Prevalence of Metallo-β-lactamase (\textit{bla} IMP, \textit{bla} VIM, and \textit{bla} NDM) Genes among Clinical Isolates from Two Tertiary Hospitals in Yenagoa, Bayelsa State, Nigeria

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Abstract

Background and Objective. One of the major clinical problems regarding β-lactam antibiotics resistance is attributed to metallo-beta-lactamases (MBL), which are group of enzymes that is a subset of beta lactamases belonging to group B of Ambler classification that causes hydrolysis of carbapenems. The study was conducted to verify the prevalence of Metallo β-lactamase (MBL); Active on imipenem (\textit{imp})\textsuperscript{1}, Verona Integron-Mediated Metallo-β-lactamase(\textit{vim}), and the New Delhi Metallo-β-lactamase(\textit{ndm}) among Gram-negative clinical isolates in two tertiary hospitals.

Method. 153 clinical specimens were collected from Urine, Wound swabs, Endocervical swabs, High vaginal swabs, Throat swabs, Eye swab and Ear swabs. Antimicrobial susceptibility testing was performed by conventional disk diffusion method and MBL-phenotypic detection was uncovered by standard bacteriological techniques, the MBL genes were amplified using pre-determined conditions set on an AB19700 Applied Biosystem thermal cycler.

Results. Bacterial isolates (n = 99) were isolated from the clinical samples, of which 25 (25.3%), 14 (14.1%), 3 (3%), 37 (37.4%), 17 (17.2%) and 3 (3%) were \textit{E. coli}, \textit{Klebsiella spp}, \textit{Proteus spp}, \textit{P. aeruginosa}, \textit{S. aureus} and \textit{Streptococcus spp}, isolates respectively. The susceptibility pattern of the Gram negative isolates revealed that resistance were 87.34%, 82.28%, 75.95%, 72.15%, 68.35%, 65.58%, 64.46%, 63.29%, 58.23% and 56.96% to Nalidixic acid, Amoxicillin/clavulanic acid, Co-trimoxazole, Cefuroxim, Ampicillin, Ciprofloxacin, Gentamicin, Pefloxacin, Streptomycin and Ofloxacin respectively. Eight species were identified as MBL producer by Polymerase Chain Reaction (PCR) method. Gene VIM with 6(75%) was the predominant gene, followed by NDM gene with 2(25%) among the positive species. No IMP gene was detected. Four (66.67%) of the VIM were detected among \textit{Pseudomonas aeruginosa}, while 1(16.67%) each were detected among \textit{E. coli} and\textit{Klebsiella spp}.The two (100%) of the NDM were detected among the \textit{klesbiellasp.spp}.

Conclusion. \textit{vim}, \textit{ndm} are present in some of our hospitals isolates and are associated with multiple drug resistance. There is need for proper infectious control measures should be taken in-order to prevent outbreaks of MBL-producing Gram negative bacterial isolate in our environment.

Key words: Metallo-β-lactamase (MBL) genes, Multidrug resistance, Clinical isolates, Gram-negative Bacilli, Yenagoa

I. INTRODUCTION

Because of the uniqueness and specificity of peptidoglycan to bacteria cell wall, β-lactam antibiotics, in terms of selectivity, are among the most frequently used antimicrobial agents and an increasing incidence of resistance to these drugs is of public health concern. The search for newer efficacious drugs led to the discovery and introduction of carbapenems which were found to be potent against all multiple drug resistant (MDR) Gram negative bacteria and in combination with their negligible toxicity to the host. In the recent past, the carbapenems became the golden last resort antibiotics for the treatment of MDR Gram negative bacterial infections. Nevertheless, the development of carbapenem resistance (CR) in \textit{Enterobacteriaceae} is of great concern because apparently there will be no next line of antibiotics to be used against carbapenemase producing (CP) \textit{Enterobacteriaceae}. [1] MDR has left less efficient antibiotics to take care of these expensive hard to treat life threatening infections[2, 3, 4, 5, 6].

Carbapenems act as inhibitors for many β-lactamases by reacting with an active site serine and forming a long-lived acyl-enzyme intermediate[7, 8].

