

**Original article:**

## **Detection of metallo beta lactamase producing pseudomonas aeruginosa in various clinical samples received from Rajindra Hospital, Patiala**

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### **ABSTRACT**

**Introduction:** Pseudomonas aeruginosa has acquired a new metallo- $\beta$ -lactamase (MBL) resistance gene responsible for increased resistance to fluoroquinolones, cephalosporins and carbapenems. Thus, it is essential to know the antibiotic sensitivity pattern and follow the antibiotic policy.

**Objectives:** The objective of this study isto detect MBL production in clinical isolates by imipenem-EDTA Combined Disk Test, imipenem-EDTA Double Disk Synergy Test and Modified Hodge test.

**Material and Methods:** The study was done on various clinical samples like pus, urine, wounds, sputum from patients admitted to Rajindra Hospital, Patiala during January 2015 to December 2015. A total of 100 consecutive isolates of P. aeruginosa were subjected to susceptibility testing by disc diffusion assay. IMP drug resistant strains were screened for MBL production.

**Results:** Ciprofloxacin resistance was seen in 64% isolates followed by piperacillin-tazobactam in 38%. Resistance toward amikacin, ceftazidime, and cefepime were noted in 44%, 82%, and 86% isolates, respectively. In 12%, IMP resistance was observed. All IMP resistant strains (n = 12) were screened for MBL production. All the 12 isolates (100%) were MBL producers. MBL producing strains were isolated from trauma cases 25%, 16.66% each were from patients with diabetes and malignancy, and 8.33% each from burns and tuberculosis patients. Among MBL producers ticarcillin-clavulanate, ceftazidime, cefepime, meropenem and imipenem showed 100% resistance each; ciprofloxacin 91.66%, gentamicin 83.33%, piperacillin-tazobactam 75%, amikacin 66.66% and aztreonam 58.33%. **Conclusion:** Emergence of P. aeruginosa as MBL producer is becoming a therapeutic challenge. There is a need to implement routine antibiotic surveillance and judicious use of antibiotics.

**Keywords:**IMP-Imipenem, MBL-Metallo- $\beta$ -lactamase

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### **INTRODUCTION**

Pseudomonas aeruginosa continues to be a major cause of opportunistic nosocomial infections, causing around 9-10% of hospital infections. Infections in hospital or other healthcare settings can be localized, as in catheter-related urinary tract infection, infected ulcers, bedsores, burns and eye-

infections. P.aeruginosa septicaemia or necrotizing pneumonia, though uncommon, is associated with a high mortality rate in neutropenic patients. Patients in critical care units are at particular risk. P.aeruginosa is the second most common cause of ventilator-associated pneumonia.<sup>[1]</sup>

The first MBL was reported from *Bacillus cereus* in the 1960's and since then 18 MBLs have been described in different Gram-negative bacteria. Production of most of these MBLs is chromosomally encoded and did not pose a serious threat of spread to other bacteria. However in 1991, the first plasmid-mediated MBL, IMP-1 from *Pseudomonas aeruginosa* was reported from Japan, while another type of acquired metallo-beta-lactamase, VIM-1 was first reported from Italy in 1999.<sup>[1]</sup>

### **Characteristics of metallo- $\beta$ -lactamase (MBL)<sup>[2]</sup>**

Metallo  $\beta$ -lactamases require zinc for their catalytic activity. Their activity is inhibited by metal chelators such as EDTA and thiol compounds. Metallo- $\beta$ -lactamases hydrolyse all  $\beta$ -lactam antibiotics including penicillins, cephalosporins and carbapenems with exception of aztreonam(monobactam). MBL producing strains are not susceptible to serine  $\beta$ -lactamase inhibitors (e.g.clavulanate).

### **MATERIAL AND METHODS**

In the present study 100 isolates of *Pseudomonas aeruginosa* were obtained from various clinical specimens like pus, urine, burn, wound, sputum, endotracheal aspirate, pleural fluid and

cerebrospinal fluid from indoor patients admitted to Rajindra Hospital, Patiala. These isolates were studied for detection of prevalence of MBL production including their antibiogram.

**Detection of MBL production:** Imipenem resistant isolates were further tested for MBL production by the following three phenotypic methods:

#### **a. Modified Hodge Test:**

The modified Hodge test (MHT) had originally been described by CDC (Centre for Disease Control, Atlanta) for carbapenemase detection in Enterobacteriaceae.

