Prevalence of Extended Spectrum Beta-Lactamase-Producing *Escherichia Coli* Isolated from Selected Health Facilities in Makurdi

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Abstract:- **The worldwide prevalence of extended-spectrum-beta-lactamase -producing *Enterobacteriaceae* (ESBL-E) is increasing, making the need for ESBL detection more urgent. In this study we investigated the presence of ESBL in 400 isolates of *Escherichia coli* from urine, stool, blood, wound swabs, throat swabs and sputum specimens collected from 6 selected health facilities (2 primary, 2 secondary and 2 tertiary) in Makurdi local government council.Standard microbiological methods were used for isolation, characterization and identification of *E. coli*. The presence of ESBL was determined using the double disc synergy method. Disc susceptibility test was performed on all isolates using standard techniques.The isolates showed high level of resistance to all the antibiotics tested except mipenem. Highest resistance was to penicillin 392(98.0%) followed by ceftriaxone 385(96.3%). The isolates showed least resistance to mipenem 02(0.5%). Out of the 400 isolates examined, 64 (16.0%) carried ESBL genes. Isolates from blood specimens (n = 5; 26.3%) harboured the highest percentage of ESBL genes followed by wound swabs isolates 9(17.3%). No ESBL gene was recovered from throat swabs (n = 0; 0.0%). There exists no significant difference between ESBL-producing *E. coli* andvarious clinical specimens (p > 0.05).Among the males, isolates from those between 45.0 and 58.0 years old harboured the highest percentage (18.8%; n= 6) of ESBL-producing *E. coli* isolates, while among the females, those within the age group 31.0 to 44.0 years harboured the highest percentage (25.0%; n=13). Benue State University Teaching Hospital (BSUTH),a tertiary care facility harboured the highest percentage of ESBL-producing *Escherichia coli* isolates, 29 (19.7%) and was followed by General Hospital (GH) 10(18.9%) which is a secondary care facility. There is no significant association between ESBL and health facilities (p=0.39).**

Key words: **ESBL, Antibiotic resistance Beta-lactamase, Makurdi, *Escherichia coli*.**

I. INTRODUCTION

Extended Spectrum b-lactamases (ESBLs) are mutant, plasmid-mediated b-lactamases which are derived from older, broad-spectrum β-lactamases (*e.g.*, TEM-1, TEM-2, SHV-1). They have extended substrate profile which allows hydrolysis of all cephalosporins, penicillins, and aztreonam (Phillipon*et al*., 1989). Clinical outcome data indicates that ESBLs significantly complicates therapeutic procedures when involved in infections and they lead to increased mortality. When detected they always indicate the need for use of appropriate antibacterial agents. Failure to detect ESBL production by routine disk-diffusion tests has been well documented (Tenover*et al*., 1999; Paterson *et al*., 1999).

Many clinical laboratories are not fully aware of the importance of ESBLs and how to detect them; laboratories may also lack the resources to detect these resistance mechanisms. This lack of understanding or resources is responsible for a continuing failure to respond appropriately to prevent the rapid worldwide dissemination of pathogens possessing these b-lactamases. The consequence has been avoidable therapeutic failures in patients who received inappropriate antibiotics (Venezia *et al*., 1995).

Risk factors that have been associated with ESBL production include old age (> 65 years), gender, previous use of β-lactam antibiotics and fluoroquinolones, (Knusden *et al*., 2014). Antibiotics use in Nigeria is unregulated and antibiotics are sold freely over the counter without prescriptions (Okeke *et al*., 1999). Where they are prescribed, extended–spectrum cephalosporins and fluoroquinolones are widely prescribed and used as broad–spectrum antibiotics and remain the drugs of choice to treat infections caused by various Gram negative pathogens (Ogbolu *et al*., 2011). These indicate that ESBL producing organisms may be present in Nigeria.