The most common mechanism of resistance is the production of β-lactamases, including enzymes of Ambler classes A, D, and B, with their genes being often associated with mobile genetic elements such as plasmids[9]. Carbapenem resistance caused by acquiring the MBLs is considered to be more serious than other resistance mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics except monobactams[10]. β-Lactamases are classified by various [11, 12, 13, 14], however, the most valuable is that of Bush &
throughout Nigeria will influence decision making among this study. As understanding the current status of CR southern, Nigeria, in particular, Yenagoa, thus the need for available scanty information about CR prevalence in south- other parts of Nigeria[23–27], but in general, there is none, if Studies have reported the existence of CP bacteria spatially in genetic unit such as plasmid[22].

Among other enzymes are group 2 enzymes (including ESBLs, Klebsiella pneumoniae carbapenemase [KPC], and OXA enzymes) are active on a wider range of substrates, extending as broadly as the extended-spectrum cephalosporins and monobactams (ESBL). Metallo-β-lactamases (MBLs) comprise group 3 (Ambler class B); these include IMP (active on imipenem), VIM (Verona integron-encoded MBL), and NDM (New Delhi MBL), among others. MBLs are characterized by the requirement for zinc ions in their active site, which can be useful diagnostically, as chelators like EDTA inhibit MBL activity by binding zinc. MBLs demonstrate broad-spectrum β-lactamase activity, including carbapenemase activity, but are not active against monobactams. This particular characteristic is not often clinically useful, as most MBL producers also produce other β-lactamases that result in monobactam resistance. CP-CRE enzymes are found in groups 2 and 3, and the diversity of β-lactamases resulting in monobactam resistance. CP-CRE enzymes are biologically instructors that codes for the enzyme metallo-beta lactamase, the genes coding for beta-lactamase are generally termed bla, followed by the name of the specific enzyme belonging to a superfamily of enzymes with wide catalytic diversity [21]. For example, blaIMP-type, blaVIM-type, blaSPM-type, and blaNDM-type. MBL genes are located on mobile genetic unit such as plasmid[22].

Studies have reported the existence of CP bacteria spatially in other parts of Nigeria[23–27], but in general, there is none, if available scanty information about CR prevalence in south- southern, Nigeria, in particular, Yenagoa, thus the need for this study. As understanding the current status of CR throughout Nigeria will influence decision making among stakeholders about the rational use of carbapenems. Therefore, this current molecular study of CP bacteria in the Niger Delta region, will be highlighting the carbapenemases genes, CR Knowledge gap and future research interventions to address CR in south-southern, Nigeria.

II. MATERIALS AND METHODS

Study Location

This study was conducted in two tertiary hospitals namely: the Federal Medical Centre (FMC), Yenagoa and Niger Delta University Teaching hospital (NDUTH), Okolobiri, all in Yenagoa Local Government area of Bayelsa state. Yenagoa is geographically located within latitude 4 15 North, 5 23 South, latitude 5 22 West and 6 45 East.

Ethical Clearance

Ethical clearance was obtained from the Ethical committee of the two hospitals mentioned above.

Sample Collection, Isolation and Identification

One hundred and fifty-three (153) clinical specimens namely: urine, high vaginal swab, throat swab, wound swab, eye swab, ear swab and endocervical swabs were collected from consented patients attending the Microbiology Laboratory units of both hospitals. All the samples were transported to the Microbiology Laboratory Unit of Niger Delta University, Wilberforce Island, for bacteriological analysis according to standard guidelines. Samples were aseptically inoculated in Blood agar and MacConkey agar (Oxoid, UK) and incubated aerobically at 37°C for 18-24 h prior to bacterial isolation [28]. Following overnight incubation at 37°C, isolates were sub-cultured for pure isolates. The isolates were identified using microbiological, biochemical methods (in API20E kits) and were also confirmed for MBL genes by PCR.

