



Extended Spectrum Beta-Lactamases Producers among Gram-negative Bacteria from Clinical and Environmental Sources in Two Tertiary Hospitals in Makurdi, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FBO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OEF supervised the work. Both authors read and approved the final manuscript.

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ABSTRACT

Extended Spectrum Beta-Lactamases (ESBLs) enzymes are produced by many Gram-negative bacteria to nearly all antibiotics. Clinical bacteria isolates were obtained from various clinical samples from the two healthcare facilities. Sewer wastewater and sediments were also collected from both hospitals using standard sampling techniques and bacteria isolated using pour plate technique. Multiple drug resistant patterns were determined using disc diffusion technique. Antibiotics sensitivity of the isolates was verified using disc diffusion method. Detection of ESBL producing bacteria was done using double disc synergy test. Data obtained were analysed using descriptive statistics. Clinical bacteria (403) were obtained, out of which 299 were confirmed Gram-negative, 218 from Federal Medical Centre (FMC) and 81 from Benue State Teaching Hospital (BSUTH). Thirty-nine Gram-negative bacteria were also isolated from the environmental samples. The ESBL producers in clinical isolates were 69 (FMC) and 42 (BSUTH) while in environmental isolates they were eight and four respectively. Out of a total of 338 environmental and clinical

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isolates from the two hospitals, 216 shown resistance/intermediate resistance to Ceftazidime. Of these 216, 123(36.39%) were positive for ESBLs production. From FMC, ESBLs producing bacteria are highly sensitive to imipenem with sensitivity frequency of 62 while they were highly resistant to ceftazidime with a frequency of 52. Age groups 0-5 had the highest percentage distribution of 21.43% and 10.14% from BSUTH and FMC respectively. ESBLs producing bacteria showed multidrug resistance.

Keywords: Gram-negative bacteria; antibiotic resistance; extended spectrum β -lactamases.

1. INTRODUCTION

Sir Alexander Fleming as far back as 1945 raised alarm that overuse of drug due to public demand could lead to abuse [1,2]. Despite warnings regarding overuse, antibiotics are overprescribed worldwide and vary from country to country as well as within the same country [3]. In Nigeria, antibiotics use and distribution are available over the counter without a prescription (unregulated). This lack of regulation results in easy accessibility and cheaply, promoting overuse. The ability to purchase such products online has also made them accessible in countries where antibiotics are regulated. Consequently, antimicrobial-resistant bacteria pose severe public health threat that may lead to reduction in effectiveness of antibiotic treatment [4]. Consumers' resident microflora may become resistant when antibiotics accumulate in animal tissues when ingested [5,6]. Treatment failure in humans is linked to infections caused by resistant microbes, which usually lead to higher cost, prolonged treatment period and at the end, lead to the death of the patients [7]. These resistant bacteria not only colonize patients, also they can be transmitted to staff and family members.

Extended-spectrum beta-lactamases (ESBLs) produced by Gram-negative bacteria evolved as a result of substitutions of amino acids leading to third generation cephalosporins [4]. They cause high number of deaths per year as a result of resistance to variety of pathogens [8,9]. Some ESBL-producing Enterobacteriaceae are resistant to nearly all antibiotics in the penicillin and cephalosporin classes which lead to the use of carbapenems as drug of last resort. Extended spectrum beta-lactamases (ESBLs) are enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin. They are therefore effective against beta-lactam antibiotics like ceftazidime, ceftriaxone, cefotaxime and oxyimino monobactams [10]. Carbapenems and cephamycine are effective against ESBL producer strains. Generally,

ESBLs are inhibited by clavulanic acid and tazobactam. ESBLs are found in Gram-negative bacteria, especially in enterobacteriaceae and *Pseudomonas aeruginosa* [11,10]. ESBLs were first described in the 1980s in *Klebsiella* species and later detected in *E. coli* and other genera in the Enterobacteriaceae family, soon after the introduction of third-generation cephalosporins in clinical practice [12].

Extended spectrum beta-lactamases are undergoing continuous mutation, causing the development of new enzymes showing expanded substrate profiles. At present, there are more than 300 different ESBL variants. Temoniera (TEM) and sulphhydryl variable (SHV) were the major types. Though, CTX-M type (predominantly hydrolyzed cefotaxime) is increasingly becoming important [13]. The variants derived from TEM and SHV enzymes and CTX-M-ESBLs (derived from other sources) are defined as "classical ESBLs". Various β -lactamases conferring wider resistance than their parent types, for example OXA-types (OXA-ESBLs) and mutant AmpC-types (plasmid-borne AmpC-like enzymes, such as DHA), with increased activity against oxyimino-cephalosporins and with resistance to oxacillin and ceftoxitin, respectively, were "ESBLs of growing importance" [14].

