Original Article

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# Plasmid Profile Analysis and bla $_{VIM}$ Gene Detection of Metalo β-lactamase (MBL) Producing *Pseudomonas aeruginosa* Isolates from Clinical Samples

Microbiology Section

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## ABSTRACT

**Introduction:** *Pseudomonas aeruginosa* is a frequent colonizer of hospitalized patients. They are responsible for serious infections such as meningitis, urological infections, septicemia and pneumonia. Carbapenem resistance of *Pseudomonas aeruginosa* is currently increasingly reported which is often mediated by production of metallo-β-lactamase (MBL). Multidrug resistant *Pseudomonas aeruginosa* isolates may involve reduced cell wall permeability, production of chromosomal and plasmid mediated β lactamases, aminoglycosides modifying enzymes and an active multidrug efflux mechanism.

**Objective:** This study is aimed to detect the presence and the nature of plasmids among metallo-β-lactamase producing *Pseudomonas aeruginosa* isolates. Also to detect the presence of bla $_{VIM}$ gene from these isolates.

**Materials and Methods:** Clinical isolates of *Pseudomonas aeruginosa* showing the metalo-β-lactamase enzyme (MBL) production were isolated. The MBL production was confirmed by three different methods. From the MBL producing isolates plasmid extraction was done by alkaline lysis method. Plasmid positive isolates were subjected for bla$_{VIM}$ gene detection by PCR method.

**Results:** Two thousand seventy six clinical samples yielded 316 (15.22%) *Pseudomonas aeruginosa* isolates, out of which 141 (44.62%) were multidrug resistant. Among them 25 (17.73%) were metallo-β-lactamase enzyme producers. Plasmids were extracted from 18 out of 25 isolates tested. Five out of 18 isolates were positive for the bla$_{VIM}$ gene detection by the PCR amplification.

**Conclusion:** The MBL producers were susceptible to polymyxin /colistin with MIC ranging from 0.5 – 2µg/ml. Molecular detection of specific genes bla $_{VIM}$ were positive among the carbapenem resistant isolates.

**Keywords:** Bla$_{VIM}$, Carbapenem resistance, Metallo-β-lactamase enzyme producers, *Pseudomonas aeruginosa*, Plasmids

## INTRODUCTION

*Pseudomonas aeruginosa* is a frequent colonizer of hospitalized patients. They are responsible for serious infections such as meningitis, urological infections, septicemia and pneumonia. The indiscriminate use of antimicrobial drugs, particularly in hospitalized patients, lead to persistent growth of drug resistant bacteria. The emergence of multidrug resistance presents a serious problem for the control of clinical infections [1].

The multidrug resistant *Pseudomonas aeruginosa* (MDRPA) isolates may involve reduced cell wall permeability, production of chromosomal and plasmid mediated β-lactamases [2], aminoglycosides modifying enzymes [3] and an active multidrug efflux mechanism [4]. Carbapenem resistance of *Pseudomonas aeruginosa* is currently increasingly reported. It is often mediated by production of metallo-β-lactamase (MBL) a class of B type of beta- lactamases that require bivalent metal ions, usually zinc for the activity.

The practical importance of bacterial plasmids was first recognized in the early 1960's when transferable drug resistance was discovered [5]. Acquired metallo-β-lactamases have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze with exception of aztreonam, all β lactams including carbapenems and also their genes are carried on highly mobile elements, allowing easy dissemination. Such strains are not susceptible to therapeutic serine β lactamase inhibitors (such as clavulanate and sulfones) [6].

Although a number of different mechanisms of resistance to carbapenems have been reported, most clinically significant carbapenem resistance has been associated with plasmid mediated acquisition of genes encoding either class B metallo-β-lactamases or carbapenem hydrolyzing class D OXA type –β- lactamases, representing the most important and wide spread mechanism of carbapenem resistance [7]. This study is aimed to characterize the nature of plasmids among metallo-β-lactamase producing *Pseudomonas aeruginosa* isolates from the clinical samples and to detect the presence of bla$_{VIM}$ gene by PCR amplification procedure and to study the prevalence of the gene in our tertiary care hospital.

## MATERIALS AND METHODS

From January 2011 to December 2011, the study was conducted in a tertiary care hospital in Chennai India. A total of 2076 clinical samples which includes the specimens from wound infection, pus discharge, blood, body fluids, central venous catheter tip, urine, sputum, broncho alveolar lavage were processed and the multidrug resistant *Pseudomonas aeruginosa (*resistant to more than 3 categories of drugs) nearly 141 strains were isolated. Among these isolates the MBL producers were confirmed by the following methods: a) the E – strip method (Radianz biotechnologies) using the Ceftazidime and Ceftazidime + EDTA (Franco MRG et al.,) [8] b) Combined disc method using Imepenem and Imepenem +EDTA by disc diffusion method (Young D et al.,) [9] and c). The Modified Hodge test (Lee et al.,) [10].