**Procedure:** In MHT, a lawn culture of 1:10 dilution of 0.5 McFarland's standard *E. coli* ATCC 25922 broth was done on a Mueller Hinton agar plate. A 10 $\mu$ g imipenem disk was placed in the centre of plate and 10 $\mu$ l of 50mM zinc sulphate solution was added to imipenem disk. Imipenem resistant *P. aeruginosa* was streaked from edge of the disk to the periphery of the plate in 4 different directions. After overnight incubation, the plates were observed for presence of a cloverleaf shaped zone of inhibition and the plates with such zones were interpreted as positive Modified Hodge test.

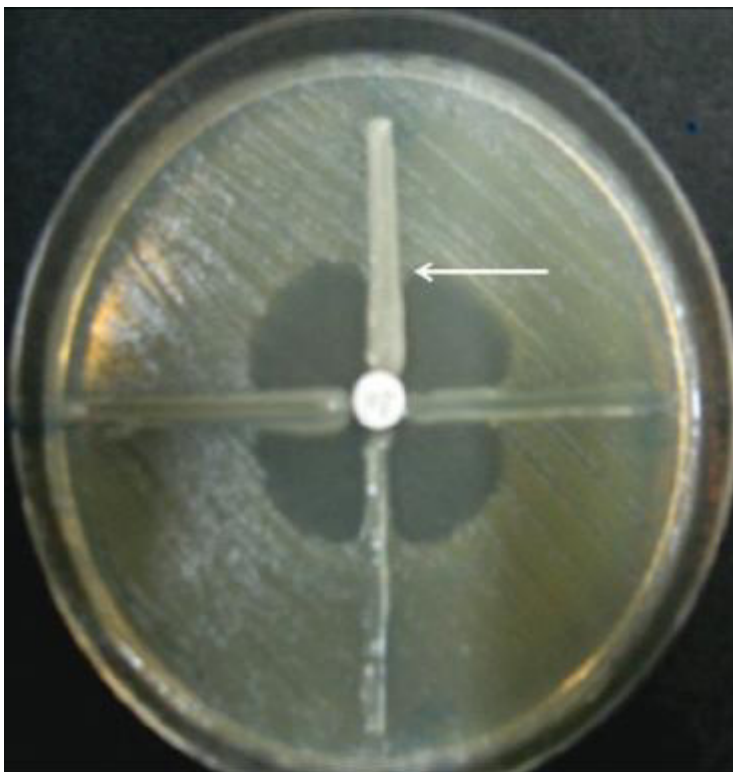
<sup>[6]</sup> (Picture 1)

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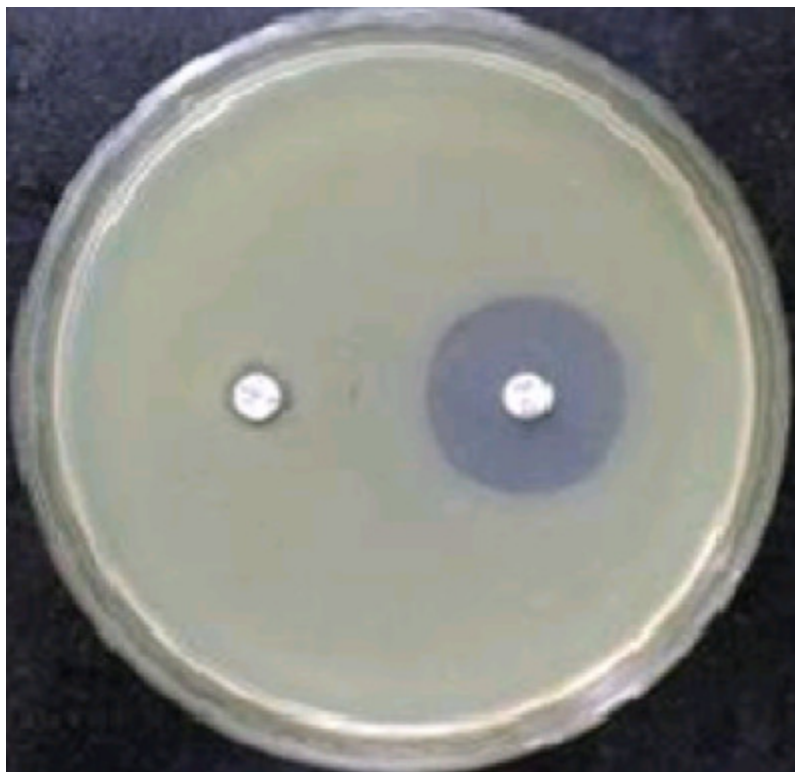
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**Picture 1: Positive Modified Hodge Test (MHT)with a clover leaf indentation**

