



Original Research Article

Antimicrobial Resistance Profile of *Pseudomonas Aeruginosa* Isolates with Special Reference to Metallo- β -Lactamase Producers

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Received: 29/01/2015

Revised: 21/02/2015

Accepted: 24/02/2015

ABSTRACT

Introduction: Acquired metallo- β -lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β -lactams including carbapenems.

Materials and methods: A total of 274 consecutive, non-repetitive isolates of *Pseudomonas aeruginosa* identified by conventional method over a period of one year were screened for carbapenem resistance by using imipenem disk. Antimicrobial susceptibility of the isolates was performed by disk-diffusion method according to CLSI guidelines. The imipenem resistant isolates were subjected to combined disk test (imipenem and EDTA) and MBL-E test for confirmation of MBL production.

Results: Imipenem resistance was observed in 31 (12.55%) *P. aeruginosa*. Of these 28 isolates were MBL positive on combined disk method. Among 28, 23 isolates were positive by MBL-E test. Incidence of MBL producing *P. aeruginosa* was 23 (8.39%). Majority were from pus (74%). Maximum numbers of isolates were from surgical wards (30%). Apart from cephalosporin's, high rate of resistance was also observed against aminoglycosides (Gentamicin 86.95%, Amikacin 56.52%) and fluoroquinolones (Ciprofloxacin 82.6%, Levofloxacin 69%) unlike MBL non-producing strains which is statistically significant ($P < 0.05$).

Conclusion: Detection of MBL-producing *P. aeruginosa* strain by phenotypic methods is recommended at diagnostic centers for prevention of the spread of these multi-drug resistant isolates.

Key words: *P. aeruginosa*, imipenem resistant, MBL E-test, multidrug resistant.

INTRODUCTION

Acquired metallo- β -lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze with the exception of aztreonam, all β -lactams including carbapenems. [1]

P. aeruginosa, producing MBL, was first reported from Japan in 1991 and since

then has been described from various parts of the world, including Asia, Europe, Australia, South America and North America. [2]

The present study was undertaken to detect metallo- β -lactamase (MBL) in *Pseudomonas aeruginosa* isolates obtained from hospitalized patients by phenotypic

methods and to find out their antimicrobial resistance profile.

MATERIALS AND METHODS

A prospective study, where in all the consecutive non-repetitive isolates of *Pseudomonas aeruginosa* from various clinical samples obtained from patients admitted to a tertiary care hospital over a period of one year were included in the study. The isolates were identified as *P. aeruginosa* by conventional method. [3]

Inclusion criteria:

- *Pseudomonas aeruginosa* isolates from samples obtained from hospitalized patients.

Exclusion criteria:

- *Pseudomonas aeruginosa* isolates obtained from patients attending outpatient department.
- Repetitive samples from the same patients.

Antimicrobial susceptibility of the isolates was performed by disk-diffusion method according to CLSI guidelines. [4] Resistance to carbapenem was detected by using imipenem disk. Imipenem resistant isolates were selected for detection of MBL production by imipenem-EDTA combined-disk test and E-test (imipenem and imipenem+EDTA). [5]

The imipenem (IMP)-EDTA combined-disk test was performed as described by Yong *et al.* Two 10 µg imipenem disks (Hi-media) were placed on the Mueller- Hinton agar plate inoculated with test strains, and an amount of 10 µl of EDTA solution was added to one of them to obtain the desired concentration of 750 µg. The inhibition zones of the imipenem and imipenem+EDTA disks were compared. If the increase in inhibition zone with imipenem+EDTA is ≥ 7 mm than the imipenem disk alone, it was considered as MBL positive. [5]

The E-test was done using E-Test MBL strip (HiMedia laboratory Pvt. Limited, Mumbai, India) containing a double-sided seven-dilution range of imipenem (4 to 256 µg/mL) and imipenem (1 to 64 µg/mL) in combination with a fixed concentration of EDTA. MIC ratio of imipenem /imipenem +EDTA of > 8 dilutions were interpreted as positive for MBL production. [6]

Comparison of Antibiotic resistance pattern of metallo-β-lactamase positive and metallo-β-lactamase negative *Pseudomonas aeruginosa* was done by using Chi-square (χ^2) Test.

