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PHENOTYPIC DETECTION OF MULTI-DRUG RESISTANT MBL-PRODUCING GRAM-NEGATIVE BACTERIA ISOLATED FROM CLINICAL SAMPLES OF PATIENTS IN HOSPITALS IN AKWA IBOM STATE, NIGERIA

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ABSTRACT

Metallo-beta-lactamase (MBL)-producing Gram-negative bacteria (GNB) continue to be a bane in the treatment of clinical infections in both community and hospital settings. Prompt detection of multidrug resistant (MDR) strains using antimicrobial susceptibility testing (AST) and MBL detection are vital for therapeutic options. The aim of this study was to determinine the pevalence, distribution and antibiotic susceptibility of MDR and MBL-producing GNB from clinical samples in health facilities in Akwa Ibom State. A total of 480 samples comprising wound, urine and blood were collected aseptically from eligible in- and out-patients for the study and GNB were recovered from the samples using standard bacteriological techniques. The identification of isolated GNB, AST and detection of MBL-producing GNB were done using VITEK®2 COMPACT (Biomerieux) automated system, Modified Kirby Bauer disc diffusion method and IMP+EDTA CDT phenotypic method, respectively. Gram-negative bacterial growth was detected in 135 (77.1%.) cases with *Escherichia coli* (20.7%), *Klebsiella pneumoniae* (17.8%) and *Burkholderia cepacia* (14.1%) being the most preponderant isolates. Urine yielded more GNB, 45.2% than other samples. The isolates were sensitive to gentamicin (63%), imipenem (54.8%), and ofloxacin (46.7%) but showed high resistance to sulfamethoxazole-trimethoprim (78.5%), ceftriaxone (74.1%) and aztreonam (66.7%). The overall prevalence of MDR was 60% with the highest recorded in University of Uyo Teaching Hospital (UUTH), 64.8%. The overall prevalence of MBL producers was 39.3% with *H. alvei*, *M. morgannii*, *P. mirabilis*, *R. radiobacter* and *P. aeruginosa* being the majority, mostly from urine samples (47.5%) and UUTH health facility (43.7%). All MBL-producing GNB were MDR strains. Seven strains were pan-drug resistant. A combination of robust antibiotic and MBL screening of drug resistant GNB is essential for effective therapeutic decisions. Also, rational use of antibiotics, review of antibiotic usage policies and increased surveillance of MBL-producing GNB is strongly advocated.

Keywords: Metallo-beta-lactamase, Gram-negative bacteria, clinical isolates

INTRODUCTION

Metallo-beta-lactamase (MBL)-producing Gram-negative bacteria constitute a significant public health problem globally, owing to their multi-drug resistance (MDR) capabilities (Hirsch and Tam, 2010). Metallo-beta-lactamases are enzymes that belong to the Ambler's class B group of β-lactamases that have increased potential to hydrolyse a wide variety of beta-lactams, such as Penicillins and their derivatives, Cephalosporins including those with oxyimino side chain, Cephamycins, Oxapenems and Cabapenems (Zhao and Hu, 2011; Zubair and Iregbu, 2018). The production of these enzymes by Gram-negative pathogenic bacteria, especially among those that belong to Enterobacteriaceae family and non-glucose fermenters, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, is of great clinical and epidemiological relevance with respect to antimicrobial chemotherapy and management of Gram-negative infections in hospitals and community settings (Poirel *et al.*, 2000).

Beta-lactam antibiotics are among the most commonly used classes of antimicrobial agents in treatment of infections because of their favourable characteristics such as, broad spectrum of antibacterial activity and their potent lethal effect on growing bacteria (Sauvage *et al.*, 2008; Livermore, 2012). Conversely, many bacteria have evolved defense mechanisms to resist the lethal effects of these drugs (Bush and Fisher, 2011). Due to widespread beta-lactam antimicrobial use, easy purchase, wrong prescription by non-clinicians and self-medications, bacterial resistance has been on the increase and now represents a serious public health threat (Fisher *et al.*, 2005; Babic *et al.*, 2006; CDC, 2015). There are several mechanisms by which pathogenic bacteria acquire resistance to β-lactam drugs. These include efflux or porin loss, reduced permeability, altered transpeptidases by mutation or recombination, and inactivation by β-lactamases (Pfeifer *et al.*, 2011; Hakenbeck *et al.*, 2012).

Several studies on co-carriage of MDR genes by Gram-negative bacteria isolates have been carried out, with increasing reports on development of acquired resistance to a number of clinically useful classes of antimicrobial agents other than beta-lactams, such as Aminoglycosides, Fluoroquinolones, Trimethoprime, Sulfonamides, Tetracyclines and Polymyxins (Hancock, 2000; Sharma *et al.*, 2013, Brooks *et al.*, 2013). The introduction of

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carbapenems into clinical practice represented a great advance for the treatment of infections caused by MBL-producing Gram-negative bacteria. This is because of their broad spectrum of activity and stability to hydrolysis by most beta lactamases (Hemalatha *et al.,* 2005). However, this scenario has changed with the emergence of carbapenem resistant strains especially among Enterobacteriaceae and non-fermenting Gram-negative bacilli (NFGNB) such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Chaudhary *et al.,* 2008).

Although there are various antimicrobial resistance mechanisms, which may be either intrinsic or acquired, the production of MBLs is one of the most important resistance mechanisms by which Gram-negative organisms acquire Carbapenem resistance. Resistance of Gram-negative bacteria to Carbapenems (imipenems, ertapenems and meropenems) due to acquisition of MBL genes results is the emergence of Multidrug resistance (MDR) and Pandrug resistance (PDR) Gram-negative bacterial infections in hospitals and community settings. They are the major cause of nosocomial infections and community-acquired infections, and are closely associated with high mortality, multiple morbidities, prolonged hospitalization and increased cost as a result of limited antimicrobial therapeutic options for infected patients (Bush and Fisher, 2011; CDC, 2015). This problem is even more challenging in developing countries including Nigeria.

There are currently no clinically available inhibitors to block MBL action. Thus, the dissemination of the genes encoding these enzymes remains an important cause of resistance in Gram-negative bacteria and a serious public health concern (Chollom *et al.,* 2012). The genes that code for these resistances are carried on mobile genetic elements such as plasmids, chromosomes and transposons, and have been shown to be either transported by integrons or packaged by gene cassettes (Sharma *et al.,* 2013).

There are multiple types of MBLs due to their genetic variability. Based on amino acid sequence homology, the different types of MBLs that belong to Ambler class B group of beta-lactamases include: The Verona integron-encoded metallo-beta-lactamases (VIM), Imipenemase (IMP), Sau Paulo metallo-beta-lactamase (SPM), Germane Imipenemase (GIM), New Delhi metallo-beta-lactamase (NDM-1), Seoul Imipenemase (SIM) and Dutch Imipenemase (DIM-1) (Tolemann *et al.,* 2002; Sedighi *et al.,* 2014). The MBLs are zinc-based enzymes whose catalytic activity does not proceed via a covalent intermediate but rather through direct attack of a hydroxide ion that is stabilized by zinc in the active site. They are inhibited by metal chelators such as EDTA and thiol compounds (Wang *et al.,* 1999; Crowder *et al.,* 2006).

