International Journal of Research in Medical Sciences
Sinha S et al. Int J Res Med Sci. 2018 Sep;6(9):3099-3104
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20183652

Original Research Article

Prevalence of metallo-beta-lactamases (MBLS) producing *Pseudomonas aeruginosa* in hospitalized patients in rural tertiary care hospital in Uttar Pradesh, India

Sarita Sinha, Amit Singh*, Rajesh Kumar Verma, Dharmendra Prasad Singh, Sunita Kumari

Department of Microbiology, UPUMS, Saifai, Etawah, Uttar Pradesh, India

Received: 05 July 2018
Accepted: 31 July 2018

***Correspondence:**
Dr. Amit Singh,
E-mail: dramitsingh.uprims@gmail.com

ABSTRACT

Background: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections due to MBLs production with limited therapeutic options, higher rate of colonization is encountered in hospitalized patient streated with broad spectrum antibiotics. This study was conducted with an aim to know the prevalence of Carbapenem resistant MBLs producing strains of *Pseudomonas aeruginosa* in hospitalized patients.
Methods: A total of 14700 samples were obtained from various wards during Jan 2016 to June 2017, were screened for *P. aeruginosa* by conventional culture and biochemical tests. All confirmed *P. aeruginosa* isolates were further subjected to Modified Kirby- Bauer disc diffusion test as per CLSI guidelines. All IPM resistant isolates were screened for MBL production by DDST, CDST, MHT and E-test MBL.
Results: Atotal of 1423were identified as *P. aeruginosa*. The isolation rate of *P. aeruginosa* at our hospital was 9.7%. Among these, 130(9.1%) isolates were IPM resistant. A total of 111 (85.4%) were MBL positive by CDST and E-test, 92 (70.5%) by DDST and 80 (61.5%) by MHT. The prevalence of MBL producing *P. aeruginosa* was 111/1423(7.8%) while among IPM resistant *P. aeruginosa*, its prevalence was 111/130 (85.4%).
Conclusions: The study documents presence of nosocomial MBL producing *P. aeruginosa* strains in our Institute. E-test and CDST were superior to DDST and MHT for detection of MBLs.

Keywords: Carbapenem, CDST, DDST, E-test MBL, IPM, MBLs, MHT

INTRODUCTION

Pseudomonas aeruginosa is a clinically troublesome gram-negative pathogen that causes nosocomial outbreaks and opportunistic infections. High mortality rate has been encountered due to resistance to many currently available antibiotics like carbapenems and others antibiotics. The mechanism involved in resistance of *P. aeruginosa* are over expression of efflux pump, acquisition of extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs).[1]

The use of carbapenems has been hampered by the emergence of strains that produce metallo-beta-lactamase, an enzyme that is able to hydrolyze and inactivate this class of antibiotics. MBLs belong to Ambler class B and have the ability to hydrolyze a wide variety of beta-lactam agents such as penicillins, cephalosporins and carbapenems MBLs are unique in requiring the presence of Zn^{+} ion in the active site of the enzyme and are inhibited by chelating agents such as EDTA.[2-3] Phenotypic tests like CDST, DDST, MHT, E-

test MBL are available for the detection of MBLs productions.[4]

Several MBLs genes were reported including IMP, VIM, SPM, GIM, SIM, NDM. These genes are usually inserted in mobile element facilitating the exchange of these resistance genes among several bacterial species.[3] Spread of MBLs producing strain have been observed worldwide and in India also. Increase in mortality rate have been documented in patients having MBL producing *Pseudomonas aeruginosa* infections.[5]

The present study aimed at detection the prevalence of MBLs in isolates of imipenem resistant *P. aeruginosa* from various inpatient department and to determine the best phenotypic test for detection of MBLs.

METHODS

Inclusion criteria

- Adult and pediatric in-patients from various wards of UPUMS, Saifai, Etawah, UP.
- Gram negative bacilli isolates which are oxidase positive were only be included in the study.

Exclusion criteria

- Adult and pediatric out-patients of UPUMS, Saifai, Etawah, UP.
- Stool specimens and throat swabs.
- Gram negative bacilli which were oxidase negative and gram-positive organisms were excluded from the study.

Study period and sample size

It was a prospective observational study conducted at Department of Microbiology, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, UP, India. All the sample from June 2016-2017 of one year were processed for the study. The consent was taken from each patients during enrolment in this study.

Identification and antimicrobial susceptibility testing

Culture, Gram's stain and biochemical identification were performed by conventional method.