Extended spectrum beta-lactamases are an important cause of transferable multidrug resistance in Gram-negative bacteria throughout the world. These bacteria have spread rapidly and have become a serious threat to human health worldwide. Determination of ESBL genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology and risk factors associated with these infections [15,16]. Conventional methods for resistance detection rely on phenotypic identification based on bacterial growth inhibition in disk diffusion or dilution tests, which are cost-effective but which usually, require two days to complete [17]. A faster more reliable detection

method is the determination of antibiotic susceptibility for positive antimicrobial therapy outcomes which would significantly influence subsequent procedures and actions [18]. This study was designed to determine ESBLs producing Gram-negative bacteria in clinical and environmental isolates of two tertiary health care facilities in Makurdi, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Site/Collection

Approval was obtained from the two hospitals before the commencement of the study. Ethical approval was obtained from the government of Benue State of Nigeria. Ministry of Health and Human Services with reference number MOH/STA/204/VOL.1/31. Clinical isolates (Stock culture) were obtained from the laboratory benches of the Medical Microbiology Department by 10th March to 3rd June, 2016. And samples of wastewater and wastewater sediments were collected in the month of September, 2016 from sewers (gutters) from the two tertiary hospitals. Sites selected for the study were drains from various wards which includes; The theatre, Female surgical ward, Pediatric ward, Male and female medical ward, Amenity ward (ward block), Resident doctors hostel and cafeteria, Laboratory (Chemical pathology, Microbiology, Hematology and Histopathology), Administrative block, and Accident and Emergency ward (A and E) BSUTH, FMC samples site include Laboratory, A ward (Male ward 18yrs and above), Gynecology ward, Theatre and Female ward. Samples were subculture routinely onto slants prepared from nutrient agar.

2.2 Waste Water and Sediment Samples

Wastewater and sediment samples were collected in the month of September, 2016. The water samples were collected into sterile bottles from the various units aseptically by using disposable micro pipette at each collection unit. Samples were safely transported by road to the laboratory, and immediately analysed.

Sediments were collected by wearing gloves and using clean hand trowel from different wastewater sampling sites to scoop sediments from the bottom of the sewers and introduced into sterile Bijou bottles. The trowel was properly cleaned using alcohol (ethanol) before using in another site to avoid contamination.

2.3 Clinical Bacterial Isolates

Clinical bacterial (Gram-negative multidrug resistant stock culture) isolates were collected from stocks from the laboratory benches of the Department of Medical Microbiology of the hospitals listed above. Collection of clinical isolates was done between 10 March and 3 June, 2016. Samples identities were confirmed using different laboratory synthetic media and biochemical tests was done using API 20E. The clinical samples were from samples of body fluid (urine and blood samples), stool and swab (high vaginal swab, endocervical, wound, ear and sputum samples), swab (high vaginal, endocervical, wound, ear and sputum samples) and stool samples.

2.4 Isolation of β -lactam Resistant Gram-negative Bacteria from Environmental Sources

Beta lactam resistant Gram-negative bacteria were isolated from wastewater and wastewater sediments. This was done by supplementing Peptone water with Ampicillin antimicrobial susceptibility test disc 10 $\mu\text{g/ml}$ (Oxoid) as described by Tan et al. [19]. Stock solution of peptone water was prepared according to manufacturer instructions. 5 ml each was dispensed into an incubating bottle and sterilized at 121°C for 15 minutes and allowed to cool. The sterile ampicillin discs 10 $\mu\text{g/ml}$ were aseptically introduced into the sterile peptone water at 50°C to a final concentration of 60 $\mu\text{g/ml}$.

Water: For the wastewater samples, 1 ml was introduced into the sterile incubating bottles containing the sterile peptone water supplemented with ampicillin discs 60 $\mu\text{g/ml}$ and incubated for 18-24h at 37°C.

Sediments: Serial dilutions were carried out with the sediment samples and 1 ml of 10⁻¹ diluent was introduced into the sterile peptone water supplemented with ampicillin discs 60 $\mu\text{g/ml}$ and also incubated for 18-24 hours at 37°C.

