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Prevalence and Molecular Characteristics of Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Pathogens from Patients in a Tertiary Care Hospital in Nigeria

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Abstract
This study determined the prevalence of extended spectrum β-lactamase (ESBL) producing Gram negative bacilli (GNB) and its genetic variants in clinical infections in our setting. A total of 359 non-duplicate GNB were recovered from various clinical samples which were aseptically collected and processed following standard microbiological methods. Antibiotic susceptibility testing was carried out by standard disk diffusion method. ESBLs producers were confirmed by combination disk test and their genetic variants determined by polymerase chain reaction-based protocols. Among 359 GNB, 94 (26.2%) produced ESBL which were mainly distributed across genera as Citrobacter (n=27; 28.7%), Escherichia (n=25; 26.6%), Klebsiella (n=14; 14.9%) Enterobacter (n=12; 12.8%) and Proteus (n=5; 5.3%). Urine was the main source of ESBL producers (n-35; 37.2%) but ESBL production was most prevalent among isolates from sputum (35.7%). Among bacterial species, Klebsiella pneuminiae had the highest prevalence of ESBL-producing phenotypes (44.8%), followed by Enterobacter cloacae (38.5%), Citrobacter freundii (37.7%), Enterobacter aerogenes (36.8%) and Escherichia coli (29.8%). Seventeen bacteria (19.8%) had single ESBL genes while 69 (80.2%) had multiple genes of which 24 harboured bla_{TEM}, bla_{SHV} and bla_{CTX-M}, 40 harboured bla_{CTX-M} and bla_{TEM}, three haboured bla_{CTX-M} and bla_{SHV} and two haboured bla_{TEM} and bla_{SHV}. Among the ESBL-producing strains, bla_{CTX-M} was the most common harboured gene (74; 78.7%), closely followed by bla_{TEM} (72; 76.6%). This study reveals a high prevalence of ESBL-producing bacteria which could complicate antibiotic treatment of clinical infections. There is a need for continuous antibiotic resistance surveillance to inform improved antibiotic stewardship and infection prevention and control.
Keywords: *ESBL, Gram-negative bacilli, CTX-M, TEM, SHV, Hospital.*

Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes that hydrolyze oxyimino-cephalosporins, penicillins and aztreonam. ESBL producing Gram negative bacteria (ESBL-GNB) have been isolated from hospital and community settings.[1] The rising prevalence of ESBL-GNB infections has raised serious concerns worldwide. As of today, nearly all the continents, including Africa have reported on the occurrence of ESBL producers. Although, the exact prevalence of ESBL is unknown in this country, previous reports have however shown the value to vary from 0-80%.[2-4] High prevalence of ESBL complicates antibiotic therapy and interferes with empirical therapy resulting in increased morbidity and mortality.[5] Patients with an ESBL-GNB infection are in danger of treatment failure due to the delay that is usually encountered before the appropriate therapy is administered.[5]

In the last 30 years, diverse variants of ESBL enzymes (CTX-M, TEM and SHV) have been detected in different bacterial species. Among the different types, the CTX-M group predominates worldwide.[6] CTX-M enzymes have been widely reported in Gram negative bacteria, and have caused different clinical infections. Reports from surveillance studies from many countries have shown that *E. coli* producing CTX-M-β-lactamases showed high resistance to several other classes of antimicrobial agents.[7]

The global increase in resistant bacteria in community and hospital settings that threatens the ability to successfully treat patients, underscores the need for sustained antimicrobial resistance surveillance, rational drug prescription and prudent infection control measures, and novel therapeutic options.[7] Sustained antimicrobial resistance surveillance is crucial for the treatment of infections, implementation of resistance control measures and prevention of the dissemination of resistant organisms in the hospital and community. In Africa, very few studies have reported on the prevalence of ESBL in Gram-negative bacilli, and the burden of associated infections continues to increase due to lack of affordable second choice antibiotics. Also, most hospital laboratories do not regularly screen for ESBL-producing bacteria. All these have contributed to preventable treatment failures and outbreaks of multidrug resistant organisms that require highly expensive control efforts. Therefore, this study was conducted to determine the prevalence of ESBL producing GNB and its genetic variants in clinical infections in our setting.