A previous study from Benin City, Southern-Nigeria reported a prevalence of 2.7 % of ESBL-producing Gram negative bacteria from blood stream infections and surgical wounds (Omoregie *et al*., 2010). A more recent study from Benin – City by Ogefere*et al*. (2015) reported a prevalence 44.3%. Another study by Ogbolu *et al*. (2010) reported a prevalence of 20.9% of ESBL-producing organisms from South-West Nigeria.

The prevalence of ESBLs-producing bacteria is unknown in this region; therefore, this current study aimed at evaluating the prevalence of ESBL-producing *Escherichia coli* from selected health facilities, using a phenotypic detection

procedure based on the combined disk diffusion method. This method is the cheapest strategy to meet the local demands in resource constrained settings like Benue State compared to more expensive and unaffordable genotypic detection techniques (Saeidi*et al*., 2014). In addition, little data exist about the level of ESBL-producers of certain members of the Enterobacteriaceae family here. Therefore, this study also will isolate and detect ESBL-producing *Escherichia coli* bacteria from clinical specimens of patients admitted at these selected health facilities. This will offer evidence on the reality of ESBL prevalence in the health facilities in Makurdi environment and will represent valuable help to control this emerging problem.

II. MATERIALS AND METHODS

A total of 400 clinical specimens were collected in this cross-sectional study, conducted in about one year (February to December, 2017) involving 52 wound swabs, 200 urine specimens, 110 stool, 19 blood, 7 throat swabs and 12 sputum specimens from 216 female and 184 male patients with mean age of 28.1± 16.8 years, who were attending various health facilities in Makurdi metropolis. Presumptive *E.coli* isolates from these specimens were identified by standard microbiological methods-colonial morphology, Gram reaction, biochemical-TSI, indole and motility.

Antibiotic susceptibility testing was performed by the Kirby Bauer disk diffusion method, using Mueller-Hinton agar, according to BSAC guidelines. The following agents were tested-Penicillin, ceftazidime, cefotaxime, ceftriaxone, Cefuroxime, ciprofloxacin, chloramphenicol, gentamycin and mipenem. ATCC strain 25922 *E. coli* was used as control strain and the breakpoint was determined by measurement of zone of inhibition. The isolates were subjected to cefotaxime (30 μg) and ceftazidime (30 μg) antibiotic disks alone and in combination with amoxicillin and clavulanic acid (10 μg) (Oxoid; Basingstoke, UK) to determine the presence of ESBLs by the disc diffusion method on Müller-Hinton agar plates, using respective bacterial suspensions with the turbidity adjusted to a 0.5 McFarland standard. Plates were also incubated at 37°C for 24 hours.

Results were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI), where interpretive criteria for ESBL activity were based on an increase of $\geq$ 5 mm in the diameter of the inhibition zone around disks containing clavulanic acid as compared to the diameters of the inhibition zone around disks free of this beta-lactamase inhibitor (CLSI, 2014).

III. RESULTS

Out of the 400 isolates examined, 64 (16.0%) carried ESBL genes. Isolates from blood specimens (n = 5; 26.3%) harboured the highest percentage of ESBL genes followed by wound swabs isolates 9(17.3%). No ESBL gene was recovered from throat swabs (n = 0; 0.0%). There is no significant difference between ESBL-producing *E. coli* andvarious clinical specimens ($p > 0.05$). ESBL-producing *Escherichia coli* isolates showed high level resistance to all antibacterial agents tested. Highest resistance was to penicillin (98.0%), followed by ceftriaxone (96.3%). Mipenem (0.5%) had the least resistance (Table 1). Mipenem is known to be stable to beta-lactamases. Other classes of antibiotics; fluoroquinolones (ciprofloxacin), aminoglycosides (gentamycin), and chloramphenicols were also strongly resisted by the ESBL-producing *Escherichia coli* bacterium. Chatterjee *et al*. (2012) reported that beta-lactamases are encoded by mobile genes which often code resistance to cephalosporins, fluoroquinolones, aminoglycosides and other classes of antibiotics. Female patients within the age group 31-44 years (25.0%; n=13) harboured higher percentage of ESBL infections than males 18.8% (n=6) between ages 45-58 years old. There is no significant difference between gender and ESBL production ($p = 0.49$).

