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High Occurrence of blaCTX-M Extended-spectrum-beta-lactamase Genes in Gram-negative Clinical Isolates from a Tertiary Hospital, South-South Nigeria

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Abstract

The extended-spectrum-beta-lactamase (ESBL) producing Gram-negative bacteria (GNB) has been implicated in the global spread of multi-drug resistance (MDR) genes leading to limited therapeutic options. This study aimed to determine the frequency of *bla*CTX-M and *bla*SHV ESBL genes in clinical isolates from patients with GNB infection in Akwa Ibom State, Nigeria. A cross-sectional study of patients having various infections was conducted at the University of Uyo Teaching Hospital, Uyo. Clinical samples were cultured by standard bacteriological methods and isolates identified using VITEK-2 protocols. Gram-negative bacteria identified were screened for antibiotics sensitivity, ESBL production and possession of ESBL genes using Kirby-Bauer disc diffusion, double disc synergy test and polymerase chain reaction, respectively. Out of 180 clinical samples of urine, blood and wound, 71 consecutive non-repetitive GNB were isolated of which 29 (%) were ESBL producers. The GNB recovered from the samples were 35 (58.3%), 22 (36.7%) and 14 (23.3%), of which 12 (34.3%), 9 (40.9%) and 8 (57.1%), were ESBL producers, respectively. *Escherichia coli* was the most prevalent GNB and the highest ESBL producer (14.1%). Susceptibility test showed moderately high resistance of GNB to trimethoprim-sulfamethoxazole (59.1%), ceftazidime (56.3%) and cefotaxime (54.9%). Of the selected 25 ESBL-producers, 15 (60%) possessed the *bla*CTX-M genes while one (4%) harboured the *bla*SHV gene. The *bla*CTX-M detection rates in wound, blood and urine were 24%, 20% and 16%, respectively. Isolates with the *bla*CTX-M genes were *E. coli*, *S. fonticola, K. pneumoniae, P. mirabilis, A. baumannii*, *K. oxytoca*, *B. cepacia, E. cloacae* and *P. aeruginosa* while *Serratia fonticola* carried the *bla*SHV gene. The implication of *bla*CTX-M genes in MDR could be associated with treatment failures in patients with GNB infections. Antimicrobial stewardship to guide appropriate and prudent use of antibiotics is advocated.

Keywords: *bla*CTX-M genes, multidrug resistance, extended-spectrum-beta-lactamase, antibiotics, Gram-negative bacteria infection

Introduction

Gram-negative bacteria infections (GNBIs) caused by ESBL-producing organisms belonging to Enterobacteriaceae family are an emerging problem worldwide with concomitant increase in empirical treatment failure, hospital bill, rate of morbidity and mortality (Jayanti *et al.,* 2018). Presently, antibiotic resistance is a growing problem in Gram-negative bacteria (GNB) and one of the most important issues in the clinical setting is the emerging multidrug resistance Enterobacteriaceae, including non-lactose fermenting Gram-negative bacilli (NLFGB) like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Bush, 2010). Infections caused by these organisms are managed with beta-lactam antibiotics, which are the drug of choice for the first-line therapeutic treatment. However, rapid increase of resistance to these agents has been reported frequently across the globe (Kanamori *et al.,* 2011; Shadid *et al.,* 2011; Umo *et al.,* 2021). Among the GNB, there have been reports on an increased incidence and prevalence of ESBLs (Perez *et al.,* 2007; Silva-Sanchez *et al.,* 2019).