Methods

Study Setting, data collection, sample processing

The approval for this cross-sectional hospital based study was obtained from the Ethics Committee of Ladoke Akintola University Teaching Hospital (LTH) Osogbo, Nigeria (Protocol Number- LTH/REC/2015/06/05/210). Three hundred and fifty-nine consecutive non-duplicate Gram negative bacilli were recovered from the Diagnostic Microbiology Laboratory of the hospital over a period of 6 months (January to July 2016). The bacteria were isolated from diverse clinical samples of patients in the hospital wards comprising Urine (n=159), Wound (n=105), Joint Aspirate (n=1), Blood (n=46), CSF (n=10), E.C swab (n=1), Ear swab (n=1), Sequestrum (n=1), Sputum (n=28) and Stool (n=7). They were aseptically collected and processed following standard microbiological methods for laboratory investigations of clinical specimens.[8] All isolates were identified by colonial morphology, standard biochemical tests including the use of Microbact^{TM}GNB 24E identification kit (Oxoid, England). Pertinent clinical and demographic information were obtained from hospital records of individual patient with the aid of proforma designed for the study.

Antimicrobial Susceptibility Test

All isolates were tested against gentamicin (10µg), ampicillin (10µg), amoxicillin- clavulanate (20/10µg), amikacin (10µg), ciprofloxacin (5µg), meropenem (10µg), ceftazidime (30µg),

cefotaxime (30µg), cefuroxime (30µg), cefepime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cotrimoxazole (1.25/23.75µg), and piperacillin-tazobactam (100/10µg) (Oxoid, England) using the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standard Institute (CLSI). Zones of inhibition diameters were measured and interpreted using the guidelines.[9]

Determination of Extended Spectrum Beta-Lactamase production

Phenotypic confirmatory test to detect ESBL production among Gram- negative bacilli was carried out on isolates which showed resistance to one or more of the tested third generation. The tests was done by combination disk test (CDT) according to the methods described in CLSI guidelines.[9]

Molecular Identification of ESBLs

Molecular detection of ESBL genes was carried out by Multiplex Polymerase Chain Reaction (PCR) only on isolates that were phenotypically confirmed to produce ESBLs. DNA extraction was done using boiling method. Two colonies of test organisms were emulsified into a 5ml peptone broth which was incubated overnight. A 1ml aliquot of the culture was centrifuged at 10,000 rpm for two minutes in a micro-centrifuge (Biorad, USA). The pellet was boiled for 10 min in 100µl of sterile distilled water. The DNA suspension served as template DNA for polymerase chain reaction (PCR) amplification.

Oligonucleotides primers and amplification reactions for ESBL resistance genes was adapted from Monstein *et al.*[10] and shown in Table 1. Each amplicon (5µl) was separated on a 1.5% (w/v) agarose gel in 1X Tris-Borate-EDTA (TBE) buffer. Gels were stained in 0.5 ug/mL ethidium bromide for 10 minutes, de-stained in distilled water for 20 minutes, and viewed under ultraviolet light using a UVitec trans illuminator (Avebury, Cambridge UK). The position of amplified products was estimated by the position of the 100bp molecular weight marker (Biolab, England).

Data Analysis

Data were analysed by R statistical software package (version 3.3.0). Chi- square and Fishers exact tests were used to compare discrete variables. Statistical testing was performed using 2-tailed tests. Statistical significance was set at a p-value less than or equal to 0.05.

Results

Distribution of Gram-negative bacilli Isolates among clinical specimens

Three hundred and fifty-nine Gram-negative bacilli were isolated from diverse clinical samples, comprising mainly urine (n=159; 44.3%), wound (n=105; 29.2%), blood (n=46; 12.8%), sputum (n=28; 7.8%), cerebrospinal fluid (CSF) (n=10; 2.8%) and stool (n=7; 1.9%). *Escherichia coli* (n=84; 23.4%) was predominant, followed by *Citrobacter* spp (n=81; 22.6%), *Proteus* spp (n=45; 12.5%), *Klebsiella* spp (n=43; 12%), *Pseudomonas aeruginosa* (n=39; 10.9%), *Enterobacter* spp (n=34; 9.5%), The other isolates included *Morganella morganii* (n=11, 3.1%), *Acinetobacter* spp (n=10, 2.8%) (Table 2).

