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Original Research Article

METALLO-β-LACTAMASE DETECTION IN *PSUEDOMONAS AERUGINOSA* ISOLATES FROM VARIOUS CLINICAL SAMPLES IN NCR REGION.

Jyoti Pal[1], Dakshina Bisht [2*], Agarwal R[3]

1. Phd Student, Department of Microbiology, Santosh Medical College, Ghaziabad, U.P
2. Professor and Head, Department of Microbiology, Santosh Medical College, Ghaziabad, U.P
3. Associate Professor, Department of Microbiology, Santosh Medical College, Ghaziabad ,U.P

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For Correspondence
Email ID:
dakshinabisht@hotmail.com

Abstract:
Background: Acquired drug resistance has been reported in *Pseudomonas spp* by production of plasmid mediated AmpC-β-lactamase, Extended Spectrum-β-lactamase (ESBL) and Metallo-β-lactamase (MBL) enzymes. Carbapenems, being the most potent and reserved drug for treating infections caused by multi-drug resistant bacteria especially *Pseudomonas spp* is under threat due to the emergence of MBL producing *Pseudomonas. aeruginosa*. Thus the study was undertaken to study Metallo- β-lactamase (MBL) production among isolates of *P. aeruginosa* to know their dissemination and thereby proper and judicious selection of antibiotics . **Methods:** 112 isolates of *P. aeruginosa* were obtained from various clinical samples which were subjected to susceptibility testing to antipseudomonal drugs as per CLSI guidelines. Isolates resistant to imipenem were screened for MBL production by Imepenem-EDTA Double Disc Synergy Test (DDST) test. **Result:** Of the 112 isolates of *P.aeruginosa*, 22% strains were imipenem resistant and 16% strains were MBL producers. **Conclusion:** MBL mediated carbapenem resistance in *P. aeruginosa* is a cause for concern in the therapy of critically ill patients. Simple DDST can be done to help monitoring of these emerging resistant determinants.

Keywords: Metallo-β-lactamase (MBL), *Pseudomonas aeruginosa*, Carbapenem, Imipenem (IMP), Double Disc Synergy Test (DDST) .

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that is able to survive in moist environments and is one of the causative agents of hospital-acquired infections, especially in burn patients, which is not only due to its high prevalence and severity but also because of its innate and acquired resistance to antibacterial drugs [(1)]. Further, accquired drug resistance is common in nosocomial isolates of *Pseudomonas spp*[(2)]. In addition

to the intrinsic resistance in *P. aeruginosa*, it also produces enzymes like β-lactamases, which are responsible for the wide-spread β-lactam resistance. Acquired drug resistance is reported in *Pseudomonas spp* by production of plasmid mediated AmpC-β-lactamase, Extended Spectrum-β-lactamase (ESBL) and Metallo-β-lactamase (MBL) enzymes[3]. These β-lactamases hydrolyse the amide bond of the four membered characteristic β-lactam ring, thus rendering the antimicrobial ineffective[4].

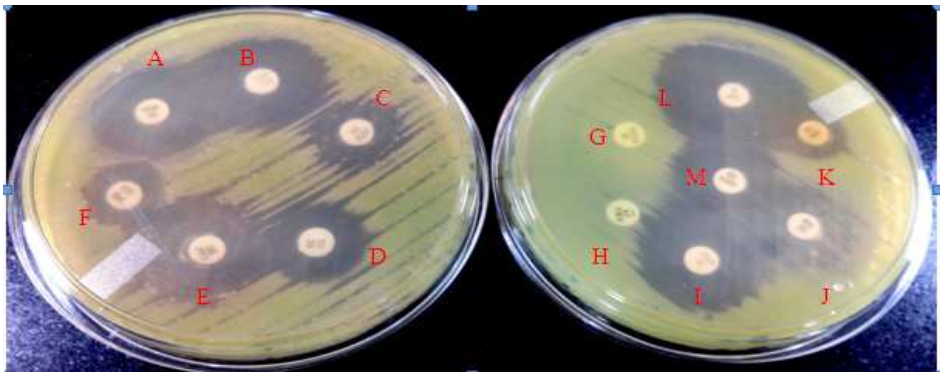
MBL are class B enzymes which hydrolyze carbapenems and are encoded by genes like IMP, VIM, etc [5]. They require divalent cations of zinc as co-factors for enzymatic activity and are universally inhibited by Ethylene Diamine Tetra-Acetic acid (EDTA) as well as other chelating agent of divalent cations usually zinc, as metal co-factors for their enzymatic activity [6]. Till now seven main types of MBL have been described throughout the world- IMP, VIM, SPM, GIM, AIM-1 [7] and NDM-1 [8] . Among them bla IMP and bla VIM are the most common types of MBLs with worldwide distribution [9]. From India only bla VIM [10], [11] and NDM-1 [12] have been reported in *P. aeruginosa* in the past.