RESULTS

A total of 274 isolates of *P. aeruginosa* recovered from various clinical samples. Of these, 179 (65.3%) were isolated from pus, 36 (13.1%) from urine, 35(12.7%) from sputum and 24 (8.75%) from ear discharge.

Imipenem resistance was observed in 31 (12.55%) isolates of *P. aeruginosa*. Twenty-eight (10%) of these isolates exhibited ≥ 7 mm zone enhancement in the combined-disk test. The zone diameters were similar and reproducible when the procedure was repeated.

MBL E-test was done on these 28 isolates which have been interpreted as MBL positive on combined-disk method. Among 28, only 23 isolates exhibited MIC ratio of imipenem: imipenem+EDTA > 8 . Five isolates were resistant to, Imipenem (MIC 256 µg/ml) and imipenem+EDTA (MIC 64 µg/ml).

Overall the incidence of MBL among *P. aeruginosa* in our hospital was 23 (8.39%). Majority of these isolates were from pus (74%) followed by urine (26%). Distribution of MBL producing *P. aeruginosa* among different wards was as follows: Surgery (30%), Orthopedics (22%),

OBG (22%), Medicine (17%) and ENT (9%).

Comparison of Antibiotic resistance pattern of metallo- β -lactamase positive and

metallo- β -lactamase negative *Pseudomonas aeruginosa* is depicted in Table – 1.

Table: - 1. Comparison of Antibiotic resistance pattern of metallo- β -lactamase positive and metallo- β -lactamase negative *Pseudomonas aeruginosa*.

Antibiotics	MBL Negative (n = 251).		MBL Positive (n = 23).		P Value
	Resistant no.	Percentage (%)	Resistant no.	Percentage (%)	
Amikacin	14	5.57%	13	56.52%	0.0
Cefepime	54	21.51%	23	100%	0.0
Ceftazidime	159	63.35%	23	100%	0.000367
Ciprofloxacin	078	31.07%	19	82.61%	0.0
Gentamicin	63	25.09%	20	86.96%	0.0
Levofloxacin	60	23.9%	16	69.57%	0.0
Imipenem	08	3.18	23	100%	0.0
Piperacillin-Tazobactam	10	4.0%	23	100%	0.0

(P < 0.00 for all the antimicrobials which is statistically significant)

DISCUSSION

Carbapenems are β -lactam antibiotics, presently considered as the most potent agents for the treatment of multi-drug resistant Gram negative bacterial infections due to the stability of these 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 resistance to this life-saving antimicrobial among *Pseudomonas aeruginosa*.

Resistance to carbapenems in *P. aeruginosa* may develop due to impermeability, which occurs due to the loss of the opr D porin, the up-regulation of an active efflux system present in these organisms, or the production of MBLs. Carbapenem hydrolyzing MBLs have been reported in several countries and emerged as the most important mechanism of carbapenem resistance.^[7]

Production of MBL by *Pseudomonas spp.* and other Gram-negative bacteria has tremendous therapeutic consequences, since these organisms also carry other multi-drug resistance genes and the only available treatment option remains the polymyxin B and colistin.

Several phenotypic methods are available for the detection of MBL producing

bacteria. All these are based on the ability of metal chelators such as EDTA and thiol-based compounds, to inhibit the activity of MBL. These tests include the double-disk synergy test using EDTA with imipenem (IMP) or ceftazidime (CAZ), 2-mercaptopropionic acid with ceftazidime or imipenem, Hodge test, a combined disk test using EDTA with ceftazidime or imipenem, the MBL E-test and micro-dilution method using EDTA and 1, 10-phenanthroline with imipenem.^[7]

The double-disk synergy test and Hodge test are often difficult and subjective to interpret. Further 2-mercaptopropionic acid and 1, 10-phenanthroline is toxic and special precaution has to be taken when working with this compounds.^[7]

Currently, no standard method for MBL detection has been proposed and despite PCR being highly accurate and reliable, its accessibility is often limited to reference laboratories. The combined-disk test using EDTA with imipenem is simple to perform and interpret and can be easily introduced into the workflow of a clinical laboratory.^[7] The E-test MBL Strip (IP-IPE) has the ability to detect metallo- β -lactamases, both chromosomally and plasmid mediated, in aerobic and anaerobic bacteria.^[6] We have screened only

carbapenem resistant isolates with combined-disk method and confirmed by MBL E-test.