Escherichia coli and *Citrobacter* were the predominant isolates from urine (54; 34% and 36; 22.6% respectively) while *Citrobacter* and *Pseudomonas aeruginosa* were the predominant isolates from wound (28; 26.7% and 19; 18.1% respectively). *Enterobacter* and *Klebsiella pneumoniae* were respectively the commonest in blood (n=10; 21.7%) and sputum (6; 21.4%). *Shigella dysenteriae* (n=3; 42.9%) and *Yersinia enterocolitica* (n=4; 57.1%) were the only isolated organisms from stool (Table 2).

Extended spectrum beta lactamase producers

Ninety four (26.2%) of 359 isolates were phenotypically confirmed to be ESBL-producing strains. Across genera, *Citrobacter* (n=27; 28.7%) was the most predominant, followed by *Escherichia coli* (n=25; 26.6%), *Klebsiella* (n=14; 14.9%), *Enterobacter* (n=12; 12.8%) and *Proteus* (n=5; 5.3%). However among species, *Klebsiella pneumoniae* had the highest prevalence of ESBL-

producing phenotype (44.8%), followed by *Enterobacter cloacae* (38.5%), *Citrobacter freundii* (37.7%), *Enterobacter aerogenes* (36.8%) and *Escherichia coli* (29.8%) (Table 3). Although, the ESBL producers were mostly from urine isolates (n=35; 37.2%) (Figure 1), ESBL-producing phenotype was most prevalent among isolates from sputum (35.7%) (Table 3). Prevalence of ESBL producers was also high among isolates from other specimens including wound (27.6%) and blood (23.9%).

Prevalence of antibacterial resistance among ESBL- and non-ESBL-producing isolates

As shown in Table 4, ESBL-producing isolates showed significantly higher resistance to antibiotics such as amikacin, ampicillin, amoxiclav, ciprofloxacin, ceftriaxone ceftazidime, cefotaxime, cefuroxime, cefepime, gentamicin, cotrimoxazole and piperacillin-tazobactam

Prevalence of ESBL genes in Gram-negative isolates

Of 94 isolates that exhibited ESBL phenotype, 86 had one or more of ESBL genes sought while eight strains did not. Among the strains that harboured the genes, 17 (19.8%) harboured single genes, comprising 7 strains (8.1%) with CTX-M gene, 6 (6.9%) strains with TEM gene and 4 strains (4.7%) with SHV gene. Sixty nine (80.2%) bacterial species had multiple genes; 24 harboured all the three variants (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$), 40 haboured *bla*$_{CTX-M}$ and *bla*$_{TEM}$, three haboured *bla*$_{SHV}$ and *bla*$_{CTX-M}$, and two haboured *bla*$_{SHV}$ and *bla*$_{TEM}$ (Table 5). In all, *bla*$_{CTX-M}$ was the most common gene harboured by the ESBL phenotype (74; 78.7%), closely followed by *bla*$_{TEM}$ (72; 76.6%). The least common was *bla*$_{SHV}$ (33; 35.1%).

In the 25 isolates of ESBL-producing *E. coli*, five had single gene: one harboured each of *bla*$_{TEM}$ and *bla*$_{SHV}$ while three haboured *bla*$_{CTX-M}$. Of the 17 *E. coli* strains that harboured multiple genes, 10 haboured combined *bla*$_{CTX-M}$ and *bla*$_{TEM}$ and seven haboured the three determinants. About 91% of the 22 gene-habouring *E. coli* had *bla*$_{CTX-M}$ either as single gene (3; 13.6%) or in associated with other bla genes (17; 77.3%). Each of the ESBL-producing phenotype of *Citrobacter freundii* harboured one or more determinant genes; among them, 4.3% (1/23) harboured the genes each for TEM alone and TEM combined with SHV, 21.7% (5/23) harboured all the three genes, and 91.3% (21/23) haboured gene for CTX-M combined with one or more other genes. Eleven (83.6%) of the 13 ESBL-producing *Klebsiella pneumoniae* phenotypes harboured the determinant genes all of which had bla gene for CTX-M either as single gene (3; 27.3%) or in combination with other genes (8; 72.2%). Each of the single strain of *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and *Klebsiella oxytoca* haboured bla gene for CTX-M combined with TEM and SHV, single bla gene for TEM and single bla gene for SHV respectively (Table 5).

