# Study of Metallo-beta lactamase producing Pseudomonas aeruginosa in Pravara Rural Hospital

Bhalerao D S*, Roushani S*, Kinikar A G**, Akhter I***

## Abstract

*Carbepenem resistance is an emerging threat in the infections caused by gram negative pathogens. Detection of Metallo-beta lactamases (MBL) producing Pseudomonas is critical in preventing their widespread dissemination. Study was aimed at detection of Metallo-beta-lactamase in clinical isolates of Ps. aeruginosa by phenotypic methods in the Department of Microbiology, Rural Medical College, Loni. Out of eighty clinical isolates fifty four were subjected for MBL production by three different methods (i) Imipenem-EDTA double disc synergy method(DDST) (ii) Imipenem-EDTA combined disc test (iii) EDTA disc potentiation test using four cephalosporins. The MBL production was found to be 66.7%. This presents a therapeutic challenge to the clinicians and calls for judicious use of antibiotics.*

**Key words:** *Ps.aeruginosa, MBL detection, Carbepenem resistance*

## Introduction

In the past few decades *Pseudomonas aeruginosa* has been recognised as a pathogen in variety of infections and also an emerging nosocomial pathogen. *Ps. aeruginosa* shows intrinsic resistance to a variety of antimicrobials, including Beta lactams. The emergence of the MBL's in Pseudomonas species is fast becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher cephalosporins. Moreover, treatment options are either not available or very expensive and may be toxic with poor outcomes.[1]

In view of the above, this study was undertaken to screen metallo beta-lactamase (MBL) production amongst multidrug resistant *Pseudomonas aeruginosa*.

* Professor, Dept of Microbiology, RMC, Loni

** Assist Prof, Dept of Microbiology, RMC, Loni

** Tutor, Dept of Microbiology, RMC, Loni

**Address for correspondence:**
Dr D.S.Bhaleroa , Professor, Dept of Microbiology, RMC, Pravara Institute of Medical Sciences, Loni
Email: microbiology@pmtpims.org

## Material and Methods

The study was conducted in the Department of Microbiology, Rural Medical College, Loni in 2009-2010. A total of 80 consecutive isolates of *Pseudomonas aeruginosa* obtained from various samples of the patients admitted in Pravara Rural Hospital were included in the study. All isolates were non duplicate. The isolates were identified as *Ps. aeruginosa* by conventional methods.[2] Routine antibiotic disc sensitivity testing was done with penicillin, ampicillin , gentamicin, amikacin, ceftazidime, cefepime, cephotaxime, ciprofloxacin, chloramphenicol, cefuroxime, cefpodoxime, norfloxacin, nitrofurantoin, cephalexin, cephazolin, sparfloxacin and cotrimoxazole. All the strains showing resistance to multiple drugs were tested for Imipenem susceptibility. Imipenem resistant isolates were further screened for MBL production.[3-6]

1) Imipenem-Ethylenediaminetetraacetic acid (EDTA) double disc synergy test: Test organisms were inoculated on the plates with Muller Hinton

agar as recommended by the CLSI (Clinical laboratory standards institute). An imipenem (10 microgram) disc was placed 20mm centre to centre from blank disc containing 10 microL of 0.5M EDTA (750 microgram). Enhancement of the zone of inhibition in the area between imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as positive (Fig1).

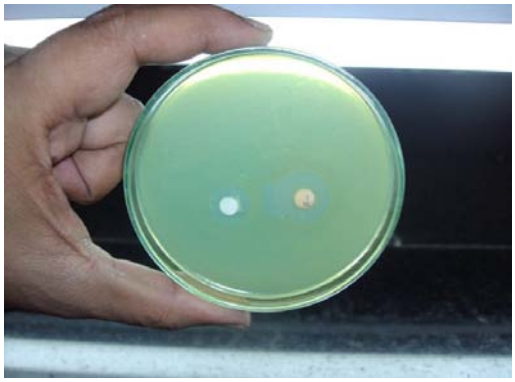

Fig 1: DDST positive

2) Imipenem (IPM)-EDTA combined disc test: Test organisms were inoculated on to the plates with Muller Hinton agar as recommended by the CLSI. Two 10 microgram imipenem discs were placed on the plates and appropriate amount of 10 micrlitre of EDTA solution were added to one of them to obtain the desired conc. of 750 microgram. The inhibition zones were compared after16-18 hrs of incubation at 35ºC. In combined disc test, if the increase in inhibition zone was more than 7mm than the imipenem disc alone, it was considered as MBL positive. (Fig 2)