Metallo beta-lactamase-producing Gram-negative bacteria have been reported as important cause of nosocomial and community-acquired infections including urinary tract infections (UTIs), surgical wound infections, pyogenic infections, cystic fibrosis, septicemia, pneumonia, meningitis, bacteremia, endocarditis, osteomyelitis, pyelonephritis, enterocolotis, puerperal sepsis, peritonitis and soft tissue infection among others (Samuel *et al.,* 2010; Chollom *et al.,* 2012). The prevalence rates of these infections have been reported within and outside the country by many researchers: 11.23% by Ajuba et al. (2020) in Anambra, 8.5% by Oduyebo et al. (2016) in Lagos, 8.6% by Abdullahi et al. (2017) in Kano and 36.1% by Adam and Elhag (2018) in Sudan. Thus, early detection of MBL producing organisms is of great importance to mitigate easy dissemination of infectious resistant phenotypes and also for prompt implementation of effective therapeutic control measures.

Several phenotypic methods are available for the detection of MBL producing bacteria. Currently, the most frequently used tests are the double-disk synergy test using EDTA with Imipenem, the combined disk (CD) assay, the modified Hodge test, the MBL E-test and a microdilution method using EDTA and 1,10-phenanthroline with IMP. All these methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds to inhibit the activity of MBLs. Variation in test results have also been observed depending on the methodology employed, the beta-lactam substrate used, the presence of MBL inhibitors (IMBL), the bacterial genus tested and the local prevalence of MBL types (Yong *et al.,* 2002). This present study was to determine the prevalence of multi-drug resistant MBL-producing Gram-negative bacteria isolated from wound, urine and blood samples of patients attending 3 major health facilities in Akwa Ibom State.

MATERIALS AND METHODS

Study Design/Study Population: This was a descriptive cross-sectional study, carried out during November 2021 – December 2022 at three different hospitals in Akwa Ibom State, Nigeria. A total of 480 patients, comprising in- and out-patients of all gender attending University of Uyo Teaching Hospital, Uyo, Immanuel Hospital, Eket and General Hospital, Ikot Ekpene, all in Akwa Ibom State were recruited in the study after obtaining a written informed consent from them.

Sample Size Determination: The sample size was determined based on a single size estimation using the prevalence rate of 52% that was taken from a study carried out at Bayelsa by Abdu et al. (2019). The calculated sample size was based on the formula adopted by Godden B. (2004): $Ss = Z^2xPx(1-P)/d^2$ where Z is a standard value equivalent to 1.96 at 95% confidence interval, P is the maximum expected prevalence rate and d is the margin sample of error at 95% confidence level. With 10% attrition rate, the total sample size used for the study was 480.

Ethical Approval: Ethical approval was sought and obtained from the Research Ethics committees of University of Uyo Teaching Hospital and Akwa Ibom State Ministry of Health before the study commences.

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Sample Collection and Processing: One hundred and sixty (160) samples each of wound, urine and blood were collected aseptically from eligible patients for the study. Samples were processed following standard bacteriological methods. Blood samples (2mls) were cultured on Brain Heart Infusion (BHI) broth (Oxoid, UK) prepared in sterile blood culture bottles. Bottles showing bacterial growth or turbidity were subcultured on MacConkey agar (Oxoid, UK) for selective isolation and presumptive identification of Gram-negative bacteria. Urine and wound samples were cultured on MacConkey agar and incubated at 37ºC for 24 hours. Purification of isolates was done on Mueller Hinton Agar (Oxoid, UK) following standard procedure.

Biochemical Identification of Gram-negative Bacterial Isolates: All Gram-negative bacterial isolates previously identified by Gram staining were further identified biochemically using GN VITEK®2 cards on a VITEK®2 COMPACT (Biomerieux) automated machine. The GN card is based on established biochemical methods for 47 biochemical tests consisting of 64 test substrates and newly developed substrates for a single isolate.

Antibiotic Susceptibility Test: Isolates prepared in suspension equivalent to 0.5 McFarland turbidity standard were used to inoculated already prepared MHA plates. Standard antibiotics discs (Oxoid, UK) of gentamicin (10µg), imipenem (10µg), augmentin (amoxicillin 20µg/clavulanate 10µg), ciprofloxacin (10µg), cefotaxime (30µg), ceftazidime (30µg), aztreonam (30µg), cefepime (30µg), trimethoprim-sulfamethoxazole (2.5µg), ceftriaxone (30µg) and ofloxacin (5µg) were placed on a 90 mm petri dish in accordance with the Modified Kirby Bauer Disc diffusion method. The CLSI guideline (2021) was used for the interpretation of inhibition zones while *E. coli* (ATCC 29522) strain was used for quality control.

Determination of Multi-drug Resistance (MDR) and Multiple Antibiotic Resistance Index (MARI): An isolate was considered MDR if it resisted more than two different classes of antibiotics tested against it. The MAR indexes of the isolates were calculated as the ratio of number of antibiotics to which an organism is resistant to total number of antibiotics to which the organism has been exposed, denoted by the formula: **a/b** (Saba *et al.*, 2011).

Phenotypic Detection of Metallo-Beta-lactamase (MBL) Production: All Gram-negative isolates resistant to imipenem in the initial screening test were considered potential MBL producers and were further selected for confirmation using Imipenem+EDTA combined disc test (IMP+EDTA CDT) as described previously by Yong *et al.*, (2012). Overnight culture of the test organism was diluted in peptone water to 10^5cfu/ml and inoculated on MHA plate using cotton swab stick. Two imipenem discs (10µg) were placed at a distance of 4-5cm from each other on the plate, and appropriate amounts of 10µl of 0.5M EDTA solution was added to one of them. The diameter of the inhibition zones of the imipenem and Imipenem+EDTA discs were compared after 16-18hrs of incubation aerobically at 35ºC. Isolates with enhancement zone size of more than or equal to 7mm between IMP+EDTA disc compared to IMP disc alone were considered as MBL positive (IMP+EDTA ≥7mm).

Statistical Analysis: The statistical analysis was performed using SPSS software version 25.0 (IBM Corp., USA). Results obtained were presented as descriptive statistics in tables and percentages. Univariate analysis was performed separately for each of the variables. P-values were calculated using the Chi-Square test for categorical variables. Odds ratio (OR) and 95% confidence interval (CI) were calculated for binomial variables at univariate level. A p-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

The distribution of the various bacterial isolates according to their Gram staining reactions is shown in Figure 1. A total of 175 bacterial isolates were obtained giving the overall prevalence distribution of isolated bacterial isolates in the study area to be 36.5%. Of the 175 isolates, 40 (22.9%) were Gram positive bacteria while 135 (77.1%) were Gram negative bacteria.

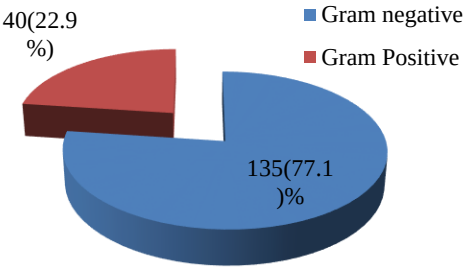

Figure 1: Distribution of Isolated Organisms in the Study area Based on Gram Staining Results

The frequency distribution of Gram-negative bacterial isolates among clinical samples in Akwa Ibom State is indicated in Table 1. The results showed the preponderance of *Escherichia coli*, (20.7%), followed by *Klebsiella pneumoniae*, (17.8%) and *Burkholderia cepacia*, (14.1%) in Akwa Ibom State. Urine samples yielded more Gram-negative bacteria, 61 (45.2%) than blood, 39 (28.9%) and wound, 35 (25.9%) samples.