Table 1: Oligonucleotides Primers and Amplification Reactions for ESBL Resistance Genes

Target gene	Name	Primer Sequence	Amplicon Size	Amplification reactions	References
bla$_{CTX-M}$	CTX-M-F	TTGCGATGTGCAGTACCAGTAA	754bp	Initial denaturation at 94°C for 3 mins, followed by 35 cycle of denaturation at 94°C for 45 secs, annealing at 60°C for 30 secs and extension at 72°C for 1 min, and a final extension at 72°C for 3 mins	[15]
	CTX-M-R	CGAATATCGTTGGTGGTGCCATA			
bla$_{SHV}$	SHV-F	ATTTGTCGCTTCTTTACTCGC	294bp		
	SHV-R	TTTATGGCGTTACCTTTGACC			
bla$_{TEM}$	TEM-F	ATGAGTATTCAACATTTCCGTG	404bp		
	TEM-R	TTACCAATGCTTAATCAGTGAG			

Table 2: Distribution of Gram -negative isolates among clinical specimens

Bacterial species	Clinical Specimens										
	Urine n=159	Wound n=105	Joint Aspirate n=1	Blood n=46	CSF n=10	Endocervical swab n=1	Ear swab n=1	Sequestrum n=1	Sputum n=28	Stool n=7	Total n (%)
Acinetobacter baumanii	0	0	0	0	0	0	0	0	2	0	2 (0.6)
Acinetobacter johnsonii	3	2	0	0	0	0	0	0	3	0	8 (2.2)
Total *Acinetobacter* spp	**3**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**10 (2.8)**
Citrobacter diversus	1	1	0	0	0	0	0	0	0	0	2 (0.6)
Citrobacter freundii	28	21	0	5	2	1	0	0	4	0	61 (17.0)
Citrobacter koseri	6	5	0	3	0	0	0	0	0	0	14 (3.9)
Citrobacter sedlakii	1	1	0	0	0	0	0	0	2	0	4 (1.1)
Total *Citrobacter* spp	**36**	**28**	**0**	**8**	**2**	**1**	**0**	**0**	**6**	**0**	**81(22.6)**
Enterobacter aerogenes	6	7	0	4	0	0	0	0	2	0	19 (5.3)
Enterobacter agglomerans	0	0	0	1	0	0	0	0	1	0	2 (0.6)
Enterobacter cloacae	3	2	0	5	3	0	0	0	0	0	13 (3.6)
Total *Enterobacter* spp	**9**	**9**	**0**	**10**	**3**	**0**	**0**	**0**	**3**	**0**	**34 (9.5)**
Escherichia coli	54	18	0	8	3	0	0	1	0	0	84 (23.4)
Hafnia alvei	3	0	0	0	0	0	0	0	0	0	3 (0.8)
Klebsiella oxytoca	7	3	0	2	2	0	0	0	0	0	14 (3.9)
Klebsiella pneumonia	11	7	0	5	0	0	0	0	6	0	29 (8.1)
Total *Klebsiella* spp	**18**	**10**	**0**	**7**	**2**	**0**	**0**	**0**	**6**	**0**	**43(12.0)**
Morganella morganii	5	3	0	3	0	0	0	0	0	0	11 (3.1)
Proteus mirabilis	17	8	0	4	0	0	0	0	0	0	29 (8.1)
Proteus vulgaris	5	8	0	3	0	0	0	0	0	0	16 (4.5)
Total *Proteus* spp	**22**	**16**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**45(12.5)**
Pseudomonas aeruginosa	9	19	1	3	0	0	1	0	6	0	39 (11.5)
Shigella dysenteriae	0	0	0	0	0	0	0	0	0	3	3 (0.8)
Stenotrophomonas maltophilia	0	0	0	0	0	0	0	0	2	0	2 (0.6)
Yersinia enterocolitica	0	0	0	0	0	0	0	0	0	4	4 (1.1)