Susceptibility Testing

Antimicrobial susceptibility test to Ampicillin (AMP:10μg), Ampicillin/oxacillin (APX: 30μg), Amoxicillin (AML: 25μg), Amoxicillin/clavulanic acid (AUG: 30μg), Cefazidime (CEF: 30μg), Cephalexin (CEP: 30μg), Chloramphenicol (CHL: 30μg); Ciprofloxacin (CIP: 5μg), Cotrimoxazole (COT: 25μg), Erythromycin (ERY: 15μg), Gentamicin (GEN: 10μg), Levofloxacin (LEV: 5μg), Nalidixic acid (NAL: 30μg), Norfloxacin (NOR: 5μg), Ofloxacin (OFL: 5μg), Perfloroxacin (PER: 5μg), Rifampicin (RIF: 25μg), and Streptomycin (STR: 10μg)(Oxoid, UK) was performed by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Merck, Germany) based on Clinical Laboratory Standards Institute (CLSI) Guidelines[29]. Escherichia coli ATCC 25922 was used as the quality control strain.

Molecular Analysis of MDL:

Resistant isolates were used to investigate blaVIM, blaIMP, and blaNDM genes by PCR. For DNA extraction, boiling method was applied. First, three to five colonies were extracted from
fresh culture medium and then suspension was prepared using 200 mL of distilled water boiled at 100°C for 10 minutes. Next, it was centrifuged at 14000 rpm for 5 minutes. The supernatant containing DNA was transferred to new Eppendorf tubes for PCR in order to amplify MBL genes, blaVIM, blalMP, and bla-NDM. The following primers, Table 1 (Inqaba Biotech, South Africa) were used to amplify MBL genes [30]. The extracted bacterial DNA was quantified using a table top NanoDrop 1000, with a pre-installed NanoDrop 1000 software connected to a computer system, 2μl of the extracted bacteria DNA was placed on the lower pedestal with the higher pedestal dropped, the purity and the quantity of the extracted DNA was determined.

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer</th>
<th>Sequence (5’ – 3’)</th>
<th>Length of product (bp)</th>
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<tr>
<td>blaVIM</td>
<td>blaVIM</td>
<td>F: CGAATGCGGACCAG/</td>
<td>390</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGGTTTTGGTCGCAAT</td>
<td></td>
</tr>
<tr>
<td>blaIMP</td>
<td>blaIMP</td>
<td>F: GGTITAAACAAACAACACCACCU/</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGAATAGAATGCGTTAACCTC</td>
<td></td>
</tr>
<tr>
<td>blaNDM</td>
<td>blaNDM</td>
<td>F: GGTITGGCCGATCTGGTTTTC/</td>
<td>621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGAATGCGCTCATCAGATC</td>
<td></td>
</tr>
</tbody>
</table>

**Amplification of Metallo Beta- Lactamase Genes**

Amplification was carried out with the following thermal cycling conditions: 5 minutes at 94°C and 36 cycles of amplification consisting of 1 minute at 94°C, 1 minute at 52–56°C, and 1 minute at 72°C, with 5 minutes at 72°C for the final extension. PCR product bands were analyzed after electrophoresis on a 1.5% agarose gel at 120 V for 20 minutes in 1X Tris-boric EDTA containing ethidium bromide and the result was checked under ultraviolet transilluminator.

**Statistical Analysis**

The data were analysed using SPSSversion 23.0 (SPSS, Chicago, IL, USA) with chi-square test and one-way analysis of variance. The differences in data were considered statistically significant at p<0.05.

**III. RESULTS**

Seventy-seven specimens were obtained from the Niger Delta University Teaching Hospital (53.33%) and seventy-six from the Federal Medical Centre (49.67%). Table 2 shows gender-wise distribution of collected specimens. As shown, one-hundred and one (66.01%) were obtained from the female patients, while fifty-two were from their male counterparts (33.99%). Of the 99 bacteria isolates recovered, 79(79.80%) were Gram-negative rods, while 20(20.20%) were Gram-positive cocci. The age range of the patients was 0 to 99 years. As shown in table 3, the isolates were obtained from patients in different age groups: 21–30 years (39.39%) being the predominant. This is closely followed by 31–34 (25.25%), 11-20 years (17.17%), 41-50 years (7.07%), 0–10, 51-60 (3.03%) each. Five (5.05%) isolates were isolated from patients more than sixty years of age. Table 4 reveals bacterial isolates as recovered from each specimen collected. As shown, fifty-five (55.56%) were isolated from urine, 32(32.32%) from wound, 3(3.03) each from Endocervical and throat swabs, while 2 (2.02%) from high vaginal, ear and eye swabs separately. Of the 99 bacterial isolates identified, Pseudomonas aeruginosa was the most common with 37(37.37%), while E. coli, S. aureus, and Klebsiella spp., are with 25(25.25%), 17(17.17%), and 14(14.14%) respectively. Proteus and Streptococcus spps., are the least isolates with 3(3.03%) each.

