Original Research Article

Study of Metallo-beta-lactamases (MBLs) producing pseudomonas aeruginosa in various clinical samples in tertiary care hospital

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Abstract

Pseudomonas is the epitome of an opportunistic pathogen, often causing Hospital acquired infection. It is well known that poor outcome occurs with serious indications due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. Therefore, detection of these MBL producing *P. aeruginosa* is crucial for optimal treatment of critically ill patients and to prevent the spread of resistance. Various clinical samples were obtained from patients admitted in hospital or attending OPD. Antimicrobial sensitivity was performed by Kirby –bauer disk diffusion method. Metallo-beta–lactamase production was detected by combined disk method, double disk synergy test method, by epsilometer test (E-test). Out of 590 P. aeruginosa isolates, 200(33.89%) were resistant to Imipenem.84.50% were found to be MBL producers by combined disk method. The no of MBL positive isolates from burn was significant. The hospital stay was significantly longer. All MBL producers were resistant to common antibiotics. However, they were sensitive to polymyxin B (100%), colistin (100%).The given cost constraints of E-test a simple screening test like combined disk test may be used. In absence of therapeutic MBL inhibitors polymixins, colistin may have retained activity against the isolates may be used for the treatment of MDR *P. aeruginosa* infections.

Key Words: metallobetalactamases, pseudomonas aeruginosa.

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INTRODUCTION

Pseudomonas is the epitome of an opportunistic pathogen, often causing Hospital acquired infection. *P.aeruginosa* infection is prevalent among patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous drug users. It causes spectrum of infections which include septicaemia, urinary tract infections, pneumonia, chronic lung infection, endocarditis, dermatitis and osteochondritis.[1] Carbapenems are the antimicrobials of choice for severe *Pseudomonas* infections. However, resistance to this novel antimicrobial is increasing worldwide. Carbapenem resistance in *Pseudomonas aeruginosa* is most commonly due to production of metallo-beta-lactamases (MBLs).[2] The benefits of early MBLs detection include timely implementation of strict infection control practices and treatment with alternative effective antimicrobials.[3] This study was undertaken to find the prevalence and antimicrobial sensitivity pattern of MBLs producing *Pseudomonas aeruginosa* in a tertiary care center and to know the different phenotypic methods.

MATERIALS AND METHODS

After approval from institutional ethical committee, present study was undertaken for period from December 2014 - May 2015 in the diagnostic laboratory of tertiary care hospital. The non repetitive and consecutive isolates were obtained from various clinical samples of all age group and both sexes. All samples were collected using strict aseptic precautions and immediately transported to

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the laboratory as per guidelines for transportation of clinical specimens for aerobic bacteriology.[4] A total of 590 P.*aeruginosa* were isolated. All the specimens were processed according to the standard microbiological technique

1. Direct smear study:
2. Culture: All samples were inoculated onto MacConkey agar and blood agar. The media were incubated aerobically overnight at 37ºc and observed for growth on the next day.[5]
3. Antimicrobial Susceptibility Tests: After identification of *P.aeruginosa*, these isolates were subjected to antimicrobial sensitivity testing by Kirby Bauer disc diffusion method according to CLSI and results were recorded and interpreted as per CLSI recommendations.[6]

All the disc were made available commercially (Hi-Media, Mumbai).The routine antimicrobial susceptibility tests were put up for a gentamicin (10 µg), Ticarcillin/clavulanic acid (75/10 ug) Tobramycin (10 ug), Lomefloxacin (10 ug) Ofloxacin (5 ug) Cefoperazone (75 ug) Cefepime (30µg), Ciprofloxacin (5µg), Piperacillin–Tazobactam (30µg), Imipenem/ Meropenem (10 µg), Colistin (10 µg), Polymyxin B(50 µg). Resistance to Imipenem was used as a screening marker for Metallobetalactamases production.[7]

Phenotypic Confirmatory Test for Metallobetalactamases production

1. Imipenem - EDTA combined disc method
2. Imipenem - EDTA double disc synergy test
3. MBL- E-test
4. Imipenem – EDTA combined disc method[2]:-

Procedure

Imipenem –EDTA combined disc method was performed as described by Yong *et al*.[8] Sterile cotton swab were dipped in the 0.5 Mcfarland broth and plated as a lawn culture on Mueller Hinton agar. After allowing it to dry for five minutes, two imipenem discs, one with 0.5 M EDTA and the other a plain imipenem disc, were placed approximately 30mm apart on the surface of agar plates. The plates were incubated 16-18 hours at 35ºC.

Interpretation

An increase in zone diameter of ≥ 7mm around imipenem+EDTA disc in comparison to imipenem disc alone indicated production of MBLs.