Table 3: Table Prevalence of ESBL producers among the Gram negative Isolates

Organisms			Prevalence among Isolates Within Clinical Specimens									
	Prevalence within Total species	Prevalence within Total ESBL Isolates	Urine n=159	Wound n=105	Joint Aspirate n=1	Blood n=46	CSF n=10	E.C swab n=1	Ear swab n=1	Sequestrum n=1	Sputum n=28	Stool n=7
Acinetobacter baumanii	1/2(50)	1/94(1.1)	0	0	0	0	0	0	0	0	1/2(50)	0
Acinetobacter johnsonii	0/8 (0)	0/94	0	0	0	0	0	0	0	0	0	0
T otal *Acinetobacter* spp	**1/10 (10)**	**1/94 (1.1)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1/5(20)**	**0**
Citrobacter diversus	0/2 (0)	0/94	0	0	0	0	0	0	0	0	0	0
Citrobacter freundii	23/61(37.7)	23/94(24.5)	8/28(28.6)	9/21(42.9)	0	2/5(40)	1/2(50)	1/1(100)	0	0	2/4(50)	0
Citrobacter koseri	2/14(14.3)	2/94(2.1)	1/6(16.7)	1/5(20)	0	1/3(33.3)	0	0	0	0	0	0
Citrobacter sedlakii	2/4 (50)	2/94(2.1)	0	1/1(100)	0	0	0	0	0	0	1/2(50)	0
Total *Citrobacter* spp	**27/81(33.3)**	**27/94(28.7)**	**9/36(25)**	**11/35(30.6)**	**0**	**3/8(37.5)**	**1/2(50)**	**1/1(100)**	**0**	**0**	**3/6(50)**	**0**
Enterobacter aerogenes	7/19(36.8)	7/94(7.4)	4/6(66.7)	2/7(28.6)	0	0	0	0	0	0	1/2(50)	0
Enterobacter agglomerans	0/2	0/94	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	5/13 (38.5)	5/94(5.3)	1/3(33.3)	2/2(100)	0	2/5(40)	0	0	0	0	0	0
Total *Enterobacter* spp	**12/34(35.3)**	**12/94(12.8)**	**5/9**	**4/8**	**0**	**2/10**	**0**	**0**	**0**	**0**	**1/3(33.3)**	**0**
Escherichia coli	25/84(29.8)	25/94(26.6)	13/54(22.5)	8/18(44.4)	0	2/8 (25)	1/3(33.3)	0	0	1/1(100)	0	0
Hafni aalvei	0/3	0/94	0	0	0	0	0	0	0	0	0	0
Klebsiella oxytoca	1/14(7.1)	1/94(1.1)	0	0	0	1/2 (50)	2	0	0	0	0	0
Klebsiella pneumoniae	13/29(44.8)	13/94(13.8)	5/11(45.5)	3/7(42.9)	0	2/5(27.3)	0	0	0	0	3/6(50)	0
Total *Klebsiella* spp	**14/43(32.6)**	**14/94(14.9)**	**5/18(27.8)**	**3/10(30)**	**0**	**3/7(42.9)**	**2/2(100)**	**0**	**0**	**0**	**3/6(50)**	**0**
Morganella morganii	3/11 (27.3)	3/94(3.2)	0/5	1/3(33.3)	0	2/3(66.7)	0	0	0	0	0	0
Proteus mirabilis	2/29 (6.9)	2/94(2.1)	1/17(5.9)	1/8(12.5)	0	0	0	0	0	0	0	0
Proteus vulgaris	3/16 (18.8)	3/94(3.2)	1/5(20)	1/8(12.5)	0	1/3(33.3)	0	0	0	0	0	0
Total *Proteus* spp	**5/45(11.1)**	**5/94(5.3)**	**2/22(4.5)**	**2/26(12.5)**	**0**	**1/7(14.3)**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudomonas aeruginosa	1/39(2.6)	1/94(1.1)	1/9(11.1)	0	0	0	0	0	0	0	0	0
Shigella d ysenteriae	2/3(66.7)	2/94(2.1)	0	0	0	0	0	0	0	0	0	2/3(66.7)
Stenotrophomonas maltophilia	2/2 (100)	2/94(2.1)	0	0	0	0	0	0	0	0	2/2(100)	0
Yersinia enterocolitica	2/4 (50)	2/94(2.1)	0	0	0	0	0	0	0	0	0	2/4
Total	**94/359 (26.2)**	**94/94(100)**	**35/159 (22)**	**29/105 (27.6)**	**0/1 (0)**	**11/46 (23.9)**	**3/10 (30)**	**1/1 (100)**	**0/1 (0)**	**1/1 (100)**	**10/28 (35.7)**	**4/7 (57.1)**

