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Prevalence of Extended Spectrum Beta-lactamase-producing Bacteria in Patients Attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka

Umezulora Blessing Ijeoma, Orji Michael Uchenna, Okafor Ugochukwu Chukwuma*, Adinchezor Chukwuma Charles

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

Abstract

The present study was undertaken to determine the prevalence of extended-spectrum *beta*-lactamase-producing bacteria isolated from in-patients and out-patients that attended Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka for treatment. A total of eight hundred and forty (840) clinical specimens comprising; urine (50%), uro-genital specimens (high vaginal swab (25.4%), semen (8.0%) and urethral swab (1.9%)), sputum (7.0%), wound swab (5.6%), pus (1.3%) and ear swab (0.8%), were collected from patients who attended this hospital between January to November, 2016. The antimicrobial resistance profile of the isolates was performed using standard disk diffusion technique. Phenotypic detection of ESBL-producing bacterial species was performed using Double Disk Synergy test (DDST) method. The prevalence of ESBL-producing bacteria with regards to demographic factors was evaluated. Statistical evaluation of the results was carried out using SPSS Statistical Software Package version 21.0. Results were expressed as means, frequencies and percentages. Chi-square was used to determine the level of significance of groups of categorical variables with *P* values<0.05 considered significant. The bacteria species isolated were; Gram-negative bacteria: *Escherichia coli* (22.0%), *Klebsiella* species (6.3%), *Proteus* spp. (3.7%), *Citrobacter* spp. (1.8%), *Pseudomonas* spp. (1.5%), *Enterobacter* spp. (1.1%), *Providencia rettgeri* (0.1%) and *Shigella flexneri* (0.1%); Gram-positive bacteria: *Staphylococcus* spp. (17.1%), *Streptococcus* spp. (6.3%) and *Enterococcus* species (4.3%). Antimicrobial resistance profiles of the isolated bacterial species showed lowest resistance to Meropenem (MEM) and highest resistance to ceftriaxone (CTR). A low prevalence (8.3%) of ESBL-producing Gram-negative bacteria with a tendency for multi-drugs resistance and a higher prevalence in the in-patients were observed. The differences observed among the subjects were statistically significant at p<0.05 level. No Gram positive bacteria produced ESBL. The differences observed in the relationship between ESBL producing bacteria with respect to age, sex and type of specimen were not statistically significant at p<0.05 level. Meropenem was the most active antibacterial agent and can be suggested as the drug of choice from this study.

Keywords

Antibiotic Resistance, *Beta*-lactam Antibiotics, Prevalence, Bacteria, Awka

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1. Introduction

Antibiotic resistance has become a worldwide problem with serious consequences on the treatment of infectious diseases and public health. Beta-lactam antimicrobial agents, an important group of drugs that are commonly used for the treatment of bacterial infections include; the Penicillins, Cephalosporins, Carbapenems and Monobactams. Among the wide array of antibiotics, beta-lactam antibiotics are the most varied and widely used agents accounting for over 50% of all

* Corresponding author
E-mail address: uc.okafor@unizik.edu.ng (O. U. Chukwuma)

systemic antibiotics in use [1].

Beta-lactamases are a family of enzymes produced by many Gram-negative and Gram-positive bacteria that usually inactivate beta-lactam antibiotics by breaking their beta-lactam ring. The continuous inactivation, by hydrolysis of the beta-lactam ring of these antibiotics by these beta-lactamases, account for many failures of antimicrobial therapy used in treatment of bacterial infections [2].

An important and emerging resistance mechanism among Gram-negative bacilli especially those belonging to the family Enterobacteriaceaeis the production of extended spectrum beta-lactamase (ESBL). Extended spectrum beta-lactamases (ESBLs) are plasmid-encoded beta-lactamases that hydrolyze extended-spectrum (or third generation) cephalosporins with oxyimino side chain (e.g. cefotaxime, ceftriaxone, ceftazidime) as well as oxyiminomonobactams (e.g. aztreonam). They however, do not affect the cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (eg. imipenem, meropenem and etrapenem), but are inhibited by certain molecules called beta-lactamase inhibitors (e.g. clavulanic acid, tazobactam) [3].