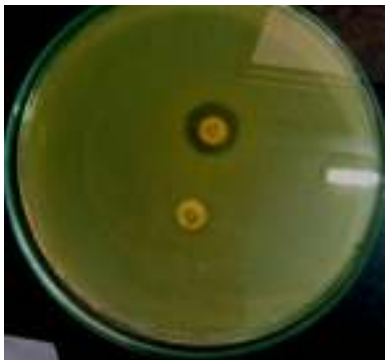

Figure 1: Imipenem–EDTA combined disc method showing Imipenem + EDTA disc produced ≥ 7mm larger zone of inhibition than the imipenem disc

Imipenem - EDTA Double Disc Synergy Test[2,7]

Procedure: Imipenem: EDTA Double disc Synergy method was performed as described by Lee *et al*.[7] Sterile cotton swab was dipped in 0.5 Mcfarland broth and a lawn culture made on Mueller Hinton agar. After allowing it to dry for five minutes. An imipenem discs (10µg) disc was placed 20mm centre to centre from a blank disc containing 10µL of 0.5 M EDTA (750µg). The plates were incubated 16-18 hours at 37ºC.

Interpretation: Enhancement of the zone of inhibition in the area between imipenem and EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBLs production.

Figure 2: Imipenem-EDTA Double Disc Synergy Test showing large synergistic zone of inhibition between imepenem and EDTA.

MBL E-Test[9]: Procedure: The E test MBL strip containing imipenem (4 to 256µg/ml) on one side and imipenem-EDTA (1 to 64 µg/ml) other side was applied on the muller hinton agar inoculated with 0.5 mcfarland growth. The plates were incubated overnight at 37ºC.

Interpretation: MIC ratio of IP (Imipenem)/IPE (Imipenem-EDTA) of > 8 presence of phantom zone and distortion of ellipse were interpreted as positive results.

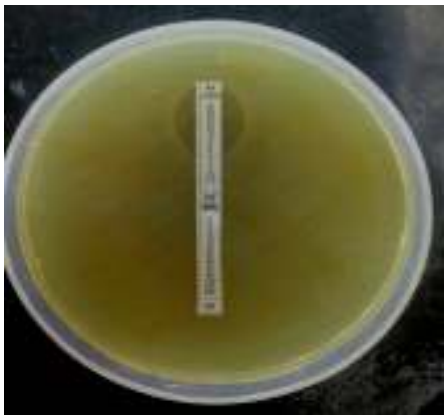

Figure 3: MBL E test showing MIC ratio of IP (Imipenem) / IPE (Impenem-EDTA) > 8.

RESULTS AND OBSERVATIONS

This Laboratory based prospective study included 590 P.*aeruginosa* isolates obtained from various clinical samples. Based on screening criteria for MBLs production, Out of 590 P.*aeruginosa* isolates, 200 (33%) were imipenem resistant. Imipenem resistant strains were subjected to the "E Test" which was taken to be the gold standard for MBLs production. Out of 200 Imipenem resistant, 29 isolates were E Test negative. 171 (85.50%) of the Imipenem producers were E test positive which were definite MBLs producers. So 171 Imipenem resistant strains were definite MBLs producers P.*aeruginosa* Maximum MBLs producing P. *aeruginosa* Isolates were isolated from pus swab (69.60%), followed by Urine (17.55%), blood (7.01%), ET secretion (4.09%), sputum (1.75%).

Table 1: Shows Antimicrobial susceptibility pattern in Imipenem resistant and Imipenem sensitive producing P.*aeruginosa* isolates

Antimicrobial agents	Imipenem resistant P.*aeruginosa* n(%)	Imipenem sensitive P.*aeruginosa* n(%)
Gentamicin	0	246 (63.07%)
Ceftazidime	6 (3%)	164 (42.05%)
Cefepime	52 (26%)	226 (57.9%)
Piperacillin+Tazobactam	68 (34%)	202 (51.7%)
Ticarcillin+clavulnic acid	40 (20%)	183 (47%)
Tobramycin	0	79 (20.4%)
Lomefloxacin	0	45 (50.56%)
Ofloxacin	0	34 (38.20%)
Cefoperazone	0	107 (27.5%)
Ciprofloxacin	21 (10.5%)	159 (40.77%)
Polymyxin B	200 (100%)	390 (100%)
Colistin	200 (100%)	390 (100%)

Lomefloxacin and Ofloxacin were tested for urine sample (imipenem resistance, n=35) (imipenem sensitive, n=89) Imipenem resistant isolates were more resistant to multiple antibiotics as compared to Imipenem sensitive. E test was taken as the gold standard out of 200 imipenem resistant strain 171 were positive by MBL E test and used to Evaluate the performance of, Im-EDTA combine disc test and Im-EDTA double disc synergy test.

Table 2: Shows performance of the different Phenotypic methods for detection of Metallo-betalactamase.