Antimicrobial susceptibility testing

After the identification, all *Pseudomonas aeruginosa* isolates were further tested for the first and second line antibiotics by Modified Kirby-Bauer disc diffusion test as per CLSI guidelines. Discs of Hi-Media were used for susuceptibility imipenem (10µg) amikacin (30µg) cefotaxime (30µg) ceftazidime (30µg) meropenem (10µg) tobramycin (30µg) ertapenem (10µg) piperacillin (100µg) piperacillin/tazobactum(100/10µg) gentamicin (10µg) ciprofloxacin (5µg). All IMP resistant *Pseudomonas aeruginosa* isolates were stocked in semisolid agar tubes and used for further phenotypic characterization.

Phenotypic screening methods for detection of beta-lactamase production

The combined disk test imipenem-EDTA

The IMP-EDTA combined disk test performed as the test organisms inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two 10µg imipenem disks (Hi-Media) placed on the plate, and appropriate amounts of 10µl of EDTA solution added to one of them to obtain the desired concentration (750µg). The inhibition zones of the imipenem and imipenem-EDTA disks was compared after 16-18 hours of incubation in air at 35°C. Increase inhibition zone with the imipenem and EDTA disk was ≥7mm than the imipenem disc alone, was considered as MBL positive (Figure 1).

Figure 1: Combined disk test using imipenem and imipenem+EDTA, IMP+EDTA disk showing ≥7mm larger zone of inhibition than imipenem disk alone.

The Double disk synergy test

Test organisms inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An imipenem (10µg) disc is placed 20 mm center to center from a blank disc containing 10µL of 0.5 M EDTA (750µg). The plate was incubated in ambient air, at 35±2°C for 16-20 hours. An enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug interpreted as a positive result (Figure 2).

The Modified Hodge test

It was performed according to the Clinical and Laboratory Standards Institute (CSLI) guidelines. In brief, and incubated at 35±2°C for 2-4 hr. The turbidity of the growth was adjusted to 0.5 McFarland standards using fresh broth. A 1:10 dilution of this broth was Prepared in saline. Inoculum was then swabbed to over

the dry surface of a Mueller-Hinton agar plate so as to obtain a lawn culture. Allowed the plates to dry for 3-10 minutes and then placed a 10 microgram Imipenem disc at the centre of plate. Using a hole or swab, took 3-5 colonies of test organism from the blood agar plate and inoculated in a straight line out from the edge of the disc. The streak should be at least 20-25mm in length. Repeated the same with the QC strain in another direction. QC strain (*Klebsiella pneumoniae* BAA2156) was used in the test. A clover-leaf like identation of the zone of the inhibition of the indicator a strain along the streak of inoculum of the test or QC strain was taken as a positive screening test for carbepenemase production (Figure 3).

Figure 2: Double disk synergy test showing zone enhancement between Imipenem and EDTA disk.

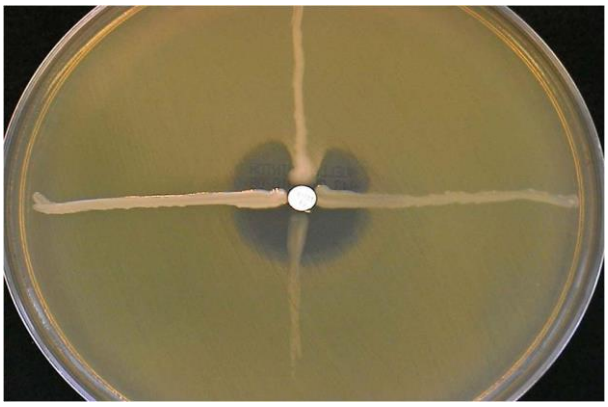

Figure 3: Clover leaf formation in modified Hodge test.

E-test MBL

A lawn culture of *Pseudomonas aeruginosa*, 0.5 MacFarland's standard was made on Mueller Hinton agar. The E-test MBL strip (Hi-Media) containing a double sided two dilution range of IMP (4 to 256µg/mL) and IMP (1 to 64µg/mL) in combination with a fixed concentration of EDTA was placed on the inoculated plate and incubated at 37°C overnight. MIC ratio of IMP (imepenem)/IMP-E (imepenem-EDTA) of >8 or >3 log2 dilution indicates MBL production.[6,7]

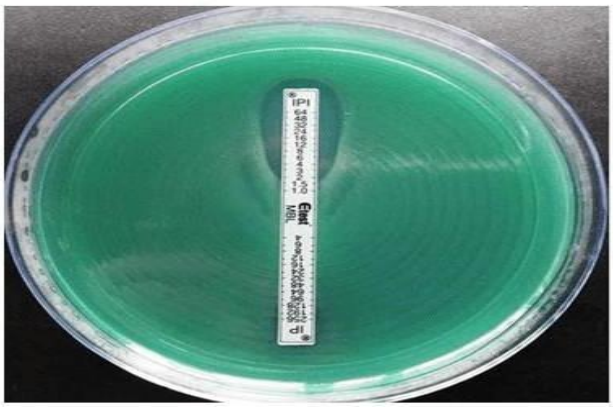

Figure 4: E-test MBL showing MIC of IP/IPI ≥8.

