Original Article

Phenotypic Detection of Extended-Spectrum β -lactamase Produced by *Escherichia coli* Isolates from Health facilities in Otukpo, Benue State Nigeria

Utulo, PS¹, Umeh, EU,² Gberikon, GM,² and Abba, PO.^{3*}

¹Department of Microbiology, Federal College of Education, Kano, Nigeria

²Department of Microbiology, Federal University of Agriculture, Makurdi, Nigeria.

³Department of Medical Microbiology/Parasitology, Benue State University Teaching Hospital, Makurdi, Nigeria.

ABSTRACT

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*Correspondence:

Dr. Abba, PO Department of
Medical
Microbiology/Parasitology,
Benue State University Teaching
Hospital, P.M.B. 102131,
Makurdi;
Tel: +2348034056504.
Email:ogorpaul1969@gmail.co
m

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Beta-lactamase production by *Escherichia coli* and other bacteria is one of the most important mechanism of resistance to beta-lactam antibiotics such as Penicillins and Cephalosporins which are the most commonly used antibiotics in the treatment of bacterial infections in Hospitals. Resistance to antibiotics is emerging worldwide as a threat to human health resulting in limitations of therapeutic options. Susceptibility tests of microorganisms to antimicrobial agents do not reveal production of Extended-Spectrum β -lactamase, hence the need to detect their presence by phenotypic or molecular techniques. This study is aimed at determining the prevalence of ESBL-production by *Escherichia coli* isolates from clinical samples collected from some selected health facilities in Otukpo, using phenotypic detection. Four hundred (400) specimens were collected from four different health facilities in Otukpo. Specimens collected included urine, blood, stool and wound swabs from 222 females and 178 males. Standard microbiological methods were employed for isolation, identification and characterization of *Escherichia coli* isolates from these specimens. Antibiotic susceptibility of the *E. coli* isolates was determined using Kirby-Bauer disc diffusion method while double disc synergy test (DDST) was used for ESBL production. Sixty-eight, (17%) of the 400 specimens tested for ESBL production were positive. Isolates from stool 22(22.7%) had the highest prevalence of ESBL-producing *E. coli*, followed by isolates from blood 4(21.4%). Urine isolates had the least prevalence 31(13.8%). Isolates from female subjects, 38(17.1%) had higher positivity rate than their male counterpart 30(16.9%). Otukpo General Hospital had the highest prevalence 20(20.0%) of ESBL followed by Otia Hospital 18(18.0%). Comprehensive health center, Otukpo had the least prevalence 14(14.0%). There is a high prevalence of ESBL production by *Escherichia coli* isolated from health facilities in Otukpo. Routine drug resistance surveillance therefore has become necessary to guide the appropriate and judicious antibiotic use.

Keywords: Antimicrobial resistance, ESBL, Phenotypic detection, *Escherichia coli*, Otukpo.

INTRODUCTION

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escheria that is commonly found in the lower intestine of warm-blooded organisms¹. Extended spectrum beta-lactamases (ESBLs) are enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporins. ESBL was first reported in 1983 from Germany in isolates of Klebsiella pneumonia¹. The frequency of ESBL production is high among E. coli and Klebsiella species. This has created a worldwide problem resulting in treatment failure.

ESBLs significantly complicate therapeutic procedures when involved in infections and they lead to increased mortality. Any time ESBLs are 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.^{2,3} Many clinical laboratories are yet to be aware of the importance of ESBLs. Some that are aware lack the resources to detect them. 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 beta-lactamases⁴

Some conditions have been observed to be risk factors for contracting infections from ESBL-producing pathogens. These include old age (> 65 years), gender, previous use of beta-lactam antibiotics and fluoroquinolones⁵. It has also been observed that health care interactions such as transfers from one hospital to another are identified risk factor for acquiring infections due to ESBL producers⁶.

In Nigeria where antibiotic use is unregulated, and where extended spectrum cephalosporins and fluoroquinolones are widely prescribed as broad-spectrum antibiotics, ESBL-producing microorganisms may be prevalent.

Studies from various parts of Nigeria have reported varying prevalences: ⁷ reported a prevalence of 2.7%

from studies conducted on blood stream infections in Benin-city, Southern Nigeria. A prevalence of 44.3% was reported by another researcher from Benin-city⁸. ⁹reported 20.9% from South-West Nigeria. A very recent study by ¹⁰ reported a prevalence of 16.0% from studies conducted on isolates from various clinical specimens obtained from different health facilities in Makurdi, Benue State.

Interactions with laboratory personnel from across Benue State reveal that bacteriological investigations for clinical specimens stop at the routine disk-diffusion antimicrobial susceptibility tests. Inability of this technique to detect resistance to antimicrobials by ESBL-producers has since been established³. Phenotypic detection of ESBL production by double disk synergy test is a simple technique that does not require high tech equipment and advanced expertise. The method is cheap and can be employed to meet local demands of resource-poor health facilities such as Otukpo and other cities in Benue State. Outcomes obtained from here will be compared with what has been gotten from other places and may reveal the extent of spread of this public health threat in this locality.