The antibiotic resistance profile of the seventy-nine-Gram negative bacteria isolated is demonstrated in Figure 1. As shown, the highest patterns of resistance among the isolates to tested antibiotics was to nalidixic acid 69(87.34%), while resistance was 67(84.81%) to ampicillin and amoxicillin each, Amoxicillin/clavulanic acid was 65(82.88%) each. Sixty (75.95%) each to ciprofloxacin and co-trimoxazole. Ceftazidime and cephalexin, 57(72.15%) each, 51(64.56%) to gentamicin, 50 (63.29%) each to norfloxacin and perfloxacin, 46(58.95%) to streptomycin, 45(56.96%) each to levofloxacin and ofloxacin.

PCR was performed on the gram-negative isolates to investigate the prevalence rate of Metallo-beta-Lactamase (blaVIM, blaNDM and blalMP) genes (Figure 2a and b). As shown in Figure 3,8 (10.13%) isolates (E. coli, Klebsiella spp., 3; P. aeruginosa 4) were found to harbour metallo-beta-lactamase genes. Among these isolates, six (7.60%) presenting 75% VIM and two (2.53%) representing 25% NDM genes were found, while none of the IMP genes were present. Among the six VIM genes found, 4(66.67%) were found among P. aeruginosa isolates, while 1 (16.67%) each were among E. coli and Klebsiella isolates. All the two blalNDM were found among Klebsiella spp. Table 5 shows the antibiogram and the source of the isolates harbouring the Metallo-beta-lactamase genes. As shown 4 (66.67%) of the blaVIM-gen genes were from the wound isolates, while the remaining two(33.33%) were urine isolates. The two isolates harbouring blaNMD genes were all isolated from the urine specimens. These eight isolates were resistant against minimum of seven of the studied antibiotics.

**IV. DISCUSSION**

It is an endless task managing infections caused by MDR Enterobacteriaceae, due to their resistance against antimicrobial agents. Diverse antibiotics together with beta
lactams, aminoglycosides and quinolones are worthwhile to treat the infections caused by these groups of bacteria. However, antibiotic resistance pattern of these bacteria is transforming rapidly. The Metallo-β-lactamases (MBL) are carbapenem-hydrolyzing enzymes produced by various Gram-negative bacteria and which gives them astonishing capability to resist the antimicrobial action of carbapenems, thus leading to emerging reports of the development and widespread of multidrug resistance genes amongst pathogenic bacteria in both the community and hospital environments [31, 32, 33, 34, 35]. Studies have shown that the resistance of Gram-negative bacteria against antibiotics oscillates, especially concerning imipenem[36]. Carbapenems and beta lactam antibiotics are considerably used to treat infections due to multidrug-resistant bacteria since they are resistant against most of the beta lactamases and have great membrane permeability[37]. However, in the last decades, alerts about an increase amongst Gram-negative resistance against antimicrobial agents are reported because these organisms have various self-protective ways against these antibiotics.