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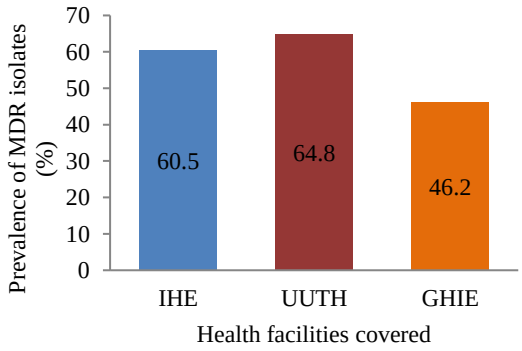
Table 1: Frequency Distribution of Gram-negative Bacterial Isolates Among Clinical Samples in Akwa Ibom State

Isolates	**Urine sample (%)**	**Wound sample (%)**	**Blood sample (%)**	**Total (%)**
Escherichia coli (n=28)	17(60.7)	8(28.6)	3(10.7)	28(20.7)
Serratia fonticola (n=8)	1(12.5)	6(75.0)	1(12.5)	8(5.9)
Serratia ficaria (n=3)	0(0)	0(0)	3(100)	3(2.2)
Klebsiella pneumoniae (n=24)	12(50)	9(37.5)	3(12.5)	24(17.8)
Klebsiella oxytoca (n=3)	3(100)	0(0)	0(0)	3(2.2)
Burkholderia cepacia (n=19)	4(21.1)	7(36.8)	8(42.1)	19(14.1)
Burkholderia stabilis (n=1)	0(0)	0(0)	1(100)	1(0.7)
Proteus mirabilis (n=5)	2(40)	0(0)	3(60)	5(3.7)
Pseudomonas aeruginosa (n=7)	7(100)	0(0)	0(0)	7(5.2)
Enterobacter aerogenes (n=4)	4(100)	0(0)	0(0)	4(3.0)
Enterobacter cloacae dissolvens (n=6)	0(0)	2(33.3)	4(66.7)	6(4.7)
Enterobacter cloacae cloacae (n=1)	0(0)	0(0)	1(100)	1(0.7)
Enterobacter kobei (n=2)	0(0)	0(0)	2(100)	2(1.5)
Enterobacter hormaechei (n=3)	0(0)	3(100)	0(0)	3(2.2)
Enterobacter cloaecae complex (n=7)	0(0)	0(0)	7(100)	7(5.2)
Citrobacter freundi (n=1)	1(100)	0(0)	0(0)	1(0.7)
Rhizobium radiobacter (n=4)	3(75)	0(0)	1(25)	4(3.0)
Acinetobacter baumannii (n=4)	2(50)	0(0)	2(50)	4(3.0)
Morganella morgannii (n=2)	2(100)	0(0)	0(0)	2(1.5)
Hafnia alvei (n=3)	3(100)	0(0)	0(0)	3(2.2)
TOTAL	**61(45.2)**	**35(25.9)**	**39(28.9)**	**135(100)**

The antibiotic susceptibility profile of isolated Gram-negative bacteria to commonly used antibiotics is shown in Table 2. The results revealed gentamicin (63%), imipenem (54.8%), and ofloxacin (46.7%) as the most effective antibiotics for the treatment of infections caused by the Gram-negative bacteria. The most resistant antibiotics are trimethoprim-sulfamethoxazole (78.5%), ceftriaxone (74.1%) and aztreonam (66.7%).

The frequency distribution of MDR Gram-negative bacterial isolates according to health facilities is presented in Figure 3. The results revealed the highest prevalence of MDR Gram-negative bacteria in UUTH to be 46 (64.8%) followed by IHE 23 (60.5%) while the least was recorded in GHIE 12 (46.2%). The overall prevalence of MDR Gram-negative bacteria in Akwa Ibom State was 60%. There was no statistically significant association between the distribution of MDR-Gram-negative bacteria and health facilities ($\chi 2=2.76$; $p=0.252$).



(Key: IHE=Immanuel Hospital, Eket; UUTH=University of Uyo Teaching Hospital; GHIE=General Hospital, Ikot Ekpene)

Figure 3: Frequency Distribution of Multi-drug Resistant (MDR) Gram-negative bacterial Isolates According to Health Facilities

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The distribution of MBL-producing Gram-negative bacterial strain among the isolates in the study area is shown in Table 3. Out of 128 Gram-negative bacterial strains, 53 (39.3%) were MBL producers. All strains of *H. alvei* and *M. morgannii* produced MBL (100%). The percentage of MBL-production was also high among *P. mirabilis* 4 (80%), *R. radiobacter* 3 (75%) and *P. aeruginosa* 4 (66.7%) isolates.

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Table 2: Antibiotic Susceptibility Profile of Gram-negative Bacterial Isolates in the Study Area

Organism	Antibiotic discs (µg/dics)											
	IPM (%)		CIP (%)		SXT (%)		OFX (%)		ATM (%)		CRO (%)	
	S	R	S	R	S	R	S	R	S	R	S	R
E. coli (n=28)	18(64.3)	10(35.7)	4(14.3)	18(64.3)	6(21.4)	22(78.6)	14(50.0)	14(50.0)	10(35.7)	18(64.3)	6(21.4)	22(78.6)
S. fonticola (n=8)	5(62.5)	2(25.0)	3(37.5)	5(62.5)	0(0)	7(87.5)	0(0)	5(62.5)	4(50.0)	4(50.0)	0(0)	8(100)
S. ficaria (n=3)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)	0(0)	3(100)
K. pneumoniae (n=24)	12(50.0)	4(16.7)	6(25.0)	16(64.0)	4(16.7)	18(75.0)	6(25.0)	16(64.0)	12(50.0)	12(50.0)	12(50.0)	10(41.7)
K. oxytoca (n=3)	2(66.7)	1(33.3)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)
B. cepacia(n=19)	10(52.6)	6(31.6)	4(21.1)	15(78.9)	0(0)	12(63.2)	7(36.8)	8(42.1)	6(31.6)	10(52.6)	7(36.8)	12(63.2)
B. stabilis (n=1)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
P. mirabilis (n=5)	0(0)	2(40)	3(60)	2(40)	0(0)	5(100)	3(60)	0(0)	3(60)	2(40)	3(60)	2(40)
P. aeruginosa (n=7)	2(28.6)	2(28.6)	4(57.1)	2(28.6)	0(0)	4(57.1)	5(71.4)	2(28.6)	2(28.6)	2(28.6)	0(0)	7(100)
E. aerogenes (n=4)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)
E. cloacae dissolvens (n=6)	6(100)	0(0)	2(33.3)	2(33.3)	0(0)	6(100)	6(100)	0(0)	0(0)	6(100)	0(0)	4(66.7)
E. cloacae cloacae (n=1)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
E. kobei (n=2)	2(100)	0(0)	0(0)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)	2(100)	0(0)	2(100)
E. hormaechei (n=3)	3(100)	0(0)	3(100)	0(0)	0(0)	3(100)	3(100)	0(0)	0(0)	3(100)	0(0)	3(100)
E. cloaecae complex (n=7)	7(100)	0(0)	2(28.6)	2(28.6)	0(0)	7(100)	7(100)	0(0)	0(0)	7(100)	2(28.6)	5(71.4)
C. freundi (n=1)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)
R. radiobacter (n=4)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)
A. baumannii (n=4)	2(50)	0(0)	0(0)	4(100)	0(0)	2(50)	4(100)	0(0)	0(0)	4(100)	0(0)	4(100)
M. morgannii (n=2)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)
H. alvei (n=3)	0(0)	3(100)	0(0)	3(100)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	0(0)	3(100)	0(0)	3(100)
TOTAL (%)	**74(54.8)**	**41(30.4)**	**31(23.0)**	**84(62.2)**	**11(8.1)**	**106(78.5)**	**63(46.7)**	**61(45.2)**	**38(28.1)**	**90(66.7)**	**31(23.0)**	**100(74.1)**