The 18-24 h incubated water and sediments samples above were subsequently streaked on MacConkey agar with the aid of sterile wire loop and incubated at 37°C for 18-24 h. This was done for all the wastewater and sediments samples.

2.5 Selection for Ceftazidime Resistant Bacteria Isolates Using Disc Diffusion Method

Antibiotic testing was carried out on both β -lactam resistant organisms selected on MaConkey agar and all the Gram-negative confirmed clinical bacteria isolates collected from the two hospitals, Benue State University Teaching Hospital and Federal Medical Center Makurdi using the Kirby-Bauer method [20].

The 18-24 hours old culture of each isolate was introduced into a sterile test tube containing normal saline (5 ml) and its turbidity adjusted to match 0.5 MacFarland standards. Sterile cotton swab stick was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a Mueller-Hinton agar plates (HIMEDIA, INDIA). After the agar surface has dried for about 5 minutes, Ceftazidime 30 μ g, antibiotic discs (Oxoid) were placed on the inoculated plate using sterile forceps. The plates were allowed to remain on the bench for 1 hour for a period of pre-incubation diffusion and incubated at 35°C. After 16-18 h of incubation, the diameters of the zones of inhibition were measured with meter rule and recorded in millimeter (mm). This was done for all the isolates selected.

The CLSI [21] standard was used for the interpretation of the zone of inhibition of the selected antibiotic discs used.

2.6 Detection and Selection of Extended-spectrum β -lactamase (ESBL) Producers Using Double Disc Synergy Test (DDST)

Mueller-Hinton agar (HIMEDIA, INDIA) was prepared according to the manufacturer's instruction, sterilized and poured into petri dishes. Antibiotic testing were carried out on the Ceftazidime resistant isolates from the previous sensitivity test.

An 18-24 hours old culture of each Ceftazidime resistant isolate was introduced into a sterile test tube containing normal saline (5 ml) and its turbidity adjusted to match 0.5 MacFarland standard. A sterile cotton swab stick was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of

a Mueller-Hinton agar plate (HIMEDIA, INDIA). After the agar surface has dried for about 5 minutes, Amoxicillin/Clavulanate antibiotic disc is placed at the Centre of the plate while Ceftazidime, Cefepime, and Aztreonam antibiotic discs were placed at a distance of 20 mm each away from the Amoxicillin/Clavulanate antibiotic discs on the inoculated plate using sterile forceps. The plate was immediately incubated at 37°C. After 16-18 hours of incubation, plate was observed for a corkscrew or key hole shapes. This was done for all the isolates resistant to ceftazidime.

3. RESULTS

Out of a total of 338 environmental and clinical isolates from the two hospitals, 216(55.67%) shown resistance/intermediate resistance to Ceftazidime. Of these 123(56.94%) were positive for ESBLs production (Table 1).

From FMC, ESBLs producing bacteria are highly sensitive to imipenem with sensitivity frequency of 62 while they are highly resistant to ceftazidime with a frequency of 52. The least sensitive antibiotic is ceftazidime with a distribution of 3, and the least resistant is imipenem with a distribution of 11. In BSUTH, the highest susceptibility is imipenem with a distribution of 36 while the least is ceftazidime with a distribution of 1. The highest resistant is ceftriaxone with a distribution of 28 and the least resistant is also imipenem with a distribution of 2. In both hospitals, imipenem has the highest susceptibility with a sensitivity of 98 while the least is ceftazidime with a sensitivity of 4. Ceftazidime has the highest resistant of 83 while the least is imipenem with a resistant of 13. Antibiotics are statistically significant (Table 2). The level of resistance was higher than sensitivity. Bacteria were not statistically significant (Table 4).

In Table 6, for frequency distribution from BSUTH, a total of 6 bacteria were sensitive while 14 were resistant while in FMC a total of 9 bacteria were sensitive and 28 were resistant. The rate of resistance is higher than sensitivity with frequencies of 42 and 15 respectively. Antibiotics were statistically significant in both hospitals. The most susceptible bacteria is *Proteus vulgaris* with a frequency rate of 5 while the highest resistance is *Citrobacter koseri* and *Proteus vulgaris* with a frequency of 9 each. Bacteria are not statistically significant (Table 7).