In this current study, the prevalence of MBL genes amongst our clinical isolates is 10.13% (8 of 79). This is baffling because, carbapenems is not a routine prescriptive drug in our set up. And one wonders “why” and “how” (what source?). Nevertheless, this outcome suggests that resistance due to other β-lactam drugs and isolates that are carbapenems source?). Nevertheless, this outcome suggests that resistance due to other β-lactam drugs and isolates that are carbapenems resistant could also harbour MBL genes [38, 39, 40]. To answer some of our worries, Olowo-okere et al. [41] in 2019 shed some insights by documenting that numerous situations may warrant the isolation of MBL harbouring Enterobacteriaceae among patients with no history of previous exposure to carbapenems. These includes:

1. Cross-resistance between carbapenem and other β-lactam antibiotics which may result in independent emergence of carbapenems resistance Enterobacteriaceae. Hence, the extensive use of Beta-lactam antibiotics (cephalosporins and penicillins) in our hospitals, resistance to which has been well documented [42, 43, 44, 45, 46].

2. Might have been imported into our region by patients returning from other part of the country or countries where MBL harbouring Enterobacteriaceae is endemic. For instance, documentations of importation of antibiotic resistant bacteria across geographical border has been reported [47]. Recently, Leangapiichert et al. [48], revealed high prevalence of antibiotic resistant bacteria at hajj camps which could be traced to be imported by pilgrimage to their respective countries on return.

3. The localisation of most carbapenemase genes on highly ambulatory genetic element may assists the ease of acquisition and transmission of acquired carbapenem resistance among bacterial isolates within a hospital set up[49].

When compared with existing prevalence rates, our report is significantly lower than some existing reports as documented in Romania, India and Egypt by Mereuţă et al. [50], Amudhan et al. [51], Nelly, et al.[52], they reported 43.4%, 51.4% and 52.2% respectively. However, it is higher than the prevalence rate of 2.5% reported in another Nigerian study conducted in Central Nigeria by Zubar and Iregbu[24]. This outcome is a worrying result, which probably might be due to the overwhelming antibiotics (in particular β-lactam and its derivatives) use patterns in our set up. As uncovered by this present study, MBL producers were 21.73%(3 of 25) for Klebsiella spp., 10.81%(4 of 37) for Pseudomonas aeruginosa while only one (4.00%) of 25 were confirmed for all isolated E.coli. These observations were in contrast to earlier documented results were Pseudomonas species was reported as the most frequent MBL producer among Gram negative bacteria isolates [53, 54]. Regarding MBL genes, the gel electrophoresis revealed 390bp for blaIMP, 621bp for blaNDM and 232 for blaIMP together with positive and negative band in Gram negative bacterial isolates, this was in agreement with the documented records[20]. From the present study, two types of MBL genes blaVIM and blaNDM were isolated, while there was no amplification in blaIMP genes. Consequently, making this study a representation of the first report on the prevalence of MBL (blaVIM and blaNDM) encoding genes among Gram-negative isolates in Bayelsa state, Nigeria. This recorded pattern of MBL genes is however dissimilar to previous studies that recovered bla VIM only [24], blaVIM and blaIMP[55] and blaVIM, blaNDM and blaIMP[56]. This outcome however substantiates earlier reports that documented that different areas in the world recorded inconsistency amongst MBL genes due to the variation of geographical circulating strains[38, 40].

