Original Research Article

Extended-Spectrum Beta-Lactamase (ESBL)–Producing Gram-negative Isolates from Urine and Wound Specimens in a Tertiary Health Facility in Southern Nigeria

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Abstract

Purpose: To determine the prevalence of extended spectrum beta lactamase (ESBL) producing Gram negative bacteria causing wound and urinary tract infections among in- and out-patients in a tertiary health facility.
Methods: The presence of ESBL was determined among 230 Gram negative bacilli isolated from wound (105) and urine (125) specimens from in- and out-patients who attended University of Benin Teaching Hospital (UBTH) for treatment using the double disc synergy method. Disc susceptibility test was performed on all isolates using standard techniques.
Results: There was no significant difference in the prevalence of ESBL production between isolates from wound (47.6 %) and urine (41.6 %) as well as between in-patients (48.8 %) and out–patients (39.38 %). Enterobacter species were the most prevalent producers of ESBL from both wound and urine specimens as well as from both in- and out–patients. Bacterial isolates that produced ESBL were more resistant to amoxicillin-clavulanate, ceftazidime, ceftriaxone, cefotaxime, gentamicin, ciprofloxacin and ofloxacin compared to non-ESBL producers.
Conclusion: A high prevalence (44.3%) of ESBL producing Gram–negative bacteria was observed among the patients, with Enterobacter species being the most prevalent. Prudent use of antibacterial agents is advocated to stem the tide.

Keywords: Extended-spectrum beta-lactamase, Enterobacter species, Wound, Urine, Gram negative bacteria

INTRODUCTION

Antibiotic resistance of bacteria is commonly seen in daily medical practice with multi-drug resistant Gram negative bacteria posing the greatest threat to human health [1]. Beta lactam antibiotics are the most predominantly prescribed antibiotics to treat bacterial infections, especially in Nigeria hospitals [1,2]. β-lactamases are major defense of Gram negative bacteria against β-lactam antibiotics [3]. Extended spectrum β-

lactamases (ESBL), a type of β-lactamase, are typically inhibitor-susceptible β-lactamase that hydrolyze penicillins, cephalosporins and aztreonam [3], and are encoded by mobile gene [4]. These genes often code resistance to cephalosporins and other antibiotics such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimetroprim [5].

Infections caused by ESBL-producing Gram negative bacteria are associated with increased morbidity and mortality which is linked to inappropriate or delayed antimicrobial treatment [6]. Risk factors that have been associated with ESBL production include old age (> 65 years), male gender, previous use of β-lactam antibiotics and fluoroquinolones amongst others [6,7]. In Nigeria, extended–spectrum cephalosporins and fluoroquinolones are widely used as broad–spectrum antibiotics and remain the drugs of choice to treat infections caused by various Gram negative pathogens [8]. Also, antibiotics use in Nigeria is unregulated and over the counter sales of antibiotics without prescriptions are rife [9-11]. These indicate that ESBL producing organisms may be rife in Nigeria and indeed reports of ESBL–producing Gram negative bacteria exists [11].

A previous study from Benin City, Nigeria reported a prevalence of 2.7 % of ESBL producing Gram negative bacteria from blood stream infections and surgical wounds [12]. However, that study, only evaluated ceftazidime as the only indicator of ESBL production. Ceftazidime is a good indicator of TEM (Temoniera) and SHV (Sulphydryl) type of ESBL and not the CTX–M (Cefotaximase – Munich) type [13]. The CTX–M type is detected with the use of cefotaxime [13]. It has been reported that Gram–negative bacteria cause a significant number of infections in Nigerian hospitals and represent the majority of both wound and urinary isolates, which form the largest group of specimens received in microbiology laboratories [11]. These will indicate that the study of Omoregie *et al* [12], may have underestimated the true prevalence of ESBL. Against this background, this study aimed to determine the prevalence of ESBL among Gram negative bacteria causing wound and urinary tract infections among community and hospital isolates. The susceptibility patterns of all the isolates were also determined.

EXPERIMENTAL

Bacterial isolates

A total of 230 consecutive non–repetitive bacterial isolates from wound (105) and urinary tract infections (125) collected from patients attending University of Benin Teaching Hospital, Benin City (UBTH) were used for this study. One hundred and twenty three (123) of these isolates were recovered from in–patients while 107 isolates were from out–patients. The isolates

included *Escherichia coli, Klebsiella* species, *Citrobacter* species, *Enterobacter* species, *Proteus mirabilis, Proteus vulgaris, Providencia* species, *Acinetobacter* species and *Pseudomonas aeruginosa*. All isolates were identified using standard techniques [14].