Table 4: Comparison of resistance of ESBL and non ESBL producing Gram-negative bacilli.

Antibiotics	ESBL producers (%) n=94	Non-ESBL-producers (%) n=265	P-value
Amikacin	29(30.9)	20(7.6)	0.001
Ampicillin	62(66)	147(55.5)	0.022
Amoxiclav	75(79.8)	120(45.3)	0.001
Ciprofloxacin	81(86.2)	79(29.8)	0.001
Ceftriaxone	93(98.9)	62(23.4)	0.001
Ceftazidime	77(81.9)	30(11.3)	0.001
Cefotaxime	90(95.7)	4(1.5)	0.001
Cefuroxime	60(63.80)	57(21.5)	0.001
Cefepime	73(77.7)	21(7.9)	0.001
Cefoxitin	26(27.7)	68(25.7)	0.620
Gentamycin	70(74.4)	79(29.8)	0.001
Meropenem	1(1.1)	10(3.8)	0.3101
Cotrimoxazole	92(97.9)	197(74.3)	0.001
Piperacillin-tazobactam	15(15.9)	13 (4.9)	0.004

Table 5: Total prevalence of ESBL genes

Organisms	Phenotypes	CTX-M	SHV	TEM	CTX-M/SHV	CTX-M/TEM	TEM/SHV	CTX-M/SHV/TEM	Total
Acinetobacter baumanii	1	-	1	-	-	-	-	-	1
Acinetobacter johnsonii	0	-	-	-	-	-	-	-	0
Citrobacter diversus	0	-	-	-	-	-	-	-	0
Citrobacter freundii	23	-	-	1	2	14	1	5	23
Citrobacter koseri	2	-	1	-	-	-	-	-	1
Citrobacter sedlakii	2	-	-	-	1	-	1	-	2
Enterobacter aerogenes	7	1	-	-	-	4	-	2	7
Enterobacter agglomerans	0	-	-	-	-	-	-	-	0
Enterobacter cloacae	5	-	-	-	-	3	-	2	5
Escherichia coli	25	3	1	1	-	10	-	7	22
Hafnia alvei	0	-	-	-	-	-	-	-	0
Klebsiella oxytoca	1	-	-	1	-	-	-	-	1
Klebsiella pneumoniae	13	3	-	-	-	6	-	2	11
Morganella morganii	3	-	-	-	-	-	-	3	3
Proteus mirabilis	2	-	-	1	-	1	-	-	2
Proteus vulgaris	3	-	-	1	-	1	-	-	2
Pseudomonas aeruginosa	1	-	-	-	-	-	-	1	1
Shigella dysenteriae	2	-	-	1	-	1	-	-	2
Stenotrophomonas maltophilia	2	-	1	-	-	-	-	-	1
Yersinia enterocolitica	2	-	-	-	-	-	-	2	2
Total	**94**	**7**	**4**	**6**	**3**	**40**	**2**	**24**	**86**