In this study, most of the *P. aeruginosa* strains have revealed resistance to routinely used antibiotics. Among the aminoglycosides, resistance to amikacin was seen in 18.24%, gentamicin in 30.29% and tobramycin 16.78% of isolates. Different studies have reported amikacin resistance ranging from 3% to 91.2%, [8-12] gentamicin from 47% to 95.75% [10,12,13] and tobramycin 11.9% to 70%. [14,12,15,10]

We found 35.4% isolates resistant to ciprofloxacin. Other studies have reported resistance ranging from 37% to 91.31%. [10,15,12,16,13]

Resistance to ceftazidime was 66.42%. Relatively less resistance was observed with piperacillin-tazobactam (12.04%). The range of ceftazidime resistance quoted by other studies is from 10% to 85.5%. [9,10,14,15,12,16] and piperacillin-tazobactam is 5% to 35.71%. [9,10,15] Imipenem resistance was observed in 31 (12.55%) isolates of *P. aeruginosa*

Twenty-eight (10%) of these isolates were MBL positive by combined-disk test. Only 23 (8.39%) isolates were confirmed to be MBL producers by MBL E-test. Five isolates were resistant to imipenem (MIC 256 µg/ml) and imipenem+EDTA (64µg/ml). These resistant 5 isolates could be due to some other mechanism involved, such as permeability mutation via the loss of porins or the up-regulation of efflux systems.

Thus, MBL producing *P. aeruginosa* was observed in 8.39%. Many different studies have reported MBL producing strains, of imipenem resistance isolates by combined-disk test, ranging from 4% to 100%. [17,11,10,18]

The unique problem with MBL is their unrivalled broad spectrum resistance profile. These MBL positive strains are

multi-drug resistant, being resistant to the agent in two or more of the following antimicrobial categories: β-lactam-antibiotics, carbapenem, aminoglycosides and fluoroquinolones. However, they remain susceptible to polymyxins. [11]

In our study *P. aeruginosa* exhibited maximum resistance to ceftazidime 182 (66.42%) and least to imipenem 31 (11.31%). All (100%) were sensitive to polymyxin B.

A total of 122 (44.52%) isolates were multi-drug resistant. Prevalence of multi-drug resistance in imipenem-resistant up to 87% has been quoted by other study. [19] MBL producing strains were found to be 100% resistant to cefepime, ceftazidime and imipenem. A high rate of resistance has been observed against aminoglycosides (gentamicin 86.95% and amikacin 56.52%) and fluoroquinolones (ciprofloxacin 82.6%, levofloxacin 69%). However, MBL non-producing strains showed lower resistance to these antibiotics which is statistically significant. (Table: 1)

The identification of MBL producing *P. aeruginosa* in clinical samples has clinical implications because such strains were more likely to cause invasive disease and associated with higher hospital case-fatality rates compared with other imipenem-resistant strains.

The accurate identification and reporting of MBL-producing *P. aeruginosa* will aid infection control practitioners in preventing the spread of this multi-drug resistant isolates. [11]

CONCLUSION

Detection and confirmation of MBL-producing *P. aeruginosa* strain by phenotypic methods is recommended at diagnostic centers for prevention of the spread of these multi-drug resistant isolates.

Authors Contributions:

Shankar Mahesh K:- Definition of intellectual content, literature search, manuscript preparation and manuscript editing.

Thipperudraswamy T:- Data acquisition, data analysis, and statistical analysis.

Nadgir Shobha D:- Concept design and manuscript review.

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How to cite this article: Shankar MK, Thipperudraswamy T, Nadgir SD. Antimicrobial resistance profile of *Pseudomonas aeruginosa* isolates with special reference to metallo- β -lactamase producers. Int J Health Sci Res. 2015; 5(3):137-142.

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