The ESBLs are plasmid-mediated enzymes that have the ability to hydrolyze beta-lactam antibiotics including 3^{rd} generation cephalosporins and monobactams, e.g. aztreonam (Abhilash *et al.,* 2010). This class A group of beta-lactamase can be inhibited by a beta-lactamase inhibitor such as clavulanic acid (Zamani *et al.,* 2015). The evolution of ESBLs is due to mutations in genes for common plasmid-mediated beta-lactamases * CTX-M, TEM and SHV enzymes that alter the amino acid configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta-lactamases for oxyimino cephalosporins (for example, ceftizoxime, cefotaxime, ceftriaxone and cefepime) (Jacoby, 2012). Their prevalence has also been noted globally both in the community and hospital settings (Abhilash *et al.,* 2010; Soltan-Dallal *et al.,* 2011; Umo *et al.,* 2021). Until the year 2000, SHV and TEM types were thought to be the most prominent ESBLs; however, since 2000, CTX-M types have emerged as new forms of ESBL that, unlike TEM and SHV, exhibit greater activity against cefotaxime than other oxyimino-bata-lactam substrates (Chong *et al.,* 2011). The CTX-M enzymes are distantly related to TEM or SHV beta-lactamases having approximately 40% identity with more than 80 variants identified. The CTX-M-15, CTX-M-14, CTX-M-13 and CTX-M-2 are the most widespread in Gram-negative clinical isolates (Hudson *et al.,* 2014).

There are reports on the high incidence of ESBL and their involvement in the global spread of multi-drug resistance, especially among members of Enterobacteriaceae and NLFGNB (Uyanga *et al.,* 2019; Umo *et al.,* 2021). The prevalent beta-lactamase genes vary in different countries and regions of the world. For instance, ESBLs with *bla*TEM and *bla*CTX-M as the prevalent genes have been reported in Southern Ecuador (Delgado *et al.,* 2016), *bla*CTX-M in Shiraz Iran (Zamani *et al.,* 2015), and *bla*SHV and *bla*CTX-M in Akwa Ibom State (Uyanga *et al.,* 2019), among others. In recent times, the production of ESBLs has become one of the most important mechanisms of antimicrobial resistance (AMR) encountered in hospital and community settings (Sharma *et al.,* 2013). Due to frequent report on increased resistance to beta-lactam antibiotics posed by ESBL-producing clinical isolates, treatment of GNBIs such as wound infections and urinary tract infections are becoming increasingly cumbersome (Bush, 2010; Jayanti *et al.,* 2018). Detection of these genes in ESBL-producing GNB isolates by molecular methods and their sensitivity pattern can give valuable insight regarding its epidemiology and helps clinicians in making informed decisions for the reasonable treatment of infections (Jayanti *et al.,* 2018). This study was carried out to determine the prevalence of ESBL production and the frequency of *bla*CTX-M and *bla*SHV genes in clinical isolates from patients with GNBI at University of Uyo Teaching Hospital in Akwa Ibom State, Nigeria.

Materials and Methods

Study Area/Study Design`

A hospital-based cross-sectional study was conducted over a period of eight (8) months from June 2022 to February 2023 at University of Uyo Teaching Hospital (UUTH). The hospital is a 500-bedded tertiary care hospital in Uyo, Akwa Ibom State. Uyo is the state capital with the population of 436,606 (Nigeria Population Commission, 2006). The people of Uyo are the * Ibibios* whose occupations are primarily farming and trading with a few plying their trade in the civil service. The latitude and longitude of Uyo, Nigeria is 5.038963 and 7.909470, respectively. It has an elevation of 65m, 213 feet above sea level with GPS coordinates of 5° 2′20.266″ N and 7° 54′ 34.092″ E. The study hospital is a referral center with Accident/Emergency (A&E) unit, Surgical Ward, Intensive Care Unit (ICU), Gynaecology, Orthopaedic, Paediatric, Ophthalmology, Blood Bank, and other specialties.

Ethical Considerations

Approval to conduct the study was obtained from Health Research Ethics Committee (HREC) of the University of Uyo Teaching Hospital (UUTH), Uyo with HREC assigned NO: UUTH/AD/S/96/VOL.XXI/629. Written informed consent was obtained from all eligible subjects before their inclusion in the study.

Isolation and Identification of Bacterial Isolates

A total of 71 consecutive non-repetitive Gram-negative bacteria were isolated from clinical samples such as blood (n=60), urine (n=60) and wound (n=60). Samples were received in the Microbiology Laboratory from patients suspected or previously diagnosed with wound infection, bacteremia and urinary tract infection, and processed by standard bacteriological methods. The Gram-negative bacteria isolates were identified and confirmed by using standard culture and Vitek®2 automated systems.