MATERIALS AND METHODS

This study was carried in Otukpo local Government Area of Benue State, which is located in the middle belt region of Nigeria. It has a latitude of 6° 49' 0" N and longitude of 8° 40' 0" E with a population projection of 359,600 according to National Bureau of Statistics.

The study examined four hundred clinical specimens involving 224 urine, 97 stool, 65 wound swabs and 14 blood samples collected from 222 female and 178 male patients (1:1.2 female to male ratio) that attended selected health facilities in Otukpo metropolis. The demographics of these patients, including age, sex and the health facility attended were reviewed and ethical clearance was obtained from the ethics review

committee of Benue State Hospitals Management Board (HMB). These specimens were inoculated on to various culture media to isolate *Escherichia coli* bacteria which were presumptively identified by standard microbiological and biochemical methods.

Extended Spectrum β -lactamase (ESBL) production was tested for in *Escherichia coli* isolates by using combination disk method based on the inhibitory effect of clavulanic acid according to Clinical Laboratory Standards Institute¹¹ criteria. The clinical isolates were tested for their susceptibility to selected antimicrobials which included β -lactam antibiotics like penicillin, ceftazidim, cefotaxime, cefixime and imipenem and non- β -lactam antibiotics such as gentamycin and ciprofloxacin using Kirby Bauer disk diffusion method.

The inoculated plates for the Double Disk Synergy Test (DDST) for the detection of ESBL and antibiotic susceptibility test were incubated at 37°C for 24 hours. Results were interpreted according to⁴ guidelines, where interpretive criteria for ESBL activity was based on an increase of greater or equal to 5mm in diameter of the inhibition zone around disk containing clavulanic acid as compared to the diameters of the inhibition zone around disks free of clavulanic acid. Data obtained from the study was analyzed using Statistical Package for Social Sciences (SPSS) for Windows version 16.0 (Armon. K. NY: IBM Corp). Simple frequencies and cross tabulations were

applied, and association between ESBL producing organisms and patient demographics was determined using Chi square test at significant level of $p=0.05$.

RESULTS

The study revealed that sixty-eight (68) patients comprising 38 female (17.1%) and 30 male (16.9%) were infected with ESBL-producing *Escherichia coli*. This gave a prevalence of 17% for both sexes (table 1) Highest percentage of ESBLs were recovered from the isolates of stool specimens (22.7%), followed by isolates from blood specimens (21.4%). Isolates from urine specimens harboured the lowest percentage of ESBLs (13.8%), table 2.

Table 3 showed that penicillin, one of the oldest antibiotics discovered was the most resisted (93.8%; $n=375$). This was followed closely by gentamycin 359(89.8%), which is a non- β -lactam antibiotic. Table 3 further revealed that the ESBL-producing isolates of *Escherichia coli* were most susceptible to imipenem 321(80.1%), a carbapenem. Cefixime, a β -lactam antibiotic came next in the susceptibility profile as the most potent against ESBL-producing *Escherichia coli* bacteria.

Table 4 revealed that Otukpo General Hospital, 20(20.0%) had the highest prevalence of ESBL-producing *E. coli*, followed by Otia Hospital 18(18.0%). The least prevalence was found in Otukpo Comprehensive Health Centre 14(14.0%).

Table 1: Distribution of ESBL producing *E.coli* According to Sex

	ESBL producing <i>E.coli</i>		Total (%)
	Negative (%)	Positive (%)	
Sex			
Female	184 (82.9)	38(17.1)	222(100.0)
Male	148 (83.1)	30 (16.9)	178 (100.0)
Total	332(83.0)	68 (17.0)	400 (100.0)

Chi square = 0.005; *df* = 1; *p* = 0.944

Table 2: Distribution of ESBL producing *E. coli* According to types of specimens

Specimens	ESBL producing <i>E. coli</i>		Total (%)
	Absent (%)	Present (%)	
Blood	11 (78.6)	3 (21.4)	14 (100.0)
Stool	75 (77.3)	22 (22.7)	97 (100.0)
Urine	193 (86.2)	31 (13.8)	224 (100.0)
Wound swab	53 (81.5)	12 (18.5)	65 (100.0)
Total	332 (83.0)	67 (17.0)	400 (100.0)