Bacterial species that produce ESBL are referred to as ESBL-producing bacteria. The majority of ESBL-producing strains are *Klebsiellapneumoniae*, *Klebsiellaoxytoca* and *Escherichia coli*. A few other types of bacteria that have been less frequently associated with ESBLs production includes *Acinetobacter*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, and *Shigella* species [4]. ESBL-producing organisms are clinically relevant and remain an important cause of therapy failure when cephalosporins are used, even when the bacteria appear to be sensitive to these agents by routine susceptibility testing [5].

ESBL-producing strains have been isolated from urine, blood, Uro-genital tract samples, stool, respiratory tract samples, throat swab, ear swab, pus, wound swabs and aspirates [6-8]. They apparently have a worldwide distribution. Rate of isolation varies greatly worldwide and within geographic areas and is rapidly changing over time.

The prevalence of bacteria producing ESBLs varies worldwide, with reports from North America, South America, Europe, Africa, and Asia [9]. Surveillance studies are available in Europe, North America, and Asia, but there is no summarized research published on the situation in Africa. The continuous rise in the prevalence of ESBLs-producing bacterial strains has caused many outbreaks worldwide [10-14]. In comparison with the rest of the world, there is generally a lack of comprehensive data regarding ESBL-producing Enterobacteriaceae in African countries. However, there is sufficient evidence to highlight the prevalence of ESBLs in Africa. Several outbreaks of infections with ESBL-producing*Klebsiella* spp. have been reported from South Africa [13], but no national surveillance figures have been published. Outbreaks of *Klebsiella* infections with strains resistant to third-generation cephalosporins have been reported in Nigeria and Kenya but there is no published surveillance data documentation of ESBL production [11, 12].

This study was undertaken to determine the prevalence of Extended Spectrum Beta-lactamase-producing bacteria in clinical patients attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State.

2. Materials and Methods

2.1. Study Population and Inclusion Criteria

A total of eight hundred and thirteen, (813) patients comprising; five hundred and twenty seven 527 out-patients (not admitted) and two hundred and eighty six, 286 in-patients (admitted), that attended the hospital between January to November, 2016, participated in the study. Patients were considered with respect to their ages (1-93 years), gender (male/female), location (out- or in-patient) and type of specimens collected from the patients.

2.2. Ethical Approval

Ethical approval was obtained from the Ethical Committee of the hospital management before the commencement of the study. Oral consent was given by the patients after they were assured that their personal identities would not be linked to any data.

2.3. Samples Collection

Proper collection of clinical specimens to avoid contamination with organisms of the normal microbiota and prompt transport to the laboratory for processing were pre-analytical steps that were keys to organism recovery. With the assistance of certified laboratory scientists, a total of eight hundred and forty (840) samples were collected following guidelines described by [15]. The specimens were collected during the period from January to November, 2016. Specimens were; urine - 420, Uro-genital specimens (high vaginal swab- 213, semen- 67, urethral swab – 16), sputum - 59, wound swab -47, pus -11 and ear swab -7.

2.4. Inoculation of Samples and Identification of Bacterial Isolates

Bacterial isolation was carried out as described by [16]. Collected samples were inoculated by streaking on media plates. After the inoculations, the plates were aerobically incubated at 37°C for 24 hours. After incubation, the plates

were carefully observed for bacterial. Using a properly flamed wire loop, observed bacterial colonies were aseptically sub-cultured and incubated again aerobically at 37°C for 24 hours, in order to obtain pure cultures of the bacterial colonies. All the media that were used for microbial isolation were prepared according to the manufacturers' instructions, sterilized in an autoclave at 121°C and 15 psi for 15 minutes and aerobically incubated at 37°C for 24 hours without inoculation to ascertain their sterility before they were used for the isolation of microorganisms. The media that were used were: Eosin methylene blue (EMB), Nutrient agar (NA), MacConkey agar and Blood agar.

