Prevalence of Metallo-β-lactamase (*bla*IMP, blaVIM, and *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 (*bla*IMP), Verona Integron-Mediated Metallo-β-lactamase(*bla*VIM), and the New Delhi Metallo-β-lactamase(*bla*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 wasperformed by conventional disk diffusion method andMBL-phenotypic detection was uncovered by standard bacteriological techniques, the MBL genes were amplified using pre-determined conditions set on an AB19700 Applied Bio-system 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 *E. coli*, *Klebsiella spp*, *Proteus spp*, *P. aeruginosa*, *S. aureus* and *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, Cefuroxime, Ampicillin, Ciprofloxacin, Gentamicin, Pefloxacin, Streptomycin and Ofloxacin respectively. Eight species were identified as MBL producerby 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 *Pseudomonas aeruginosa*, while 1(16.67%) each were detected among *E. coli* and*Klebsiella spp.*The two (100%) of the NDM were detected among the *klebsiellaspp.*isolates.

Conclusion. blaVIM, blaNDM 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 isolated 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 *Enterobacteriaceae* is of great concern because apparentlythere will be no next line of antibiotics to be use against carbapenemase producing (CP) *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 &

Jacob[15],which specify both their functional and structural profiles.

At present, the high prevalence of carbapenem resistant Enterobacteriaceae (CRE) isolates globally is most significant among *Klebsiellapneumoniae* and *Escherichiacoli* isolates in hospitals, community-associated infections and vets subsequently to a huge burden to the health care system [3, 11;16-19].Ambler class B beta lactamases which are termed as Metallo beta Lactamases consisting of NDM, VIM, IMP, SPM, GIM, SIM, KHM, AIM, DIM, SMB, TMB and FIM [12].IMP, VIM and NDM plasmid mediated Metallo beta lactamases are of worldwide occurrence possibly because the genes that code for them are located on mobile genetic elements [12], carbapenem hydrolyzing class D beta lactamases (CHDLs) encompass various group of oxacillinases (OXA) with hydrolytic activity of amino and carboxy penicillins[20].Genetically, determinants of CR have been classified into: Ambler class A beta lactamases which include; KPC, GES/IBC, SME, NMC-A, IMI and SFC [11 – 14].Enzymes in groups 1 and 2 (Ambler class C or A and D, respectively) contain an active site serine, but group 1 (primarily AmpC enzymes) members demonstrate preferential hydrolysis of cephalosporins whereas 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) and the carbapenems (KPC and OXA-48-like). 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 enzyme types contributes to difficulty in both detection and treatment [15] The Metallo-beta lactamase genes are biological 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, ^{bla}IMP-type^{bla}VIM-type, ^{bla}SPM-type and ^{bla}NDM-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 conductedin two tertiary hospitals namely: theFederal 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^{o}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/cloxacillin (APX: 30µg), Amoxicillin (AML: 25µg), Amoxicillin/clavulanic acid (AUG: 30µg), Ceftazidime (CEF: 30µg), Cephalexin (CEP: 30µg); Chloramphenicol (CHL: 30µg); Ciprofloxacin (CIP: 5µg), Co-trimoxazole (COT: 25µg), Erythromycin (ERY: 15µg), Gentamicin (GEN: 10µg), Levofloxacin (LEV: 5µg), Nalidixic acid (NAL: 30µg), Norfloxacin (NOR: 5µg), Ofloxacin (OFL: 5µg), Perfloxacin (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: ^{bla}VIM, ^{bla}IMP, and ^{bla}NDM Genes by PCR

Resistant isolates were used to investigate ^{bla}VIM, ^{bla}IMP, and ^{bla}NDM 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, blaIMP, 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 1. Primer for *bla*VIM, *bla*IMP and *bla*NDM in this study (Poirel *et al*., 2011). [20]

Target Gene	Primer	Sequence (5' – 3')	Length of product (bp)
blaVIM	blaVIM	F: CGAATGCGCACCAG/ R: TGGTGTTTGGTCGCAAT	390
*bla*IMP	*bla*IMP	F: GTTTAACAAAACAACCACC/ R: GGAATAGAATGGCTTAACTCTC	232
*bla*NDM	*bla*NDM	F: GGTTTGGCGATCTGGTTTTC/ R: CGGAATGGCTCATCACGATC	621

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 andone-way analysis of variance. The differences in data wereconsidered statistically significant at $p<0.05$.