Key: IPM=Imipenem; SXT=Trimethoprim-sulfamethoxazole; OFX=Ofloxacin; CIP=Ciprofloxacin; CRO=Ceftriaxone; ATM=Aztreonam

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Table 2: Antibiotic Susceptibility Profile of Gram-negative Bacterial Isolates in the Study Area (Contd.)

Organisms	Antibiotic discs (µg/dics)									
	CTX (%)		CN (%)		CAZ (%)		AMC (%)		FEP (%)	
	S	R	S	R	S	R	S	R	S	R
E. coli (n=28)	6(21.4)	20(71.4)	22(78.6)	6(21.4)	10(35.7)	16(57.1)	6(21.4)	16(57.1)	10(35.7)	16(57.1)
S. fonticola (n=8)	4(50.0)	4(50.0)	4(50.0)	4(50.0)	3(37.5)	4(50.0)	2(25.0)	0(0)	3(37.5)	5(62.5)
S. ficaria (n=3)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	0(0)	0(0)	3(100)	0(0)
K. pneumoniae (n=24)	13(54.2)	8(33.3)	14(59.3)	10(41.7)	12(50.0)	12(50.0)	4(16.7)	10(41.7)	12(50.0)	10(41.7)
K. oxytoca (n=3)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)
B. cepacia (n=19)	9(47.4)	10(52.6)	13(68.4)	6(31.6)	6(31.6)	12(63.2)	6(31.6)	8(42.1)	5(26.3)	14(73.7)
B. stabilis (n=1)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)
P. mirabilis (n=5)	3(60)	2(40)	3(60)	2(40)	3(60)	0(0)	3(60)	2(40)	3(60)	2(40)
P. aeruginosa (n=7)	3(42.9)	4(57.1)	5(71.4)	2(28.6)	2(28.6)	4(57.1)	7(100)	0(0)	2(28.6)	4(57.1)
E. aerogenes (n=4)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)
E. cloacae dissolvens (n=6)	0(0)	6(100)	6(100)	0(0)	2(33.3)	2(33.3)	5(83.3)	0(0)	2(33.3)	4(66.7)
E. cloacae cloacae (n=1)	1(100)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)
E. kobei (n=2)	2(100)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)	0(0)
E. hormaechei (n=3)	0(0)	3(100)	3(100)	0(0)	3(100)	0(0)	0(0)	0(0)	0(0)	3(100)
E. cloaecae complex (n=7)	5(71.4)	2(28.6)	7(100)	0(0)	3(42.9)	4(57.1)	3(42.9)	0(0)	3(42.9)	4(57.1)
C. freundi (n=1)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)
R. radiobacter (n=4)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)
A. baumannii (n=4)	0(0)	4(100)	0(0)	4(100)	0(0)	4(100)	0(0)	0(0)	2(50)	0(0)
M. morgannii (n=2)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)
H. alvei (n=3)	0(0)	3(100)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	0(0)	2(66.7)	1(33.3)	2(66.7)
TOTAL (%)	**50(37.0)**	**80(59.3)**	**85(63.0)**	**49(36.3)**	**49(36.3)**	**77(57.0)**	**38(28.1)**	**52(38.5)**	**51(37.8)**	**77(57.0)**

Key: FEP=Cefepime; AMC=Amoxicillin-clavulanate; CAZ=Ceftazidime; CN=Gentamicin; CTX=Cefotaxime

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Table 3: Distribution of MBL-producing Gram-negative Bacterial Strain in the Study Area

Isolate	No. screened	No. MBL-producer (%)
Escherichia coli	28	13(46.4)
Burkolderia cepacia	19	8(42.1)
Klebsiella pneumoniae	24	5(20.8)
Seratia ficaria	3	0(0.0)
Pseudomonas aeruginosa	7	4(66.7)
Serratia fonticola	8	4(57.1)
Burkelderia stabilis	1	0(0.0)
Enterobacter kobei	2	0(0.0)
Hafnia alvei	3	3(100)
Proteus mirabilis	5	4(80.0)
Citrobacter freundi	1	0(0.0)
Enterobacter cloacae cloacae	1	1(100)
Morganella morgannii	2	2(100)
Enterobacter hormaechei	3	0(0.0)
Acinetobacter baumannii	4	2(50.0)
E. cloacae dissolvens	6	0(0.0)
Enterobacter aerogenes	4	2(50)
Rhizobium radiobacter	4	3(75.0)
Klebsiella oxytoca	3	2(66.7)
Enterobacter cloaecae complex	7	0(0.0)
Total	**135**	**53(39.3)**

Key: MBL = metallo-beta-lactamase

The distribution of MBL-producing Gram-negative bacterial isolates among clinical samples is indicated in Figure 4. Out of 53 MBL-producers, 29 (47.5%) were recovered from urine, 15 (46.9%) from wound and 9 (21.4%) from blood samples.

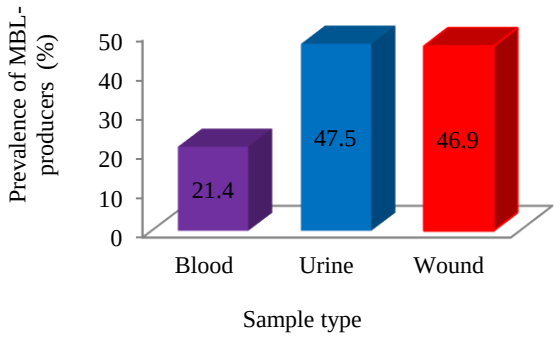

Figure 4: Distribution of Metallo-beta-lactamase (MBL)-producing Clinical Bacterial Isolates Among Clinical Samples

The distribution of MBL-producing Gram-negative bacteria according to health facilities is shown in Table 4. The highest prevalence of MBL-producing Gram-negative bacteria was recorded in UUTH, 31 (43.7%) followed by GHIE 9 (34.6%) and IHE 13 (34.2%). There was no statistically significant association between the prevalence of MBL-producing Gram-negative bacteria and health facilities (p=0.544).