**b. Imipenem (IMP)-EDTA Combined Disk Test (CDT):**

A lawn culture of the test strain was done on MH agar plates (opacity adjusted to 0.5 McFarland's standard). Two 10µg imipenem disks were placed on inoculated plates wide apart and 10µl of 50mM of zinc sulphate solution was added to each of the imipenem disks. Then, 5µl of 0.5M EDTA solution was added to one imipenem disk. After overnight incubation, an increase in zone size of  $\geq 7$ mm around the imipenem-EDTA disk as compared to the imipenem only disk was recorded as a positive result.<sup>[8]</sup> (Picture 2)



**Picture 2: Positive Combined Disk Test (CDT) showing an increase in zone size of  $\geq 7$ mm around the imipenem-EDTA disk as compared to the imipenem only disk.**

**c. IMP-EDTA Double Disk Synergy Test (DDST):**

Test organisms were inoculated onto plates with Mueller Hinton agar as recommended by the CLSI. An overnight broth culture of the test strain (opacity adjusted to 0.5 McFarland opacity standard) was inoculated on an MHA plate. After drying, a 10 $\mu$ g imipenem disk and a blank filter paper disk (6mm in diameter, Whartmann filter paper no.2) were placed 10mm apart from edge to edge. 10 $\mu$ l of 50mM zinc sulphate solution was added to the 10 $\mu$ g imipenem disk. Then, 10 $\mu$ l of 0.5mM EDTA solution was applied to the blank filter paper disk. After overnight incubation, the presence of an enlarged zone of inhibition towards the EDTA disk was interpreted as DDST positive.<sup>[6]</sup> (Picture 3)



**Picture 3: Positive Double Disk Synergy Test(DDST) showing an enlarged zone of inhibition towards the EDTA disk.**

The results of the various tests were compiled, tabulated and statistically analysed.