	Im-EDTA combine disc test	Im-EDTA double disc synergy test
Positive	169	135
Negative	2	36
Sensitivity	98.83%	76.94%

Im-EDTA combine disc test was 98.83% sensitive for MBLs. Whereas the Im- EDTA double disc synergy test showed the lower sensitivity as compare to Im-EDTA combine disc test (76.94%).

DISCUSSION

Development of antibiotic resistance is a major concern in the management of bacterial infection. The beta-lactamases are the major defense system of gram-negative bacteria against beta-lactam antibiotics. Metallo-beta-lactamases have potent hydrolyzing activity not only against carbapenem but also against other β-lactam antibiotics such as penicillins and cephalosporin[10] *Pseudomonas aeruginosa* producing MBLs was first reported from Japan in 1991.[11] In India metallo-beta-lactamase producing P.*aeruginosa* were first reported in 2002.[12] Carbapenems are often used as antibiotics of last resort for treating infections due to multidrug resistant Gram-negative bacilli. Therefore, detection of MBLs-producing P. *aeruginosa* is crucial for the optimal treatment of patients and to control the spread of resistance.[10] Present study was based on study of metallo-betalactamases producing P.*aeruginosa* in various clinical samples in tertiary care centre. A total of 590 isolates of P.*aeruginosa* were obtained from various clinical samples. Based on screening criteria for MBLs production, Out of 590 P.*aeruginosa* isolates, 200 (33.89%) were imipenem resistant. In India Imipenem resistance among P.*aeruginosa* range from 10 to 42%. Mehul *et al*[13] in 2011 reported low Imipenem resistance i.e.5.30%. Bashir *et al*[14] reported 13.42% of imipenem resistance. Kumar R *et al*[15] reported 30% of imipenem resistance in 2014. All imipenem resistant P. *aeruginosa* were evaluated for MBLs production by different phenotypic techniques. The "E" test was taken to be gold standard because it is very sensitive in detection of MBLs in P.*aeruginosa*.71 Out of 200 Imipenem resistant P.*aeruginosa*, (85.50%) were Metallo-beta-lactamases positive by MBL- E- test. In present study prevalence of MBLs producing P.*aeruginosa* was computed to be 28.98% (171 out of 590). It vary from centre to centre in country. In India Jayakumar *et al*[16] in 2007 from Coimbatore reported low prevalence i.e. 2.4%. The

occurrence of MBLs positive isolates pose not only therapeutic problem but also a serious concern for infection control management. As a result of being difficult to detect, such organisms pose significant risks particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBLs gene transfer with other pathogens in the hospital.[14] Percentage of imipenem resistant and MBLs prevalence in various studies within India is shown below.

Table 3:

Sr no	Auther	Year	Imipenem resistant percentage	MBLs%
1	Varaiya *et al*[12]	2008	26%	20%
2	Bashir *et al*[15]	2011	13.42%	11.60%
3	Kumar R *et al*[17]	2014	30%	18%
4	Biradar S *et al*[18]	2015	32%	25%
5	Present study	2016	33.89%	28.98%

This variation of MBLs prevalence may be because every institute uses different antibiotic prescribing policies and other associated risk factors. To prevent spread of MBLs producing P.*aeruginosa* in health care setting, it is important to develop antibiotic policy which needs to be followed and monitored regularly. We found MBL –E-test to be sensitive for detection of MBLs on P.*aeruginosa*. Another study by S Ranjan detected 36.36% of Imipenem resistant isolates by E test.[19] The E test MBLs strip is specifically designed to detect as many as clinically relevant MBLs as possible.[20] This novel method could be used by clinical laboratories to monitor the emergence of metallo-beta-lactamase in a range of clinically significant bacteria and by the surveillance laboratories to establish the spread of enzymes. Study by Walsh *et al* reported 94% detection rate.[9] Another study by Behra *et al*[3] Lee *et al*[7] and Khosravi *et al*[20] reported 100% detection rate. Evaluating the different phenotypic methods for detection of MBLs in P.*aeruginosa*, combined disc is more superior than Im-EDTA double disk synergy test and Im-EDTA combined disc and E test were found to be equally sensitive for MBLs detection. The E test is very expensive and costly. Due to cost constraints and availability, a simple screening method like Im-EDTA combined disc method can be used. This technique is very easy, economical and can be incorporated into routine testing of any Microbiology Laboratory. In our study, all MBLs producing P.*aeruginosa* were sensitive to polymixin B and Colistin. To overcome the problems of emergence and spread of multidrug resistant P.*aeruginosa*, the infection control practices, aggressive routine surveillance of antibiotic resistance and the rational use of antibiotic policy in the hospital should be implemented.