Antibiotic Susceptibility Testing (AST)

The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing of the isolates after incubation at 37 °C for 24 hours on Mueller-Hinton agar plates as recommended by the Clinical Laboratory Standard Institute (CLSI, 2023). Overnight culture of the test isolates prepared in Bijou bottles and adjusted to 0.5 McFarland turbidity standard were used to test for the sensitivity of the following antibiotics: ceftazidime (30µg), aztreonam (30µg), cefepime (30µg), trimethoprim-sulfamethoxazole (2.5µg), ceftriaxone (30µg) and ofloxacin (5µg) gentamicin (10µg), imipenem (10µg), augmentin (amoxicillin 20µg/clavulanate 10µg), ciprofloxacin (10µg), cefotaxime (30µg), (Oxoid, UK). *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for susceptibility testing. The AST results were interpreted in accordance with the CLSI guidelines and interpretive criteria (CLSI, 2023).

Phenotypic Screening for ESBL Production

The production of ESBL was confirmed phenotypically in those Gram-negative isolates that showed reduced susceptibility to the third-generation cephalosporins (cefotaxime, ceftazidime and ceftriaxone) in the initial screening test using the double disc synergy test in accordance with the CLSI guidelines (CLSI, 2023).

Bacterial DNA Extraction

Bacterial DNA extraction was carried out using ZR Bacterial DNA Mini-Prep Extraction kit supplied by Inqaba South Africa according to the manufactures instruction. The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer (Thermo Scientific, Inqaba Biotec).

DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer (Thermo Scientific, Inqaba Biotec). The software of the equipment was launched by double-clicking on the Nanodrop icon. The equipment was initialized with 2 µL of sterile distilled water and blanked using PCR water. Two microliters of the extracted DNA were loaded. The higher pedestal was lowered onto the lower pedestal to make contact with the extracted DNA on the lower pedestal. By clicking on the measure button, the DNA concentration was determined.

Detection of ESBL Genes Types: *bla*SHV and *bla*CTX-M by PCR

The amplification of *bla*TEM and *bla*CTX-M genes was done using specific primers listed in Table 1. The reaction was performed on ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions for *bla*CTX-M were as follows: Initial denaturation, 94ºC for 5 minutes; denaturation, 94ºC for 40 seconds; annealing 52 °C for 45 seconds; extension, 68ºC for 45 seconds for 35 cycles and final extension, 68ºC for 5 minutes. For the *bla*SHV gene, the PCR conditions were: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 55ºC for 40 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The PCR product was separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and resolved under a UV trans-illuminator. The amplicon size was extrapolated using a 100bp molecular marker.

Table 1: Primers for PCR amplification of *bla*SHV and *bla*CTX-M genes

Gene	Target	Primer sequence (5′ - 3′)	Amplicon size (bp)
*bla*CTX-M	β-lactam	F: 5- CGCTTTGCGATGTGCAG -3'	550
		R: 5′-ACCGCGATATCGTTGGT -3′	
*bla*SHV	β-lactam	F: 5-TTTCGTGTCGCCCTTA TTCC-3'	401
		5′- ATCGTTGTCAGAAGTAAGTTGG -3′	

Results

The antibiotic susceptibility patterns of Gram-negative bacteria isolated from clinical samples at UUTH is presented in Table 2. The results showed imipenem (73.2%), ofloxacin (70.4%) and gentamicin (69.0) as the most effective antibiotics against the test isolates. The highest level of resistance of the isolates to trimethoprim-sulfamethoxazole (59.1%), ceftazidime (56.3%) and cefotaxime (54.9%) antibiotics was recorded in this study.