Nosocomial infections by *P. aeruginosa* are escalating and importantly the production of MBL is a matter of concern.Thus the present study was conducted to detect the prevalence of MBL producing *P.aeruginosa*.

Material and Methods

112 isolates of *P. aeruginosa* were obtained from various clinical samples of pus /wound swab, urine, blood, respiratory secretion and pleural fluid. *P. aeruginosa* strains isolated were characterized by morphology, pigment production and standard biochemical tests. Antimicrobial susceptibility of all isolates was performed on Muller Hinton Agar plates by Kirby bauer disc diffusion method according to CLSI guidelines [13]. *P.aeruginosa* ATCC 27853 was used as control.

Antibiotic sensitivity of all the isolated strains was put up for Amikacin (30 µg), Tobramicin (10µg), Cefoperazone /sulbactam (75µg), Cefepime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), Azteronam (30µg), Piperacillin/ tazobactam (100/10 µg), Gentamicin (10µg) and Polymixin B (300 units).



A. Tobramicin, **B.**Piperacillin/tazobactam, **C.** Azteronam , **D.** Ceftriaxone, **E.** Ceftazidime, **F.** Polymyxin B, **G.** Meropenem, **I.** Cefepime, **J.** Amikacin, **K.** Cefoperazone/sulbactam, **L .**Ciprofloxacin**, H.** Imipenem, **M.** Gentamicin M. Polymyxin B

Fig-1 Antibiotic sensitivity pattern.

MBL production by *P. aeruginosa* was suspected when the strain was found to be resistant to imipenem and these were subjected further for the detection of MBL production using IMP- EDTA combined Double Disc Synergy Test method.

Detection of MBL

Imipenem- EDTA Double Disc Synergy: The Double Disc Synergy test was performed as described by Yong et al [(14)]. The organisms were inoculated onto plates of Mueller-Hinton agar as recommended by CLSI. Two 10 µg imipenem discs (Becton Dickinson) were placed on the plate and appropriate amounts of 10 µl of EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the imipenem and imipenem EDTA discs were compared after 16-18 h of incubation at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was 7mm than the Imipenem disc alone, it was consider as MBL positive [(15)].

