mins. Then, the supernatants were transferred to fresh 1.5 ml micro-centrifuge tubes and stored at -20 °C in a deep freezer for PCR analysis.

### Multiplex PCR (Polymerase Chain Reaction) Amplification

A multiplex PCR reaction was carried out to identify bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, and bla<sub>TEM</sub> (as shown in table 1). PCR was performed in DNA thermal cycler using Maxima Hot Start PCR Master Mix kit (2x), (Cat. No K1051) according to the manufacturer's instruction. It is a ready-to-use mix containing Maxima hot start Taq DNA polymerase, optimized hot-start PCR buffer, MgCl<sub>2</sub>, and ddNTPs. The reaction was performed in a 20 µl volume containing 2 µM Maxima hot start PCR master mix (2x), the primers (0.2 µM each), and 2 µl of extracted DNA. Nuclease-free water was used to make up 20 µl volumes. The amplification profile was done according to Jemima and Verghese's (2008) and negative control was amplified with each run (Jemima and Verghese, 2008).

**Table 1:** Primers Used in this Study

GENE TARGET	PRIMER	SEQUENCE (5' TO 3' AS SYNTHESIZED)	PRODUCT SIZE (bp)	ANNEALING TEMP (°C)
SHV	SHV-F	ATGCGTTATATTCGCCTGT	753	55
	SHV-R	TGCTTTGTTATTCGGGCCAA		
CTX-M	CTX-M-F	TTGCGATGTGCAGTACCAGTA	543	62
	CTX-M-R	CGATATCGTTGGTGGTGCCAT		
TEM	TEM-F	AAACGCTGGTCAAAGTA	822	43
	TEM-R	AGCGATCTGTCTATCCT		

### Agarose Gel Electrophoresis of Amplification Product

The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 80 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining and a 100 bp DNA ladder was used as the DNA molecular weight standard.

### RESULTS

A total of 150 swab samples were processed and the isolates were identified using the Gram staining procedure. The identified

*Enterobacteriaceae* were confirmed using GNB 24E Microbact kit (Oxoid, UK) and 78 isolates were obtained. Of the 78 isolates, eight different organisms were identified. *Klebsiella ozaenae* had the highest number of occurrence- 23 (29.4%) and other isolates obtained in their order of prevalence are: *Klebsiella pneumoniae* 14 (17.9%), *Escherichia coli* 10 (12.8%), *Raoultella ornithinolytica* 9 (11.5%), *Pantoea agglomerans* 8 (10.3%), *Pseudomonas mallei* 6 (7.7%), *Proteus mirabilis* 7 (7.7%) and *Escherichia fergusonii* 2 (2.6%) being the least. Table 2 shows the distribution of the different ESBL-producing isolates and their encoding genes

across the two hospital centres in this study.

Of the 78 isolates identified, 17 ESBL positive isolates were confirmed using double disc synergy test and were subjected to molecular analysis for the confirmation of resistant genes. The results showed the highest percentage of 13 (76.5%) for CTX-M gene followed by 3 (17.6%) for TEM gene; 3 (17.6%) isolates showed no bla gene when analysed using PCR. However, the coexistence of bla<sub>CTX-M</sub> and bla<sub>TEM</sub> gene was detected in 2 (11.8%) of the 17 ESBL-producing isolates. Figure 1 shows a picture of a typical phenotypic ESBL producing isolate. Table 2 and figure 2 show the distribution of genes of the ESBL positive isolates.

A total of 13 antibiotics were used to test for

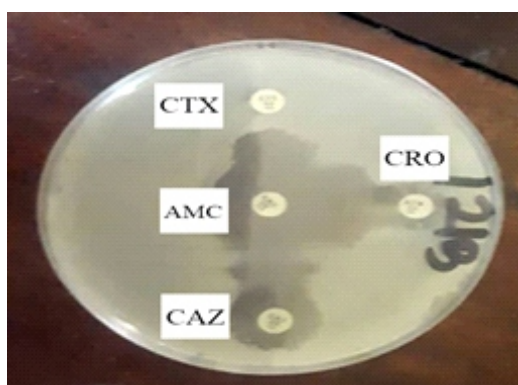
ESBL and non-ESBL producing isolates (Table 3, Figure 3). Antibiotics susceptibility profile of the ESBL producing isolates includes tigecycline 16 (94.1%), colistin 14 (82.3%), meropenem 14 (82.3%), imipenem 14 (82.3%), gentamicin 13 (76.5%), ceftazidime 13 (76.5%), amoxicillin-clavulanic acid 10 (58.8%), tetracycline 10 (58.8%), cefotaxime 8 (47.1%), chloramphenicol 7 (41.2%), aztreonam 6 (35.3%), ciprofloxacin 5 (29.4%) and ceftriaxone 3 (17.6%). The antibiotics resistance profile of the ESBL producing isolates showed the highest resistance of 14 (82.4%) to ceftriaxone and the least resistance to tigecycline 1 (5.9%). The antibiotics profile of both ESBL and non-ESBL producing isolates (Figure 2) shows that imipenem is the most susceptible antibiotics (92.3%) and ceftriaxone is the least susceptible antibiotics (26.9%).