Chi square = 4.097; df = 3; p = 0.25

Table 3: Antimicrobial susceptibility pattern of ESBL-producing *E. coli*

Antibiotics	ESBL-producing <i>E. coli</i>		Total (%)
	Susceptible (%)	Resistant (%)	
Penicillin	25 (6.2)	375 (93.8)	400 (100.0)
Ceftazidime	82 (20.5)	318 (79.5)	400 (100.0)
Cefotaxime	87 (21.8)	313 (78.3)	400 (100.0)
Gentamycin	41 (10.25)	359 (89.8)	400 (100.0)
Cefixime	199 (49.8)	201 (50.1)	400 (100.0)
Ciprofloxacin	105 (26.3)	296 (73.8)	400 (100.0)
Imipenem	321 (80.1)	79 (19.8)	400 (100.0)

Table 4: Distribution of ESBL producing *E. coli* according to Health Facilities

Health facility	ESBL producing <i>E. coli</i>		Total (%)
	Negative (%)	Positive (%)	
General hospital	80 (80.0)	20 (20.0)	100 (100.0)
Otukpo Comm. Health Centre	86 (86.0)	14 (14.0)	100 (100.0)
Otia hospital	82 (82.0)	18 (18.0)	100 (100.0)
St. Daniel hospital	84 (84.0)	16 (16.0)	100 (100.0)
Total	332 (83.0)	68 (17.0)	400 (100.0)

Chi square = 1.417; df = 3; p = 0.70

DISCUSSION

The spread of ESBL-producing organisms has defied geographical locations. The prevalence of 17.0 obtained in this study location which is a semi-urban setting is even slightly higher than 16.0% reported by ¹⁰ from studies conducted in Makurdi city, an urban setting. The finding is also at variance with other previous works such as ¹² who reported 18.6% from Jos plateau; 15.8% by ⁷ from Benin-city and 36.8%, by ¹³ from Kano, north-West Nigeria. This has demonstrated that continuous monitoring of antibiotics prescribed by health professionals and the scaling up of infection control measures are absolutely required throughout all levels of healthcare. Epidemiologic data on ESBL-producing organisms in Benue State are still scanty. There is therefore need for sustained health education programme to the populace on this public health threat. Our results showed

that isolates from stool specimens harboured the highest percentage of ESBLs. This is not quite unexpected for semi-urban setting like Otukpo, where sanitary habits may be far from excellent. Open defecation may still be practiced as some households may be still be lacking proper toilet systems. Sources of water which may just be open or covered wells and streams which easily get contaminated by fecal matter. The results of this study disagree with the report of ¹¹ who recovered most ESBLs from isolates of blood specimens in Makurdi metropolis, Benue State. This result also disagreed with the findings of ¹⁴ who reported highest percentage of ESBLs in isolates of urine specimens in a study he conducted in Enugu, South-East of Nigeria. The result also is in contrast with ¹⁵ who reported a prevalence of 18.5% from urine specimens.

Antimicrobial susceptibility test results showed highest resistance to penicillin (93.8%; n = 375) followed by gentamycin (89.8%; n = 359). Penicillin, being one of the earliest antibiotics discovered some centuries ago has actually been overused and abused over time. Penicillin powder has been used on open wounds by rural folks and health professionals have used the

injection to treat all manner of infections ranging from sexually transmitted infections to systemic infections. Its easy accessibility and sale over the counters in patent medicine stores and pharmacy shops might have contributed to the high resistance. Non-human use of antibiotics, especially in poultry and other livestock production which eventually get consumed by man are also contributory factors to multidrug resistance¹⁶.

Imipenem, (80.1%; n = 321) a carbapenem is the most active antibiotic against ESBL-producing *Escherichia coli*, followed by cefixime (49.8%; n = 199). Since only 19.8% of all isolates of ESBL-producing *Escherichia coli* are resistant to imipenem, this agent becomes the best treatment for serious infections by ESBL-producing enterobacteriaceae. This finding agrees with¹⁷ who reported 100% susceptibility to imipenem by ESBL-producing *Escherichia coli*. Further research into the molecular determination of resistance genes by ESBL-producing isolates from these specimens will give a clearer picture of resistance patterns by the organism.

CONCLUSION

There is decreased susceptibility of pathogenic microorganisms to readily available drugs. With the slow pace in the development of new antimicrobials, there is need for proper utilization of available antibiotics and accurate detection and reporting of antibiotic resistance in order to keep multidrug resistant bacteria (MDR) under control. The use of carbapenems such as imipenem should be done with utmost caution as the study has shown that it remains the most potent antibiotic for treatment of infections caused by ESBL-producing organisms.

Recommendation

Findings from this study have revealed that antimicrobial surveillance has become imperative in all health facilities. Health professionals who prescribe antibiotics need to be properly trained on the art of

prescription. Use of beta-lactam antibiotics as broad spectrum drugs needs to be checked.

Limitations

Data for this study was obtained only from consenting patients that attended the selected health facilities during the period of the study. If data was gotten from the community and from more respondents, the prevalence may be higher. Also isolates were only subjected to phenotypic screening only. Molecular determination may yield higher prevalence.

Conflict of interests

The authors declare no conflict of interest.

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