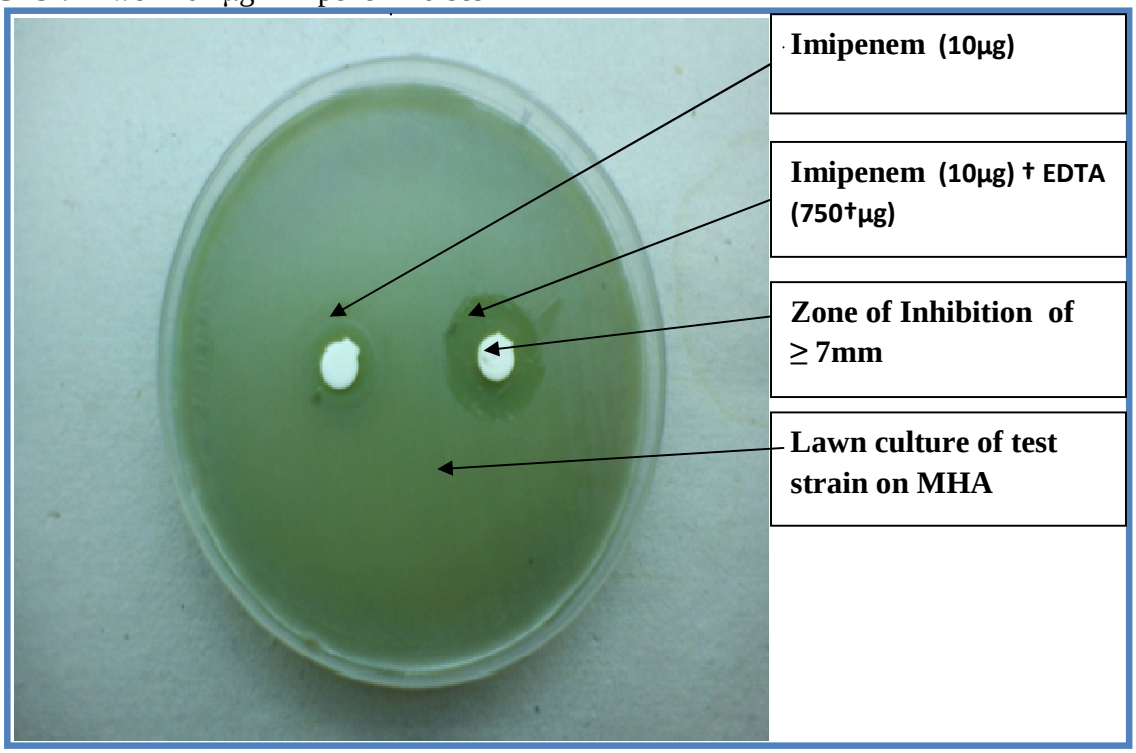


Fig-2 Imipenem† Imipenem EDTA double disc Synergy test showing difference in the inhibition zones between the two discs is >7 mm represents MBL production

Results

Of the 112 (28%) strains of *P. aeruginosa* isolated 25 (22%) strains were Imipenem resistant. The antibiotic sensitivity pattern of Imipenem resistant strains observed were sensitive to Ceftriaxone (5.6%), Ceftazidime (7%), Ciprofloxacin (8%), Cefoperazone/Sulbactam (11%), Gentamicin (11.5%), Cefepime (12%), Tobramicin (12%), Aztreonam (25%), Amikacin (26%), Piperacillin/ tazobactam (30%) and 100% sensitive to Polymixin B as shown in Graph1. None of the isolates were sensitive to Imipenem and Meropenem.

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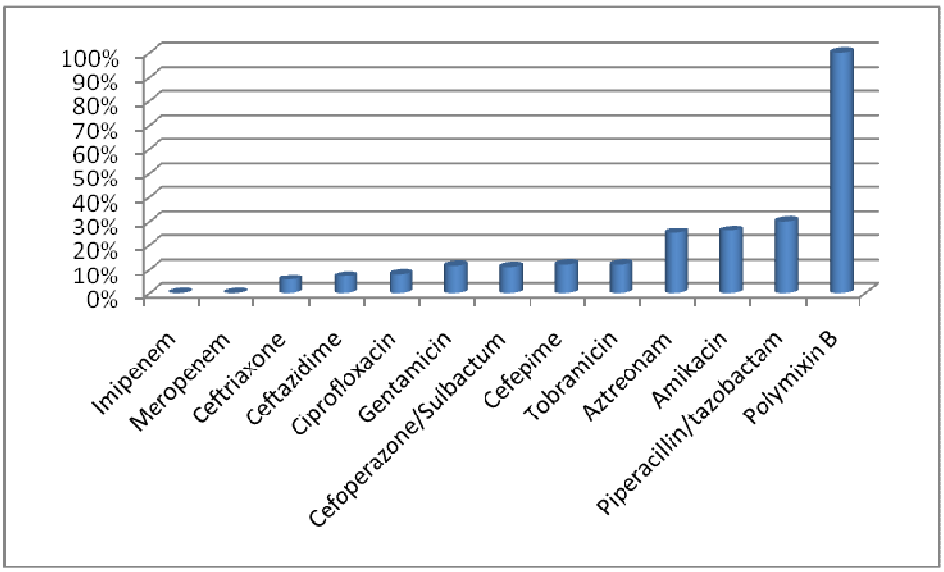

Graph-1 . Antibiotic sensitivity pattern of Imipenem Resistant strains.

Amongst the 112 non-repetitive strains of *P.aeruginosa*, 25(22.3%) strains showed resistance to carbapenem while 18(16%) strains were found to be MBL producers. Amongst MBL producing isolates, maximum number of MBL producing isolates were found from pus / wound swab (50 %) followed by respiratory secretions (27.7%) and urine (22.2%) as shown in Table.1

Table-1. Isolated strains of *P. aeruginosa* from different clinical samples.

Samples	Pseudomonas Isolates (N=112) (28%)	Carbapenem Resistant (N=25) (22%)	MBL producers (N=18) (16%)
Pus	54	14	9 (50%)
Respiratory Secretions	24	5	5 (27.7%)
Urine	28	6	4 (22.2%)
Blood	4	0	0
Pleural fluid	2	0	0

Discussion

P. aeruginosa constitutes a great public health concern, particularly because of the limited therapeutic options available for this pathogen. MBL has been detected with increasing frequency in *P. aeruginosa* worldwide and has been frequently implicated in serious nosocomial infections and outbreaks. [16] Imipenem and meropenem are used routinely for the treatment of nosocomial infections but increasing resistance to these antibiotics, has limited their effectiveness. MBL displays a mobile nature and often co-exists with other resistance determinants, resulting in multidrug resistance (MDR) or a pan-resistance profile. Furthermore, the detection of these carbapenemases is

difficult, which together with the clinical unavailability of MBL inhibitors makes the MBL resistance a major therapeutic and public health problem.