The bacterial isolates that were obtained from the cultures were identified based on morphological, cultural as well as biochemical characteristics. The biochemical tests were carried out as described in [17]. Gram stain, Voges-Proskauer (VP) test, urease test, catalase test, triple sugar iron agar test (TSI), hydrogen sulphide production test, indole test, motility test, citrate utilization test, oxidase test and gene sequencing were used for bacterial identification.

2.5. 16S rRNA Region Sequencing Analysis

The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 reaction mixture by using a *EF-Taq* (SolGent, Korea) as follows: activation of Taq polymerase at 95°C for 2 minutes, 35 cycles of 95°C for 1 minute, 55°C, and 72°C for 1minutes each were performed, finishing with a 10-minute step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was carried out in accordance with the approved guidelines for antimicrobial susceptibility testing specified by [18], using Kirby Bauer's modified method for antimicrobial susceptibility tests.

2.7. Turbidity Standard Equivalent to 0.5 McFarland

This was prepared by the addition of 1ml of concentrated Sulphuric acid to 99ml of water, mixing them thoroughly to produce a 1% v/v solution. On the other hand, a 1% v/v solution of Barium Chloride was prepared by dissolving 0.5g of dihydrate barium Chloride ($BaCl_2.H_2O$) in 49.5ml of distilled water. Using a Pasteur pipette, 0.6ml of the Barium Chloride solution was measured and poured into 99.4ml of the Sulphuric acid solution and mixed properly. Finally, a small volume of the turbid solution was transferred into a capped tube and stored at room temperature in the laboratory for use as 0.5 McFarland standard solution [17].

2.8. Screening and Confirmation of ESBLs Production by Bacterial Isolates

Suspended bacterial colonies adjusted to 0.5McFarland's Standard of turbidity were inoculated on Mueller Hinton agar. Third generation cephalosporin antibacterial agents (cefotaxime - CTX, ceftazidime - CAZ and ceftriaxone - CTR) were aseptically placed on the inoculated media and incubated at 35ºC for 24 hours. After incubation, plates were observed for resistance to any of the antibiotics. The clinical and Laboratory Standards Institute (CLSI) guidelines for screening and Confirmatory test of bacterial isolates for ESBLs production presented were used for the test interpretation. According to CLSI guidelines, bacterial species showing zone of inhibition of ≤22 mm for ceftazidime, ≤27 mm for cefotaxime, and ≤25 mm for ceftriaxone were selected for ESBL confirmatory test. The phenotypic confirmatory test for ESBL production was carried out using the double disc synergy test method. Suspended bacterial colonies adjusted to 0.5 McFarland standard of turbidity were with the help of sterile swab sticks, picked from the inoculated tubes. The immersed swab sticks were properly drained to remove excess fluid and to minimize loads of bacterial colonies. They were subsequently used to inoculate Mueller Hinton media. A clavulanate-containing beta-lactam antibacterial agent (amoxicillin-clavulanate) was placed 25mm centre to centre, in-between Cefotaxime (CTX) and Ceftazidime (CAZ). Plates were then incubated at 35°C for 24 hours. After 24-hour incubation, plates were observed for synergy, which when present, the organism was regarded as an ESBL-producing bacteria (ESBL-positive). Synergy is confirmed whenever there is an enhanced zone of inhibition extending towards the disk containing the clavulanic acid, confirming the presence of ESBL. If the zone of inhibition is not extending towards the clavulanate containing disk, the organism is ESBL-negative.