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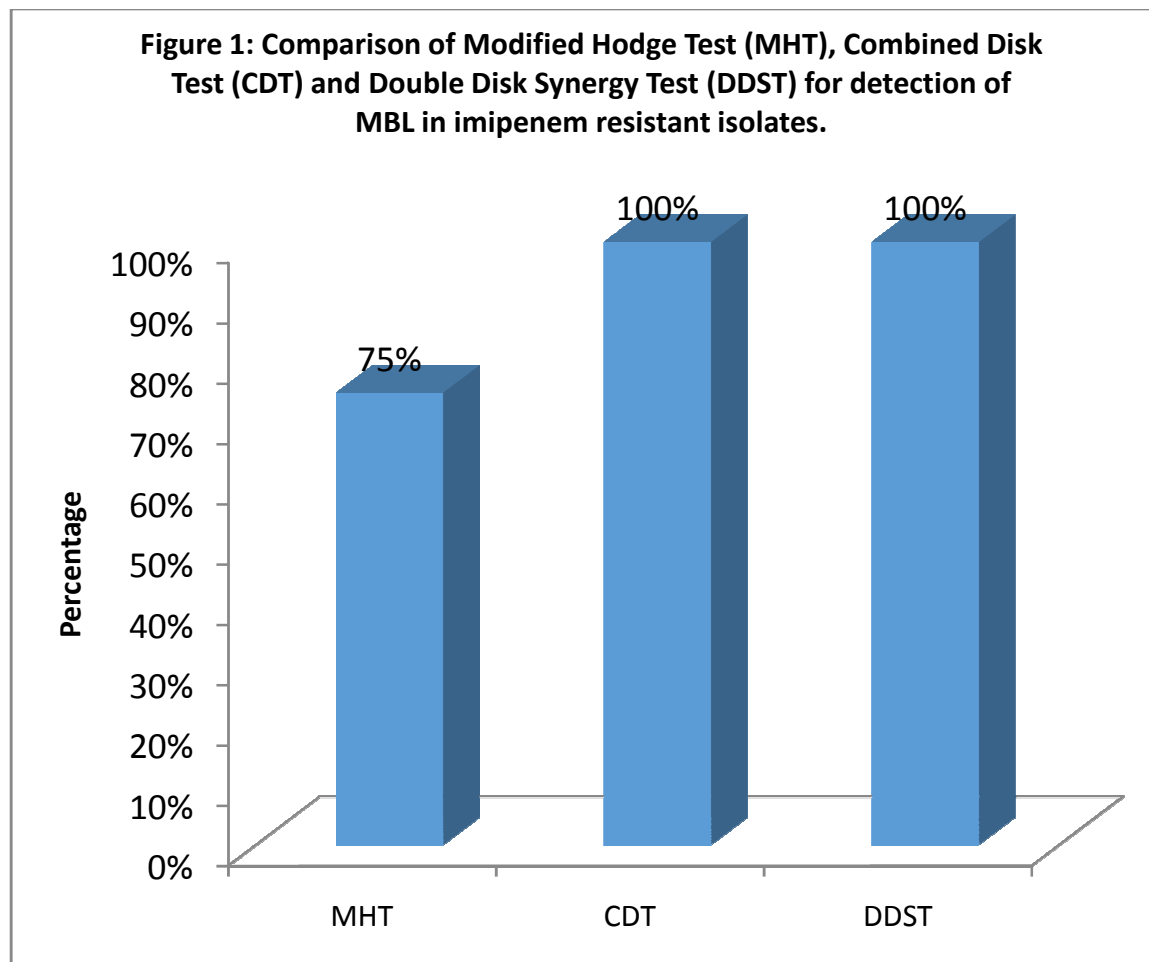
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