The presence of multidrug resistant *P. aeruginosa* is an increasing trend, rendering many antimicrobial agents ineffective [16]. A study by Andréa Lucena et al reported sensitivity to Amikacin(15%), Gentamicin (7%), Cefepime (9%), Ceftazidime (13%), Ciprofloxacin (8%), Imipenem (0%), Meropenem (3%), and Polymyxin (89%)[17]. However, our study showed maximum sensitivity to Polymyxin B(100%) followed by Piperacillin/Tazobactam (30%), Amikacin (26%), Aztreonam (25%) and Cefepime (12%).

Due to the fact that MBL hydrolyse virtually all classes of β lactamase, their continuous spread will be a clinical catastrophe[18]. With global increase in the types of MBL early detection is crucial[19]. Carbapenems are β-lactam antibiotics, presently considered as the most potent agents of treatment of multidrug resistant gram negative bacterial infections due to the stability of the agents against the majority of β-lactamases and their high rate of permeation through bacterial outer membranes. However, in the last decade there have been increasing reports of carbapenem resistance to this life-saving antimicrobial in *P.aeruginosa*[20]. Carbapenem hydrolysing MBLs have been reported in several countries and have emerged as the most important mechanism of carbapenem resistance.[21,22].

A study by Behra et al showed 85.7% MBL production among *P. aeruginosa* isolates.[19] Agamy et al reported 41% [23] while Simit et al, showed only 6.06% of MBL production[24] .However, a study conducted by Shashikala et al[25] reported 20.7% carbapenem resistant *P. aeruginosa* isolates from endotracheal aspirates showing indwelling devices as major risk factors for the development of resistance while Ami Varaiya et al[26] reported 25% . Our study showed 16% MBL production of which maximum number were from pus / wound swab (50 %). In a similar study by P. Vasundhara et al[27] 40 % of MBL production was reported from pus samples of the total 36% MBL producing strains.

Emergence of MBL-producing *P. aeruginosa* in this hospital reflects excessive use of carbapenems and selective antibiotic pressure. Therefore, a strict antibiotic policy should be followed in every hospital to prevent further spread of MBLs . Clinicians should be made aware of the problem of MBLs, so that they can prescribe antibiotics judiciously. As most MBL-producing organisms are multidrug resistant [28] this might pose a therapeutic challenge to clinicians as well as to microbiologists. Timely implementation of proper infection control practices reduce, eliminate and prevent establishment of antibiotic-resistant organisms as the nosocomial flora. [29].

The development of simple screening tests designed to detect acquired MBL production is a crucial step towards large scale monitoring of these emerging resistant determinants.

Conclusion

Thus our study underlines the unique problem of MBL mediated resistance and documents that MBL producers are present in the community. To overcome the problem of emergence and spread of multi drug resistant *P.aeruginosa*, it is therefore necessary to know their prevalence to help detect emerging trends and to adopt a rational for antibiotic use. Use of simple screening test like DDST can be important in early detection and large scale monitoring of the emerging

resistant determinants and help in appropriate antimicrobial therapy and avoid the development and dissemination of these multi drug resistant strains.

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Resources for the study were self-funded as there was no funding for the study.

Conflict of Interest

The authors declare there is no conflict of interest.