3)EDTA disc potentiation using cephalosporins: Test organisms were inoculated on Muller Hinton agar as described for the standard disc diffusion test. A filter paper, Whatman no.2 blank disc was placed and then following cephalosporins-Ceftazidime, Ceftizoxime, Cefotaxime and cefepime were placed


Fig 2: DDST Combined Positive

25mm center to center from blank disc. Ten microL of 0.5 M EDTA was added to the blank disc and plates were incubated at 35ºC overnight. Enhancement of the zone of inhibition in the area between the EDTA disc and any one of the four cephalosporin disc in comparison with the zone of inhibition on the far side of the drug was interpreted as positive. ATCC 27853 Pseudomonas aeruginosa was used as negative control.[3, 4, 5, 6] Preparation of 0.5 M EDTA solution : 186.1g of disodium EDTA was dissolved in 1000 ml of D.W and pH adjusted to 8 by using NaOH. The mixture was sterilized by autoclaving.

3-2.jpg

## Results

There were 80 isolates of *Ps. aeruginosa* in the study. Out of 80 isolates 54 Imipenem resistant isolates were screened for MBL production. 15 were isolated from pus, 13 from urine, 10 from blood, 5 from pleural fluid, 4 from vaginal swab, 3 from ICD catheter and 1 each from sputum, CSF, ear discharge & conjunctiva. All the strains exhibited resistance to most of the drugs (Table 1). Out of 54 Imipenem resistant isolates tested for MBL production 36 isolates exhibited more than 7mm zone size enhancement by the combined disc method whereas only 27 gave positive result by DDST and 6 isolates by EDTA disc potentiation method. Zone sizes were similar and reproducible when the procedure was repeated. Those isolates positive by DDST, were also positive by combined disc test for MBL production. The results with EDTA disc potentiation with four cephalosporins were not encouraging. Only six isolates showed zone enhancement towards EDTA disc in one or two antibiotics (Table 2).

Table 1: Antibiotic susceptibility testing showing resistance to various drugs

| ANTIBIOTIC | RESISTANCE |
|---|---|
| Penicillin | 100% |
| Ampicillin | 92.86% |
| Amikacin | 97.06% |
| Chloramphenicol | 93.75% |
| Ceftazidime | 96.43% |
| Ciprofloxacin | 76.92% |
| Cephalexin | 100% |
| Cephazolin | 97.56% |
| Cephotaxime | 100% |
| Cefuroxime | 75% |
| Cefpodoxime | 98% |
| Cefepime | 92% |
| Gentamicin | 85.71% |
| Norfoxacin | 73.27% |
| Nitrofurantoin | 100% |
| Nalidixic acid | 100% |
| Cotrimaxazole | 95.24% |
| Sparfloxacin | 84% |

## Discussion

*Pseudomonas aeruginosa* is known to cause a variety of infections like urinarytract infection, wound infections, septicemia and device infections is now emerging as MBL producer and is spreading it in a horizontal way to members of Enterobacteriacae.The injudicious or overuse of broad spectrum antibiotics has extended the incidence of MBL production in the community as well.[7] These facts need to caution our clinicians and draw their attention towards the indiscrete use of antibiotics.In this study the imipenem resistance in *Ps.aeruginosa* was found to be 67.5% compared to 26% by A.Varaiya, 69% by Behera et al, 59.52% by S.Irfan.30% by Gupta et al and only 8.05% by Agrawal et al.[8,9,10,11]

In our study, by different methods,66.7% (36/ 54 isolates) were found to be MBL producers. The remaining negative isolates may have other mechanism of resistance such as impermeability of outer membrane and or active efflux mechanism. A similar study carried out by Behera et al showed 48 isolates out of 64 as MBL producers (76.19%) while 25 out of 25 i.e,100% isolates were MBL producers in the study conducted by S.Irfan et al.[8,9]

Table 2: Comparison of methods for detection of MBL Ps. aeruginosa.

| | Total isolates tested (n=54) | | |
|---|---|---|---|
| Test | DDST test | IMP-EDTA combined test | EDTA disc potentiation |
| ATCC 27853 Ps. aeruginosa | Negative | Negative | Negative |
| Isolates | 27 (50%) | 36 (66.7%) | 06 (11.1%) |