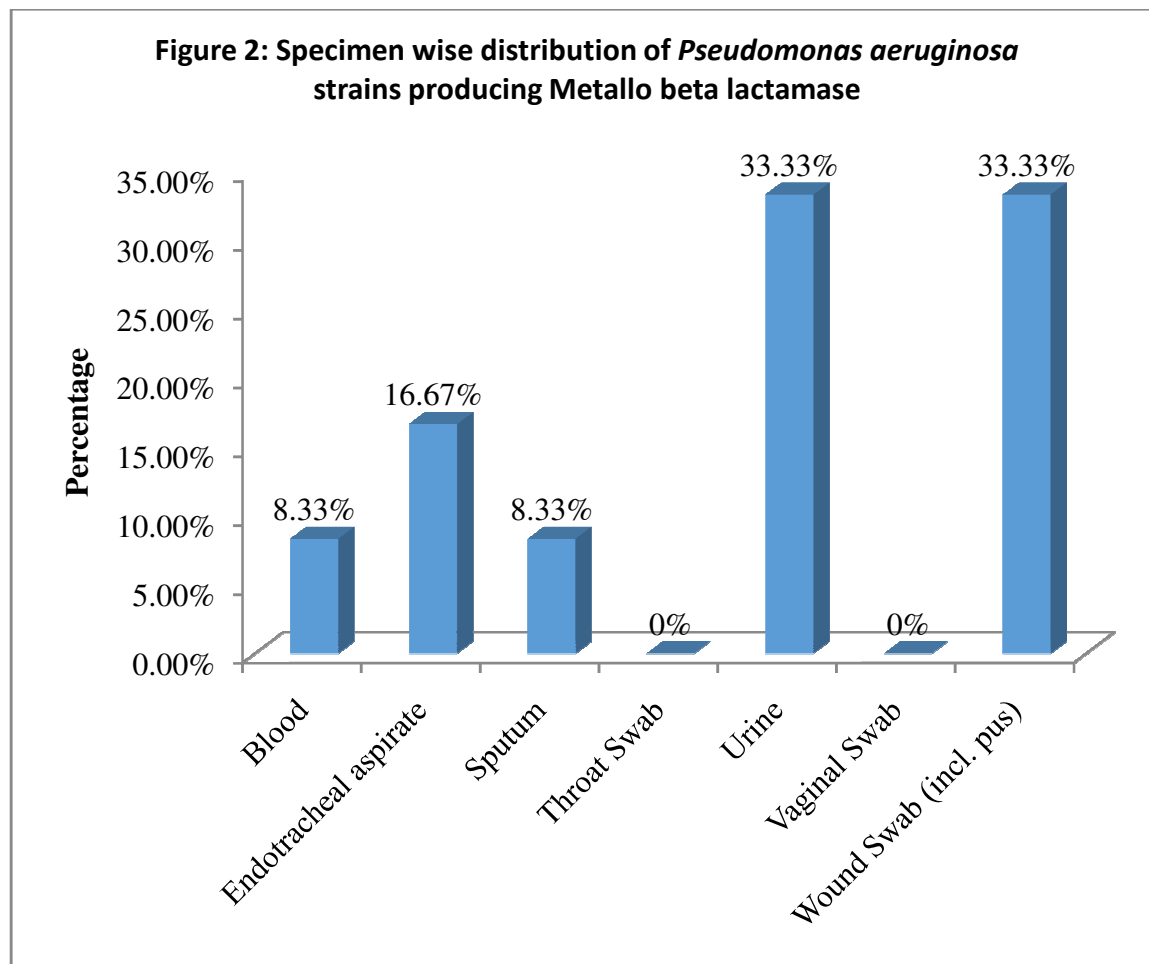
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**OBSERVATIONS AND RESULTS**