A tertiary health facility, Benue State University Teaching Hospital, Makurdi (19.7%; n=29) harboured the highest percentage of ESBL production, followed by General Hospital, Makurdi, (18.9%; n=10) a secondary health facility.There is no significant difference between ESBL production and health facilities ($p = 0.39$).

Table 1: Susceptibility Profile of Isolated *Escherichia coli* to Antibiotics (N=400)

Antibiotics	Abbreviations	Disc content	Resistance (%)	Susceptible (%)
Penicillin	P	10µg	392(98.0)	8(2.0)
Ceftriaxone	CRO	30 µg	385(96.3)	15(3.8)
Cefuroxime	CXM	30 µg	376(94.0)	24(6.0)
Cefotaxime	CTX	30 µg	371(92.8)	29(7.2)
Chloramphenicol	C	30 µg	348(87.0)	52(13.0)
Ciprofloxacin	CIP	5 µg	336(84.0)	64(16.0)
Ceftazidime	CAZ	30 µg	333(83.3)	67(16.8)
Gentamycin	CN	10 µg	331(82.7)	49(14.8)
Mipenem	MIP	10 µg	02(0.5)	398(99.5)

Table 2: Distribution of ESBL-producing *Escherichia coli* by Clinical Specimens

	ESBL DETECTION		
Specimen	Number positive (%)	Number negative (%)	Total number examined (%)
Blood	5(26.3)	14(73.7)	19(100)
Wound swabs	9(17.3)	43(82.7)	52(100.0)
Urine	33(16.5)	167(83.5)	200(100.0)
Stool	16(14.5)	94(85.5)	110(100.0)
Sputum	1(8.3)	11(91.7)	12(100.0)
Throat swabs	0(0.0)	7(100)	7(100.0)
Total	64(16.0)	336(84.0)	400(100.0)

$\chi^2 = 3.64$; df = 5; p = 0.60

Table 3: Distribution of ESBL-producing *Escherichia coli* by Age and Sex

		ESBL detection					
		Absent	Present				
Sex	Age (yrs)	Number of isolates (%)	Number of isolates (%)	Total (%)	χ2	df	P value
Male	≤ 16	41(93.2)	3(6.8)	44(100.0)			
	17.0 - 30.0	41(87.2)	6(12.8)	47(100.0)			
	31.0 - 44.0	42(82.4)	9(17.6)	51(100.0)	3.29	4	0.51
	45.0 - 58.0	26(81.3)	6(18.8)	32(100.0)			
	≥59	9(90.0)	1(10.0)	10(100.0)			
	Total	**159(86.4)**	**25(13.6)**	**184(100.0)**			
Female	≤ 16	52(82.5)	11(17.5)	63(100.0)			
	17.0 - 30.0	61(83.6)	12(16.4)	73(100.0)			
	31.0 - 44.0	39(75.0)	13(25.0)	52(100.0)			
	45.0 - 58.0	19(86.4)	3(13.6)	22(100.0)	3.45	4	0.49
	≥59	6(100.0)	0(0.0)	6(100.0)			
	Total	**177(81.9)**	**39(18.1)**	**216(100.0)**			
	Grand total	**336(84.0)**	**64(16.0)**	**400(100.0)**			

Table 4: Distribution of ESBL According to Health Facilities.

	ESBL DETECTION		
	ABSENT	PRESENT	
HEALTH FACILITIES	NUMBER (%)	NUMBER (%)	TOTAL (%)
BSUTH	118(80.3)	29(19.7)	147(100.0)
GH	43(81.1)	10(18.9)	53(100.0)
BMMC	25(83.3)	5(16.7)	30(100.0)
FMC	86(86.0)	14(14.0)	100(100.0)
FSP	36(90.0)	4(10.0)	40(100.0)
PHC	28(93.3)	2(6.7)	30(100.0)
TOTAL	**336(84.0)**	**64(16.0)**	**400(100.0)**

$\chi^2 = 5.17$; DF =5; p = 0.39.