Table 2: Antibiotic susceptibility patterns of Gram-negative clinical isolates in UUTH, Akwa Ibom State (n=71)

Antimicrobial agent (disc conc.)	Number (%) of susceptible/resistant isolates		
	S	I	R
IMP (10μg)	52(73.2)	2(2.8)	17(24.0)
SXT (2.5μg)	20(28.2)	9(12.7)	42(59.1)
OFX (5μg)	50(70.4)	1(1.4)	20(28.2)
FEP (30μg)	28(39.4)	13(18.3)	30(42.3)
AMC (20μg/10μg)	39(54.9)	9(12.7)	23(32.4)
CIP (10μg)	36(50.7)	10(14.1)	25(35.2)
CTX (30μg)	24(33.8)	8(11.3)	39(54.9)
CRO (30μg)	24(33.8)	9(12.7)	38(53.5)
ATM (30μg)	33(46.5)	7(9.9)	31(43.6)
CAZ (30μg)	27(38.0)	4(5.6)	40(56.3)
CN (10μg)	49(69.0)	5(7.0)	17(24.0)

IMP: Imipenem, SXT: Trimethoprim-sulfamethoxazole, OFX: Ofloxacin, FEP: cefepime, AMC: Amoxicillin-clavulanic acid, CIP: Ciprofloxacin, CTX: Cefotaxime, CRO: Ceftriaxone, ATM: Aztreonam, CAZ: Ceftazidime, CN: Gentamicin.

The prevalence of ESBL production among clinical isolates from UUTH is shown in Table 3. The results revealed a 40.8% prevalence of ESBL in UUTH. Out of 71 GNB, E. coli 10 (14.1%) had the highest ESBL production followed by *S. fonticola* 5 (7.0%) and *P. mirabilis* 3 (4.2%). Isolates such as *K. pnuemoniae*, *K. oxytoca*, *A. baumanii* and *B. cepacia* had the same ESBL production rate (2.8%). The least ESBL producers were *Enterobacter cloacae, Pseudomonas aeruginosa,* and *Citrobacter freundi* with ESBL production rate of 1.4%.

Table 3: Prevalence of ESBL production among clinical isolates from UUTH

Bacterial isolates	No. of isolates	No. of ESBL positive (%)
Serratia fonticola	8	5(7.0)
Klebsiella pneumoniae	14	2(2.8)
Klebsiella oxytoca	3	2(2.8)
Escherichia coli	20	10(14.1)
Proteus mirabilis	5	3(4.2)
Acinetobacter baumannii	3	2(2.8)
Burholderia cepacia	11	2(2.8)
Enterobacter cloacae	2	1(1.4)
Citrobacter freundi	2	1(1.4)
Pseudomonas aeruginosa	3	1(1.4)
Total	**71**	**29(40.8)**

The distribution of ESBL genes in clinical samples from patients in the study area is presented in Table 4. Isolates from blood samples had the highest prevalence of ESBL production (57.1%) while *bla*CTX-M genes were mostly detected in isolates from wound samples (24%). Of the 25 ESBL-producing isolates screened for the presence of *bla*CTX-M and *bla*SHV genes, 15 harboured the *bla*CTX-M genes while only one isolate from wound sample had the *bla*SHV gene. The overall prevalence of *bla*CTX-M and *bla*SHV genes among clinical isolates in this study was 60% and 4%, respectively.

Table 4: Distribution of ESBL genes in clinical samples

Sample	No. of GNB (%)	ESBL positive (%)	No. tested by PCR = 25	
			***bla*SHV (%)**	***bla*CTX-M (%)**
Urine	35 (58.3)	12(34.3)	0(0)	4(16.0)
Blood	14 (23.3)	8(57.1)	0(0)	5(20.0)
Wound	22 (36.7)	9(40.9)	1(4.0)	6(24.0)
Total	**71 (39.4)**	**29(40.8)**	**1(4.0)**	**15(60.0)**

GNB: Gram-negative bacteria, ESBL: Extended spectrum beta-lactamase.