The plasmids were extracted from 25 MBL producers by the alkaline lysis method (Sambrook J et al.,) [11] and subjected to gel documentation by the electrophoresis and the band patterns of isolated plasmids were analysed. (S.J Du et al.,) [12].

### Extraction of plasmid (Sambrook et al.,) [11]

Plasmid DNA isolation by the alkaline lysis of cells is a 'mini- prep' procedure which yields clean DNA quickly and easily. Organism

grown from a single colony in a suitable medium such as Luria broth was tested for plasmid presence. An aliquot of the culture was used for preparing the plasmid DNA after the culture has been grown overnight at 37$^0$C.

About 1.5 ml of well grown culture was transferred in a microfuge tube. After subjecting for centrifuge at 10,000 rpm for 5 minutes, the supernatant was discarded. Another 1.5 ml of culture was subjected for centrifugation and the supernatant was removed. The ice cold solution 1 (Solution 1 contains glucose, Tris and EDTA -Ethylene diamine tetra acetic acid and glucose) 0.2 ml was added to the pellet and cells were resuspended. The solution 2 (Solution 2 contains NaOH and Sodium dodecyl sulphate SDS) 0.4 ml was added and left at room temperature for 5 minutes. After 5 minutes the ice cold solution 3 (Sodium acetate) 0.3 ml was added and tubes were incubated for 10 minutes. Then tubes were centrifuged for 5 minutes. The supernatant was transferred into a fresh micro centrifuge tube, using the clean disposal transfer pipette. The precipitate were left undisturbed, this fractional step separates the plasmid DNA from the cellular debris and chromosomal DNA in the pellet. The remainder of the centrifuge tube filled with isopropanol, left at room temp for 2 minutes, the tubes were centrifuged for 5 minutes, and a milky white pellet at the bottom after removing the supernatant was obtained. Later 1 ml of ice cold ethanol 70% was added and placed in spin for 1 minute. The tubes were dried for 5 min, 50 µl TE (Tris -EDTA) was added to tubes, the DNA were ready for use and stored at -20$^0$C. The electrophoresis was done on molten agarose (1.2%) gel after placing the combs and Tris EDTA (TE) buffer was poured in the tank in such a way that the buffer covers the gel surface. The wells were loaded with 5 µl plasmid preparation and 2 µl bromophenol blue tracking dye. The appropriate molecular weight marker was loaded in other well. The electrophoresis was carried out at 50-70 V for 2.5 hrs. After completion of the run, the gel carefully removed and placed on the UV trans-illuminator and the DNA bands were examined.

The plasmid positive isolates were analyzed for bla-$_{VIM}$ gene by PCR method using the following primers, bla-$_{VIM}$ VIM 1A-5'- TCT ACA TGA CCG CGT CTG TC-3' and VIM 1B- 5'-TGT GCT TTG ACA ACG TTC GC-3' (Giakkoupi P., et al.,) [13].

## RESULTS

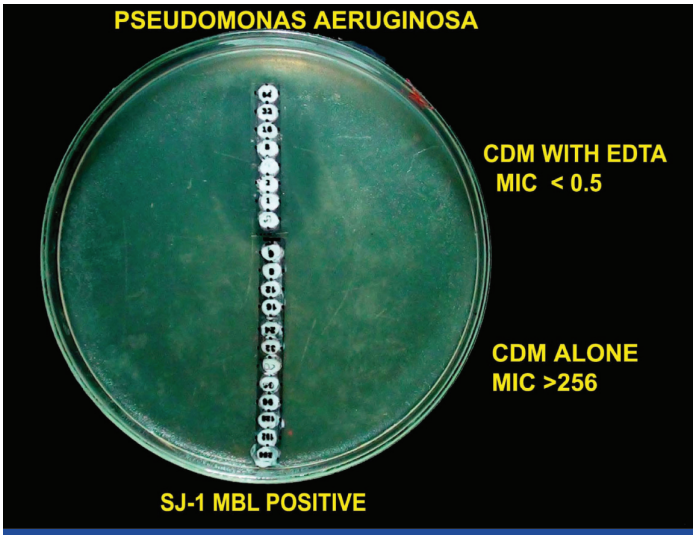
They yielded 316 (15.22%) *Pseudomonas aeruginosa* isolates, out of which 141 (44.62%) were multidrug resistant. Among the multidrug resistant isolates of *Pseudomonas aeruginosa* 94 strains were ceftazidime resistant [Table/Fig-1]. Nearly 69/94 (73.4%) were imepenem susceptible, 30/94 (31.9%) were imepenem resistant. The MBL producers were susceptible to MIC ranging from 0.5 – 2µg/ml. MBL– enzyme production was detected in the carbapenem resistant strains by three different methods [Table/Fig-2]. Out of 30 strains tested 25 (25/141, 17.73%) were metallobetalactamase (MBL) enzyme producers [Table/ Fig-3].