2.9. Evaluation of the Prevalence of ESBL-producing Bacteria with Regards to Demographic Factors

The prevalence of ESBL-producing bacteria with regards to demographic factors (age, gender), was carried out using information obtained from the patients' hospital folder, laboratory test request forms and through direct interactions

with the participants. Also, the prevalence of ESBL-producing bacteria with regards to location of patients and sample types was considered.

2.10. Statistical Evaluation of the Results

Data were analyzed using SPSS Statistical software Package version 21.0. The statistical tools used were Chi-Square, McNemar, Friedman, Wilcoxon Signed Ranks and NPar tests. P values of <0.05 was considered to indicate statistically significant differences.

3. Results

Table 1. Frequency and percentage distribution of bacteria isolated from samples.

Study samples	Total samples examined	Total positive samples (%)	Total bacteria Gram positive (%)	Total bacteria Gram negative (%)
Urine	420	285 (33.9)	137 (16.3)	158 (18.8)
Urogenital specimens	296	106 (12.6)	21 (2.5)	97 (11.5)
Sputum	59	48 (5.7)	25 (3.0)	29 (3.5)
Wound swab	47	47 (5.6)	34 (4.0)	21 (2.5)
Pus	11	8 (1.0)	9 (1.1)	2 (0.2)
Ear swab	7	5 (0.6)	7 (0.8)	1 (0.1)
Total	840	499 (59.4)	233 (27.7)	308 (36.7)

Table 2. Frequency and percentage distribution of isolated bacterial species from study samples.

Bacterial isolates	Number of bacteria identified	Percentage (%) distribution From total samples
Gram-negative bacteria		
Escherichia coli	185	22.0
*Klebsiella*spp.	53	6.3
Proteus spp.	31	3.7
Citrobacter spp.	15	1.8
Pseudomonas spp.	13	1.5
Enterobacter spp.	9	1.1
Providenciarettgeri	1	0.1
Shigellaflexneri	1	0.1
Gram-positive bacteria		
Staphylococcus spp.	144	17.1
Streptococcus spp.	53	6.3
Enterococcus spp.	36	4.3
Total	541	64.4

Note: total samples examined=840.

Table 3. Distribution of the isolated bacterial species according to gender of Patients.

Bacterial isolates	Gender of study participants	
	Male (%)	Female (%)
Gram-negative bacteria		
Escherichia coli	38 (4.5)	147 (17.5)
*Klebsiella*spp.	29 (3.5)	24 (2.9)
Proteus spp.	14 (1.7)	17 (2.0)
Citrobacter spp.	3 (0.4)	12 (1.4)
Pseudomonas spp.	9 (1.1)	4 (0.5)
Enterobacter spp.	3 (0.4)	6 (0.7)
Providenciarettgeri	1 (0.1)	0 (0)
Shigellaflexneri	1 (0.1)	0 (0)
Total	98 (11.7)	210 (25.0)
Gram-positive bacteria		
Staphylococcus spp.	55 (6.5)	89 (10.6)
Streptococcus spp.	28 (3.3)	25 (3.0)
Enterococcus spp.	14 (1.7)	22 (2.6)
Total	97 (11.5)	136 (16.2)

Note: total samples examined=840.

Table 4. Inhibition zones diameter for antimicrobial susceptibility tests for susceptibility/resistance of the bacterial isolates to third generation cephalosporins and other antibacterial agents.