The distribution of *bla*CTX-M and *bla*SHV genes in ESBL-producing strains is presented in Table 5. The result showed *Serratia fonticola* as the only wound isolate that carry the *bla*SHV gene. Of the 15 isolates that harbor the *bla*CTX-M gene, 6, 5 and 4 were from wound, blood and urine, respectively. Among them include 3 *E. coli* isolates, 2 isolates each of *S. fonticola, K. pneumonia, P. mirabilis, A. baumannii*, and 1 isolate each of *K. oxytoca*, *B. cepacia, E. cloacae* and *P. aeruginosa.*

Table 5: Distribution of *bla*CTX-M and *bla*SHV genes in ESBL-producing strains

Table 5: Distribution of *bla*CTX-M and *bla*SHV genes in ESBL-producing strains

Bacterial species	Sample ID	Sample type	ESBL genes detected	
			***bla*CTX-M**	***bla*SHV**
Serratia fonticola	1	Blood	+ve	-ve
Proteus mirabilis	8	Blood	+ve	-ve
Burkolderia cepacia	14	Blood	+ve	-ve
Enterobacter cloacae	18	Blood	+ve	-ve
Acinetobacter baumannii	21	Blood	+ve	-ve
Klebsiella pneumoniae	3	Wound	+ve	-ve
Serratia fonticola	4	Wound	+ve	+ve
Escherichia coli	6	Wound	+ve	-ve
Klebsiella pneumoniae	11	Wound	+ve	-ve
Escherichia coli	13	Wound	+ve	-ve
Pseudomonas aeruginosa	19	Wound	+ve	-ve
Klebsiella oxytoca	5	Urine	+ve	-ve
Escherichia coli	7	Urine	+ve	-ve
Acinetobacter baumannii	9	Urine	+ve	-ve
Proteus mirabilis	16	Urine	+ve	-ve

The agarose gel micrographs of *bla*CTX-M and *bla*SHV genes are shown in Figures 1 and 2, respectively. The result in Figure 1 shows the 550 base pairs of amplified *bla*CTX-M genes in clinical isolates (Lanes 1-21), while that in Figure 2 represents the 401 base pairs of amplified *bla*SHV gene found only in *Serratia fonticola* isolated from patient with wound infection in UUTH.

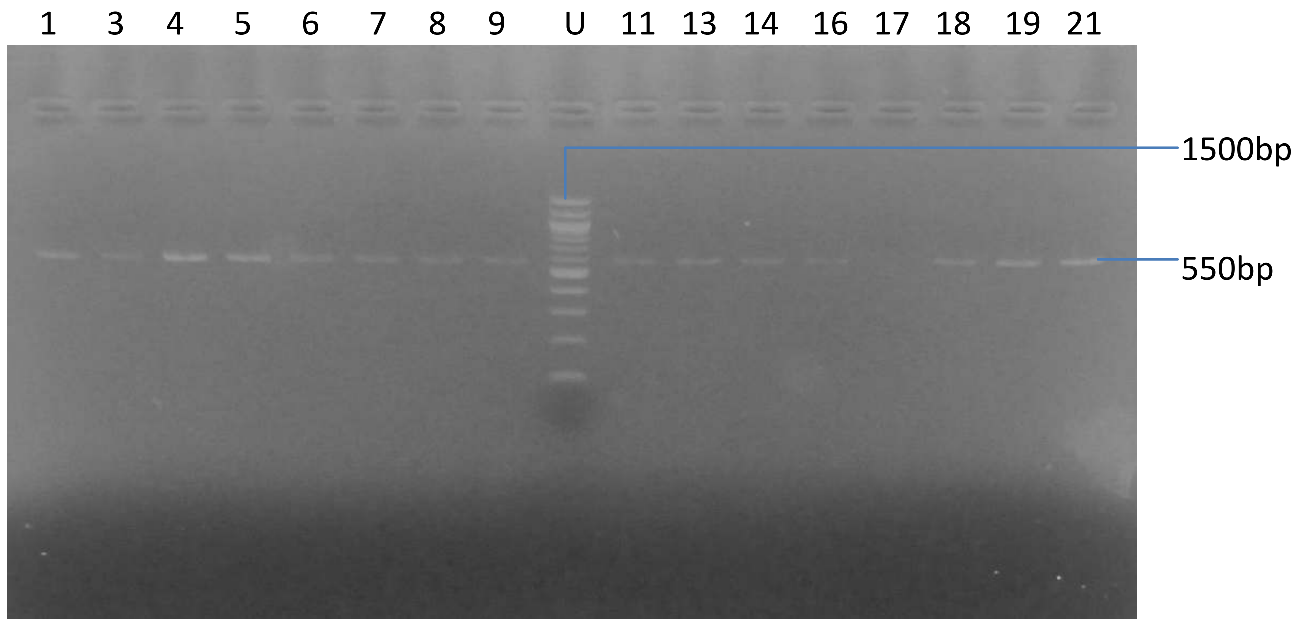

Figure 1: Agarose gel electrophoresis of some selected bacterial isolates. Lane 1-21 represents CTX-M gene bands (550bp). Lane U represents the 100bp DNA ladder.