**Table 2:** Distribution of the Different ESBL Producing Isolates and their Encoding Genes across the Two Hospital Centres in this Study

S/N	Isolate name	Sample Location	Source of Isolates	Phenotypic presence of ESBL	Bla-gene
1	<i>Klebsiella ozaenae</i>	Randle	Patient	Yes	CTX-M and TEM
2	<i>Klebsiella pneumoniae</i>	Randle	Fomite	Yes	CTX-M
3	<i>Klebsiella pneumoniae</i>	LUTH	Patient	Yes	CTX-M
4	<i>Raoultella ornithinolytica</i>	LUTH	Patient	Yes	CTX-M
5	<i>Raoultella ornithinolytica</i>	LUTH	Patient	Yes	CTX-M
6	<i>Raoultella ornithinolytica</i>	Randle	Health care worker	Yes	TEM
7	<i>Klebsiella ozaenae</i>	LUTH	Fomite	Yes	NONE
8	<i>Klebsiella pneumoniae</i>	LUTH	Patient	Yes	CTX-M
9	<i>Klebsiella ozaenae</i>	Randle	Fomite	Yes	CTX-M and TEM
10	<i>Klebsiella ozaenae</i>	Randle	Patient	Yes	CTX-M
11	<i>Klebsiella ozaenae</i>	LUTH	Fomite	Yes	CTX-M
12	<i>Pseudomonas mallei</i>	Randle	Fomite	Yes	CTX-M
13	<i>Klebsiella ozaenae</i>	LUTH	Patient	Yes	CTX-M
14	<i>Pantoea agglomerans</i>	LUTH	Fomite	Yes	CTX-M
15	<i>Proteus mirabilis</i>	Randle	Health care worker	Yes	CTX-M
16	<i>Klebsiella ozaenae</i>	Randle	Health care worker	Yes	NONE
17	<i>Klebsiella pneumoniae</i>	Randle	Health care worker	Yes	NONE

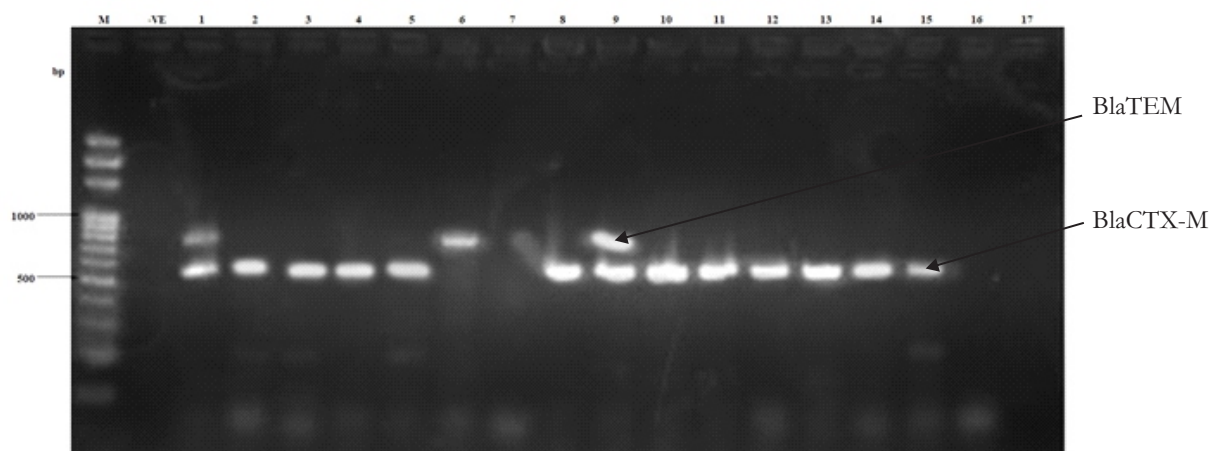
**Table 3:** Antibiotics Susceptibility Profile of all the Isolates

