

“Prevalence of Metallo-betalactamases (MBL) producing *Pseudomonas aeruginosa* in a Tertiary care Hospital.”

Dr Anil Rajput*, Dr Bhavin Prajapati **, Dr.Bimal Chauhan***, Dr Atit Shah****, Dr Toral Trivedi*****, Dr Mina Kadam*****

Abstract:

Introduction: *Pseudomonas aeruginosa* isolates are responsible for outbreaks of nosocomial infection in different parts of the world. These isolates have also been responsible for serious infections such as septicemia and pneumonia. MBL production is significant problem in hospital isolates of *Pseudomonas aeruginosa*. *P. aeruginosa* is a pathogen associated with numerous nosocomial infections in immunocompromised patients. The present study was conducted at our hospital with an aim to know the prevalence of Carbapenem resistance & production of metallo-betalactamases producing strain of *Pseudomonas aeruginosa* in our hospital.

Material & Method: This study was conducted at our hospital during April 2010 to October 2011. Total 116 strain of *Pseudomonas* isolated from different samples like pus, swab, Urine, ET secretion etc were evaluated for Carbapenems resistance & MBL production. Various methods has been recommended for screening MBL producing strain like modified Hodge test, EDTA impregnated Imipenem disk & EDTA impregnated Meropenem disk, disk potentiation test with EDTA. We use disk potentiation test with EDTA impregnated Imipenem disk in this study.

Conclusion: In our study out of 116 isolates 20(17.2%) isolates are resistant to imipenem & Meropenem. We have confirmed 14(12%) isolates are MBL producers by disk potentiation test. In India prevalence of MBLs range from 7 – 65% with a recent study reporting 34% occurrence. The early detection of MBL producing *P. aeruginosa* may help in appropriate antimicrobial therapy and avoid the development & dissemination of these multidrug resistance strains. So all isolates of *P. aeruginosa* resistant to imipenem should be screened for MBL production. Combined disk diffusion or disk potentiation test should be introduced in every clinical microbiology laboratory in order to aid infection control.

Key words: Metallo-betalactamases, Carbapenem, Disk potentiation test.

Introduction:

Pseudomonas aeruginosa isolates are responsible for outbreaks of nosocomial infection in different parts of the world. These isolates have also been responsible for serious infections such as septicemia and pneumonia. While isolates producing ESBL remain sensitive to Carbapenems, Carbapenemase producing isolates are resistant to all antibiotics except colistin & tigecyclin

heralds an era of untreatable condition¹. Resistance to Carbapenem is predominantly mediated by MBL, a class B type of betalactamases that recognize bivalent metal ions². Hence widespread surveillance is required to prevent this scenario. The present study was conducted with an aim to know the prevalence of Carbapenem resistance & production of metallo-betalactamases producing strain of *Pseudomonas aeruginosa* in our hospital.

Material & method:

This study was conducted at our hospital during April 2010 to October 2011. Total 116 strain of *Pseudomonas* isolated from different samples like pus, swab, Urine, Blood, ET

* Assistant Professor, ** Tutor,
*** Associate Professor, *****Assistant Professor,
*****Professor
Department of Microbiology,
AMC MET Medical College, L.G.Hospital, Ahmedabad.
*** Assistant Professor, Smt.NHL MMC, Ahmedabad.

secretion, sputum etc were evaluated for Carbapenems resistance & MBL production. Identification of organism was done by the standard laboratory technique³. Antimicrobial sensitivity testing was performed on Muller-Hinton agar plates with commercially available disks (Himedia) by Kirby Bauer disk diffusion method & interpreted as per CLSI recommendation (M100-S16) 2006⁴. MBL producing pseudomonas was suspected when the isolate was resistant to meropenem & imipenem. Various methods has been recommended for screening MBL producing strain like modified Hodge test, EDTA impregnated Imipenem disk⁵ & EDTA impregnated Meropenem disk, disk potentiation test with EDTA⁶. We use disk potentiation test with EDTA impregnated disk in this study. For MIC detection of imipenem the E test strip⁷ and micro dilution plate method⁸ is recommended. However MIC was not done in this study for the cost constraint.