Table 4: Distribution of Metallo-beta-lactamase (MBL)-producing Gram-negative Bacteria According to Health Facilities

Facility	No. screened	No. MBL-producer (%)	Statistical analysis	
			Chi-Square	P-value
UUTH	71	31(43.7)		
GHIE	26	9(34.6)	1.218	0.544
IHE	38	13(34.2)		
Total	**135**	**53 (39.3)**		

Key: UUTH=University of Uyo Teaching Hospital; GHIE=General Hospital, Ikot Ekpene; IHE=Immanuel Hospital, Eket

The multidrug resistant pattern and MAR index of MBL-producing strains in the study area is shown in Table 5. Some of the isolated organisms exhibited similar resistance pattern to some drug combinations, e.g., UR28 (*Pseudomonas aeruginosa*) and W3 (*Serratia fonticola*) had same resistant drug combination (IPM, SXT, OFX, FEP, CIP, CTX, CRO, CAZ, CN). Seven (7) strains: *E. coli*-U2263, *B. cepacia*-U46, *B. cepacia*-B2000, *H. alvei*-U001 from IHE, *E. aerogenes*-U1, *R. radiobacter*-U13 from IHE and *K. oxytoca*-U9 from UUTH were resistant to all the 11 antibiotics tested. In total, 53 strains were MDR having multiple antibiotic resistance index (MARI) ranging from 0.3 to 1 and drug combinations ranging from 3 to 11.

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Table 5: Multidrug resistant pattern and MAR index of MBL-producing strains in the study area

MBL-producers	Strains	Resistant drug combination	No. drug combinations	MARI
P. mirabilis	UR5	IPM,SXT,CRO	3	0.3
B. cepacia	B1961	IPM,CIPATM,CAZ	4	0.4
E. cloacae cloacae	B11	IPM,SXT,CRO,CAZ	4	0.4
P. aeruginosa	U3-IHE	IPM,SXT,FEP,CTX,CRO	5	0.5
S. fonticola	BR002	IPM,SXT,OFX,CIP,CRO	5	0.5
H. alvei	U8-IHE	IPM,CIP,CTX,CRO,ATM	5	0.5
K. pneumonia	W32	IPM,AMC,CRO,ATM,CAZ	5	0.5
E. coli	W30	IPM,AMC,CIP,CTX,CRO,CAZ	6	0.5
E. coli	U2263	IPM,AMC,CTX,CRO,ATM,CAZ	6	0.5
P. aeruginosa, S. fonticola	UR28, W3	IPM,SXT,OFX,FEP,CIP,CTX,CRO,CAZ,CN	9	0.8
P. mirabilis	B1974	IPM,SXT,OFX,FEP,AMC,CIP,CTX,CRO,CN	9	0.8
B. cepacia	W12	IPM,SXT,FEP,AMC,CIP,CTX,CRO,ATM,CN	9	0.8
K. pneumonia	W2-IHE	IPM,SXT,OFX,CIP,CTX,CRO,ATM,CAZ,CN	9	0.8
M. morgannii	U2-IHE	IPM,SXT,OFX,FEP,CIPCTX,CRO,ATM,CAZ,CN	10	0.9
K. pneumonia	W2315	IPM,SXT,OFX,AMC,CIP,CTX,CRO,ATM,CAZ,CN	10	0.9
E. coli	U48	IPM,SXT,OFX,FEP,AMC,CIP,CTX,CRO,ATM,CAZ	10	0.9
E. coli, B. cepacia, B. cepacia, H. alvei, E. aerogenes, R. radiobacter, K. oxytoca	U2263, U46, B2000, U001-IHE, U1, U13-IHE, U9	IPM,SXT,OFX,FEP,AMC,CIP,CTX,CRO,ATM,CAZ,CN	11	1

Key: IPM=Imipenem; SXT=Trimethoprim-sulfamethoxazole; OFX=Ofloxacin; FEP=Cefepime; AMC=Amoxicillin-clavulanate; CIP=Ciprofloxacin; CTX=Cefotaxime; CRO=Ceftriaxone; ATM=Aztreonam; CAZ=Ceftazidime; CN=Gentamicin; MBL=metallo beta-lactamase, MARI=Multiple Antibiotic Resistance Index

The prevalence of multi-drug resistant Gram-negative bacteria in relation to metallo-beta-lactamase production in the study area is presented in Table 6. The result shows that 100% of MBL-producers and 35.4% of non-MBL-producers were MDR.

Table 6: Prevalence of MDR Gram-negative Bacteria in Relation to MBL Production

Phenotypic characteristic	No. of isolates	No. MDR (%)
MBL	53	53(100)
Non-MBL	82	29(35.4)
Total	**135**	**82(60.7)**

DISCUSSION

The increase in Gram-negative broad-spectrum antibiotic resistance is of grave public health significance, especially as there are few available antibiotics against metallo-beta-lactamase producers. Metallo-beta-lactamases (MBLs) are zinc-based enzymes that mediate resistance to virtually all classes of beta-lactam antibiotics, including carbapenems. To curtail the spread of MDR phenotypes and to augment treatment outcomes, early detection of MBL-producing Gram-negative bacteria is imperative. In this study, out of the 480 clinical samples cultured, 175 (36.5%) yielded significant bacterial growth, of which 22.9% were Gram-positive while 77.1% were Gram-negative bacteria. This result is dissimilar to that obtained from a recent study in Sentinel health care facilities in Lagos State by Chukwu *et al*. (2022), in which 51.7% Gram-positive and 48.3% Gram-negative bacteria were reported but close to 65% Gram-negative isolates and 35% Gram-positive isolates gotten from clinical samples in a tertiary care hospital in India (Divyashanthi *et al*., 2014). The observed variation in the proportion of isolated bacteria could be due to differences in the isolation methods and sample collection techniques which could affect bacteria yields.

The percentage occurrence of Gram-negative bacteria was highest in urine, 45.2% compared to other clinical samples. The most commonly isolated uropathogens were *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Blood samples yielded 28.9% of Gram-negative bacteria of which *Burkholderia cepacia*, *Enterobacter cloacae complex* and *Enterobacter cloacae dissolvens* were most commonly isolated, while the most preponderant isolates in wound cultures were *K. pneumoniae*, *E. coli* and *Serratia fonticola* with a prevalence of 25.9%. This is consistent with the report of Onwuezobe et al. (2019) in Akwa Ibom State, same study area, in which *E. coli* and *K. pneumoniae* were the most prevalent uropathogens. However, previously reported prevalent blood and wound pathogens by other researchers greatly differ. Abdu et al. (2020) reported *K. pneumoniae* and *S. marcescens* as the most frequently isolated blood pathogens in Yenagoa while Pondei et al. (2013) reported more of *P. aeruginosa* and coliforms (except *E. coli*) from wound at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria. Mwakalinga *et al.* (2022) in Tanzania reported *Pseudomonas aeruginosa* and *E. coli* as the most prevalent wound pathogens while Wangoye (2022) in Uganda reported the preponderance of *Klebsiella spp*. and *Proteus spp.* in wound. These observed differences may depict

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evidence of local and regional variability in the rate of bacterial infections indicating the prevalent bacteria in the study areas.