Organisms	Antibacterial agents/ Average diameter ofthezones of inhibition (mm)									
	CTX	CAZ	CTR	CN	OFX	CXC	AMC	CIP	CMX	MEM
Gram-negative bacteria										
E. coli	14.1	10.8	8.3	8.6	8.3	5.3	8.0	9.2	8.3	9.8
Klebsiella spp.	8.5	8.2	6.4	8.5	7.7	3.1	6.6	8.1	7.9	4.9
Proteus spp.	8.1	6.5	5.1	8.7	9.0	1.5	2.6	7.2	3.8	10.8
Citrobacter spp.	1.6	1.1	0.6	4.7	5.3	1.3	1.0	6.0	2.3	8.3
Pseudomonas spp.	1.7	1.3	0.3	8.8	8.5	2.3	2.3	8.5	2.5	5.6
Enterobacter spp.	0.6	0.6	0.6	7.4	4.6	1.9	3.1	6.4	2.9	7.4
Providenciarettgeri	0	0	0	0	0	0	0	0	0	0
Shigellaflexneri	0	0	0	0	0	0	0	17.0	0	14.0
Gram-positive bacteria										
Staphylococcus spp.	3.6	5.0	3.8	8.9	6.5	5.6	7.0	9.3	7.0	12.1
Streptococcus spp.	6.3	6.0	4.3	6.8	5.9	5.1	8.5	8.7	5.7	6.3
Enterococcus spp.	4.0	5.3	3.4	7.1	5.1	4.4	7.1	6.9	5.7	8.3

Table 5. The diameter of the zone of inhibition for sensitivity/resistance to third generation cephalosporins at the initial screening for ESBLs production by Gram negative bacterial isolates.

Organisms	Third generation Cephalosporin antibiotics/average diameter of the zones of inhibition (mm)		
	CTX	CAZ	CTR
E. coli	16.6	12.3	10.2
Klebsiella spp.	8.5	8.2	6.4
Proteus spp.	10.4	9.2	7.1
Citrobacter spp.	1.6	1.1	0.6
Pseudomonas spp.	1.7	1.3	0.3
Enterobacter spp.	0.6	0.6	0
Providenciarettgeri	0	0	0
Shigellaflexneri	0	0	0

Table 6. Bacteria species resistant to third generation cephalosporin antibiotics at the initial screening for ESBL production by Gram-negative bacteria isolates.

Organisms	Resistance to 3rd generation antibacterial agents (%)		
	Cefotaxime (CTX)	Ceftazidime (CAZ)	Ceftriaxone (CTR)
Escherichia coli	135 (16.3)	153 (18.2)	162 (19.3)
*Klebsiella*spp.	53 (6.3)	53 (6.3)	53 (6.3)
Proteus spp.	23 (2.7)	27 (3.2)	30 (3.6)
*Citrobacter*spp.	15 (1.8)	15 (1.8)	15 (1.8)
Pseudomonas spp.	13 (1.5)	13 (1.5)	13 (1.5)
*Enterobacter*spp.	8 (1.0)	8 (1.0)	8 (1.0)
Providenciarettgeri	1 (0.1)	1 (0.1)	1 (0.1)
Shigellaflexneri	1 (0.1)	1 (0.1)	1 (0.1)
Total	249 (29.6)	271 (32.3)	283 (33.7)

Table 7. Prevalence of extended spectrum beta-lactamase (ESBL) –producing Gram-negative bacteria species.

Organisms	Total isolated	Number ESBL positive (%) (+)
Escherichia coli	185	30 (3.6)
*Klebsiella*species	53	18 (2.1)
Proteus Species	31	10 (1.2)
Citrobacter species	15	3 (0.4)
Pseudomonas species	13	4 (0.5)
*Enterobacter*species	9	3 (0.4)
Providenciarettgeri	1	1 (0.1)
Shigellaflexneri	1	1 (0.1)
Total	308	70 (8.3)

P<0.05.
Figures in parenthesis represent percentages (%).
N. B: total samples examined=840.

Table 8. The prevalence of ESBL-producing Gram-negative bacteria with regards to sex of study participants.