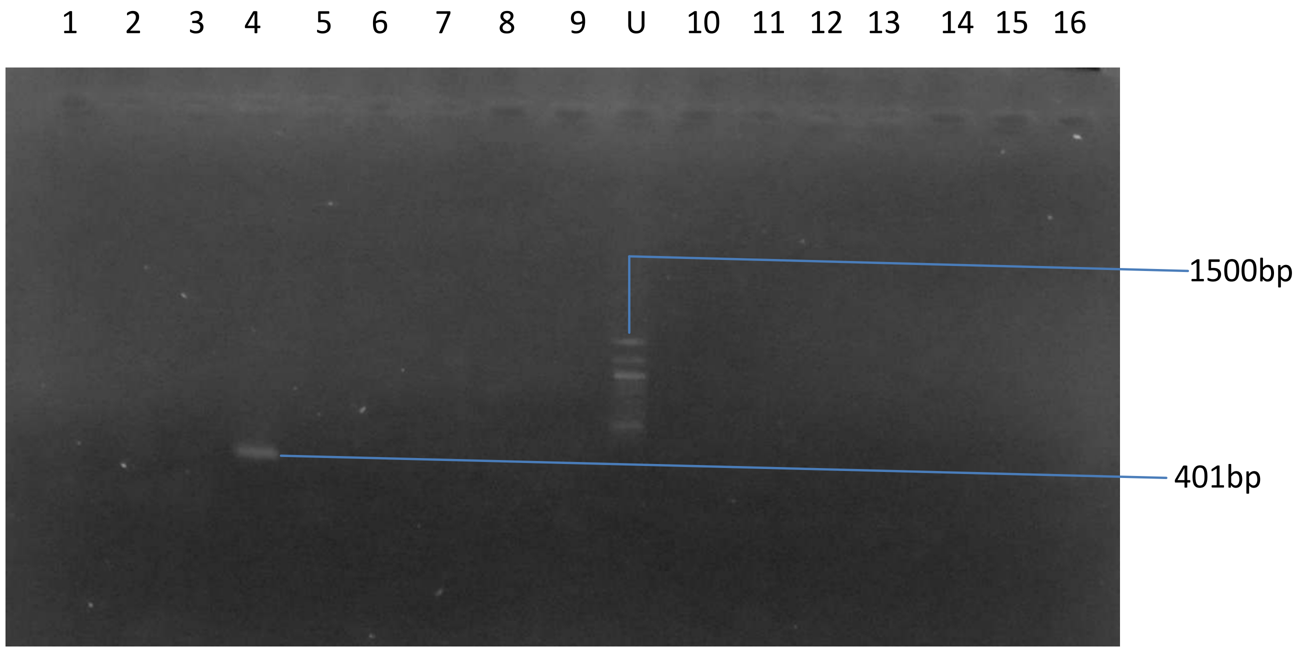

Figure 2: Agarose gel electrophoresis of some selected bacterial isolates. Lane 5 represents SHV gene bands (401bp). Lane U represents the 100bp DNA ladder.