Age groups 0-5 (From FMC) had the highest percentage distribution of 10.14% while age groups 51-55, 61-65 and 71-75 had the lowest percentage distribution of 1.45% each (Table 8). From BSUTH, age group 0-5 also recorded highest percentage distribution of 21.43% while age groups 31-35, 51-55, 76-80 and 91-95 had the lowest percentage distribution of 2.38% each (Table 9). There was also problem of improper documentation from the two hospitals, problem of no documentation (No data) and that of

unclassified age group (AD), The problem of no data was higher in FMC with 43.48% compare to that of BSUTH with 2.38% while that of unclassified age group (AD) was higher in BSUTH with 14.29% compared to that of FMC of 5.80% (Tables 8 and 9).

From Table 10 ESBLs producing bacteria show multidrug resistant with almost all bacteria showing multiple resistant to two or more antibiotics.

Table 1. Number of ESBLs-producing gram-negative bacteria from environmental and clinical samples

No. of Isolates from environmental and clinical samples	Antibiotic tested	No. resistant	ESBLs Producers No. (%)
338	Ceftazidime	216	123(36.39)

Key: ESBLs – Extended Spectrum Beta-lactamases

Table 2. Antibiotic susceptibility profile of extended-spectrum beta-lactamases positive bacteria from the two hospitals

Hospitals	Antibiotic	Susceptibility profile			Total No.
		S No.	I No.	R No.	
BSUTH	Ceftazidime	1	10	31	42
	Ceftriaxone	10	4	28	42
	Ciprofloxacin	16	1	25	42
	Ertapenem	23	4	15	42
	Imipenem	36	4	2	42
Total		86	23	101	210
FMC	Ceftazidime	3	14	52	69
	Ceftriaxone	14	8	47	69
	Ciprofloxacin	22	3	36	61
	Ertapenem	39	14	16	69
	Imipenem	62	4	11	77
Total		140	43	162	345
Total	Ceftazidime	4	24	83	111
	Ceftriaxone	24	12	75	111
	Ciprofloxacin	38	4	61	103
	Ertapenem	62	18	31	111
	Imipenem	98	8	13	119
Total		226	66	263	555

BSUTH: $\chi^2 = 123.221, df = 8, p = 0.00$

FMC: $\chi^2 = 77.915, df = 8, p = 0.00$

Total: $\chi^2 = 196.095, df = 8, p = 0.00$

Key: S – Sensitive, I – Intermediate, R – Resistant

Table 3. Frequency of Extended-spectrum Beta-lactamases Positive Bacteria Compared to Different Antibiotics from BSUTH and FMC

Hospitals	ESBL positive bacteria	Antibiotics					Total
		Ceftazidime	Ceftriazone	Ciprofloxacin	Ertapenem	Imipenem	
BSUTH	<i>E. coli</i>	33.00	33.00	33.00	33.00	33.00	165.00
	<i>Klebsiellapneumoniae</i>	5.00	5.00	5.00	5.00	5.00	25.00
	<i>Pseudomonas sp.</i>	4.00	4.00	4.00	4.00	4.00	20.00
	Total	42.00	42.00	42.00	42.00	42.00	210.00
FMC	<i>E. coli</i>	29.00	29.00	26.00	29.00	32.00	145.00
	<i>Klebsiellapneumoniae</i>	8.00	8.00	8.00	8.00	8.00	40.00
	<i>Proteus sp.</i>	2.00	2.00	2.00	2.00	2.00	10.00
	<i>Pseudomonas sp.</i>	30.00	30.00	25.00	30.00	35.00	150.00
	Total	69.00	69.00	61.00	69.00	77.00	345.00
Total	<i>E. coli</i>	62.00	62.00	59.00	62.00	65.00	310.00
	<i>Klebsiellapneumoniae</i>	13.00	13.00	13.00	13.00	13.00	65.00
	<i>Proteus sp.</i>	2.00	2.00	2.00	2.00	2.00	10.00
	<i>Pseudomonas sp.</i>	34.00	34.00	29.00	34.00	39.00	170.00
	Total	111.00	111.00	103.00	111.00	119.00	555.00

BSUTH: $\chi^2 = 0.00$, $df = 8$, $p = 1.00$

FMC: $\chi^2 = 0.438$, $df = 12$, $p = 1.00$

Total: $\chi^2 = 0.611$, $df = 12$, $p = 1.00$

Table 4. Antibiotic susceptibility profile of extended-spectrum beta-lactamases positive bacteria from clinical samples in BSUTH and FMC

ESBL Positive Gram-negative Bacteria	Susceptibility profile			Total
	S	I	R	
<i>E. coli</i>	131	40	139	310
<i>Klebsiellapneumoniae</i>	24	11	30	65
<i>Proteus sp.</i>	6	0	4	10
<i>Pseudomonas sp.</i>	65	15	90	170
Total	226	66	263	555