Bacterial isolates	Sex /no. of ESBL-positive isolates (%)	
	Female	Male
Escherichia coli	20 (2.4)	10 (1.2)

Bacterial isolates	Sex /no. of ESBL-positive isolates (%)	
	Female	Male
*Klebsiella*species	8 (1.0)	10 (1.2)
Proteus species	4 (0.5)	6 (0.7)
Citrobacter species	3 (0.4)	0 (0)
Pseudomonas species	2 (0.2)	2 (0.2)
*Enterobacter*species	1 (0.1)	2 (0.2)
Providenciarettgeri	0 (0)	1 (0.1)
Shigellaflexneri	0 (0)	1 (0.1)
Total	38 (4.5)	32 (3.8)

P value=0.207.
NB: Total samples examined=840.

Table 9. The prevalence of ESBL-producing Gram-negative bacteria with regards to study specimens.

Organisms	Specimens/number ESBL positive (%)				
	Urine	Urogenital specimens	Sputum	Wound swab	Pus
Escherichia coli	20 (2.4)	7 (0.8)	0 (0)	2 (0.2)	1 (0.1)
*Klebsiella*species	9 (1.1)	0 (0)	9 (1.1)	0 (0)	0 (0)
Proteus Species	4 (0.5)	3 (0.4)	0 (0)	3 (0.4)	0 (0)
Citrobacter species	3 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas species	0 (0)	0 (0)	0 (0)	3 (0.4)	1 (0.1)
*Enterobacter*species	2 (0.2)	1 (0.1)	0 (0)	0 (0)	0
Providenciarettgeri	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Shigellaflexneri	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Total	40 (4.8)	11 (1.3)	9 (1.1)	8 (1.0)	2 (0.2)

P value=0.134.
N. B: Total samples examined=840.

Table 10. Resistance profiles of the ESBL producing Gram-negative bacteria species to antibacterial agents tested.

ESBL positive bacteria	Resistance to Antibacterial agents %									
	CTX	CAZ	CTR	CN	OFX	CXC	AMC	CIP	CMX	MEM
Escherichia coli n=30	30 (3.6)	30 (3.6)	30 (3.6)	29 (3.5)	29 (3.5)	29 (3.5)	30 (3.6)	29 (3.5)	29 (3.5)	08 (1.0)
Klebsiella species n=18	18 (2.1)	18 (2.1)	18 (2.1)	17 (2.0)	17 (2.0)	17 (2.0)	17 (2.0)	17 (2.0)	18 (2.1)	10 (1.2)
Proteus species n=10	9 (1.1)	10 (1.2)	10 (1.2)	8 (1.0)	8 (1.0)	9 (1.1)	9 (1.1)	8 (1.0)	8 (1.0)	1 (0.1)
Citrobacter species n=3	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	0 (0)
Pseudomonas species n=4	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	4 (0.5)	2 (0.2)
Enterobacter species n=3	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)	3 (0.4)
Providenciarettgeri n=1	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)
Shigellaflexneri n=1	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	0 (0)	1 (0.1)	0 (0)
Total tested=70	69 (8.2)	70 (8.3)	70 (8.3)	66 (7.9)	66 (7.9)	67 (8.0)	68 (8.1)	65 (7.7)	67 (8.0)	25 (3.0)

P value=0.000 (appendix VII).
CTX (30µg)=Cefotaxime CAZ (30µg)=Ceftazidime CTR (30µg)=Ceftriaxone CN=Gentamicin.
OFX=Ofloxacin CXC=Cloxacillin AMC=Augmentin CIP=Ciprofloxacin CMX=Cefuroxime MEM=Meropenem.

4. Discussion

This study investigated the prevalence of ESBL-producing bacteria in clinical specimens comprising urine, uro-genital specimens (high vaginal swab, semen and urethral swab), sputum, wound swab, pus and ear swabs. These specimens were obtained from in-patients and out-patients that attended Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka for treatment between January to November, 2016. Out of the eight hundred and forty (840) clinical specimens obtained, the urine specimen had the highest number, 420 (50.0%). [19], urine specimens are the most frequent specimens received in the microbiology laboratory. This agrees with one study by [20] showing similar observations. Five hundred and forty-one, 541 (64.4%) bacterial species comprising both Gram-negative, 308 (36.7%) and Gram-positive, 233 (27.7%) bacteria were identified from four hundred and ninety-nine, 499 (59.4%) specimens that were positive with bacterial isolates. This may suggest that there were more bacterial species than samples positive with bacteria. This was because some of the specimens had more than one bacterial isolates. Again, more Gram-negative than Gram-positive bacteria was reported in this study. This observation agrees with the findings of [21-23] that more Gram-negative than Gram-positive bacteria were isolated from various clinical specimens examined.