The disk potentiation test⁶

A lawn culture of the test strain was done on Muller Hinton agar plates (opacity adjusted to 0.5 McFarland's standard). Two 10mg Imipenem disk were placed on inoculated plates wide apart & 10microlitre of 50mM zinc solution was added to each of the Imipenem disk. Then 5microlitre of 0.5M EDTA solution was added to one Imipenem disk. After overnight incubation, an increase in zone size of ≥ 7 mm around the Imipenem-EDTA disk as compared to the Imipenem only disk was considered as a positive result for the presence of MBL.(Figure:1)

Results& Discussion:

We have isolated 116 Pseudomonas aeruginosa from different clinical samples during the study period. In our study out of 116 isolates 20(17.2%) isolates are resistant to imipenem & Meropenem. We have confirmed

14(12%) isolates are MBL producers by disk potentiation test. In India prevalence of MBLs range from 7 – 65%⁹ with a recent study reporting 34% occurrence¹⁰.

In our study the prevalence of MBL producing P.aeruginosa strains was 12%, which is similar to studies conducted by Attal RO et al (11.4%)¹¹, Navneeth et al (12%),¹² Mendiratta et al (8.2%),¹³ Hemlata et al (14%)⁵ and Agrawal et al (8.05%)¹⁴ respectively from different parts of India.

Out of the 14MBL producing P.aeruginosa strains, 6(42.9%) were isolated from pus and wound swab and 3(21.4%) were isolated from urine & sputum samples. (Table: 2)

Carbapenems are the only reliable active antibiotics against many multiresistant gram negative pathogens particularly those with extended spectrum betalactamases (ESBLs) and AmpC enzymes¹⁵.The emergence of Carbapenemase producing strain is therefore a major concern.

MBL production is significant problem in hospital isolates of Pseudomonas aeruginosa. P aeruginosa is a pathogen associated with numerous nosocomial infections in immunocompromised patients¹⁶.With increasing isolation of ESBL producing isolates & wide spread use of Carbapenems also increases the problem of MBL production. A simple screening test using combined disk diffusion test, disk potentiation test has been very useful to screen this problem which is also validated by various studies¹⁷.

1st MBL producing Pseudomonas was reported from Japan in 1991 & since then it has been described from various parts of the worlds, including Asia, Europe, Australia, South America & North America.^(18,19)

In some countries MBLs in Pseudomonas constitute about 20% of all nosocomial isolates.⁷

Most nosocomial infections are preventable by simple means even when resources are scarce, but require an institutional approach with immediate & long term measures.²⁰

This emergence of Carbapenemase in this era leads to requirement of strict statutory guidelines implanting intervention for limiting inappropriate uses of antibiotics. Ignorance of rational antibiotics prescribing principles, lack of awareness of the problem of the alarming rise in the multiresistance & pharmaceutical promotion are possible combining factors leading to unnecessary antimicrobial usage. Inadequate infection control is further compounding the problem.

The early detection of MBL producing *P. aeruginosa* may help in appropriate antimicrobial therapy and avoid the development & dissemination of these multidrug resistance strains. So all isolates of *P. aeruginosa* resistant to imipenem should be screened for MBL production. Combined disk diffusion or disk potentiation test should be introduced in every clinical microbiology laboratory in order to aid infection control.

Reference:

- 1) Livermore DM. Has the era of untreatable infection arrived? *J. Antimicrob Chemother* 2009;64:29-36
- 2) Bush K. β lactamases of increasing clinical importance. *Curr Pharm Des* 1999;5:839-45
- 3) Forbes BA, Sham DF, Weissfeld AS. Bailey and Scott's diagnostic Microbiology, 12th Ed. New York: 2007: 340-350.
- 4) Clinical and laboratory standards Institute, Performances standards for antimicrobial disk test, M100-S16, 2006
- 5) Hemalatha V, Sekar U, Kamat V. Detection of metallo- β -lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2005; 122:148-52
- 6) Young D, Lee K, Yum J H, Shin H B, Resselinism, Chong Y. Imipenem –EDTA disk method for differentiating of metallo- β -lactamase producing clinical isolates of *Pseudomonas* spp. And *Acinetobacter* spp. *J Clin Microbiol* 2002;40:3798-801.
- 7) Walsh TR, Toleman MA, Pirel L, Nordmann P. Metallo- β -lactamase; the quiet before the storm? *Clin Microbiol Rev* 2005;18:306-25
- 8) Migliavacca R, Docquire JD, Mugnaioli C, Amicosante G, Daturi R, Lee K et al. Simple Microdilution test for the detection of metallo- β -lactamase production in *Pseudomonas aeruginosa*. *J Clin Microbiol* 2002;40:4388-90.
- 9) Pitout J D, Gregson DB, Poirel L, McClure J A, Le P, Church DI. Detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamases in a large centralized laboratory. *J Clin Microbiol* 2005;43:3129-35
- 10) Castanheira M, Bell J M, Turnidge JD, Mathai D, Jones RN. Carbapenem resistance among *Pseudomonas aeruginosa* strains from India: Evidence for Nationwide endemicity of Multiple Metallo- β -lactamase Clones (VIM-2, -5, -6 and -11) and the Newly characterized VIM-18. *Antimicrob Agents Chemother* 2009; 53:1225-7.
- 11) Attal RO, Basak S, et al; Metallobetalactamase producing *Pseudomonas aeruginosa*: An emerging threat to clinicians. *Journal of clinical and Diagnostic Resaech*, 2010 August(4)2691-2696
- 12) Navneeth B, Shridaran D, Sahay D, Belwadi MRS, A preliminary study on metallobetalactamases producing of *P. aeruginosa* in hospitalized patients. *Indian J Med Res* 2002;116;264-7
- 13) Mendiratta DK, Deotale V, Narang P, Metallobetalactamases producing of *P. aeruginosa* in hospital from rural area. *Indian J Med Res* 2005;121: 701-3
- 14) Agrawal G, Lodhi RB, Kamalakar UP, Khadse RK, Jalgaokar SV. Study of metallobetalactamases production in clinical isolates of *P. aeruginosa*. *Indian J Med Microbiol* 2008;26(4):349-51.
- 15) Pitout JD, Laupland KB. Extended spectrum beta-lactamases producing Enterobacteriaceae: An emerging public health concern. *Lancet Infect Dis* 2008;8:159-66
- 16) Bonfiglio G, Laksai Y, Franchino L, Amicosante G, Nicoletti G. Mechanism of β lactam- resistance amongst *Pseudomonas aeruginosa* isolated in an Italian survey. *J Antimicrob Chemother* 1998; 42:697-702

17) Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotyping techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. *Indian J Med. Microbiol* 2008;26:233-7

18) Gales Ac, Menezes LC, Silbert S, Sadar HS. Dissemination in district Brazilian regions of an epidemic Carbapenem resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamases. *J Antimicrob chemother* 2003;52:699-702

19) Lee K, Lee VG, Uh Y, Ha GY, Cho J, Chong Y, VIM- and IMP- type metallo-beta-lactamases producing *Pseudomonas* spp and *Acinetobacter* spp. In Korean hospital. *Emerg infect Dis* 2003;9:868-71

20) Sarma JB, Ahmed GU, infection control with limited resources: Why & How to make it possible? *Indian J Med Microbiol* 2010;28:11-6

Table 1: Prevalence of MBL Producing *P.aeruginosa* strains

Total no of strains	Positive for MBL(no.)	Percentage for MBL(%)
116	14	12 %

Table 2: Isolation of MBL Producing *Pseudomonas aeruginosa* from different clinical specimen.

Clinical specimen	<i>Pseudomonas aeruginosa</i> (n=116)	MBL Producing.
Pus & Wound swab	51(43.96%)	6(42.9%)
Urine	26(22.41%)	3(21.4%)
Blood	20(17.24)	0
Sputum	11(9.4%)	3(21.4%)
Body fluid	5(4.3%)	1(7.1%)
ET secretion	3(2.58%)	1(7.1%)
Total	116(100%)	14(100%)

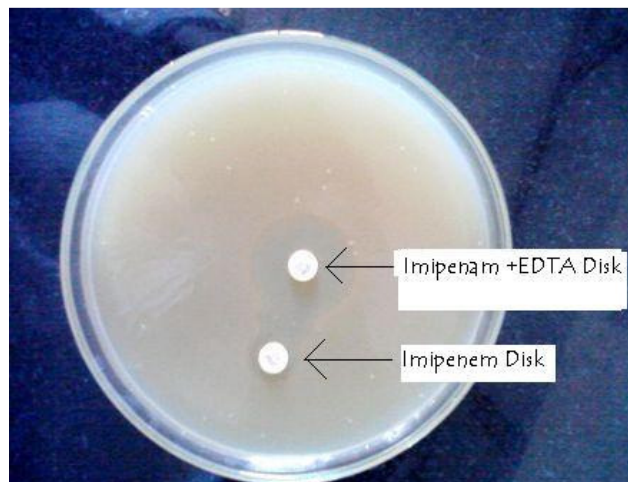


Figure 1: Disk Potentiation Test: An increase in zone size of ≥ 7 mm around the Imipenem-EDTA disk as compared to the Imipenem only disk was considered as a positive result for the presence of MBL.

Date of manuscript submission: 22 January 2012

Date of Peer review approval: 2 August 2012

Date of Publication: 5 September 2012

Conflict of Interest: Nil, Source of Support: Nil.

Date of initial approval: 5 February 2012

Date of final draft preparation: 25 August 2012