Discussion

The global increase in prevalence and dissemination of ESBL genes among pathogenic Gram-negative bacteria is of grave public health significance. This calls for a combination of robust antibiotic sensitivity screening and phenotypic technique to investigate the prevalence of MDR ESBL-producing strains and molecularly characterize ESBL genes on every suspected clinical isolate (Narayan *et al.,* 2016). The findings of this study revealed high resistance rates of Gram-negative clinical isolates to multiple drugs, especially to folate pathway antagonist and cephalosporins, as described in previous studies (Onwuezobe *et al.,* 2015; Umo *et al.,* 2021). The majority of the isolates were resistant to trimethoprim-sulfamethoxazole (59.1%), ceftazidime (56.3%), cefotaxime (54.9%) and ceftriaxone (53.5%). These findings have significant implications for empirical management of patients with wound infection, bacteremia and urinary tract infections using 3rd generation cephalosporins. Imipenem (73.2%), ofloxacin (70.4%) and gentamicin (69.0%) were observed to have the highest level of sensitivity against the test isolates. This is consistent with the findings of Onwuezobe and Etang (2018) in Uyo, Illiyasu *et al.* (2018) in Bauchi and Gharavi *et al.* (2021) in Iran. Variation in the rate of resistance of the isolates to antibiotics might be a function of misuse in such a location. It also implicates the production of ESBLs as the enzymes responsible for resistance to beta-lactams such as cefotaxime, ceftazidime and aztreonam including non-beta-lactam antibiotics like the fluoroquinolones, aminoglycosides and folate pathway inhibitors, among others, as previously reported (Jacoby and Medeiros, 1991).

In this study, the prevalence of ESBL-producing Gram-negative bacteria causing bacteremia, wound infection and urinary tract infections in University of Uyo Teaching Hospital (UUTH) in Akwa Ibom State, Nigeria was 40.8%. This is less than earlier report from the same study center (50.4%) by Umo *et al.* (2021) and at a tertiary hospital in Benin City (47.1%) by Ibadin *et al.* (2018). However, it is significantly higher than previous reports by Onwuezobe and Orok (2015) at UUTH in Akwa Ibom State (20%), Ogefere *et al.* (2019) at military hospitals in South-south Nigeria (17.1%) and Yusuf *et al.* (2013) at a tertiary hospital in Kano (15%). Previous studies outside the country had reported higher prevalence rates. For instance, studies conducted in Chadian and Ugandan tertiary hospitals had reported the prevalence of

47.42% and 89%, respectively (Andrew *et al.,* 2017; Mahamat *et al.,* 2019). The prevalence of ESBL in bacterial pathogens has been shown to vary according to geographical location and study period (Shaikh *et al.,* 2015). Lack of antibiotic surveillance policies, poor hygiene and antibiotic misuse, especially in countries with limited resources; have also been reported to contribute to the increase in ESBL prevalence and risk of multidrug resistance development by bacteria in hospital and community environments (Jaggi *et al.,* 2012).

In this study, the potential of Gram-negative bacteria to produce ESBL varied; with the highest production found in *Escherichia coli* (14.1%) followed by *Serratia fonticola* (7.0%) and *Proteus mirabilis* (4.2%). This is dissimilar to the findings of Umo *et al.* (2021) in which *K. ozanae* (66.7%), *P. gergoviae* (66.7%), *E. cloacae* (62.5%) and *P. agglomerans* (60%) were reported as the most ESBL producers. The preponderance and variations in occurrence of ESBL-producing Gram-negative bacteria may not be unconnected to the type of clinical samples. Previous studies had reported similar result, indicating the preponderance of ESBL production in *E. coli* (50%) but with lower rates in *Klebsiella spp.* (23.0%), *Enterobacter spp.* (18.5%) and *Citrobacter spp*. (6.5%) (Wadekar *et al.,* 2013; Gupta and Farooq, 2018). A much higher prevalence of ESBL production has been reported by Mishra *et al*. (Mishra *et al.,* 2012) in *E. coli* (62%) and *K. pneumoniae* isolates (73%). As reported in literature, the prevalence of ESBL-producing isolates depends on some factors such as type of species, geographic location, hospital, group of patients, type of infection and extensive abuse of antibiotics (McNulty *et al.,* 2018).

Currently, more than 70 ESBLs have been found worldwide ever since they were first identified in Western Europe. Many of the ESBL gene types have also been identified in clinical isolates in Nigeria (Jacoby and Bush, 2012). Plasmid-encoded class A TEM, SHV and CTX-M type ESBLs evolution are attributed to successive mutations in their structural genes, resulting in either single or multiple amino acid changes in the encoded enzymes (Bush and Jacoby, 1997).