The plasmids were extracted from 18 of the 25 (72%) [Table/Fig-4,5] strains subjected. Out of 18 plasmids positive *P. aeruginosa* strains tested for bla $_{VIM}$ gene by PCR, the gene was detected in 5/18 (27.77%) strains with product size 748 bp [Table/Fig-6].

## DISCUSSION

*Pseudomonas aeruginosa* plays an important aetiological role in human infections as many clinical isolates are resistant to common antibiotics [14,15]. The prevalence of *P.aeruginosa* infection in our study 316/2076 (15.22%) were similar to (18.2%) studies of Gad et al., [16]. Resistance to the quinolones was 35-45% (ciprofloxacillin 42%,ofloxacin 39.5%) similar to Corona–Nakamura et al., [17].

The E strip with the combination of a β lactam substrate and a β lactam/metallo β lactamases inhibitor is specifically designed to detect MBL [18]. The prevalence of the multidrug resistant strains of




**[Table/Fig-1]:** Carbapenamase detection of *P. aeruginosa* using E strip with Ceftazidime (CDM) and Ceftazidime + EDTA

| S. No | Test method | Total strain tested (n=30) | |
|---|---|---|---|
| | | Positive (%) | Negative (%) |
| 1 | Imepenem + ( Imepenem + EDTA) Combined disc diffusion | 83.3 % ( 25/30) | 16.6 %(5/30) |
| 2 | Modified hodge test | 60 % (18/30) | 40 %(12/30) |
| 3 | Ceftazidime + ( Ceftazidime + EDTA) E - strip method | 60 % (18/30) | 40 %(12/30) |

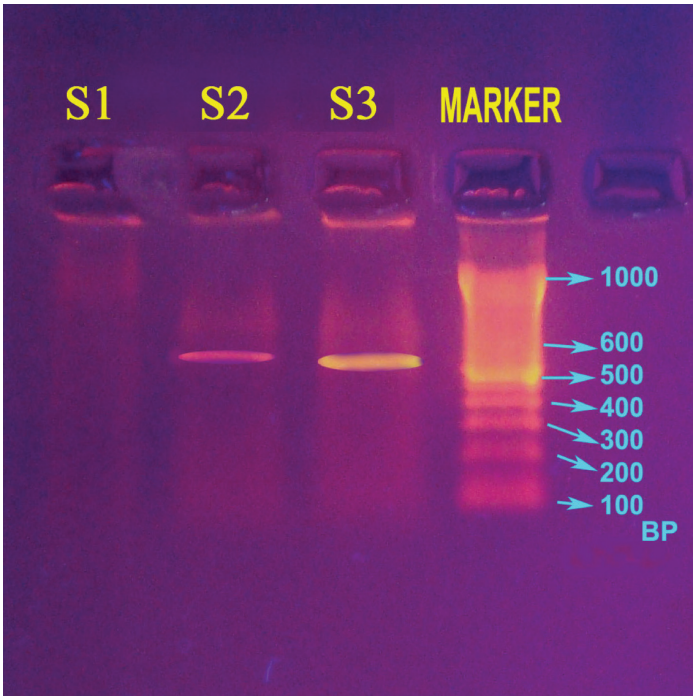
**[Table/Fig-2]:** The phenotypic detection of MBL producing *Pseudomonas aeruginosa* among the carbapenem resistant isolate

| No of Pseudomonas aeruginosa | No of MDR isolates | No of MBL Producers |
|---|---|---|
| 15.22 % (316/2076) | 44.62 % (141/316) | 17.73% ( 25/141) |