ESBL detection

The presence of ESBL was detected in all isolates using the double disc test [13]. Briefly, test organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standards. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. A disc containing 30 µg amoxicillin–clavulanate (Oxoid, England) was placed at the centre of the agar plate. A 30 µg ceftazidime disc (Oxoid, England) was placed 25 mm from the amoxicillin–clavulanate disc and another disc containing 30 µg cefotaxime (Oxoid, England) was placed on the opposite side of the amoxicillin–clavulanate disc (25 mm apart). The plates were incubated at 37 ^{o}C overnight and ESBL production was inferred as positive if there was an expansion of the zone of inhibition between the ceftazidime and amoxicillin–clavulanate disc, cefotaxime and amoxicillin–clavulanate disc or both.

Disc susceptibility testing

Disc susceptibility tests were performed on all bacterial isolates using the British Society for Antimicrobial Chemotherapy (BSAC) method [15].

Statistical analysis

The data obtained were analyzed with chi square (X^2) test and odd ratio analysis using the statistical software Instat (R) (Graph Pad Software Inc, La Jolla, CA, USA).

RESULTS

A total of 102 (44.3 %) out of the 230 isolates produced ESBL. Of these isolates that were positive for ESBL, 97 (95.1 %) were indicated by both ceftazidime and cefotaxime, while 5 (4.9 %) were indicated by cefotaxime alone. Generally and among individual isolates (with the exception of *Citrobacter* species), there was no significant difference in EBSL production among isolates from wounds and urinary tract infections. EBSL

production by *Citrobacter* species was more likely to occur from the bacteria isolated from wounds as compared to urinary tract infections. (OR = 55.000, 95 % CI = 0.828, 3654.5, *p* = 0.0476). *Enterobacter* species were the most prevalent producers of ESBL generally whether from wound, and urine specimens (Table 1).

Isolates of *Enterobacter* species from both in–patients and out–patients were the most predominant producers of ESBL in comparison to other isolates. Although Gram negative isolates from in–patients (48.8 %) had higher prevalence of ESBL production than others recovered from out–patients (39.3 %) and were more likely to produce ESBL (OR = 1.474, 95 % CI = 0.872, 2.492); this was not statistically significant (P = 0.1833) (Table 2).

The susceptibility profiles of ESBL–producing and non–ESBL–producing Gram negative bacterial isolates are shown in Table 3 and 4

respectively. Generally, isolates that produce EBSL were more resistant to the antimicrobials used compared with non–ESBL producers. Imipenem was the most active antibiotic against ESBL–producing and non–ESBL–producing Gram negative bacteria.

DISCUSSION

Of the 102 Gram negative bacteria that produced ESBL, 97 (95.1 %) were detected by both ceftazidme and cefotaxime. This will indicate that majority of the isolates producing ESBL in this study produce multiple types of ESBL such as TEM, SHV and CTX–M types. Indeed previous studies have reported both TEM, SHV and CTX–M types of ESBL in Nigeria [8,17]. Five isolates (4.9 %) produced only CTX–M type. This indicates that the CTX–M type of ESBL were more predominant in this study and agrees with previous reports from other Nigerian studies

Table 1: Prevalence of ESBL production in relation to specimen

Organism	Wound		Urine		Total (%)	
	No. tested	No. positive (%)	No. tested	No. positive (%)	No. tested	No. positive (%)
Escherichia coli	25	12 (48.0)	54	24 (44.4)	79	36 (45.6)
Klebsiella species	25	14 (56.0)	23	7 (30.4)	48	21 (43.8)
Citrobacter species*	2	2 (100.0)	5	0 (0.0)	7	2 (28.6)
Enterobacter species	1	1 (100.0)	8	7 (87.5)	9	8 (88.9)
Proteus mirabilis	12	3 (25.0)	4	2 (50.0)	16	5 (31.3)
Proteus vulgaris	8	3 (37.5)	8	3 (37.5)	16	6 (37.5)
Providencia species	2	0 (0.0)	5	1 (20.0)	7	1 (14.3)
Acinetobacter species	6	2 (33.3)	12	5 (41.7)	18	7 (44.4)
Pseudomonas aeruginosa	24	13 (54.2)	6	3 (50.0)	30	16 (53.3)
Total	105	50 (47.6)	125	52 (41.6)	230	102 (44.3)

Wound vs urine: OR 55.000, 95% CI = 0.8278, 36545; p = 0.0476 OR = odd ratio, CI = confidence interval