$X^2 = 7.434, df = 6, p = 0.28$

Key: S – Sensitive, I – Intermediate, R – Resistant

Table 5. Antibiotic susceptibility profile of extended-spectrum beta-lactamases positive bacteria from BSUTH and FMC

Hospitals	Antibiotics	Susceptibility profile			Total
		S	I	R	
BSUTH	Ceftazidime	0	-	4	4
	Ceftriaxone	0	-	4	4
	Ciprofloxacin	3	-	1	4
	Ertapenem	0	-	4	4
	Imipenem	3	-	1	4
	Total	6	-	14	20
FMC	Ceftazidime	1	0	7	8
	Ceftriaxone	1	1	6	8
	Ciprofloxacin	2	0	6	8
	Ertapenem	0	0	8	8
	Imipenem	5	2	1	8
	Total	9	3	28	40
Total	Ceftazidime	1	0	11	12
	Ceftriaxone	1	1	10	12
	Ciprofloxacin	5	0	7	12
	Ertapenem	0	0	12	12
	Imipenem	8	2	2	12
	Total	15	3	42	60

BSUTH: $X^2 = 18.770, df = 8, p = 0.02$

Total: $X^2 = 28.429, df = 8, p = 0.00$

Key: BSUTH – Benue State University teaching Hospital, FMC – Federal Medical Centre, S – Sensitive, I – Intermediate, R – Resistant

4. DISCUSSION

ESBLs prevalence in this study was recorded as 36.39% (Table 1) which is lower to study conducted globally by Ali et al. [22], Jabeen et al. [23] and Ullah et al. [24] from Pakistan, 40%, 43%, and 58.7% ESBLs producers respectively, Ahmed et al. [25] in Sudan, Rao et al. [26] in India with prevalence of 59.6% and 57.5% ESBLs producers respectively. Similar prevalence rates were recorded by Anjum and Mir [27] in Pakistan which observed 33%. In Nigeria, this is comparable to Iroha et al. [28] and Akanbi et al. [29] with prevalence of 39.8%, and 33.6% respectively. Higher prevalence were recorded by Okesola and Adeniji [30], Iroha et al.

[31]; Azehueme et al. [32] with 76.9%, 47.1%, and 58.6%, respectively. The variation in ESBLs prevalence rates reported between geographical areas, institutions and countries may be attributed to the complex epidemiology of ESBLs, specific type of bacteria involved and methods used for ESBL detection among other factors [33,34,35]. In this study, ESBL- producing *E. coli* are found to be (55.86%) which may place Nigeria on a high scale next to India according to the report by Hsueh et al. [36].

The antibiogram pattern (Table 2) showed a higher degree of resistance in ESBL producers. It also revealed that 61 ESBL producers from the two hospitals were resistant to Ciprofloxacin, one

of the most commonly used fluoroquinolone frequency in Makurdi. Contrary to norm that drugs which might be indicative of fluoroquinolones should not be prescribed fluoroquinolones being prescribed at a high routinely.

Table 6. Frequency and susceptibility profile of extended-spectrum beta-lactamases positive bacteria compared to different antibiotics from environmental samples in BSUTH and FMC

Hospitals	ESBL positive Environmental Gram-negative Bacteria	Antibiotics					Total	
		Ceftazidime	Ceftriazone	Ciprofloxacin	Ertapenem	Imipenem		
BSUTH	<i>Citrobacter koseri</i>	1	1	1	1	1	5	
	<i>Shigella sonnei</i>	1	1	1	1	1	5	
	<i>Proteus vulgaris</i>	2	2	2	2	2	10	
	Total	4	4	4	4	4	20	
FMC	<i>Citrobacter diversus</i>	1	1	1	1	1	5	
	<i>Citrobacter koseri</i>	1	1	1	1	1	5	
	<i>E. coli</i>	1	1	1	1	1	5	
	<i>Klebsiella pneumoniae</i>	1	1	1	1	1	5	
	<i>Shigella sonnei</i>	1	1	1	1	1	5	
	<i>Proteus mirabilis</i>	2	2	2	2	2	10	
	<i>Proteus vulgaris</i>	1	1	1	1	1	5	
	Total	8	8	8	8	8	40	
	Total	<i>Citrobacter diversus</i>	1	1	1	1	1	5
		<i>Citrobacter koseri</i>	2	2	2	2	2	10
<i>E. coli</i>		1	1	1	1	1	5	
<i>Klebsiella pneumoniae</i>		1	1	1	1	1	5	
<i>Shigellasonnei</i>		2	2	2	2	2	10	
<i>Proteus mirabilis</i>		2	2	2	2	2	10	
<i>Proteus vulgaris</i>		3	3	3	3	3	15	
Total		12	12	12	12	12	60	