In our study we employed three methods for MBL detection. The incidence of MBL producing Pseudomonas by DDST was found to be 50% as compared to combined disc method (66.7%). In combined test it was easier to detect extension of zone more than 7 mm and therefore ease interpretations. The EDTA disc potentiation test with four cephalosporins was not found to be useful as it could detect only 6 isolates as MBL producers. The combined disc method proved to be more sensitive over DDST and EDTA disc potentiation methods in detection of MBL producers.[12]

## Conclusion

The study focuses on the resistance in *Ps. aeruginosa* towards Imipenem. This could be troublesome. Therefore there is need for implementation of surveillance studies in routine cases and careful selection of antibiotics in individual cases as well. The Imipenem-EDTA combined disc method is more convenient and useful for MBL screening. Emergence of MBL producing *Ps.aeruginosa* in clinical strains emphasizes the need in clinical practice to follow antibiotic restriction policies tthereby avoiding

excess use of Carbapenems and other broad spectrum antibiotics. Since there are no standard guidelines for detection of MBL, the laboratories must evaluate the various methods for detection of MBL and usewhat is most suited. We believe that considering cost constrains, the combined disc test can be used as a convenient method.

## References


1. Marra AR , Pereira CA, Gales AC, Menez LC, Cal RG, De Souza JM, et al.Blood stream infections with metallo-beta lactamase producing Pseudomonas aeruginosa: Epidemiology, Microbiology and clinical outcomes. Antimicrob Agents Chemother 2006;50:388-90
2. Collee JG, Diguid JP, Fraser AG, Mackie and McCartney practical Medical Microbiology.14th ed.(Churchill Livingstone, Edinburgh) 1996
3. Clinical and Laboratory Standards Institute (CLSI).Performance standards for antimicrobial susceptibility testing, 16th informational supplement. CLSI Document M2-Ag, Wayne PA: 2006.
4. K Lee, Y S Lim, D Yong, J H Yum, and Y Chong. Evaluation of Hodge test and the Imipenem-EDTA Double Disc Synergy Test for differentiating Metallo-beta lactamase producing isolates of Pseudomonas spp.and Acinetobacter spp.Journal of Clinical Microbiology,Oct2003,p.4623-4629.
5. Clare Franklin, Lisa Liolios, and Anton Y. Peleg. Phenotypic Detection of Carbapenems-Susceptible Metallo-beta lactamase producing Gram negative bacilli in the clinical laboratory. Journal of Clinical Microbiology, Sept, 2006, 3139-3134.
6. B Behra ,P Mathur, A Das, A Kapil, V Sharma.An evaluation of four different phenotypic techniques for detection of metallo-beta lactamase producing Pseudomonas aeruginosa. Indian Journal of Medical Microbiology.(2008)26 (3):233-37
7. S Bhattacharya. ESBL from Petri dish to the patient. Indian Journal of Medical Microbiology.(2006) 24 (1):20-24
8. A Variaya, M Kulkarni, P Bhalekar, J Dogra.Incidence of Carbepenem-resistant Pseudomonas aeruginosain diabetes and cancer patients.Indian Journal of Medical Microbiology (2008)26(3):238-40
9. S Irfan, A Zafar, D Guhar, T Ahsan,R Hasan.Metallo-beta lactamase producing clinical isolates of Acinetobacter species and Pseudomonas aeruginosa from intensive care unit patients of a tertiary care hospital.Indian Journal of Medical Microbiology (2008)26(3)243-245.
10. G Agrawal, RB Lodhi, V P Kamalkar, R K Khadse., S V Jalgaonkar. Study of Metallo-beta lactamase production in clinical isolates of Pseudomonas aeruginosa. Indian Journal of Medical Microbiology(2008) 26 (4) :349-51
11. Ekta Gupta,Srujana Mohanty, Seema Sood, Bena Dhawan, Bimal K Das and
12. Aarti Kapil,Emerging resistance to carbapenems in a tertiary care hospital in North India.Indian Journal of Medical Research 124,July 2006:95-98
13. Yan J J, Wu JJ, T sai S H , Chuang C L.Comparison of the double disk,combined –disk, and E-test methods for detecting metallo-beta lactamases in gram negative bacilli.Diag Microbiol Inf Dis 2004;49:5-11