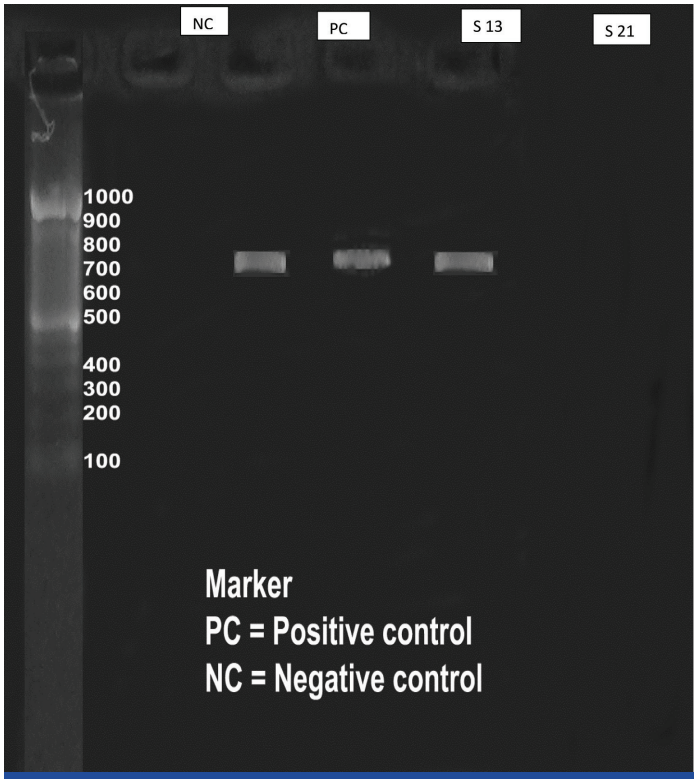
**[Table/Fig-3]:** MBL producers among *Pseudomonas aeruginosa* isolates

| Strains tested | | Positive (%) | Negative (%) |
|---|---|---|---|
| 1. | Plasmid detection (n=25) | 18 (72%) | 7 (28%) |
| 2. | bla $_{VIM}$ gene detection (n=18) | 5 (27.7%) | 13 (72.2%) |

**[Table/Fig-4]:** bla $_{VIM}$ gene and plasmid detection from MBL producing *Pseudomonas aeruginosa*




**[Table/Fig-5]:** Plasmid Analysis




**[Table/Fig-6]:** MBL bla$_{VIM}$ gene with controls by PCR amplification

our study is 44.62% and the MBL producers is 17.73% [Table/Fig 3] which are consistent with other studies (Behera et al., Kazuyoshi senda et al.,) [19,20]. Resistance to the imepenem (9.77%) is much lower when compared to the meropenem resistance (25.56 %) which is in concordance with the study by Gad et al., [16]. Though carbapenem were the treatment option for the serious bacterial infections, the increase in the resistance pattern to them by the Gram negative bacteria are reported very frequently alarming the world wide outbreaks in healthcare settings [14,15]. The carbapenem resistant strain were susceptible to polymyxin B and colistin by disc diffusion method and MIC E- strip method .

An alarming increase in resistance to various antimicrobial agents has been reported in India, studies regarding the isolation and characterization of plasmids from multidrug resistant *P. aeruginosa* strains are still elementary in our country [15]. MBL's have become a serious concern in hospitals worldwide over the past decade, they can be disseminated horizontally through transfer of resistance determinants, the integrons are frequently located in plasmids or transposons, the dissemination of which contributes to the global spread of this resistance mechanism [21]. However the result of the present study provides the evidence for the drug resistance gene located in the plasmid and the plasmid bands of the metallo-β-lactamase producer at 550 bp when read with the molecular weight ladder.

The study of J Lv et al., [22] that more than 80% of the isolated strains were multidrug resistant caused by a large plasmid of 150 kilobase (Kb), these plasmid were designated to mediate the multidrug resistance to many antibiotics and the study of Te-Li Chen, et al., [23] showing resistance pattern are being coded for plasmid mediated drug resistance than the genes encoded chromosomally were in concurrence with our study.

The plasmid detection (positive 72%, negative 28%) was similar to plasmid analysis done by Maria et al., [8], 88.2% strains plasmid positive and no plasmid was detected in 11.8% of the strains. The presence of bla $_{VIM}$ gene in the plasmids in our study (27.77%) is higher than the study by 2003 [13] [Table/Fig-6].

## CONCLUSION

Since it is impossible to predict what impact MBL genes will have on the future antimicrobial regimens, study with antibiotic drug resistance along with conjunction with antibiotic resistance gene pattern will be the future plan. And continued surveillance is essential to control the spread of this resistance further investigations are necessary to understand the detailed genetic background.

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