BSUTH: $X^2 = 0.000$, $df = 8$, $p = 1.00$

FMC: $X^2 = 0.000$, $df = 8$, $p = 1.00$

Total: $X^2 = 0.000$, $df = 8$, $p = 1.00$

Table 7. Frequency and antibiotic susceptibility profile of extended-spectrum beta-lactamases positive bacteria in environmental samples from BSUTH and FMC

ESBL positive Environmental Gram-negative Bacteria	Susceptibility profile			Total
	S	I	R	
<i>Citrobacter diversus</i>	0	1	4	5
<i>Citrobacter koseri</i>	1	0	9	10
<i>E. coli</i>	1	0	4	5
<i>Klebsiella pneumoniae</i>	1	0	4	5
<i>Shigellasonnei</i>	3	0	7	10
<i>Proteus mirabilis</i>	4	1	5	10
<i>Proteus vulgaris</i>	5	1	9	15
Total	15	3	42	60

$X^2 = 9.571$, $df = 12$, $p = 0.65$

KEY: S – Sensitive, I – Intermediate, R – Resistant

Table 8. Percentage and age distribution of ESBLs positive bacteria isolates in clinical samples collected from FMC

S/N	Age group (Year)	No of samples collected	No of ESBLs positive cases	Percentage of ESBLs positive cases (%)
1	0-5	21	7	10.14
2	6-10	7	2	2.90
3	11-15	4	2	2.90
4	16-20	7	2	2.90
5	21-25	17	4	5.80
6	26-30	26	4	5.80
7	31-35	15	2	2.90
8	36-40	15	5	7.25
9	41-45	14	4	5.80
10	46-50	3	0	0
11	51-55	7	1	1.45
12	56-60	4	0	0
13	61-65	1	1	1.45
14	66-70	2	0	0
15	71-75	3	1	1.45
16	76-80	0	0	0
17	81-85	1	0	0
18	AD	22	4	5.80
19	No Data	104	30	43.48
Total		273	69	98.57

Table 9. Percentage and age distribution of ESBLs positive bacteria isolates in clinical samples collected from BSUTH

S/N	Age group (Year)	No of samples collected	No of ESBLs positive cases	Percentage of ESBLs positive cases (%)
1	0-5	29	9	21.43
2	6-10	7	5	11.90
3	11-15	1	0	0
4	16-20	7	2	4.76
5	21-25	8	2	4.76
6	26-30	11	3	7.14
7	31-35	6	1	2.38
8	36-40	3	0	0
9	41-45	5	3	7.14
10	46-50	3	2	4.76
11	51-55	6	1	2.38
12	56-60	5	2	4.76
13	61-65	3	0	0
14	66-70	6	3	7.14
15	71-75	2	0	0
16	76-80	5	1	2.38
17	81-85	1	0	0
18	86-90	0	0	0
19	91-95	1	1	2.38
20	AD	19	6	14.29
21	No Data	4	1	2.38
Total		132	42	99.98

The Percentage and age distribution of ESBLs positive bacteria isolates among clinical samples from FMC and BSUTH (Tables 8 and 9) nearly shows that age has an appreciable influence on the incidence of ESBLs cases among clinical samples collected since the incidence of ESBLs

Table 10. Multiple antibiotic resistant pattern of ESBLs positive bacteria

Number of antibiotic classes	MAR Pattern	No. (%)
2	CAZ, CRO	6(8.70)
	CAZ, CIP	2(2.90)
	CAZ, ETP	1(1.45)
3	CAZ, CRO, ETP	9(13.09)
	CAZ, CIP, CRO	21(30.43)
	CAZ, CIP, IMP	2(2.90)
	CAZ, ETP, IMP	1(1.45)
4	CAZ, CIP, CRO, ETP	13(8.84)
	CAZ, CIP, ETP, IMP	1(1.45)
	CAZ, CRO, ETP, IMP	1(1.45)
	CIP, CRO, ETP, IMP	1(1.45)
5	CAZ, CIP, CRO, ETP, IMP	4(5.80)

Key: CAZ (Ceftazidime), CRO (Ceftriaxone), CIP (Ciprofloxacin), ETP (Ertapenem), IMP (Imipenem), MAR (Multiple Antibiotic Resistance)

positive cases was found to be more within the age group 0-5 in the two hospitals. Although there was also problem of improper documentation from the two hospitals, problem of no documentation (No data) and that of unclassified age group (AD), the problem of no data was higher in FMC 43.48% when compared to that of BSUTH with 2.38% while that of unclassified age group (AD) is higher in BSUTH with 14.29% compare to that of FMC of 5.80.