REFERENCES

1. Konemann DW, Allen SH, Janda MW, Schreckenberger PC. Winn Jr WC. The Nonfermenter gram Negative Bacilli. In colour atlas and textbook of Diagnositc Microbiology. Sixth edition, Washington C; Lippincott Williams and Wilkins; 1997:316-320.
2. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A. Detection of metallo-beta-lactamase producing Pseudomonas aeruginosa in intensive care units. Australasian Medical Journal 2013;6(12):686-93.
3. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing Pseudomonas aeruginosa. Ind J Med microbial 2008;26(3):233-37.
4. Collee JG, Marr W. Specimen collection, Culture container and media.In: Collee JG, Fraser AG, Marmion BP, Simmons A(eds.) Mackie and McCartney Practical Medical Microbiology. 14th ed Edinburgh: Churchill Livingstone; 1996: P.95-112..
5. Goven JRW. Pseudomonas, Stenotrophomonas, Burkholderia in Collee JG, Fraser AG, Marmoon BP, Sommons A. Mackel and McCartney Practical Medical Microbiology. 14th ed; Elsevier; New Delhi.413-424.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Four Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; CLSI M100-S24;2014
7. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge Test and the Imipenem-EDTA Double-Disk Synergy Test for Differentiating Metallo beta Lactamase-Producing Isolates of Pseudomonas spp. and Acinetobacter spp. J clin microbial 2003;41(10):4623–29
8. Yong D, Choi YS, Roh KH, Kim CK, Park YH, Yum JH, Lee K, Chong Y. Increasing prevalence and diversity of metallo-beta lactamases in Pseudomonas spp., Acinetobacter spp., and Enterobacteriaceae from Korea. Antimicrob Agents Chemother. 2006;50(5):1884-6.
9. Walsh TR, Bolmström A, Qwärnström A, Gales A, Walsh T R, Bolmstro A et al. Evaluation of a New Etest for Detecting Metallo- β –Lactamases in Routine Clinical Testing Evaluation of a New Etest for Detecting Metallo β Lactamases in Routine Clinical Testing.J clin microbial 2002;40(8):2755.
10. Saroj Meena, S.P. Garg, B.P. Sharma, Abhishek Binnani, B.R Bishnoi, Dhara Singh and Kavita Bansiwal. Study Of Prevalence and Antimicrobial Susceptibility Pattern Of Matello-Beta Lactamase Producing Pseudomonas aeruginosa Isolated at a Tertiary Care Institute in North West Region of Rajasthan, India. International. Journal of Current Microbiology and Applied Sciences.(2015)4(8):614-620.
11. Varaiya A, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-beta- lactamase-producing Pseudomonas aeruginosa in diabetes and cancer patients. Indian J Pathol Microbiol 2008;51(2):200-03.
12. Navaneeth BV, Sridaran D, Sahay D, Belwadi MRS. A preliminary study on metallo-β-lactamase producing P.aeruginosa in hospitalized patients. Indian J Med Res 2002; 116:264-7.
13. Chaudhari MS, Javadekar BT, Ninama G, Pandya N, Damor J. A study of Metallo-beta-lactamase producing

P.aeruginosa in clinical samples of S.S.G. Hospital. Natl J Med Res. 2011; 1(2):60-63.
14. Bashir D, Thokar MA, Bashir AF, Bashir G Zahoor D, Shabir A et al. Detection of metallo-beta-lactamase (MBL) producing P.aeruginosa at a tertiary care hospital in Kashmir. Afr J Microbiol Res 2011; 5(2):164-172.
15. Kumar R, Srivastva P, Rishi S, S S Dahiya, Hemwani K, Nirwan P.Detection And Antimicrobial Susceptibility Pattern Of Pseudomonas aeruginosa Isolates In Various Clinical Samples With Special Reference To Metallo Beta Lactamase From A Tertiary Care Hospital In Jaipur, India. National Journal Of Medical Research 2014; 4(2):128-131.
16. Jayakumar S, Appalaraju B, Prevalance of multi and pan drug resistant P.aeruginosa with respect to ESBL and MBL in tertiary care hospital. Indian J Pathology Microbiol. 2007;50(4):922-25.
17. Biradra S, C. Roopa Prevalence Of Metallo-Beta-Lactamase Producing Pseudomonas aeruginosa and its antibiogram in a tertiary care centre. Ind.J.Curr.Microbiol.App.Sci (2015);4(9):952-956.
18. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care unit. Indian J Med Microbiol. 2005; 23:189–191.
19. Ranjan S, Banshankari GS, Babu PR. Evaluation of phenotypic tests and screening markers for detection of metallo-β-lactamases in clinical isolates of Pseudomonas aeruginosa.: A prospective study. Med J DY Patil Univ 2015;8:599-605.
20. Khosravi Y, Loke MF, Chua EG, Tay ST, Vadivelu J. Phenotypic detection of metalloβ-lactamase in Imipenem –resistant Pseudomonas aeruginosa. Scientific World Journal 2012; 2012:654939.

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