RESULTS

During the study period, a total of 14700 samples (blood, urine and other tissue and body fluids) were obtained in the Department of Microbiology of UPUMS from different wards and departments of our facility. They were subsequently cultured and were assessed for presence of *P. aeruginosa*. Among these, all the imipenem resistant strains were identified and subsequently assessed for MBL producing strains. Out of a total of 14700 samples obtained in our department, a total of 1423 were *P. aeruginosa*, thus showing the prevalence of *P. aeruginosa* to be 9.7%. Among these, 130 were IPM resistant, of which a total of 111 samples were identified as MBL producing strains (Table 1).

Table 1: Identification of MBL-producing P. aeruginosa strains.

Variable	No.
Total samples obtained at department of microbiology during the study period	14700
Samples positive for *P. aeruginosa*	1423/14700 (9.7%)
Samples identified for imipenem resistance	130/1423 (9.1%)
Samples detected as MBL producing	111/130 (85.4%)

Thus, prevalence of MBL producing *P. aeruginosa* at our centre was 111/14700 (0.76%), and among total *P. aeruginosa* isolates its prevalence was 111/1430 i.e.7.8% while among imipenem resistant *P. aeruginosa*, its prevalence was 111/130 i.e. 85.4%. Age of patients ranged from 6 days (0.02 years) to 84 years, median age was 51 years and mean age was 47.31±20.48 years. Majority of patients were aged above 40 yrs (60.8%), most common age group was 61-70 years (22.3%) followed by 31-40 years (16.9%) and 51-60 years (16.2%) while least common age group was 21-30 years (8.5%) followed by 41-50 years (10.0%) (Table 2).

Table 2: Distribution of study population according to age (n=130).

Age Group	No. of patients	Percentage
≤20 yrs	18	13.8
21-30 yrs	11	8.5
31-40 yrs	22	16.9
41-50 yrs	13	10.0
51-60 yrs	21	16.2
61-70 yrs	29	22.3
>70 yrs	16	12.3

Out of 130 isolates imipenem resistant *Pseudomonas aeruginosa*, majority were males (60.0%) and rest were female (40.0%) (Table 3).

Table 3: Distribution of study population according to gender (n=130).

Gender	No. of patients	Percentage
Female	52	40.0
Male	78	60.0

Around three-fourth (n=94; 72.3%) of the patients were from FMW, MMW and PW. Most common clinical setting was PW (25.4%) followed by FMW (23.8%) and MMW (23.1%) while least common clinical setting was LR (0.8%), followed by FOW (1.5%). Out of 130 patients, 15 (11.5%) patients were from ICUs (ICU-3, NICU-5 and SICU-7). All the samples were also resistant for imipenem on disk-diffusion test (100.0%) (Table 4).

Table 4: Distribution of study population according to clinical setting (n=130).

Clinical Setting	No. of patients	Percentage
FMW	31	23.8
FOW	2	1.5
FSW	4	3.1
ICU	3	2.3
LR	1	0.8
MMW	30	23.1
MOW	8	6.2
MSW	6	4.6
NICU	5	3.8
PW	33	25.4
SICU	7	5.4

There were 93.1% ertapenem and 79.2% meropenem resistant strains too (Table5). Out of 130 imipenem resistant *P. aeruginosa* isolates, a total of 111 (85.4%) were confirmed as MBL producing (Table 8) by EDTA combined disk test.

A total of 92 (70.8%) were confirmed as MBL producing by EDTA double disk synergy test and 80 (61.5%) were confirmed as MBL producing by modified Hodge test (Table 6).

Table 5: Resistance pattern of study population.

Antibiotic	No. of patients	%
Ertapenem resistant	121	93.1
Meropenem resistant	103	79.2
Imipinem (Disk-diffusion)	130	100.0

Table 6: Phenotypic methods in detection of MBL production.

Mode of detection	No. of patients	%
EDTA combined disk test	111	85.4
EDTA double disk synergy test	92	70.8
Modified Hodge Test	80	61.5

Table 7: Findings of E-test MBL (MIC, µg/ml).

No. of specimens	Min	Max.	Median	Mean	SD
130	3	18	10.00	10.30	3.31

Table 8: Distribution of study population according to MBL production.

