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

Florence Bose Omoregbe1* and Obasola Ezekiel Fagade2

1Department of Microbiology, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
2Department of Microbiology, University of Ibadan, Nigeria.

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
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 cephemycine 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 cefoxitin, 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 μg/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 μg/ml were aseptically introduced into the sterile peptone water at 50°C to a final concentration of 60 μg/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 μg/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 μg/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 MacConkey 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 plate (HIMEDIA, INDIA). After the agar surface has dried for about 5 minutes, Ceftazidime 30 µg, antibiotic discs (Oxoid) were placed at the Centre of the 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 | S No. | I No. | R No. | Total No. |
|-----------|------------|-------|-------|-------|-----------|
| BSUTH     | Ceftazidime| 1     | 10    | 31    | 42        |
| Ceftiraxone| 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        |
| Ceftiraxone| 14        | 8     | 47    | 69    |
| Ciprofloxacin| 22      | 3     | 36    | 61    |
| Ertapenem | 39        | 14    | 16    | 69    |
| Imipenem  | 62        | 4     | 11    | 77    |
| Total     |            | 140   | 43    | 162   | 345       |

BSUTH: $X^2 = 123.221$, df = 8, p = 0.00  
FMC: $X^2 = 77.915$, df = 8, p = 0.00  
Total: $X^2 = 196.095$, 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       |  |  |  |  |  |  |  |  |  |
|-----------|------------------------|-------------------|---|---|---|---|---|---|---|---|---|
|           |                        | Ceftazidime       | Ceftiriazone | Ciprofloxacin | Ertapenem | Imipenem | Total |
| BSUTH     |                        | 33.00             | 33.00         | 33.00         | 33.00      | 33.00     | 165.00 |
|           | E. coli                | 33.00             | 33.00         | 33.00         | 33.00      | 33.00     | 165.00 |
|           | Klebsiella pneumoniae | 5.00              | 5.00          | 5.00          | 5.00       | 5.00      | 25.00  |
|           | Pseudomonas sp.        | 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       |                        | 69.00             | 69.00         | 61.00         | 69.00      | 77.00     | 345.00 |
|           | E. coli                | 29.00             | 29.00         | 26.00         | 29.00      | 32.00     | 145.00 |
|           | Klebsiella pneumoniae | 8.00              | 8.00          | 8.00          | 8.00       | 8.00      | 40.00  |
|           | Proteus sp.            | 2.00              | 2.00          | 2.00          | 2.00       | 2.00      | 10.00  |
|           | Pseudomonas sp.        | 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     |                        | 111.00            | 111.00        | 103.00        | 111.00     | 119.00    | 555.00 |

BSUTH: $X^2 = 0.00$, df = 8, $p = 1.00$
FMC: $X^2 = 0.438$, df = 12, $p = 1.00$
Total: $X^2 = 0.611$, 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 | S   | I   | R   | Total |
|-------------------------------------|-----|-----|-----|-------|
| E. coli                             | 131 | 40  | 139 | 310   |
| Klebsiella pneumoniae               | 24  | 11  | 30  | 65    |
| Proteus sp.                         | 6   | 0   | 4   | 10    |
| Pseudomonas sp.                     | 65  | 15  | 90  | 170   |
| **Total**                           | 226 | 66  | 263 | 555   |

\[ X^2 = 7.434, \text{df} = 6, p = 0.28 \]

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 | S   | I   | R   | Total |
|-----------|-------------|-----|-----|-----|-------|
| BSUTH     | Cefazidime  | 0   | -   | 4   | 4     |
|           | Ceftriaxone | 0   | -   | 4   | 4     |
|           | Ciprofloxacin | 3 | -   | 1   | 4     |
|           | Ertapenem  | 0   | -   | 4   | 4     |
|           | Imipenem   | 3   | -   | 1   | 4     |
|           | **Total**  | 6   | -   | 14  | 20    |
| FMC       | Cefazidime  | 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** | Cefazidime  | 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: \[ X^2 = 18.770, \text{df} = 8, p = 0.02 \]
Total: \[ X^2 = 28.429, \text{df} = 8, p = 0.00 \]

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

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

| Antibiotics               | ESBL positive Environmental Gram-negative Bacteria |
|---------------------------|---------------------------------------------------|
| Citrobacter koseri        | 1 1 1 1 1 1 5                                    |
| Shigella sonnei           | 1 1 1 1 1 5                                      |
| Proteus vulgaris          | 2 2 2 2 2 10                                     |
| Total                     | 4 4 4 4 4 20                                     |
| Citrobacter diversus      | 1 1 1 1 1 5                                      |
| Citrobacter koseri        | 1 1 1 1 1 5                                      |
| E. coli                   | 1 1 1 1 1 5                                      |
| Klebsiella pneumonia      | 1 1 1 1 1 5                                      |
| Shigella sonnei           | 1 1 1 1 1 5                                      |
| Proteus mirabilis         | 2 2 2 2 2 10                                     |
| Proteus vulgaris          | 1 1 1 1 1 5                                      |
| Total                     | 8 8 8 8 8 40                                     |
| Citrobacter              | 1 1 1 1 1 5                                      |
| Citrobacter koseri        | 1 1 1 1 1 5                                      |
| E. coli                   | 1 1 1 1 1 5                                      |
| Klebsiella pneumonia      | 1 1 1 1 1 5                                      |
| Shigella sonnei           | 2 2 2 2 10                                      |
| Proteus mirabilis         | 2 2 2 2 10                                      |
| Proteus vulgaris          | 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

| Susceptibility profile       | S  | I  | R  | Total |
|-----------------------------|----|----|----|-------|
| Citrobacter diversus        | 0  | 1  | 4  | 5     |
| Citrobacter koseri          | 1  | 0  | 9  | 10    |
| E. coli                     | 1  | 0  | 4  | 5     |
| Klebsiella pneumonia        | 1  | 0  | 4  | 5     |
| Shigella sonnei             | 3  | 0  | 7  | 10    |
| Proteus mirabilis           | 4  | 1  | 5  | 10    |
| Proteus vulgaris            | 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                       | 0                         | 0                                      |
| 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
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.

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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 (Ceftazidine), CRO (Ceftriaxone), CIP (Ciprofloxacin), ETP (Ertapenem), IMP (Imipenem), MAR (Multiple Antibiotic Resistance)
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