In this study, genomic DNA extraction result showed that 60% of the ESBL-producing isolates harboured the *bla*CTX-M genes. This is lower than the rates reported in Lagos teaching hospital, Nigeria (79%) by Raji *et al.* (2015) and in Shiraz, Iran (91.5%) by Zamani *et al.* (2019). The prevalence of *bla*CTX-M genes among ESBL-producing isolates was 20% in *E. coli,* 13.3% in *S. fonticola, K. pneumonie, P. mirabilis* and *A. baumannii* and 6.7% in *K. oxytoca*, *B. cepacia, E. cloacae* and *P. aeruginosa.* The study also revealed the dominance of *bla*CTX-M genes in wound pathogens (24%) followed by blood-borne pathogens (20%) with the least recorded among uropathogens (16%). The spread of *bla*CTX-M genes with a frequency of 42% among *E. coli,* 17% among *E. clocae* and 25% among *K. pneumoniae* recovered from urine samples had been reported in South-south, Nigeria (Uyanga *et al.,* 2019). The high prevalence of *bla*CTX-M type ESBL in this study lends credence to the global pandemic spread of these genes, a phenomenon that has reached epidemic proportion among members of the family Enterobacteriaceae. The ESBL production mediated by *bla*CTX-M beta-lactamase genes have been reported to be the most widespread enzymes replacing *bla*TEM and *bla*SHV genes in many parts of the world such as in the United States (Doi *et al.,* 2013), North America (Lweis *et al.,* 2007), Europe (Castanheira *et al.,* 2008), the Middle East (Al Hashem *et al.,* 2011) and Nigeria (Akinyemi *et al.,* 2015; Uyanga *et al.,* 2019).

In this study, the prevalence of *bla*SHV genes and *bla*CTX-M + *bla*SHV co-acquisition among ESBL-producing *Serratia fonticola* isolate was 4%. To the best of our knowledge, this is the first report of *bla*SHV gene detection in *serratia fonticola* as well as *bla*CTX-M + *bla*SHV co-expression in the same isolate in University of Uyo Teaching Hospital, Uyo, Akwa Ibom State. No *bla*SHV gene was detected in other ESBL-producing isolates, indicating that other determinant factors may be responsible for multidrug resistance in these isolates. The dominance of *bla*CTX-M genes as observed in this study further emphasized that this ESBL gene is the most preponderant in our state and Nigeria by extension. Recent studies have demonstrated the role of *bla*CTX-M ESBL variants in clinical infections in Nigeria (Ogbolu *et al.,* 2011; Raji *et al.,* 2015; Uyanga *et al.,* 2019). These resistance genes can easily move from one species to another with the possibility of easy interspecies transfer (Raji *et al.,* 2015). The clinical implication of this finding is that many patients with wound infection, urinary tract infection and bacteremia caused by MDR Gram-negative bacteria may stand the risk of treatment failure.

Conclusion

This study revealed *bla*CTX-M-type genes as the dominant genotype of the ESBL found among clinical isolates in University of Uyo Teaching Hospital, Uyo, Akwa Ibom State. Only one isolate co-harboured *bla*CTX-M and *bla*SHV genes. The findings of this study further demonstrated an explosive emergence of multidrug resistant phenotypes mediating *bla*CTX-M type ESBL production in pathogenic clinical isolates. Dissemination of strains harbouring *bla*CTX-M would make antibiotic therapies more difficult because of their potent hydrolytic activity against oxyimino-cephalosporins. Immediate implementation of antibiotic stewardship and surveillance policies are of prime importance to mitigate the spread of multidrug resistant Gram-negative bacteria in the hospital.

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Author's Contributions

Etang UE and Moses AE designed the study. Inyang UC and Moses EA conducted literature review and sample collection. Etang UE and Moses AE discussed the results. Akpan SS and Etang UE conducted laboratory analyses and drafted the manuscript. All authors read, edited and approved the final manuscript.

Conflict of Interest

The authors declared that there was no conflict of interest to this manuscript.

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