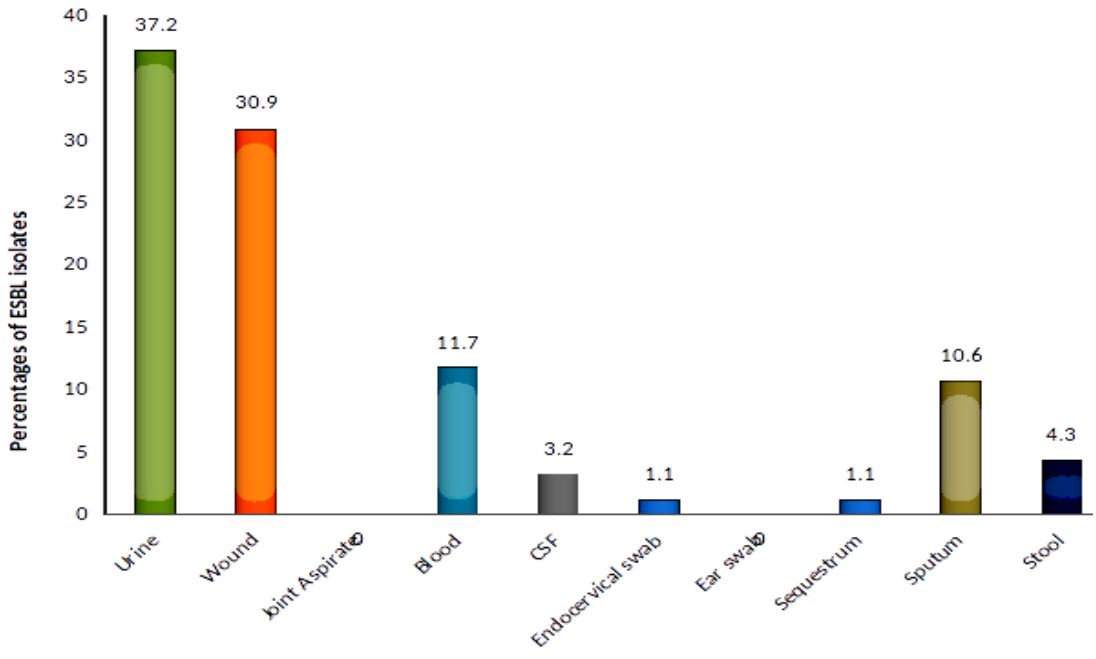

Figure 1: Distribution of ESBL-producing organisms in different clinical samples

Discussion

The occurrence of ESBLs in Gram negative organisms has been widely reported and has remained a global problem, made worse in Sub-saharan Africa by restricted access to effective antibiotics. Clinical infections with ESBL-producing bacteria have led to poor outcomes with considerable morbidity and mortality. In the present study, the prevalence of ESBL-producing strains among Gram negative bacilli was 26.2%. However, higher rates have been reported in different parts of the country particularly in cases of surgical site and orthopaedic wound infections.[3,4] The prevalence of ESBL producers is known to differ from one regions or country to another. In Asia for example, prevalence rate of 17.3% was reported among Enterobacteriaceae in a hospital-based study in Qatar,[11] while a pooled prevalence of 40% was documented in a country-wide systematic review in Parkistan.[7] The observed prevalence of ESBL in Gram-negative isolates in our study is within the rate of 10-90% previously reported by other investigators across continents.[1,12,13] Nonetheless, it is a cause for concern in view of the weak laboratory infrastructure and low capacity for effective and adequate surveillance. Rising levels of ESBL production among bacterial isolates in our environment could be as a result of the selective pressure imposed by excessive use of antibiotics caused by unguided access as well as low level and poor implementation of antimicrobial stewardship in our setting.[14]

Among the genera of bacteria isolated, ESBL-producing phenotypes that were predominant are *Citrobacter* and *Escherichia coli*, and this is because these bacteria constituted about 54% of the tested Enterobacteriaceae which accounted for over 85% of the total Gram negative bacterial isolates. Furthermore, *Klebsiella pneumoniae* as a major hospital pathogen was found to have a high prevalence rate of ESBL-producing strain in this study which is in accordance with findings from other studies.[3,11]