References

1. Adachi JA, Perego C, Graviss L, Dvorak T, Hachem R, Chemaly RF, et al. The role of interventional molecular epidemiology in controlling clonal clusters of multidrug resistant *Pseudomonas aeruginosa* in critically ill cancer patients. *Am J Infect Control*. 2009;37(6):442–6.
2. Hemalatha V, Uma S & Vijaylakshmi K. Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. Indian J Med Res .2005;122: 148-152.
3. Supriya U, Malay R S, Amitabha B. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries. 2010; 4(4): 239-242.
4. Bardford PA. Extended spectrum β-lactamase in the 21st century: The characterization, epidemiology and the detection of this important resistance threat. Clin.Microbiol.2001;14:933-951.
5. Jaykumar S, Appalraju B. The prevalence of multi and pan drug resistant *Pseudomonas aeruginosa* with respect to ESBL and MBL in a tertiary care hospital. Indian J Pathol Microbiol. 2007;50 (4): 922-25.
6. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β- lactamases The quiet before the strom?. Clin Microbio Rev. 2005; 18: 30625.
7. Gupta V. Metallo beta lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* species.Expert Opin Investig Drugs. 2008;17:131-43.
8. Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, Giesecke J. The European NDM-1 Survey Participants. New Delhi metallo-beta-lactamase producing Enterobacteriaceae: Emergence and response in Europe. Euro Surveill.2010;15.pii:19716.
9. Kouda S, Ohara M, Onodera M, Fujiue Y, Sasaki M, Kohara T, *et al*. Increased prevalence and clonal dissemination of multidrug-resistant *Pseudomonas aeruginosa* with the *bla*IMP-1 gene cassette in Hiroshima. J Antimicrob Chemother .2009;64:46-51.
10. Mariana Castanheira JM. Carbapenem Resistance among *Pseudomonas aeruginosa* Strains from India. Evidence for Nationwide Endemicity of Multiple Metallo-beta-lactamase clones (VIM-2,-5,-6-11 and the newly characterized VIM-18).Antimicrob Agents Chemother. 2009;531:225-7.
11. Manoharan A, Chatterjee S, Mathai D, SARI Study Group. Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. Indian J Med Microbiol. 2010;28:241-4.
12. Dugal S, Fernandes A. Carbapenem hydrolyzing metallo-betâ lactamases: A Review. Int J Curr Pharma Res. 2011;3:9-16.
13. Wayne PA: Clinical and Laboratory Standards Institute; 2009. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Nineteeneth Informational Supplement. CLSI document M100-S19.
14. Clinical and Laboratory standards institute (CLSI) Performance standards for antimicrobial susceptibility testing,

16th informational supplements. CLSI Document M2-A9, Wayne PA:2006.
15. Yong D,Lee K, Yum JH , Shin HB , Rossolini G M, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase producing clinical isolates of Pseudomonas spp and Acinetbacter spp. J.Clin.Microbial.2002;40:3798-3801.
16. Maltezou HC. Metallo-lactamases in Gram-negative bacteria: introducing the era of pan-resistance?Int J Antimicrob Agents. 2009;33:e1–7.
17. Andréa L, Libera M. Dalla C et al. Comparison of phenotypic tests for the detection of metallo-beta-lactamases in clinical isolates of *Pseudomonas aeruginosa* Enferm Infecc Microbiol Clin. 2014; 32(10):625–630.
18. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, MaCarkey LA, et al. Prevalence, resistance mechanisms and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2010; 45(3):1160-64.
19. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evalution of four different phenotypic techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol. 2008; 26:233-7.
20. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. Ind J Med Res. 2002;124:95-8.
21. Nordmann P, Poriel L. Emerging carbapenems in gram negative aerobes. Clin Microbial Infect. 2002;115:153-7.
22. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metall-β-lactamases producing Gram- negative bacilli in clinical laboratory. J Clin Microbiol. 2006;44:3139-44.
23. Agamy Al, Mohamed H,Shibl, Atef M,Tawfik, Abdulkader F et al. Extended-spectrum and metallo-beta-lactamases among ceftazidime-resistant *Pseudomonas aeruginosa* in Riyadh, Saudi Arabia. Journal of chemotherapy. 2012;24(2):97-100(4).
24. Simit HK, Anuradha S de et al. Prevalence and risk factors of metallo e-lactamase producing *Pseudomonas aeruginosa* and acinetobacter species in burns and surgical wards in a tertiary care hospital. Journal of laboratory physicions. 2012;vol-4: issue-1.
25. Shashikala, Kanungo R, Srinivasan S, Devi S. Emerging resistance to cabapenem in hospital acquired pseudomonas infection: a cause of concern. Indian j pharmacol. 2006; 38:287-88.
26. Ami V, Nikhil K, Manasi K, Pallavi B & Jyotsana D. Incidence of Metallo beta lactamase producing pseudomonas aeruginosa in icu patients. Indian j med res.2008;127 pp :398-402.
27. Vasundhara. P, Sreenivasulu. P et al. Prevalence of Metallo-beta lactamases producing *Pseudomonas aeruginosa* among the clinical isolates: A study from tertiary care hospital. Int. J. Curr. Microbiol.App.Sci.2015;4(4):955-961.
28. Saderi H, Lotfalipour H et al. Detection of Metallo- Beta-Lactamase producing *Pseudomonas aeruginosa* isolated from burn patients in Teheran. Iran. Lab Med .2010; 41:609-12.
29. Weber J, McManus A. Infection control in burn patients .Burns 200; 30: A16-24.