Antibiotic susceptibility testing was performed for the most commonly encountered clinical isolates in this study. The essence of this was to determine which antibiotics regimen is effective for the treatment of infections caused by these pathogens and to reduce the incidence of acquired antibiotics resistance in both community and hospital settings. The overall resistance profile of the isolates in this study revealed two definitive antibiotic susceptibility patterns: a very high resistance pattern for trimethoprim-sulfamethoxazole (78.5%), ceftriaxone (71.4%) and aztreonam (66.7%) as against high susceptibility pattern exhibited by gentamicin (63%), imipenem (54.8%) and ofloxacin (46.7%). This is consistent with findings from recent reports in the study area clearly depicting the epidemiology of bacteria pathogens and type of sensitive antobiotics that can presumptuously be used to treat similar infections (Onwuezobe and Etang, 2018 and Umo *et al.,* 2021). The high resistance pattern observed with trimethoprim-sulfamethoxazole appears to be a common pattern in Africa (particularly in Nigeria) and Europe as previously suggested (Rodriquez *et al.,* 2003; Mendes *et al.,* 2005). This may be due to the fact that the drug is one of the commonest over-the-counter drugs obtained with or without clinician's prescription for a wide variety of infections such as UTIs, GITs and respiratory infections. The same reason could be attributed to ceftriaxone, a third-generation cephalosporin (Okon *et al.,* 2014). Aztreonam is a monobactam which is indicated for use against beta-lactamase-producing Gram-negative bacteria due to its stability against both serine and zinc-based beta-lactamases. The resistance of most isolated organisms to aztreonam in this study is worrisome, reflecting its possible silent abuse therefore throwing up the need to institute effective antimicrobial surveillance system in Akwa Ibom State and environs. The high sensitivity of the aminoglycoside (gentamicin), the carbapenem (imipenem) and the fluoroquinolone (ofloxacin) in this study corroborates the findings of Umo *et al*. (2021) in the same study area, Okon *et al*. (2014) in Maiduguri and Hadadi *et al*. (2008) in Iran. The high sensitivity rates offered by these drugs signify that they could serve as alternative therapeutic options for treatment of infections caused by multidrug resistant Gram-negative bacteria.

The production of MBLs among Gram-negative constitutes a major challenge in the selection of appropriate antibiotics for the treatment of individual patients and antibiotics formulation policies. The prevalence of MBL-producing Gram-negative bacteria in this study was found to be 39.3%. This result is higher than that reported by Oduyebo *et al*. (2016) in Lagos (8.5%) and Abdullahi et al. (2017) in Kano (8.6%). The highest MBL producers were *Escherichia coli*, *Burkholderia cepacia*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia fonticola* and *Proteus mirabilis*. This correlates with the work of Abdullahi and colleagues (2017) in Kano, in which the highest MBL production was found among *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis* isolates. A study in India by Gupta and Farooq (2018) reported that majority of MBL-producing bacteria were *E. coli* and *K. pneumoniae.* Different results have been reported by other workers. For instance, Wadekar *et al*. (2013) revealed maximum MBL production in *Klebsiella spp.* and *Enterobacter spp.*, which was quite different from the present study. The differences in prevalence rates may be due to some specifics attributable to settings where studies were conducted as well as phenotypic methods of detection but worthy to be noted.

In this study, majority of MBL-producing Gram-negative bacteria were recovered from urine, 47.5% and wound samples, 46.9% by IMP+EDTA CDT phenotypic method and more predominant in clinical samples from UUTH, 43.7% than other health facilities studied. Nevertheless, no statistically significant association existed between the distribution of MBL-producers in clinical isolates (p=0.554) even as blood samples had the least MBL-producers contrary to the report by Gupta and Farooq (2018), in which the highest incidence of MBL producing isolates was reported in blood and lowest in urine samples. These findings have significant implications for empirical management of patients with Gram negative bacterial infections in this environment using carbapenems.

Multi-drug resistance among Gram-negative bacteria and MBL–producers highlight an overwhelming health and economic burden especially in low-resource settings as infection with MDR reduces the therapeutic options resulting to increased medical bill and period of stay in hospitals as well as morbidity and mortality rates in both community and hospital settings (Jit *et al.,* 2020). This study revealed a high prevalence of MDR-producing Gram-negative bacteria in UUTH, 64.8% and IHE, 60.5%, and is in agreement with recent report by Umo *et al.* (2021) in which 68.9% prevalence of MDR Gram-negative bacteria was documented in UUTH. The result is comparatively higher than that obtained in previous studies by Siwakoti *et al*. (2018) in Nepal, Gharavi *et al.* (2021) and Abdelaziz *et al*. (2021). The reason for this high prevalence of MDR in the Gram-negative bacterial isolates is not far-fetched as it may be due to irrational use of antibiotics, over-the-counter availability of drugs, self-prescription and lack of clinical microbiology laboratories to conduct antibiotic susceptibility testing before antibiotics is prescribed by physicians. Also, higher rates of MDR observed in UUTH may be due to it being a tertiary health facility where serious cases of illnesses are referred to after patients have been previously administered antibiotics compared to the general hospital (secondary healthcare facility) settings.

In this study, 60% of MDR Gram-negative bacterial isolates harbouring MBLs had multiple antibiotics resistance (MAR) index > 0.2, indicating their sources of contamination. Multiple antibiotic resistance (MAR) index is a measure of the extent of the isolates' resistance to antibiotics within the group of antibiotics used for that study. It has been reported that an isolate with a MAR index of 0.2 and above indicates

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high risk sources of contamination which is mostly associated with the human fecal source (Saba *et al.,* 2011). According to Thenmozhi *et al*. (2014), MAR index values > 0.2 indicate a very high risk source of contamination with frequent use of antibiotics while MAR index values of ≤ 0.2 indicates that the source of the bacterial isolate is more likely associated with infrequent antibiotic usage. The high MAR indices obtained in this study (0.3-1) with drug combinations ranging from 3 to 11 by MBL-producing isolates are worrisome, especially the pan-resistant Gram-negative strains. Another striking result obtained in this study that was of much interest was the observation of a PDR strain, *Burkholderia cepacia* B2000 from blood sample of a diabetic patient at UUTH showing complete resistance to imipenem+EDTA CDT. This report might just be a pointer to a bigger problem as it relates to circulation of superbug in our environment. The situation reports are furthered compounded by lack of basic facilities necessary for routine definitive isolation and identification of these dangerous bacterial pathogens, a common norm associated with resource-limited laboratories. Epidemiological data based on antibiotic resistant pattern generated by local hospital laboratories on these bacterial pathogens can go a long way to provide baseline information to guide physicians (Okon *et al.,* 2014). This calls for increased surveillance and application of remedial measures. Hence, there is need to re-emphasize that antibiotic prescriptions must strictly follow antibiotic sensitivity patterns to guide therapy options for improved patient outcome.

CONCLUSION

A prevalence of 39.3% and 60% was observed for MBL production and multidrug resistant Gram-negative bacteria in Akwa Ibom State. All isolated strains of *H. alvei* and *M. morgannii* produced MBL (100%), including *P. mirabilis*, (80%), *R. radiobacter*, (75%) and *P. aeruginosa*, (66.7%). Majority of the isolates that exhibited pandrug resistance (PDR) were from urine: *E. coli*, *B. cepacia*, *H. alvei*, *E. aerogenes* (urine), *R. radiobacter* U13-IHE and *K. oxytoca*, and *B. cepacia* from blood. Gram-negative bacterial isolates that produced MBLs were highly resistant to antibacterial agents. Cautious use of antibiotics in therapeutic management of MBL-producing Gram-negative bacterial infections in both hospital and community settings as well as a large scale surveillance study of these strains are strongly advocated.