Although, urine isolates accounted for the highest number of ESBL producers among various clinical samples examined, ESBL-producers were more commonly isolated from sputum. A study by Adeyankinnu *et al*. in the same region of the country also noted that this resistant-strain is most commonly harboured by sputum isolates.[2] Similarly, other studies, including the one by Sid Ahmed *et al*. of cases of infection among critically ill patients in Qatar, document predominance of ESBL-producing isolates in sputum.[11] This is not unexpected because pneumonia in hospitalised patients is mainly hospital-acquired and commonly caused by *Klebsiella pneumoniae* which is also an important multidrug-resistant bacteria.[15] Furthemore in this study, the high level of ESBL-producing pathogens in urine and wound specimens observed is respectively due to high number of patients with obstructive uropathy and chronic wounds which are established predisposing factors to acquisition of multi-drug resistant bacterial strains.[12,16]

Significant resistance to ampicillin, oxyimino-cephalosporins, co-trimoxazole, augmentin, ciprofloxacin, gentamicin, piperacillin-tazobactam, ciprofloxacin, cefepime and amikacin was observed in isolates that produced ESBL compared with those that did not produce the enzyme. The high level of resistance shown by ESBL-producing bacteria against commonly tested and used antibiotics could be as a result of selective pressure caused by excessive use of antimicrobial agents as a result of unrestricted access, self-prescription and poor implementation of antimicrobial stewardship programmes in Nigeria.[17] Excessive exposure to antibiotics continue to exert impactful selective pressure over the years causing bacteria to bear additional resistance genes and mechanisms that show multidrug-resistance.

Of all the antimicrobials tested, meropenem still offers an effective treatment option against ESBL-producing bacterial infections at the moment in our setting. This finding has also been previously noted by another investigator.[18] The implication of

this high susceptibility to meropenem is that about 99% of ESBL-producing GNB in this environment do not co-habour carbapenem resistance determining genes, which is an important observation considering the fact that empiric use of carbapenems is low in Nigeria at the moment because of restrictions caused by high cost and non-availability in most of the major cities. Carbapenems are considered as the last option against ESBL-producing bacteria, their use in hospital wards should therefore be guided to prolong their useful life.

The major drawback of phenotypic tests is their failure to detect ESBL-production in some strains especially if some of the enzymes fail to reach a detectable level. Molecular methods, on the other hand, give definitive identification and detection of ESBL production. We used a molecular based method (multiplex PCR) to screen all the ninety-four phenotypically detected ESBL producers for the three commonly reported families of ESBL genes. We found incidence of CTX-M to be highest in our study; CTX-M-type ESBLs have been increasingly detected and they are now the most prevalent ESBLs encountered globally especially in *Escherichia Coli* and *Klebsiella pneumoniae*[19] Our finding is also a snapshot of occurrence in Nigeria vis-à-vis the west African sub-region that there is high prevalence of ESBL production among *Escherichia coli* and *Klebsiella pneumoniae*.[13]

Most isolates haboured multiple ESBL genes; twenty-four haboured the three genes (bla TEM, CTX-M, SHV) while 45 haboured two variants of the ESBL determining genes. Our finding is not uncommon, other researchers observed that significant number of ESBL-producing strains carried multiple genes.[10,20] Carriage of multiple genes increases the spectrum of hydrolysable antibiotics by these strains, in addition, plasmids with such multiple ESBL genes act as reservoirs for horizontal transmission, and this portends grave consequences to infection control in health care settings.

Conclusion

In conclusion, the study establishes that there is high prevalence of ESBL-producers in clinical isolates in our hospital setting which could complicate antibiotic treatment of patients with infectious diseases. Meropenem was still appreciably potent to most isolates whereas they were commonly resistant to all the other antibiotics tested. We therefore propose an intensification of routine screening of clinical isolates for possible ESBL production to inform proper and timely treatment of patients infected with the strains thereby preventing further dissemination of antibiotic resistance determinants. High rate of ESBL-producing pathogens in this study provides the basis for advocacy for review, strengthening of antimicrobial stewardship and infection prevention and control in our hospitals.

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