S/N	Antibiotics	Antibiotics susceptibility	Total number of isolates antibiotics was tested on	Percentage (%) susceptibility
1	Meropenem	71	78	91.0
2	Imipenem	72	78	92.3
3	Chloramphenicol	41	78	52.6
4	Ciprofloxacin	25	78	32.1
5	Tetracycline	41	78	52.6
6	Gentamicin	44	78	56.4
7	Ceftriaxone	21	78	26.9
8	Amoxicillin-Clavulanic acid	32	78	41.0
9	Tigecycline	70	78	89.7
10	Aztreonam	32	78	41.0
11	Ceftazidime	43	78	55.1
12	Cefotaxime	34	78	43.6
13	Colistin	55	78	70.5

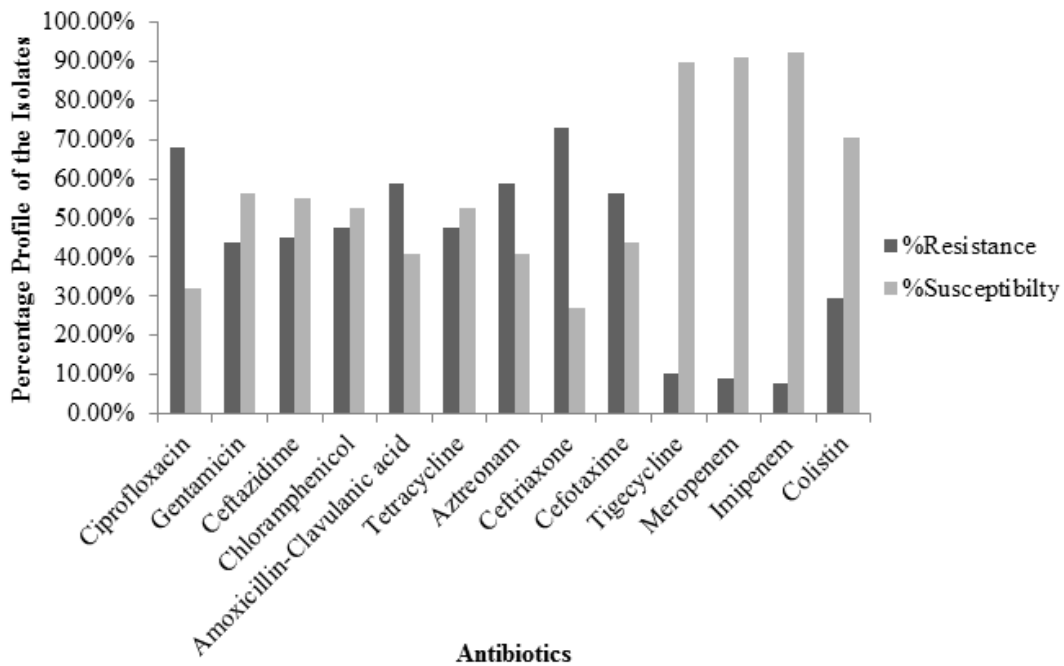
**Figure 1: ESBL-producing Organism (*Klebsiella ozaenae*)**

The cork (key shape) in-between ceftazidime, amoxicillin-clavulanic acid, and ceftriaxone indicates the synergy that occurs as a result of ESBL enzyme production.

**Abbreviation:** CAZ- Ceftazidime, AMC- Amoxicillin-Clavulanic acid, CTX- Cefotaxime, CRO-Ceftriaxone.

**Figure 2: Gene Distribution among ESBL-producing Isolates**

Lane M: DNA Marker (100bp), Lane -VE: Negative Control, Lanes 1-17- Test organisms, Lane 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14, and 15- CTX-M gene, Lane 1, 6 and 9- TEM gene, Lanes 1 and 9 shows both CTX-M and TEM gene, Lanes 7, 16 and 17- No gene was seen (the representative isolates for these genes are in table 2).



**Figure 3:** Antibiotics Profile of the ESBL and non-ESBL Producing Isolates

## DISCUSSION

The overall prevalence of ESBL producers in this study was confirmed phenotypically to be 21.7% (n=17/78 isolates). This result is similar to a recent report around the same study area by Jewoola *et al.*, (2020) that had a total carriage rate of clinical isolates of 25.3% ESBL positive isolates and is in contrast to the findings of Bajpai *et al.*, (2014) and Ramesh and Sumathi (2008) that reported ESBL resistance in India at 36.8% and 71.5% respectively. This observed variance may be attributed to differences in study design, geographical areas where the study was conducted, the procedure for specimen selection, or screening techniques used in the laboratory centres (Ashrafian *et al.*, 2013; Schechner *et al.*, 2013).

The isolates were screened for ESBL resistance using antibiotics discs. Of the 17 phenotypically positive ESBL isolates, 12 (70.5%) were detected by ceftazidime, 3 (17.6%) by cefotaxime, and 2 (11.7%) by ceftriaxone as ESBL producers. This shows that ceftazidime is the best ESBL screening agent among the three cephalosporins used in this study. This conforms to the finding of Akujobi and Ewuru (2010) who reported that ceftazidime detected 80% of the ESBL-producing isolates in their study.