MBL	No. of patients	Percentage
MBL Non-producing	19	14.6
MBL producing	111	85.4

Table 9: Clinical setting of *P. aeruginosa* producing MBL.

Clinical setting	Total	MBL non-producing (n=19)		MBL producing (n=111)	
		No.	%	No.	%
FMW	31	4	12.9	27	87.1
FOW	2	0	0.0	2	100.0
FSW	4	1	25.0	3	75.0
ICU	3	0	0.0	3	100.0
LR	1	0	0.0	1	100.0
MMW	30	5	16.7	25	83.3
MOW	8	1	12.5	7	87.5
MSW	6	1	16.7	5	83.3
NICU	5	0	0.0	5	100.0
PW	33	7	21.2	26	78.8
SICU	7	0	0.0	7	100.0

χ^2=4.800(df=10); p=0.904

On E-test, the values ranged from 3 to 18µg/ml with a median of 10 and mean of 10.30 with standard deviation of 3.31 respectively. A total of 111 samples had titer >8 and were confirmed as MBL-producing sample (Table 7).

MBL-production was observed in all the specimens from FOW, ICU, LR, NICU and SICU (100.0% each). MBL production was least common in FSW (75.0%) and PW (78.8%) (Table 9).

Statistically, association between clinical setting and MBL-producing strains was not observed to be significant.

DISCUSSION

Metallo beta lactamases producing *Pseudomonas aeruginosa* is an important nosocomial pathogen that shows resistance to beta lactam antibiotics, which are currently conserved for the treatment of MDR isolates. *P. aeruginosa* producing MBL was first reported in India in 2002. Present study covered 130 imipenem resistant *P. aeruginosa* isolates, 111 of which were MBL producing isolates. Phenotypic test of these isolates were performed. A total of 111 (85.4%) were MBL positive by CDST and E-test, 92 (70.5%) by DDST and 80 (61.5%) by MHT. The Prevalence of MBL producing *P. aeruginosa* was 111/1423 (7.8%).

The susceptibility pattern of *Pseudomonas aeruginosa* were as follows: imipenem was found to be 100.0% resistant followed by ertapenem (93.08%) and meropenem (79.23%) while amikacin was found to be least resistant (44.62%) followed by ciprofloxacin (46.15%) and tobramycin (46.15%). Several studies have documented the prevalence of MBLs among *P. aeruginosa* varying from 7.5 to 20.8%.[8,9]

Metallo-beta-lactamase producing *Pseudomonas aeruginosa* is an important nosocomial pathogen that shows resistance to all beta-lactam antibiotics except monobactams. Present study shows a quite alarming 85% MBL positive imipenem resistant *Pseudomonas aeruginosa* cases. The study revealed that prevalence rate of 0.76 % in the our centre, among total *Pseudomonas aeruginosa* isolates the prevalence rate of MBLs was 7.8%, while in among imipenem resistant *Pseudomonas aeruginosa* isolates its prevalence was 85.4% is almost comparable to the prevalence rate shown in studies done in India 9.25% and Afghanistan 6.67%. The most common source of the isolate in our study were urine, sputum (35%,25.4%) respectively, rest other isolates were recovered from pus, blood and other body fluids Viren et al, also reported maximum isolation from Urine and sputum and pus 26.7% etc.[10] Distribution of specimens may vary with various wards in the hospital as each setting has different environment. Most common clinical setting in the present study was PW (pulmonary ward), followed by FMW (female medicine ward), and MMW (male medicine ward). Gender wise male patients 60% constituted a larger groups in this study, other studies have shown similar findings.[11,12]

When factors such as age of the patients were considered, the occurrence of the isolates was higher in the age group of patients who were more than 60 yrs of age (22%), similar observation was made by Somporn et al, 29.5%, Srinivas et al 37%.[13,14] *Pseudomonas aeruginosa* infection is more common in the old age groups patients, this could be explained as due to decreased immunity, prolonged hospitalization and other associated co-morbidities in the age groups. In the present study, IMP resistant isolates were used as criteria for MBL screening. Imipenem was found to be 100.0% resistant to *P. aeruginosa* followed by ertapenem (93.08%) and meropenen (79.23%) while amikacin was found to be least resistant (44.62%) followed by ciprofloxacin (46.15%) and tobramycin (46.15%). The positivity rate among phenotypic test were combined disk test 85.4%, double disk synergy test 70.5% and Modified Hodge test 61.5%, with higher numbers of isolates producing MBL was detected by combined disk test than double disk test and modified Hodge test.