The Identification of bacterial isolates were by phenotypic testsSequence analysis of the 16S ribosomal RNA (rRNA) gene has been widely used to identify bacterial species and diagnose microbial infections. Eleven [11] different species

of bacteria comprising eight (8) Gram-negative bacteria and three (3) Gram-positive bacteria were isolated and identified in this study. Among Gram-negative bacteria, *Escherichia coli* (22.0%) species were found to be the most predominant species and this predominance of *E. coli* tally with the findings of [24]. Similarly, among Gram-positive bacteria, *Staphylococcus* species was the most predominant pathogen isolated and this predominance conforms to the observations of [22].

Urine specimens had the highest frequency of both Gram-negative bacteria, 158 (18.8%) and Gram-positive bacteria, 137 (16.3%) in this study. The finding that most bacterial species were isolated from urine specimens suggests that most of these patients may be suffering from urinary tract infections. According to [25], urinary tract infections are the most common infections in clinical practice. This is similar to the findings of [20, 26] that urine samples produced the highest frequency of bacteria species among various clinical samples examined. Again, many patients are more willing to give urine specimens than other specimens.

Findings from this study reveal that bacterial species were mainly isolated from female patients compared to male patients. Female patients generally seek more medical attention than their male counterparts due to their role in pregnancy and childbirth as most of the female participants were from the gynaecology ward. This higher prevalence among female population may be due to decrease in normal vaginal flora (*Lactobacilli*), less acidic PH of vaginal surfaces, poor hygienic condition, short and wider urethra and proximity of the female reproductive organ to the anus [27].

The values used to determine resistance was in accordance with CLSI criteria and the average values obtained have been presented as measured from the test. *Escherichia coli* had the highest diameter zone of inhibition to tests against CTX, CAZ, CTR, CN, OFX, CXC, AMC, CIP, CMX and MEM at 14.1, 10.8, 8.3, 8.6, 8.3, 5.3, 8.0, 9.2, 8.3 and 9.8 respectively. No zone of inhibition occurred in *Providencia rettgeri*a zero (0) zone of inhition to all the antibiotics tested. Meropenem, a member of the carbapenems, was the most active antibacterial agent against all the Gram-negative bacteria species. Statistical analysis of the subjects showed the differences observed to be statistically significant at $p<0.05$. This finding is similar to the report presented by (28). Other studies have also shown similar trends with the carbapenems [29, 7]. Meropenem like other carbapenems, have become widely recognized as the drug of choice for the treatment of serious infections caused by both Gram-negative and Gram-positive bacterial species. These agents are highly stable to hydrolysis by ESBLs, distributed into body tissues in high concentrations and there is no inoculum effect [30].

The prevalence of ESBL-producing bacteria was 8.3% with Escherichia coli and Klebsiella species being the most frequently involved pathogens. This disagrees with the report of [7], which says that the most frequently involved pathogen was *Enterobacter* species. Statistical analysis of the subjects showed the differences observed to be statistically significant at $p<0.05$. This prevalence is low considering the fact that majority of the bacterial species demonstrated considerable resistance to many groups of antibiotics tested. This suggests that the non-ESBL producers may possess other mechanisms of resistance to beta-lactams; inhibitor resistant beta-lactamases (AmpC), cephalosporinase over-production and production of beta-lactamases with carbapenemase activity. The high prevalence rates among *Escherichia coli, Klebsiella* species and *Enterobacter* species have been well documented in several studies [13, 7]. The prevalence is lower than the prevalence 16.0% and 44.3% reported in similar studies in South-east and South-west Nigeria respectively [6, 7]. This is much lower than the rates reported in other African countries like Egypt (42.9%), Algeria (17.7-31.4%) (31) and Ghana (49.9%) [32].