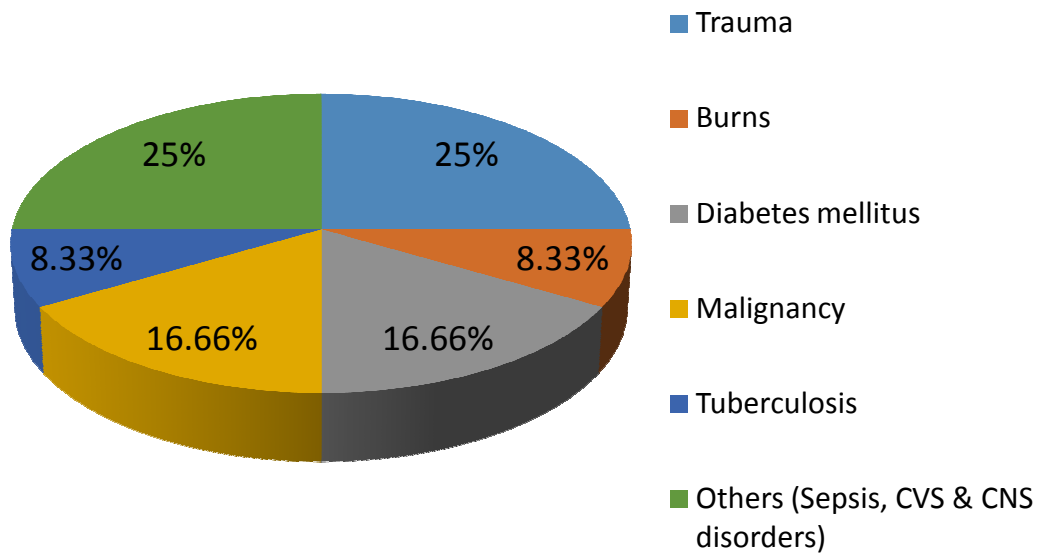


**Comparative statistical analysis of the three phenotypic tests for MBL detection**

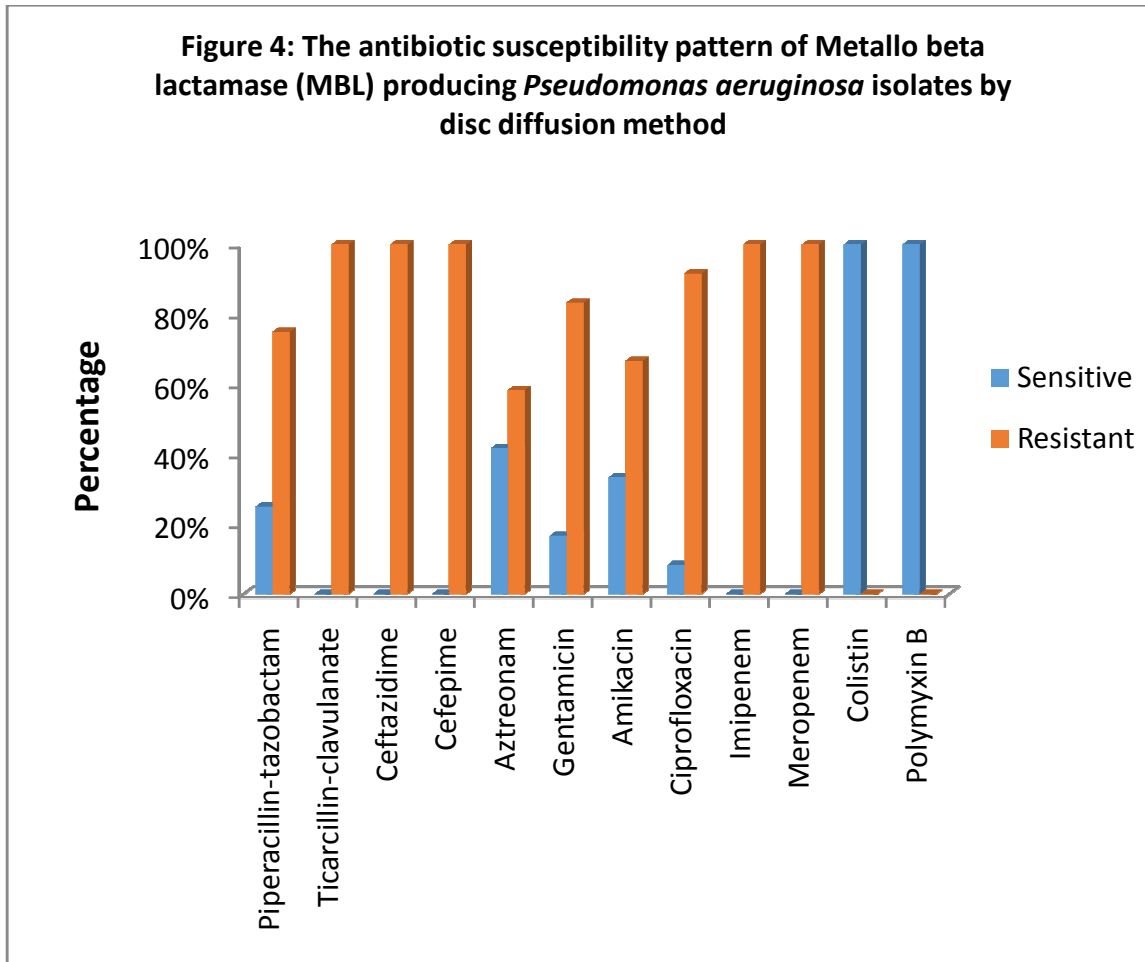
Test	Mean Rank	X <sup>2</sup>	p value	Statistical significance
MHT	1.97	6.00	0.050	Significant
CDT	2.02			
DDST	2.02			



**Figure 3: Association of risk factors with MBL production**







## DISCUSSION

In the present study, antibiogram of 100 *P.aeruginosa* isolates had shown more resistance against ticarcillin-clavulanate 92% followed by cefepime 86% and ceftazidime 82% which is similar to the observations of Behera et al<sup>[11]</sup> at AIIMS, New Delhi during April 2007 for a period of one month where resistance against ticarcillin-clavulanate was 89%, that to cefepime was 81% and ceftazidime was 70%. 12% isolates in our study were imipenem resistant and 38% were piperacillin-tazobactam resistant. These observations are in agreement with those of Angadi et al<sup>[12]</sup> who showed imipenem resistance to be

21.6% and piperacillin-tazobactam resistance to be 45.6%. As for meropenem, we observed a 12% resistance which resembles a study by Minhas et al<sup>[13]</sup> who obtained a 13.89% resistance for the drug. Prevalence of MBL in clinical isolates is found to be 12%. Other studies such as those conducted by Bashir et al<sup>[10]</sup>, Behera et al<sup>[11]</sup> reported MBL prevalence to be 11.66%, 39.56% respectively. In the present study prevalence of MBL in imipenem resistant isolates is 100%. This is in concordance with study by Chaudhari et al<sup>[2]</sup> and Attal et al<sup>[3]</sup> who showed MBL production in imipenem resistant isolates as 100% and 88.8% respectively. In the current study