REFERENCES

Abdelaziz, S. M., Aboshanab, K. M., Yahia, I. S., Yassien, M. A. and Hassouna, N. A. J. A. (2021). Correlation between the Antibiotic Resistance Genes and Susceptibility to Antibiotics among the Carbapenem-Resistant Gram-Negative Pathogens. *Antibiotics,* 10, 255.

Abdu, B. A., Egbagba, J. and Alade, T. (2020). Antimicrobial Susceptibility Pattern and Bacterial Isolates Profile in Septicaemia Suspected Patients Attending FMC. Yenagoa. *International Journal of Research and Scientific Innovation,* 7(4):134-143.

Abdullahi, S. A., Arzai, A. H., Yusuf, I., Adamu, S. M., Adamu, S., Koki, Y. A., Rabi'u, M. A. and Abbas, A. M. (2017). Molecular Detection of New Delhi Metallo-beta-lactamase 1 (NDM-1)-producing Bacterial Isolates in Kano- Northwestern Nigeria. *Annual Research and Review in Biology,* 14(4): 1-6.

Adam, M. A. and Elhag, W. I. (2018). Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. *BMC Infectious Disease,* 18:668.

Ajuba, I. M., Akujobi, C. N., Aghanya, I. N., Ushie, S. N., Okoro, A. E., Ofiaeli, R. O., Ezeama, C. J., Egbuniwe, M. C. and Okeke, M. O. (2020). Emergence of New-Delhi Metallo-beta-lactamase-1 and Oxacillinase-48 positive *Escherichia coli* in South-Eastern Nigeria. *Nigerian Journal of Medicine,* 29, 701-706.

Babic, M., Hujer, A. M. and Bonomo, R. A. (2006). What's New in Antibiotic Resistance? Focus on Beta-lactamases. *Drug Resistance Updates,* 9:142–156.

Brooks, G. F., Carrol, K. C., Butel, J. S., Morse, S. A. and Mietzner, T. A. (2013). *Jawetz, Melnick and Adelberg's Medical Microbiology.* 26th edition. McGraw Hill Companies Incorporated, USA, pp 378-800.

Bush, K. and Fisher, J. F. (2011). Epidemiological Expansion, Structural Studies, and Clinical Challenges of New β-lactamases from Gram-negative Bacteria. *Annals of Review in Microbiology*, 65:455–478.

Centers for Disease Control and Prevention (2015). About Antimicrobial Resistance. Available at: www.cdc.gov. Retrieved on April 12, 2023.

Chaudhary, U., Bhaskar, H. and Sharma, M. (2008). Imipenem-EDTA Disk Method for Rapid Identification of Metallo-β-lactamase Producing Gram-negative Bacteria. *Indian Journal of Medical Research*, 127:406-7.

Chikere, C. B., Chikere, B. O. and Omoni, V. T. (2008). Antibiogram of Clinical Isolates from a Hospital in Nigeria. Africa*n Journal of Biotechnology,* 7(24): 4359-4363.

Chollom, S. C., Agada, G. O., Gotep, J. G., Mwankon, S. E. and Okwori, A. J. (2012). Short communication: Bacteriological profile of infected surgical sites in Jos, Nigeria. *Malaysian Journal of Microbiology,* 8:285-8.

Chukwu, E. E., Awoderu, O. B., Enwuru, C. A., Afocha, E. E., Lawal, R. G., Ahmed, R. A., Olanrewaju, I., Onwuamah, C. K., Audu, R. A. and Ogunsola, F. T. (2022). High Prevalence of Resistance to Third-generation Cephalosporins Detected Among Clinical Isolates from Sentinel Healthcare Facilities in Lagos, Nigeria. *Antimicrobial Resistance and Infection Control,* 11 (134): 1-11.

Crowder, M. W., Spencer, J. and Vila, A. J. (2006). Metallo-β-lactmases: Novel Weaponry for Antibiotic Resistance in Bacteria. *Account of Chemical Research,* 39:721–728.

Divyashanthi, C. M., Adithiyakumar, S. and Bharathi, N. (2014). Study of Prevalence and Antimicrobial

Susceptibility Pattern of Bacterial Isolates in a Tertiary Care Hospital. *International Journal of Pharmacy and Pharmaceutical Science,* 7(1): 185-190.

Fisher, J. F., Meroueh, S. O. and Mobashery, S. (2005). Bacterial Resistance to Beta-lactam Antibiotics: Compelling Opportusism, Compelling Opportunity. *Chemistry Review*, 105:395–424.

Gharavi, M. J., Zarei, J., Roshani-Asl, P., Yazdanyar, Z., Sharif, M. and Rashid, N. (2021). Comprehebsive Study of Antimicrobial Susceptibility Pattern and Extended Spectrum Beta-lactamase (ESBL) Prevalence in Bacteria Isolated from Urine Samples. *Scientific Reports*, 11(1): 578.

Godden, B. (2004). Sample Size Calculation. Available at: http://www.williamgodden.com/samplesizeformula.pdf . Retrieved on April 11, 2023.

Gupta, D. and Farooq, U. (2018). Phenotypic Detection of Extended Spectrum Beta-lactamases and Metallo-beta-lactamases in Various Clinical Isolates of Enterobacteriaceae. *Scholars Journal of Applied Medical Sciences*, 6(2): 457 – 465.

Hadadi, A., Rasoulinejad, M., Maleki, Z., Yonesian, M., Shirani, A. and Kourorian, Z. (2008). Antimicrobial Resistance of Gram-negative Bacilli of Nosocomial Origin at 2 University Hospital in Iran. *Diagnostic Microbiology and Infectious Disease*, 60:301-305.

Hakenbeck, R., Bruckner, R., Denapaite, D. and Maurer, P. (2012). Molecular Mechanisms of β-lactam Resistance in *Streptococcus pneumoniae*. *Future Microbiology*, 7:395–410.

Hancock, R. E. and Speert, D. P. (2000). Antibiotic Resistance in *Pseudomonas aeruginosa*: Mechanism and Impact on Treatment. *Drug Resistance Update*, 3: 247-255.

Hemalatha, V., Sekar, U. and Kamat, V. (2005). Detection of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* in Hospitalized Patients. *Indian Journal of Medical Research*, 122:148-52.

Hirsch, E. B. and Tam, V. H. (2010). Detection and Treatment Options for *Klebsiella pneumoniae* carbapenemases (KPCs): An Emerging Cause of Multidrug-resistant Infection. *Journal of Antimicrobial Chemotherapy*, 65(6):1119-25.

Jit, M., Ng, D. H. L., Luangasanatip, N., Sandmann, F., Atkins, K. E., Robotham, J. V. and Pouwels, K. B. (2020). Quantifying the Economic Cost of Antibiotic Resistance and the Impact of Related Interventions: Rapid Methodological Review, Conceptual Framework and Recommendations for Future Studies. *BMC Medical*, 18, 38.