III. RESULTS

Seventy-seven specimens were obtained from the Niger Delta University Teaching Hospital (50.33%) 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 of more than sixty years of age. Table 4 reveals bacterial isolates as recoveredfrom 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 swabsseparately. 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 isdemonstrated 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/cloxacillin and Amoxicillin/clavulanic acid was 65(82.28%) 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 (*bla*VIM, *bla*NDM and blaIMP) genes (Figure 2a and b). As shown in Figure 3,8 (10.13%) isolates (*E. coli*1, 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 geneswere 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 *bla*NDM were found among *Klebsiella* spp. Table 5 shows the antibiogram and the source of the isolates habouring the Metallo-beta-lactamase genes. As shown 4 (66.67%) of the *bla*VIM-genes were from the wound isolates, while the remaining two(33.33%) were urine isolates. The two isolates habouring *bla*NDM 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 ourclinical 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 susceptible could also harbour MBL genes [38, 39, 40]. To answer some of our worries,Olowo-okere et al. [41] in 2019shed some insights by documenting that numerous situations may warrant the isolation of MBL habouring *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 habouring Enterobacteriaceae is endemic. For instance, documentations of importation of antibiotic resistant bacteria across geographical border has been reported [47].Recently, Leangapichart 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 ^{bla}VIM, 621bp for ^{bla}NDM and 232 for ^{bla}IMP 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 ^{bla}VIMand ^{bla} NDM were isolated, while there was no amplification in ^{bla}IMP genes. Consequently, making this study a representation of the first report on the prevalence of MBL (^{bla}VIM and ^{bla}NDM) 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],^{bla}VIM and ^{bla}IMP[55] and ^{bla}VIM, ^{bla}NDM and ^{bla}IMP[56].This outcome howeversubstantiates earlier reports that documented that different areas in the world recorded inconsistencyamongst MBL genes due to the variation of geographical circulating strains[38, 40].

Amongst the eight positive MBL genes, Verona integron Metallo-beta-lactamase (^{bla}VIM) was the most frequent gene representing6 (75%) among positive MBL genes, while ^{bla}NDM 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],Mushi[58],Zafer*et al.*[59] and Aghamiri[39],with higher frequencies of ^{bla}VIM against ^{bla}NDM (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 disagreements 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 studydetected ^{bla}IMP genes 8(36.4%), alone without detecting ^{bla}VIM nor ^{bla}NDM 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* habouring ^{bla}VIM 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 habouring *bla*NDM were uropathogenic *Klebsiella*.All the isolates habouring MBL genes were resistance to five classes of antibiotics namely: aminoglycosides, β-lactam and β-lactamase inhibitors, fluroquinolones, and Nalidixic 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*VIM and *bla*NDM) producing Gram-negative bacteria are present amongst the clinical isolates in our hospitalsset up. These arefactual,despite no historyof 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 rodsfor critical identification and ceasing the spread of these organisms in our most susceptible populations. We suggest that a targeted surveillance program should be embarkedon in 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 befortified.

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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 TolulopeOlukemiAlade; statistical analysis: Abdulrasheed B. Abdu. All authors read and approved final manuscript.

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FIGURES AND TABLES

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

Specimen	No collected	Male (%)	Female (%)
Urine	79	32 (40.5)	47(59.5)
Wound swab	32	13(40.6)	19(59.4)
Endocervical swab	15	-	15(100)
High vaginal swab	13	-	13 (100)
Throat swab	7	3 (42.9)	4 (57.1)
Ear swab	3	2 (66.7)	1(33.3)
Eye swab	4	2(50)	2(50)
Total	153	52(33.99)	101(66.01)

Table 3. Age-wise distribution of isolates from clinical specimens

Age (Years)	*E. coli* (%)	*P. aeruginosa* (%)	*Klebsiella spp* (%)	*Proteus spp*(%)	*S. aureus* (%)	*Streptococcus spp* (%)	Total (%)
0 - 10	-	-	1(33.3)	-	2(66.7)	-	3(3.03)
11 -20	6(35.3)	3(17.6)	-	1(5.9)	6(35.3)	1(5.9)	17(17.17)
21 - 30	10(25.6)	10(25.6)	6(15.4)	2(5.1)	9(23.1)	2(5.1)	39(39.39)
31 - 40	6(24)	15(60)	4(16)	-	-	-	25(25.25)
41 - 50	-	5(71.4)	2(28.6)	-	-	-	7(7.07)
51 - 60	1(33.3)	2(66.7)	-	-	-	-	3(3.03)
61 -70	-	-	1(100)	-	-	-	1(1.01)
71 - 80	-	2(100)	-	-	-	-	2(2.02)
>81	2(100)	-	-	-	-	-	2(2.02)
Total	25(25.25)	37(37.37)	14(14.14)	3(3.03)	17(17.17)	3(3.03)	99(100)