None of the Gram positive bacterial species screened was an ESBL-producer; hence the prevalence was zero 0 (0%). Several reports about ESBL-producing gram negative bacteria have been well documented [7, 6, 28]. However, no report exists about ESBL-producing Gram-positive bacteria, hence; a zero (0%) prevalence of ESBL-producing Gram positive bacteria reported from this study may not be a surprise.

The prevalence of ESBL-producing bacteria with regards to sex was evaluated. Out of the seventy (70) ESBL-producing bacteria species isolated, 38 (4.5%) occurred in female while 32 (3.8%) occurred in men. The *Shigella flexneri* and *Providencia rettgeri* isolated did not occur in female while all *Citrobacter* species that were ESBL-positive occurred only in female, none was isolated from male. ESBL-producing *E. coli* had higher value in female, 20 (2.4%) while ESBL-producing *Klebsiella* species and *Proteus* species had higher values in the males with 10 (1.6%) and 6 (0.7%) respectively. However, the differences observed was not statistically significant at $p<0.05$ this agrees with the study of [33],

The highest prevalence of ESBL-producing bacteria was from bacterial isolates from the urine specimens, 40 (4.8%) while the least was from bacterial isolates from pus, 2 (0.2%). However, there was no statistically significant difference between bacteria species that produced ESBL and the clinical specimens examined. This agrees with previous report [7], showing similar observations.

The ESBL-producing bacterial species showed multi-resistance to the antibacterial agents tested with meropenem (MEM) emerging the most active antibacterial agent with a

resistance rate of 25 (3.0%). However, Ceftaxidime (CTX) and Ceftriaxone (CTR) collectively had the lowest activities against the bacterial species, 70 (8.3%) each. This resistance was statistically significant at $p<0.05$. One of the issues surrounding the therapy of ESBL-associated infections is that even if an antibacterial agent is selected that has activity against the bacteria *in vitro* (when tested in the laboratory), clinical efficacy in patients is not always guaranteed. Some studies reported a reduction in the clinical effect against ESBL-producing bacteria with some beta-lactam agents despite testing susceptible *in vitro,* whereas, other studies have shown good clinical outcome with beta-lactam-beta-lactamase inhibitor combinations [34, 35, 36]. Despite the high rate of resistance observed, it is worthy of note that a number of the ESBL-producing bacteria were susceptible to some of the antibacterial agents, but *in vitro* susceptibilities to these agents does not guarantee good clinical outcome when applied for the treatment of ESBL infections in the study patients.

5. Conclusion

Antibiotic resistance is an important issue affecting public health. Antimicrobial resistance profiles of the isolated bacterial species showed lowest resistance to Meropenem (MEM) and highest resistance to ceftriaxone (CTR). A low prevalence (8.3%) of ESBL-producing Gram-negative bacteria with a tendency for multi-drugs resistance and a higher prevalence in the in-patients were observed. The differences observed among the subjects were statistically significant at $p<0.05$ level. No Gram positive bacteria produced ESBL. The differences observed in the relationship between ESBL producing bacteria with respect to age, sex and type of specimen were not statistically significant at $p<0.05$ level. Meropenem was the most active antibacterial agent and can be suggested as the drug of choice from this study.

6. Recommendation

The findings from this study would serve as a helpful tool in the formulation of an antibiotic policy that will help to check the irrational consumption of antibacterial agents both in the hospital and the community settings. It will also serve as a useful epidemiological tool for the management as well as a report of multi-drugs resistant (MDRs) bacterial species in our environment.

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