Livermore, D. M., Walsh, T. R., Toleman, M. and Woodford, N. (2012). Balkan NDM-1: escape or transplant? *Lancet of Infectious Disease*, 11:164.

Mendes, C., Oplustil, C., Sakagami, E., Turner, P. and Kiffer, C. (2005). MYSTIC Brazil Group. Antimicrobial susceptibility in intensive care. MYSTIC Program Brazil 2002, Brazil. *Journal of Infectious Disease*, 9:44-51.

Oduyebo, O. O., Falayi, O. M., Oshun, P. and Ett, A. O. (2016). Phenotypic Determination of Carbapenemase Producing Enterobacteriaceae Isolates from Clinical Specimens at a Tertiary Hospital in Lagos, Nigeria. *Nigerian Postgraduate Medical Journal*, 22(4): 223-227.

Okon, K. O., Balogun, S. T., Askira, U. M., Jibrin, Y. B., Aguoru, C. U., Isyaka, T. M. and Ghamba, P. E. (2014). Retrospective Analysis of Gram-Negative Bacteria Isolated at a Tertiary Hospital in Maiduguri, Nigeria. *British Microbiology Research Journal*, 4(11): 1235-1247.

Onwuezobe, I. A. and Etang, U. E. (2018). Current Antibiotic Resistance Trends of Uropathogens from Outpatients in a Nigerian Urban Health Care Facility. *International Journal of Healthcare and Medical Sciences*, 4(6): 99-104.

Onwuezobe, I. A., Ubong, E. E. and Ekuma, A. E. (2019). Correlating Urinary Tract Infection with Patients' Presenting Symptoms and Bacterial Isolation from Urine in Uyo, Nigeria. *Science Journal of Clinical Medicine*, 8(3): 21-27.

Pfeifer, Y., Wilharm, G. and Zander, E. (2011). Molecular Characterization of blaNDM-1 in an *Acinetobacter baumannii* Strain Isolated in Germany in 2007. *Journal of Antimicrobial Chemotherapy*, 66(9): 1998–2001.

Poirel, L., Le Thomas, I., Naas, T., Karim, A. and Nordmann, P. (2000). Biochemical sequence analysis of GES-1, a novel class A extended-spectrum beta-lactamase, and the class 1 integron *In* 52 from *Klebsiella pneumoniae*. *Antimicrobial Agents Chemother*apy, 44 (3): 622-632.

Pondei, K., Fente, B. G. and Oladapo, O. (2013). Current Microbial Isolates from Wound TMH Swabs, Their Culture and Sensitivity Pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria. *Tropical Medicine and Health*, 41(2): 49-53.

Rodriguez, C. H., Juarez, J., De Mier, C., Pugliese, L., Blanco, G. and Vay, C. (2003). Bacterial Resistance to Antibiotics in Gram-negative Rods Isolated from Intensive Care Units. Comparative Analysis between two Periods (1998 and 2001). *Medicina*, 63, 21-27.

Saba, R., Muhammad, F. and Shahida, H. (2011). Antibiotic Susceptibility Pattern and Multiple Antibiotic Resistances (MAR) Calculation of Extended Spectrum Beta-lactamse (ESBL) Producing *Escherichia coli* and *Klebsiella species* in Pakistan. *African Journal of Biotechnology*, 10(33): 6325-6331.

Samuel , S. O., Kayode, O. O., Musa, O. I., Nwigwe, G. C., Aboderin, A. O. and Salami, T. A. (2010). Nosocomial Infections and the Challenges of Control in Developing Countries. *African Journal of Clinical and Experimental Microbiology*, 11:102-10

Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A. and Charlier, P. (2008). The penicillin-binding proteins: structure and roled in peptidoglycan biosynthesis. *FEMS Microbiology Review*, 32:234–258.

Sedighi, M., Salehi-Abargouei, A., Oryan, G. and Faghri, J. (2014). Epidemiology of VIM-1-imipenem Resistant

Pseudomonas aeruginosa in Iran: A systematic Review and Meta-analysis. *Journal of Research in Medical Science*, 19 (9): 899-903.

Sharma, M., Pathak, S. and Srivastava, P. (2013). Prevalence and Antibiogram of Extended-spectrum Beta-lactamase Producing Gram-negative Bacteria and Further Molecular Characterization of β-lactamase-producing *Escherichia coli* and *Klebsiella spp. Journal of Clinical and Diagnostic Research*, 7 (10): 2173-2177.

Siwakoti, S., Subedi, A., Sharma, A., Baral, R., Bhattarai, N. R. and Khanal, B. (2018). Incidence and Outcomes of Multidrug-Resistant Gram-negative Bacteria Infections in Intensive Care Unit from Nepal- a Prospective Cohort Study. *Antimicrobial Resistance and Infection Control*. 7, 114.

Thenmozhi, S., Rajeswari, P., Suresh, K. T., Saipriyanga, V. and Kalpan, M. (2014). Multi-drug Resistant Patterns of Biofilm Forming *Aeromonas hydrophila* from Urine Samples. *International Journal of Pharmaceutical Science and Research*, 5(7): 2908-2918.

Toleman, M. A., Simm, A. M. and Murphy, T. A. (2002). Molecular Characterization of SPM-1, a Novel Metallo-β-lactamase Isolated in Latin America: Report from the SENTRY Antimicrobial Surveillance Programme. *Journal of Antimicrobial Agents and Chemotherapy*, 50(5): 673–679.

Umo, A. N., Etang, U. E., Ama, V. O. and Moses, A. E. (2021). Phenotypic Detection of Multi-drug Resistant Extended spectrum Beta-lactamase-producing Gram-negative Clinical Bacteria in Health Care Facilities in Akwa Ibom State, Nigeria. *World Journal of Applied Science and Technology*, 13(1): 114-123.

Wadekar, M. D., Anuradha, K. and Venkatesha, D. (2013). Phenotypic Detection of ESBL and MBL in Clinical Isolates of Enterobacteriaceae. *International Journal of Current Research and Academic Review*, 1(3):8 9 -95.

Wang, Z., Fast, W., Valentine, A. M. and Benkovic, S. J. (1999). Metallo-β-lactamase: Structure and Mechanism. *Current Opinion in Chemistry and Biology*, 3:614–622.

Wangoye, K., Mwesigye, J., Tungotyo, M. and Samba, S. T. (2022). Chronic Wound Isolates and their Minimum Inhibitory Concentrations Against Third Generation Cephalosporins at a Tertiary Hospital in Uganda. *Scientific Reports*, 12:1195-1205.

Yong, D., Lee, K., Yum, J. H., Shin, H. B., Rossolini, G. M., Chong, Y. (2002). Imipenem‐EDTA Disk Method for Differentiation of Metallo‐β‐lactamase‐producing Clinical Isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *Journal of Clinical Microbiology*, 40:3798‐801.

Zhao, W. H. and Hu, Z. Q. (2011). Epidemiology and Genetics of VIM-type Metallo-β-lactamases in Gram-negative Bacilli. *Future Microbiology*, 6(3): 317–333.

Zubair, K. O. and Iregbu, K. C. (2018). Resistance Pattern and Detection of Metallo-beta-lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital. *Niger Journal of Clinical Practice*, 21, 176-182.