The age group distribution revealed the maximum number 10.14% of ESBL producers in the age group 0-5 from FMC and 21.43% from BSUTH. This is at variance to the study of Segar et al. [35] who reported that the age group most commonly affected was within 21-30 years.

5. CONCLUSION

In conclusion, in this study majority of the ESBL enzyme producers were *E. coli*. Most of the organisms were multidrug resistant while the ESBL producers further compounded the problem by exhibiting co-resistance to other classes of antibiotics including fluoroquinolones. However, the carbapenems remain the most effective therapeutic option for ESBL producing bacteria as the isolates were highly sensitive to imipenem. The problems related to antibiotic resistances and the lack of antibiotics can only be solved or, at least, alleviated if scientists and society as a whole work together nationally and internationally pursuing diverse, coordinated approaches to menace of antibiotic resistance.

6. RECOMMENDATIONS

The wide spread of ESBLs is extremely worrisome and suggests a need for more prudent

antibiotic prescription, (for example, limitation of 3rd generation cephalosporins) and stricter infection control. Controlled use of antibiotics is also advised and physicians should try and relate with the laboratory before any of the cephalosporins are prescribed.

ETHICAL APPROVAL

Ethical approval was obtained from the government of Benue State of Nigeria. Ministry of Health and Human Services with reference number MOH/STA/204/VOL.1/31.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. *Clinical Infectious Diseases*. 2013;56(10): 1445–1450.
- Spellberg B, Gilbert DN. The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases*. 2014;59(2): 71–75.
- Centers for Disease Control and Prevention. Office of Infectious Disease Antibiotic Resistance Threats in the United States; 2013. Available: <http://www.cdc.gov/drugresistance/threat-report-2013> (Accessed January 28, 2015)
- Ittoo Danishta, Mamodelsmet, Deepa Sonatun, Yasmina Jaufferally-Fakim.

- Antibiotic resistance of *Escherichia coli* isolates from environmental and waste water samples in Mauritius. *Advance Environmental Biology*. 2010;4(1):1-9.
5. Witte W. Medical consequences of antibiotic use in agriculture. *Science*. 1998;279:996-997.
 6. Alhaj N, Mariana NS, Raha AR, Ishak Z. Prevalence of antibiotic resistance among *Escherichia coli* from different sources in Malaysia. *International Journal of Poultry Science*. 2007;6(4):293-297.
 7. Zilberberg MD, Shorr AF. Understanding cost-effectiveness. *Clinical Microbiology and Infection*. 2010;16:1707–1712.
 8. Gross M. Antibiotics in crisis. *Current Biology*. 2013;23(24):1063–1065.
 9. Lee C, Ventola MS. The antibiotic resistance crisis. *PT*. 2015;40(4):277–283.
 10. Bradford PA. Extended spectrum beta-lactamases in the 21st Century: Characterization, epidemiology and the detection of this important resistance threat. *Clinical Microbiology Reviews*. 2001;14:933-951.
 11. Nordmann P, Guibert M. Extended spectrum beta-lactamases in *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy*. 1998;42:128-131.
 12. Bali EB, Acik L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum β -lactamase produced by *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella* isolates in a Turkish hospital. *African Journal of Microbiology Research*. 2010;4(8):650–654.
 13. Lal K, Kapil A, Das BK, Sood S. Occurrence of TEM & SHV gene in extended spectrum β -lactamases (ESBLs) producing *Klebsiella* sp. isolated from tertiary care hospital. *Indian Journal of Medical Research*. 2007;125:173–178.
 14. Lee JH, Bae IK, Hee Lee S. New definitions of extended-spectrum betalactamase conferring worldwide emerging antibiotic resistance. *Medicinal Research Reviews*; 2010. (Epub Ahead of Print)
 15. Al-Agamy MHM, Shibl AM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Annual Saudi Medicine*. 2009;29(4):253–257.
 16. Oliveira CF, Salla A, Lara VM, Rieger A, Horta JA, Alves SH. Prevalence of extended-spectrum beta-lactamases-producing microorganisms in nosocomial patients and molecular characterization of the SHV type isolates. *Brasilian Journal of Microbiology*. 2010;41:278–282.
 17. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum beta-lactamases in members of the family enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *Journal of Antimicrobial Chemotherapy*. 2000;45:881-885.
 18. Grimm V, Ezaki S, Susa M, Knabbe C, Schmid RD, Bachmann TT. Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance. *Journal of Clinical Microbiology*. 2004;42: 3766-3774.
 19. Tan CW, Malcolm TT, Kuan CH, Thung TY, Chang WS, Loo YY, Rukayadi Y. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from short mackerels (*Rastrelliger brachysoma*) in Malaysia. *Frontiers in Microbiology*. 2017;8:1087.
 20. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. 1966;36:493-496.
 21. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Test 24th informational supplement. CLSI document M100-S24, Wayne Pa., CLSI; 2014.
 22. Ali AM, Rafi S, Qureshi AH. Frequency of extended beta lactamase producing gram-negative Bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi. *Journal of Ayub Medical College, Abbottabad*. 2004;16(1): 35-37.
 23. Jabeen K, Zafar A, Hassan R. Frequency and sensitivity pattern of extended spectrum beta lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. *Journal of the Pakistan Medical Association*. 2005; 55(10):436-439.
 24. Ullah F, Malik SA, Ahmed T. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. *Burns*. 2009;35(7):1020-1025.