12 out of 100 *Pseudomonas aeruginosa* isolates (12%) showed resistance to imipenem and all these imipenem resistant isolates were screened for carbapenemase production by Modified Hodge test (MHT) and were tested for MBL production by Combined Disk test (CDT) and Double Disk Synergy Test (DDST). By these methods, 9 out of 12 imipenem resistant isolates (75%) gave positive results in case of MHT and all 12 (100%) of the imipenem resistant isolates tested positive by CDT and DDST. All the isolates positive by MHT also tested positive by CDT and DDST. On comparative statistical analysis of the three phenotypic tests for MBL detection, the p value was calculated to be 0.050 which denotes significant statistical association between these three phenotypic tests and MBL production. Studies such as those by Attal et al [3] and Shivappa et al [5] reported a positivity of 88.85 and 46.7% in case of MHT for carbapenemase screening while the results of CDT and DDST are in concordance with few studies as Pandya et al [4] (CDT-96.3%; DDST-81.48%), Attal et al [3] (CDT and DDST 88.8% each). Most of the studies have shown CDT to be a more sensitive method for MBL detection as DDST has a more subjective interpretation but in our study both CDT and DDST showed equal sensitivity for the same. In this study maximum number of MBL producers were in the age group of more than 60 years i.e. 33.33% and males accounted for 58.33% and females 41.67%. The age range was found to be  $43.39 \pm 21.39$  while the median age was observed as 43 years. Bashir et al<sup>[10]</sup> reported highest number of MBL producers from patients aged above 60 years, males accounting for 51.5% and females 48.5%.

Among MBL producers wound swabs (including pus) and urine accounted for 33.33% each followed by endotracheal aspirate 16.66%, sputum and blood 8.33% each. This correlates with the study by Attal et al<sup>[3]</sup> at JNMC, Wardha during June 2008 to December 2009 who showed that among MBL producers wound swabs accounted for 43.7%, followed by urine 37.5%, sputum 6.2%, endotracheal tube secretions 6.2% and body fluids 6.2%. On statistical analysis of the present study, the p value was calculated to be 0.01 which shows statistical significance. MBL producers were maximally isolated from the surgical wards (including general surgery, orthopaedics, burns & plastic surgery and ENT wards) i.e. 50%, medical wards including TB and chest and comprised 25%, while paediatrics, obs&gynae wards and the ICU constituted 8.33% each. These results simulated those of Nandy et al<sup>[14]</sup> who reported 41.1% MBL producers from the ICU, 29.41% from surgical wards, 11.76% each from medicine and gynae wards, and 5.8% from paediatrics wards.

On statistical analysis for the current study, the p value was calculated to be 0.01 which is statistically significant.

Maximum number of MBL producing strains in this study were being isolated from cases of trauma 25%, 16.66% each were from patients with diabetes and malignancy, and 8.33% each from burns and tuberculosis patients. The rest of the MBL producers comprised of cases of sepsis, cardiovascular and neurological disorder. This observation pattern correlates with Zahoor et al<sup>[9]</sup> whereby of the 30 MBL producers, 7 (23.3%) from patients with trauma, 6 (20%) from patients with

malignancy, 5 (16.7%) were isolated from patients with burns, 4 (13.3%) from patients with sepsis, 3 (10%) from patients with diabetes, 5 (16.7%) from patients with other diagnosis. Among MBL producers resistance to ticarcillin-clavulanate, ceftazidime, cefepime, imipenem and meropenem was 100% each followed by ciprofloxacin (91.66%), Gentamicin (83.33%), piperacillin-tazobactam (75%), amikacin (66.66%) and aztreonam (58.33%). This is similar to the observations made by Bashir et al<sup>[10]</sup> who demonstrated 100% resistance to ceftazidime, gentamicin, meropenem, 90.9% to both ciprofloxacin and amikacin, 81.8% to piperacillin-tazobactam. Overall both polymyxin B and colistin showed a sensitivity of 100% in all the isolates of *Pseudomonas aeruginosa* as well as for all the MBL producing strains. This correlates with various studies such as those of Bashir et al<sup>[10]</sup>, Attal et al<sup>[3]</sup> who reported 100% sensitivity to

polymyxin B while Nandy et al<sup>[14]</sup> showed 100% sensitivity to colistin.

#### SUMMARY AND CONCLUSION

MBLs may prove to be a therapeutic challenge. The early detection of MBL-producing *P. aeruginosa* may avoid the future spread of these multidrug-resistant isolates. PCR confirmation for MBLs can be performed at a regional laboratory using the methods reported in this study

As the phenotypic methods are easier to perform, they are able to discriminate among the various beta lactamases, which the automated systems fail to do. Hence, the phenotypic methods should be regularly performed where the molecular methods are not available.

Strict infection control practices, the judicious use of antibiotics, an early detection of the MBL carriage, all will together help in extending the longevity of the carbapenems, which are the last resort antibiotics.

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