Table 2: Prevalence of ESBL production in relation to patients

Organism	In–patient		Out–patient		OR	95 % CI	P value
	No. tested	No. positive (%)	No. tested	No. positive (%)			
Escherichia coli	36	16 (44.4)	43	20 (46.5)	0.920	0.378-2.240	1.0000
Klebsiella species	29	13 (44.8)	19	8 (42.1)	1.117	0.347-3.595	1.0000
Citrobacter species	4	2 (50.0)	3	0 (0.0)	7.000	0.224-219.13	0.4286
Enterobacter species	4	3 (75.0)	5	5 (100.0)	0.212	0.007-6.822	0.4444
Proteus mirabilis	12	4 (33.3)	4	1 (25.0)	1.500	0.116-19.450	1.0000
Proteus vulgaris	6	4 (66.7)	10	2 (20.0)	8.000	0.803-79.700	0.1181
Providencia species	1	0 (0.0)	6	1 (16.7)	1.222	0.031-48.244	1.0000
Acinetobacter species	8	4 (50.0)	10	3 (30.0)	2.333	0.336-16.188	0.6305
Pseudomonas aeruginosa	23	14 (60.9)	7	2 (28.6)	3.889	0.617-24.529	0.2040
Total	123	60 (48.8)	107	42 (39.3)	1.474	0.872-2.492	0.1833

OR = odd ratio, CI = confidence interval

Table 3: Susceptibility profiles of ESBL producing isolates

Organism	Antibacterial disc (µg/disc)							
	AUG (30)	CAZ (30)	CRO (30)	CTX (30)	IPM (10)	CN (10)	CIP (5)	OFX (5)
Escherichia coli (n=36)	2 (5.6)	6 (16.7)	2 (5.6)	0 (0.0)	26 (72.2)	11 (30.6)	6 (16.7)	6 (16.7)
Klebsiella species (n=21)	1 (4.8)	1 (4.8)	1 (4.8)	2 (9.5)	11 (52.4)	0 (0.0)	3 (14.3)	1 (4.8)
Citrobacter species (n=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)
Enterobacter species (n=8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	7 (87.5)	1 (12.5)	3 (37.5)	2 (25)
Proteus mirabilis (n=5)	2 (40.0)	1 (20.0)	0 (0.0)	1 (20.0)	5 (100.0)	1 (20.0)	1 (20.0)	1 (20.0)
Proteus vulgaris (n=6)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	3 (50.0)	1 (16.7)	0 (0.0)	0 (0.0)
Providencia species (n=1)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Acinetobacter species (n=7)	0 (0.0)	1 (14.3)	1 (14.3)	0 (0.0)	3 (42.9)	1 (14.3)	0 (0.0)	0 (0.0)
Pseudomonas aeroginosa (n=16)	0 (0.0)	4 (24.0)	0 (0.0)	0 (0.0)	7 (43.8)	2 (12.5)	1 (6.3)	5 (31.3)

Number tested, AUG= Amoxicillin-clavulanate, CAZ=ceftazidime, CRO=Ceftriaxone, CTX=Cefotaxime, PM=Imipenem, CN=Gentamicin, CIP=Ciprofloxacin, OFX=Ofloxacin. Figures in parenthesis are percentages

Table 4: Susceptibility profiles of non–ESBL producing isolates

Organism	Antibacterial disc (µg/disc)							
	AUG (30)	CAZ (30)	CRO (30)	CTX (30)	IPM (10)	CN (10)	CIP (5)	OFX (5)
Escherichia coli (n=43)	5 (11.6)	24 (55.8)	16 (37.2)	24 (55.6)	37 (86.0)	19 (44.2)	17 (39.5)	18 (41.7)
Klebsiella species (n=27)	1 (3.7)	7 (25.9)	6 (22.2)	4 (14.8)	22 (81.5)	2 (7.4)	15 (55.6)	15 (55.6)
Citrobacter species (n=5)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	4 (80.0)	0 (0.0)	2 (40.0)	2 (40.0)
Enterobacter species (n=1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Proteus mirabilis (n=11)	2 (18.2)	9 (81.8)	10 (90.9)	8 (72.7)	4 (36.4)	9 (81.8)	3 (27.3)	7 (63.6)
Proteus vulgaris (n=10)	2 (20.0)	3 (30.0)	6 (60.0)	4 (40.0)	6 (60.0)	5 (50.0)	4 (40.0)	2 (20.0)
Providencia species (n=6)	1 (16.7)	4 (66.7)	4 (66.7)	4 (66.7)	5 (83.3)	3 (50.0)	2 (33.3)	2 (33.3)
Acinetobacter species (n=11)	2 (18.2)	6 (54.5)	9 (18.8)	5 (45.5)	7 (63.6)	5 (45.5)	4 (36.4)	4 (36.4)
Pseudomonas aeroginosa (n=14)	0 (0.0)	6 (42.9)	8 (57.1)	1 (7.1)	10 (71.4)	8 (57.1)	6 (42.9)	6 (42.9)