Amongst the eight positive MBL genes, Verona integron Metallo-beta-lactamase (blaVIM) was the most frequent gene representing 6 (75%) among positive MBL genes, while blaNDM was the least detected in 2 (25%). This outcome is in conformity with studies done in other African countries. For example, Uganda, Tanzania, Egypt and the Middle East, Iran by Okoche et al. [57], Mushil[58], Zafere[et al.[59] and Aghamiri[39], with higher frequencies of blaVIM against blaNDM (VIM:NDM) as: [21(10.7%):5(2.6%)], [34(15%):9(4%)], [58.3:2.1%] and [70(33%):20(9%)] correspondingly. Nonetheless, our findings were in disagreement with that found in Iraq by Anoar et al.[40] with reports of IMP as the most frequently detected genes 33(18.6%), VIM 19(10.7%), and NDM 2(1.12%), in addition, Chika et al. [55] in a south-eastern Nigerian study detected blaIMP genes 8(36.4%), alone without detecting blaVIM nor blaNDM genes. Current results revealed that there was a significant difference (p<0.05) in the prevalence of MBL production among the isolates recovered from wound and urine as against isolates from other sources. As revealed, MBL producers were of equal proportions amongst wound and urine isolates. All the four Pseudomonas aeruginosa harbouring blaVIM were from the wound source. The
clarification for this could be that *P. aeruginosa* is more prevalent in the hospital environment and are common within the soil which might have originates from the patients or visitors foots to the hospital [40]. Among the urine isolates, MBL producers were 3 (75%) for *Klebsiella* spp., and only one (25%) was uropathogenic *E. coli*. It is worthwhile to note that the two isolates harbouring *bla*<sup>NDM</sup> were uropathogenic *Klebsiella*. All the isolates harbouring MBL genes were resistance to five classes of antibiotics namely: aminoglycosides, β-lactam and β-lactamase inhibitors, fluoroquinolones, and Natidixic acid. While 7(87.5%) were resistance to co-trimoxazole. Thus, by chance, this up to date study revealed the prevalence of MBL in FMC, Yenagoa, is less than reports from other cities. Nonetheless, it should be distinguished that studies in most areas of the world, the prevalence is on the increase and if precautionary measures are not taken, there will be core clinical problems. In addition, studies have revealed that most integronesconfines VIM-1 gene coding aminoglycosides destructing enzymes, thus, intensifying this problem [60]. As a result, diagnostic methods should be routinely used to determine these species in clinical laboratories; also, synthesis of more effective antimicrobial compounds with new effecting mechanisms should be noted.

V. CONCLUSION

Concisely, MBL (*bla*<sup>VIM</sup> and *bla*<sup>NDM</sup>) producing Gram-negative bacteria are present amongst the clinical isolates in our hospital set up. These are factual, despite no history of usage of carbapenems in the set up and their presence pose a danger in our hospital. There is need for further surveillance, as well as the analysis of molecular mechanisms in MDR Gram-negative rods for critical identification and ceasing the spread of these organisms in our most susceptible populations.

We suggest that a targeted surveillance program should be embarked on critically ill and immunocompromised patients who are cared for in our tertiary healthcare settings, since the penalties of infection by MBL-producing Gram-negative bacteria are likely to lead to a disturbing score, thus, making no one to be fortified.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declared that there is none.

AUTHORS CONTRIBUTION

Study concept and design: Abdulrasheed B. Abdu, Tolulope Olukemi Alade, and Oluwayemisi Agnes Olorode; Collection and transportation of specimens: Powei Ayibakeme Joy; acquisition of data: Powei Ayibakeme Joy; analysis and interpretation of data: Abdulrasheed B. Abdu; drafting of the manuscript: Oluwayemisi Agnes Olorode; critical revision of the manuscript for important intellectual content: Abdulrasheed B. Abdu and Tolulope Olukemi Alade; statistical analysis: Abdulrasheed B. Abdu. All authors read and approved final manuscript.

REFERENCES


### FIGURES AND TABLES

#### Table 2. Gender wise Distribution of Specimen collected for the study

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No collected</th>
<th>Male (%)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>79</td>
<td>32 (40.5)</td>
<td>47 (59.5)</td>
</tr>
<tr>
<td>Wound swab</td>
<td>32</td>
<td>13 (40.6)</td>
<td>19 (59.4)</td>
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<tr>
<td>Endocervical swab</td>
<td>15</td>
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<td>15 (100)</td>
</tr>
<tr>
<td>High vaginal swab</td>
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<td>-</td>
<td>13 (100)</td>
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<tr>
<td>Throat swab</td>
<td>7</td>
<td>3 (42.9)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>Ear swab</td>
<td>3</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Eye swab</td>
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<td><strong>Total</strong></td>
<td>153</td>
<td>52 (33.99)</td>
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#### Table 3. Age-wise distribution of isolates from clinical specimens

<table>
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<tr>
<th>Age (Years)</th>
<th>E. coli (%)</th>
<th>P. aeruginosa (%)</th>
<th>Klebsiella spp (%)</th>
<th>Proteus spp (%)</th>
<th>S. aureus (%)</th>
<th>Streptococcus spp (%)</th>
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<td>0 - 10</td>
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<td>-</td>
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<td>2 (66.7)</td>
<td>-</td>
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<td>11 - 20</td>
<td>6 (35.3)</td>
<td>3 (17.6)</td>
<td>-</td>
<td>1 (5.9)</td>
<td>6 (35.3)</td>
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<td>21 - 30</td>
<td>10 (25.6)</td>
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<td>6 (15.4)</td>
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<td>31 - 40</td>
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<td>51 - 60</td>
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#### Table 4: Recovery of Bacterial Isolates by Specimen