This report is in agreement with the findings of Pitout *et al.*, (2007) that demonstrated the molecular epidemiology of CTX-M gene in the Calgary Health Region and proved that CTX-M genes are now widely distributed beta-lactamase genes among the ESBL genes in both nosocomial infection and community-acquired pathogens. The study is however in contrast to the findings of Cruz and Hedreyda, (2017) where it was reported that TEM-type is more prevalent in clinical isolates from Filipinos.

The detection of ESBL isolates in fomites and health care workers in this study suggests the possible spread of the pathogen in the hospital environment which could be a means for transmission of hospital-acquired infection (Huijbers *et al.*, 2014; Day *et al.*, 2019). In this study, a significantly low prevalence of ESBL producers was isolated from health care workers compared to patients. Good hand-washing and hygienic practices were observed among the health care workers in this study which should be emulated in every healthcare setting (Ellingson *et al.*, 2014; Sendall *et al.*, 2019). The high prevalence of bla<sub>CTX-M</sub> gene in this study is of clinical importance because of its implication against third-generation

cephalosporins (cefotaxime and ceftriaxone). Resistance to ceftazidime was also recorded supporting the report of Rossolini *et al.*, (2008).

Co-existence of multiple bla<sub>CTX-M</sub> and bla<sub>TEM</sub> gene was observed in 2 (11.7%) ESBL producing isolates but no bla<sub>SHV</sub> was detected similar to the study of Gundran *et al.*, (2019). The coexistence of different beta-lactamase genes within the same isolates has been reported by several investigators (Bai *et al.*, 2017; Gundran *et al.*, 2019) The presence of multiple ESBL resistance genes could result in retained resistance to beta-lactamases despite the reduced expression of one or two genes, therefore, making it more difficult to treat since the isolates will most likely be resistant to more antibiotics and most third-generation cephalosporins (Gundran *et al.*, 2019).

The current study supports previous reports of the spread of ceftriaxone resistance among *Enterobacteriaceae* worldwide (Goldstein *et al.*, 1995; Al Kraiem *et al.*, 2018; Gashe *et al.*, 2018). This could be due to the transfer of resistant genes by resistant organisms to their offspring by replication (vertical gene transfer) or by conjugation where the plasmids carrying the resistant gene are exchanged between the nearby organisms through a process called horizontal gene transfer (Baral *et al.*, 2012).

Colistin a last-resort antibiotic showed a resistance of 43.6% which is an increase compared to the report of Obasi *et al.*, (2018) but not up to that of Otokunefor *et al.*, (2019) that recorded 60% colistin resistance in Port Harcourt, Nigeria. This shows a worrisome spread of colistin resistance in Nigeria. The spread of these resistance genes needs to be deeply investigated and measures to overcome the widely disseminating antibiotic resistance should be researched and satisfactorily executed. Carbapenem regarded as the last resort antibiotics of choice for the treatment against infections caused by ESBL-producing isolates (Shaikh *et al.*, 2015; Rodriguez-Baño *et al.*, 2018) showed 7.7% resistance to imipenem and 9.0% resistance to meropenem. Although the rate of carbapenemase resistance in this study compared to some other studies in Nigerian hospitals might be low (Olalekan *et al.*, 2020; Oli *et al.*, 2019), it should however be noted that this study

established the presence of carbapenemase-resistant Enterobacteriaceae in clinical settings. It should also be noted that the incidence of carbapenemase-producing isolates varies from one region to another as well as from time to time across seasons (Alaka *et al.*, 2019).

Nonetheless, the study supports the report of Alaka *et al.*, (2019) that both Gram-negative and Gram-positive organisms are predominantly sensitive to carbapenems but less susceptible to third-generation cephalosporins. It is evident that strict control measures and judicious use of antibiotics in healthcare centres are needed to avoid an uncontrollable increase in resistance to these antibiotics in this region.

## CONCLUSION

This study established the presence and distribution of ESBL encoding genes within the community. Based on the results of this study, we advise that health care workers should inculcate good hygiene and management practices especially within the hospital environment and should be included in the study of detection of Extended-Spectrum Beta-Lactamases as this study shows that health care practitioners can act as a carrier of these enzymes from the hospital to the community. There is a need to put in place strict control measures in the usage of polymyxin, third-generation cephalosporins, and carbapenem antibiotics as resistance to these classes of antibiotics have been reported in high numbers in other studies and were confirmed in this study. This further reduces and limits the available treatment option for infections and could lead to the emergence of untreatable infections if preventive measures are not put in place.

## ACKNOWLEDGMENTS

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