Table 4: Recovery of Bacterial Isolates by Specimen

Specimen	Bacteria isolates						Total
	E. coli	*Klebsiella spp*	*P. aeruginosa*	*Proteus spp*	*S. aureus*	*Streptococcus spp*	
Urine	25(45.45)	14(25.45)	6(10.91)	2(3.67)	8(14.55)	-	55(55.56)
Wound swab	-	-	30(93.75)	1(3.13)	1(3.13)	-	32(32.32)
Endocervical swab	-	-	-	-	3(100)	-	3(3.03)
High vaginal swab	-	-	-	-	2(100)	-	2(2.02)
Throat swab	-	-	-	-	-	3(100)	3(3.03)
Ear swab	-	-	1(50.00)	-	1(50.00)	-	2(2.02)
Eye swab	-	-	-	-	-	2(100)	2(2.02)
Total	25(25.25)	14(14.14)	37(37.37)	3(3.03)	15(15.15)	5(5.05)	99(100)

Table 5: Antibiogram and source of isolates habouring MBL genes

Bacterial Isolate	Source	Antibiogram	MBL genes		
			VIM	NDM	IMP
P. aeruginosa	wound	AMP, AUG, CEP, CIP, GEN, NAL, PER	+	-	-
P. aeruginosa	wound	AMP, AUG, CEP, CIP, COT, GEN, NAL,OFL, PER, STR	+	-	-
P. aeruginosa	wound	AMP, AUG, CEP, COT, GEN, NAL, PER	+	-	-
P. aeruginosa	wound	AMP, AUG, CEP, CIP, COT, GEN, NAL, OFL, PER	+	-	-
Klebsiella spp.	urine	AUG, CEP, CIP, COT, NAL, OFL, STR	-	+	-
Klebsiella spp.	urine	AMP, AUG, COT, GEN, NAL, OFL, PER	-	+	-
Klebsiella spp.	urine	AMP, CEP, CIP, COT, NAL, PER	+	-	-
E. coli	urine	AMP, AUG, CIP, COT, GEN, NAL, OFL, PER, STR	+	-	-

Key: AMP: Ampicillin, AUG: Amoxicillin/clavulanic acid, CEP: Cephalexin, CIP: Ciprofloxacin, COT: Co-trimoxazole, GEN: Gentamicin, NAL: Nalidixic acid, OFL: Ofloxacin, PER: Perfloxacin, STR: Streptomycin, +: Positive, -: Negative.