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<tr>
<th>Specimen</th>
<th>E. coli</th>
<th>Klebsiella spp</th>
<th>P. aeruginosa</th>
<th>Proteus spp</th>
<th>S. aureus</th>
<th>Streptococcus spp</th>
<th>Total</th>
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<tbody>
<tr>
<td>Urine</td>
<td>25 (45.45)</td>
<td>14 (25.45)</td>
<td>6 (10.91)</td>
<td>2 (3.67)</td>
<td>8 (14.55)</td>
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<td>55 (55.56)</td>
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<tr>
<td>Wound swab</td>
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<td>-</td>
<td>30 (93.75)</td>
<td>1 (3.13)</td>
<td>1 (3.13)</td>
<td>-</td>
<td>32 (32.32)</td>
</tr>
<tr>
<td>Endocervical swab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (100)</td>
<td>-</td>
<td>3 (3.03)</td>
</tr>
<tr>
<td>High vaginal swab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (100)</td>
<td>2 (2.02)</td>
</tr>
<tr>
<td>Throat swab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (100)</td>
<td>3 (3.03)</td>
</tr>
<tr>
<td>Ear swab</td>
<td>-</td>
<td>-</td>
<td>1 (50.00)</td>
<td>-</td>
<td>1 (50.00)</td>
<td>-</td>
<td>2 (2.02)</td>
</tr>
<tr>
<td>Eye swab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (100)</td>
<td>2 (2.02)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25 (25.25)</td>
<td>14 (14.14)</td>
<td>37 (37.37)</td>
<td>3 (3.03)</td>
<td>15 (15.15)</td>
<td>5 (5.05)</td>
<td>99 (100)</td>
</tr>
</tbody>
</table>
Table 5: Antibiogram and source of isolates harbouring MBL genes

<table>
<thead>
<tr>
<th>Bacterial Isolate</th>
<th>Source</th>
<th>Antibiogram</th>
<th>MBL genes</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>VIM  NDM  IMP</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>wound</td>
<td>AMP, AUG, CEP, CIP, GEN, NAL, PER</td>
<td>+ - -</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>wound</td>
<td>AMP, AUG, CEP, CIP, GEN, NAL, OFL, PER, STR</td>
<td>+ - -</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>wound</td>
<td>AMP, AUG, CEP, GEN, NAL, PER</td>
<td>+ - -</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>wound</td>
<td>AMP, AUG, CEP, COT, GEN, NAL, OFL, PER</td>
<td>+ - -</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>urine</td>
<td>AUG, CEP, CIP, COT, NAL, OFL, STR</td>
<td>- + -</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>urine</td>
<td>AMP, AUG, COT, GEN, NAL, OFL, PER</td>
<td>- + -</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>urine</td>
<td>AMP, CEP, CIP, NAL, PER</td>
<td>+ - -</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>urine</td>
<td>AMP, AUG, CIP, COT, GEN, NAL, OFL, PER, STR</td>
<td>+ - -</td>
</tr>
</tbody>
</table>


Figure 1. Antibiotic Resistance Profile of Gram negative bacteria Isolates

Figure 2a: Agarose Gel Electrophoresis showing VIM gene.

Lane 1-10 represent the isolates where 3, 4, 5, 6, 7, 9 were positive while 1, 2, 8 and 10 showed no amplification. Lane L represent the molecular ladder.
Fig 2b: Agarose Gel Electrophoresis showing NDM gene of 691bp.
Lane 1-10 represent the isolates where 1 and 2 was positive while 3, 4, 5, 6, 7, 8, 9 and 10 showed no amplification. Lane L represent the molecular ladder.

Fig 3: Prevalence of Metallo-β-lactamase genes among Gram negative Bacterial Isolates