25. Ahmed BO, Omar AO, Asgher AH, Elhassan MM. Increasing prevalence of ESBL- producing Enterobacteriaceae in Sudan community patients with UTIs. Egyptian Academic Journal of Biological Science. 2013;5:17-24.
26. Rao SPN, Rama PS, Gurushanthapa U, Manipura R, Srinivasan K. Extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*: A multi-centric study across Kamataka. Journal of Laboratory Physicians. 2014;6: 7-13.
27. Anjum F, Mir A. Susceptibility pattern of *Pseudomonas aeruginosa* against various antibiotics. African Journal of Microbiology Resaerch. 2010;4(10):1005-1012.
28. Iroha IR, Ezeifeke GO, Amadi ES, Umezurike CR. Ocurrance of extended spectrum beta lactamase producing resistant *Escherichia coli* and *Klebsiella pneumoniae* in clinical isolates and associated risk factors. Research Journal of Biological Sciences. 2009;4:588-592.
29. Akanbi BO, Ojonuba BD, Njoku R. Detection of extended spectrum β -lactamase producing *Klebsiella pneumoniae* and *Echerichia coli* in two hospitals in the federal Cpital Territory, Abuja, Nigeria. Open Journal of Medical Microbiology. 2013;3:207-212.
30. Okesola AO, Adeniji TW. Extended spectrum beta lactamases production among clinical isolates of *Proteus* species in western Nigeria. World Journal of Medical Sciences. 2010;5:94-97.
31. Iroha IR, Amadi ES, Oji AE, Nwuzo AC, Ejik-ugwu PC. Detection of plamid borne extended spectrum beta-lactamase enzymes from blood and urine isolates of Gram-negative bacteria from a University Teaching Hospital in Nigeria. Current Research in Bacteriology. 2010;3:77-83.
32. Azekhume I, Anietie E, Moses, Samuel AD. Exteded-spectrum beta-lactamases in clinical isolates of *Echerichia coli* and *Klebsiella pneumoniae* from University of Uyo teaching hospital, Uyo-Nigeria. 2015;2(3):117-125.
33. Al-Jasser AM. Review Article: Extended-spectrum beta lactamases (ESBLs). A global problem; 2006.
34. Kaur K, Chopra S, Sheeveni S, Mahajanara G. Modified double disc synergy test to detect SSBL producing in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Journal of Clinical and Diagnostic Research. 2013;7:229-233.
35. Seger L, Kumar S, Joseph NM, Sivaraman U. Prevalence of extended spectrum beta lactamases among Enterobacteriaceae and their Antibiogram pattern from various clinical samples. Asian Journal of Pharmaceutical and Clinical Research. 2015;8:220-223.
36. Hsueh PR, Hoban DJ, Carmeli Y, Chen SY, Desikan S, Alejandria M. Consensus review of epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia–Pacific region. Journal of Infection. 2011;63:114-23.

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