Key:

BSUTH =Benue State University Teaching Hospital, Makurdi

GH = General Hospital, Makurdi

BMMC = Bishop Murray Medical Centre, Makurdi.

FMC = Federal Medical Centre, Makurdi.

FSP = Family Support clinic, Makurdi.

PHC = Primary Health Care Centre, Asase – Makurdi.

IV. DISCUSSION

Of 400 isolates tested, 64 (16.0%) produced ESBLs. This prevalence obtained from the study location is higher than 2.7% (Ogbolu *et al*. 2011) for South-West Nigeria. The value is consistent with 15.8% reported by Omoregie *et al*. (2010) from Benin-City, Southern Nigeria. Our result is however, lower than the report of another study by Ogbolu and coworkers (2013) and Aibinu *et al*. (2003) from South-West Nigeria. Our finding is also lower than 36.8% in Kano, North-West Nigeria (Yusha'u *et al*., 2010)), 59.4% in Enugu, South-East Nigeria (Iroha *et al.,* 2010). These differences may be due to variations in data and sample collection protocols, differences in thepresumptive identification of ESBL producing isolates. Furthermore, the populations investigated may differ in various socio-demographic, immune-epidemiologic and clinical parameters. Our result is very close to the report from Jos (18.6%), Plateau State also in North-Central Nigeria (Onyedible *et al.,* 2018). Isolates from blood specimens 5(26.3%) harboured the highest percentage of ESBL-producing *E. coli* in the present study, whereas in the study by Iroha *et al*. (2010), urine 35(60.3%) carried the highest percentage of ESBL-producing *E. coli*, followed by blood 23(39.6%) in Enugu, South-East Nigeria.

This will indicate that some of theseisolates of *E. coli* producing ESBL in this study may produce multiple types of ESBLs such as TEM, SHV and CTX–M types. If these ESBL resistance genes were prevalent in South and South-Western Nigeria, Makurdi in the North-Central region may not be an exception.

Previous use of antimicrobial agents, especially cephalosporins and fluoroquinolones, has been reported as risk factors associated with emergence of ESBL (Knusden*et al.,* 2014). These same antimicrobial agents are the drug of choice for treating infections with Gram-negative bacteria in Nigeria, of which the study location is a part.

ESBL enzymes have been reported to confer resistance to all penicillins and cephalosporins (Cormican*et al.,* 1996).Though some ceftriaxone, cefotaxime, ceftazidime and others showed in- vitro antimicrobial activities against some ESBL-producing *Escherichia coli* isolates, they will experience therapeutic failures because ESBL–mediated resistance is not obvious in disc or dilution susceptibility testing. It becomes imperative therefore, that ESBL detection should be carried out alongside susceptibility test if penicillins and cephalosporins are to be used for treatment. The fluoroquinolones showed moderate antibacterial activity against ESBL-producing *Escherichia coli*. A strong association has been shown between quinolone resistance and ESBL production (Lautenbach *et al*., 2001). This may explain the finding in this study.Percentages of ESBL detection was higher in the tertiary health facilities than other tiers of health facilities, though without a significant difference (p=0.39). This is consistent with health facilities referral practice, in which difficult cases are referred from primary health centres to secondary health centres, which in turn refer cases to tertiary health facilities. Bad cases therefore tend to concentrate more at tertiary health facilities. Percentages of ESBL production was higher in the female than the male sex, though without a significant difference too (p> 0.05).

V. CONCLUSION

A prevalence of 16.0% of ESBL production by *Escherichia coli* organism has been established by this study. There is need to carry out ESBL detection alongside antimicrobial susceptibility testing when penicillins and cephalosporins are to be used for treatment of infections caused by Gram-negative bacteria. Prudent use of antibacterial agents is advocated and prescription should be based on laboratory results of antimicrobial susceptibility test.

AUTHORS' CONTRIBUTION

P. O. Abba conceived, designed and executed the study; G. M. Gberikon and E. B. Agbo supervised data collection while E. U. Umeh analyzed the data and supervised manuscript writing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare none.

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