The emergence of resistance in *P. aeruginosa* is an increasing clinical problem which not only limits future therapeutic choices but is also associated with increased rates of mortality, morbidity and higher cost. The prevalence of *P. aeruginosa* often varies between communities, hospitals, therefore it is important to institute to establish system of surveillance in a hospital for appropriate therapeutic approach. Increase in antibacterial resistance *in P. aeruginosa* is a cause of concern, continuous monitoring of bacterial resistance trends should be done, and therapy should be based on antibacterial susceptibility testing.

CONCLUSION

This study shows a clearer spectrum of the current MBL producing *Pseudomonas aeruginosa* in the hospitalised patients. MDR microorganisms are accelerating and becoming major problem in the era of infectious diseases. Treatment of patients infected with MBLs resistant organisms is challenging due to the currently limited options. Such isolates also show resistant to other antibiotics. Out of a total of 14700 samples obtained in our department, total of 1423 were *P. aeruginosa* positive, thus showing the prevalence of *P. aeruginosa* to be 9.7%.

Among these, 130, a total of 111 samples were identified as MBL producing. Thus prevalence of MBL producing *P. aeruginosa* at our centre was 111/14700 (0.76%), among total *P. aeruginosa* isolates its prevalence was 111/1430 i.e. 7.8% while among imipenem resistant *P. aeruginosa*, its prevalence was 111/130 i.e. 85.4%. Majority of cases found in age above 60 yrs. Phenotypic test

The positivity rate among phenotypic test were combined disk test 85.4%, double disk synergy test 70.5% and modified Hodge test 61.5%, with higher numbers of isolates producing MBL was detected by combined disk test than double disk test and modified Hodge test.

This study found imipenem-EDTA combine disc test detected more MBLs isolates, than combined disc test, according to E-test for MBLs 111 out of 130 had titre >8 and were confirmed as MBL-producing isolates.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Valenza G, Joseph B, Elias J, Claus H, Oesterlein A, Engelhardt K, et al. First survey of metallo-β-lactamases in clinical isolates of Pseudomonas aeruginosa in a German university hospital. Antimicrobial agents and chemotherapy. 2010 Aug 1;54(8):3493-7.
2. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of Pseudomonas aeruginosa producing metallobeta-lactamases in a large centralized laboratory. J Clin Microbiol. 2005;43:3129-35.
3. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol. 2000;3:489-95.
4. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 2001;7:88-91.
5. 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 Microbiol. 2008 Jul 1;26(3):233.
6. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and imipenem-EDTA-disk synergry test for differentiating metallo- beta-lactamase producing isolates of Pseudomonas Spp and Acinetobacter spp. J clin Microbiol. 2003;414623-9.
7. Ranjan S, Banashankari 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 Dr. DY Patil University. 2015 Sep 1;8(5):599.
8. Gupta V, Datta P, Chander J. Prevalence of metallo-beta lactamase (MBL) producing Pseudomonas spp and Acinetobacter spp.in a tertiary care hospital in India. J Infect. 2006;52:311-4.
9. Basak S, Khodke M, Bose S, Mallick SK. Inducible Amp-C beta-lactamase producing Pseudomonas aeruginosa isolated in a rural hospital of central India. J Clin Diagn Res. 2009;3:1921-7.
10. Javiya VA, Ghatak SB, Patel kr, Patel JA. Antibiotic susceptiblity patterns of Pseudomonas aeuginosa at a tertiary care hospital in Gujrat, India. Indian J Pharmacol. 2008;40(5):230-4.
11. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S. Detection of metallo-beta lacatmase producing Pseudomonas aeruginosa in intensive care units. Australas Med J. 2013;6(12):686-93.
12. Tsakris A, Poulou A, Krist I, Pittaras T, Spanakis N, Pournara S, et al. Large dissemination of VIM-2 metallo-beta-lactamases producing pseudomonas aeruginosa strains causing health care-associated community onset infection. J Clin Microbiol. 2009;47(11):3524-9.
13. Samporn S, Chuntima T, Thitiya Y, Chertask D. Prevalence and antimicrobial susceptibility of Pseudomonas aeruginosa mucoid and non-mucoid type. Southeast Asia J Trop Med and Public Health. 2004;35:893-4.
14. Srinivas B, Lalitha devi D, Narasinga Rao B. A prospective study of Pseudomonas aeruginosa and its antibiogram in a teaching hospital of Rural setup. J Pharma and Biomed Sci. 2012;22(18):23-9.

Cite this article as: Sinha S, Singh A, Verma RK, Singh DP, Kumari S. Prevalence of metallo-beta-lactamases (MBLS) producing Pseudomonas aeruginosa in hospitalized patients in rural tertiary care hospital in Uttar Pradesh. Int J Res Med Sci 2018;6:3099-104.