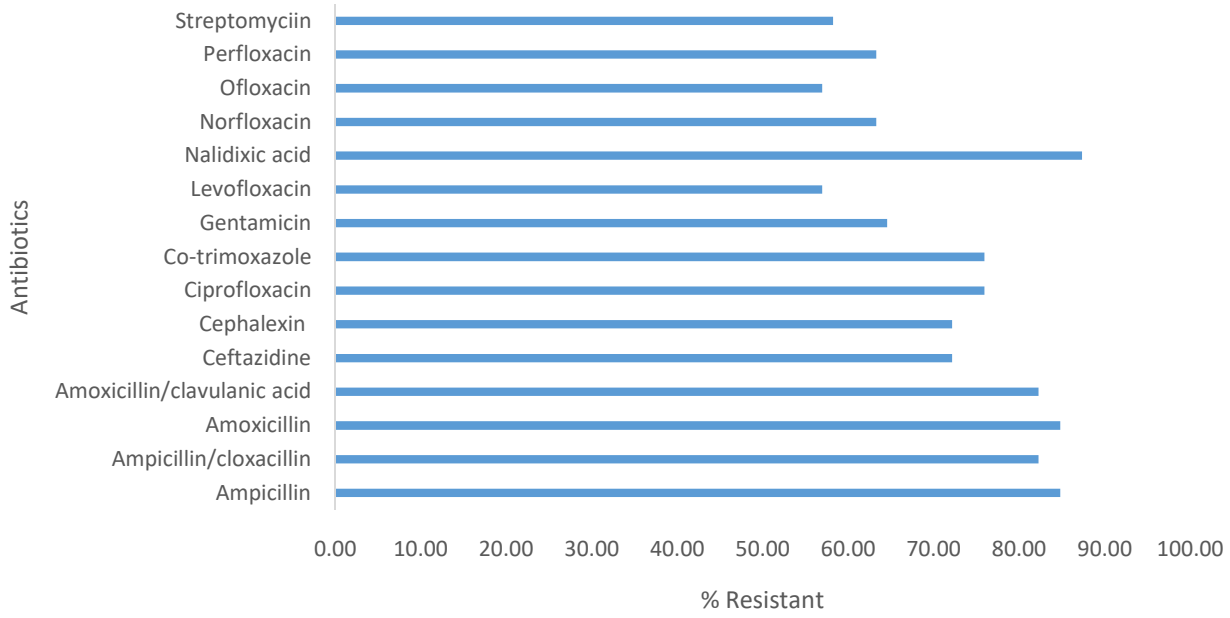

Figure 1. Antibiotic Resistance Profile of Gram negative bacteria Isolates

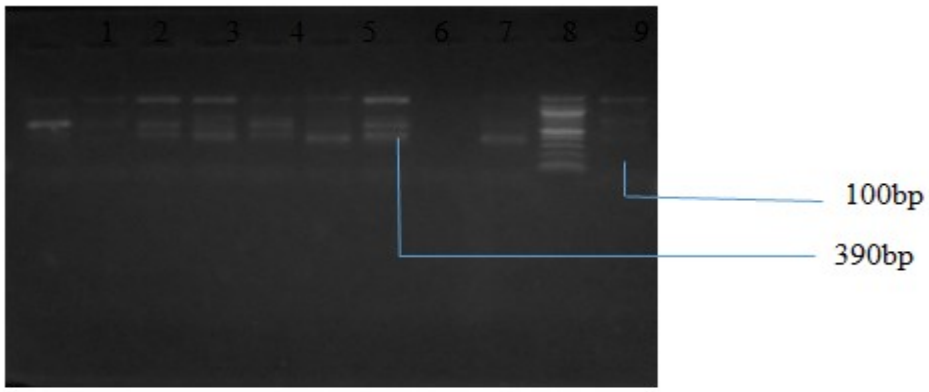

Figure 2a: Agarose Gel Electrophoresis showing VIM gene.

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.

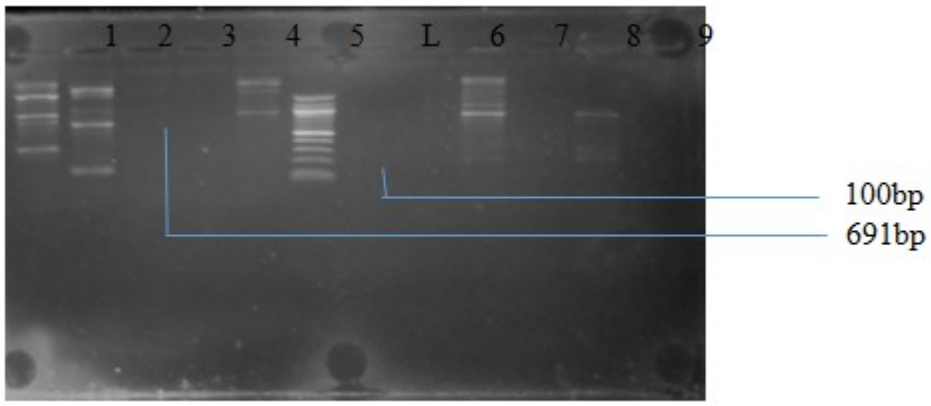

Fig 2b: Agarose Gel Electrophoresis showing NDM gene of 691bp.

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

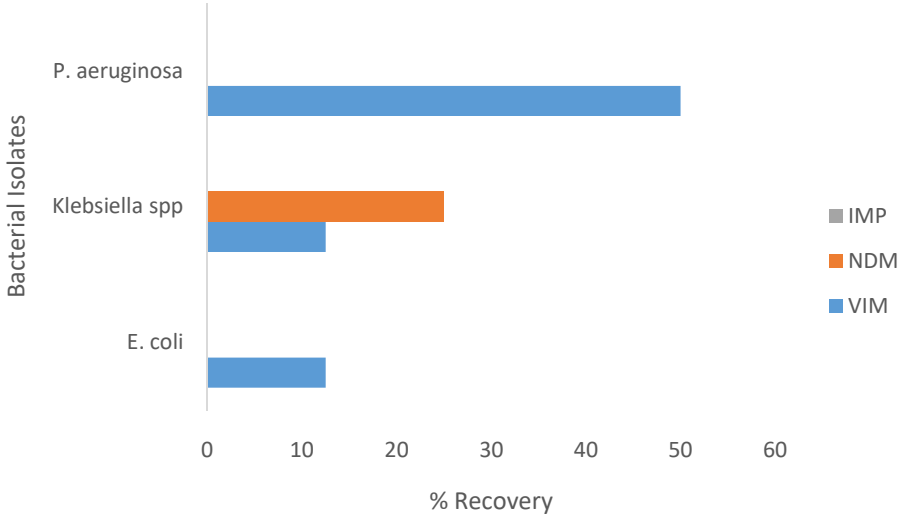

Fig 3: Prevalence of Metallo-β-lactamase genesamong Gram negative Bacterial Isolates

Key: VIM: Verona integronmetallo β- lactamase, IMP: Imipenemase, NDM: new delhimetallo β- lactamase.