Number tested, AUG= Amoxicillin-clavulanate, CAZ=ceftazidime, CRO=Ceftriaxone, CTX=Cefotaxime, PM=Imipenem, CN=Gentamicin, CIP=Ciprofloxacin, OFX=Ofloxacin. Figures in parenthesis are percentages

[11,17], where CTX–M–15 was reported to predominate [11]. CTX–M–15, CTX–M–3 and CTX–M–2 have been reported as the predominant CTX–M type of ESBL from previous studies [11,17,18]. Molecular studies are needed to determine which ESBL type predominates in our locality as the previous studies in south–west geo-political zone of Nigeria.

A total of 102 (44.3 %) of the 230 Gram negative bacteria isolates produces ESBL. This is higher than the 2.7 %, 15.8 %, 20 % and 28.9 % reported in previous studies in Nigeria [8,12,18,19]. Previous use of antimi-crobial agents, especially cephalosporins and fluoroquinolones, has been reported as risk factors associated with emergence of ESBL [6,7]. Both antimicrobial agents are the drug of choice in treating Gram negative bacterial infections [8]. Unregulated use of antibiotics and over the counter sales of antibiotics without prescription is rife in Nigeria [9-11]. The cumulative effect of these over time may have been responsible for this high prevalence.

There was no significant difference in the prevalence of ESBL production between Gram negative bacterial isolates recovered from wounds and urinary tract infections. Gram negative bacteria cause a significant number of infections in Nigerian hospitals and represent the majority of both wound and urinary isolates, which form the largest group of clinical specimens received in microbiology laboratories [11]. This indicates that unregulated use of antibiotics for treating infections from these two sites may be rife. Indeed, cephalosporins and fluoroquinolones (risk factors associated with ESBL production) are used as drugs of choice in treating infections caused by Gram negative pathogens [8] which may explain the findings in this study.

The finding that Gram negative bacteria from wound and urine specimens produce ESBL agrees with previous reports [5,19]. *Enterobacter* species were the most prevalent producers of ESBL generally and from both wounds and urinary tract infections as previously reported from this study locality [12] and disagrees with others which reported *Escherichia coli* and *Klebsiella* species as the predominant producers [20]. The reason for this is unclear. The prevalence of ESBL did not differ significantly between Gram negative bacteria recovered from in–patients and out–patients (*p* = 0.1833). An earlier study from the same facility that consisted of bacterial isolates from female reproductive tract did not observe higher antibacterial resistant

among isolates from in–patients compared to their out–patient counterparts [21]. However, it has been reported that the highest volumes of antibiotics are being prescribed and consumed in ambulatory care [22] which would have resulted in higher prevalence of ESBL among out-patients isolates. Thus, the unregulated use of antibiotics in the facility may explain the findings in this study.

The susceptibility profiles of ESBL-producing Gram negative bacteria revealed imipenem to be the most active antibacterial agent. Imipenem is not affected by ESBL and this may explain the finding in this study. Gentamicin and fluoroquinolones used in this study were poorly active against ESBL producers. Genes coding for CTX–M type of ESBL have been reported to be associated with plasmids that code for resistance to tetracycline, aminoglycosides and quinolones [8]. Phenotypically, the CTX–M types of ESBL were more prevalent in this study and this may explain the poor activity of gentamicin and the fluoroquinolones used in this study to ESBL–producing Gram negative bacteria.

Although amoxicillin–clavulanate, ceftazidime, ceftriaxone and cefotaxime showed *in vitro* activity against some ESBL–producing isolates, ESBL enzymes have been reported to confer resistance to all penicillins and cephalosporins [23]. ESBL–mediated resistance is not obvious in disc or dilution susceptibility testing but is associated with clinical failure [12,13]. This may explain the result of their disc susceptibility testing results. Susceptibility profiles of non–ESBL–producing isolates reveal poor to high activity while Imipenem was the most active antibacterial agent. The finding confirms that ESBL–producing Gram negative bacteria are more resistant than their non–ESBL–producing counter parts.

The limitation of this study is that molecular technique was not applied in the evaluation as there are some type of ESBL that can only be detected by molecular means and not the phenotypic means used in this study.

CONCLUSION

A high prevalence (44.3 %) of ESBL production among Gram negative bacteria is reported in this study. *Enterobacter* species were the predominant producers of ESBL and ESBL–producing isolates were more resistant to antibacterial agents. Prudent use of antibacterial agents is advocated.

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