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Original Article

Prevalence of Metallo-beta-lactamase Production in Imipenem-resistant *Pseudomonas* in Tertiary Care Center at Kota Region

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Abstract

Introduction: *Pseudomonas* spp. is common cause of nosocomial infection. Metallo-beta-lactamase (MBL) production is an important cause of antimicrobial drug resistance in *Pseudomonas*. Determination of their prevalence is essential to form an effective antibiotic policy and for prevention of their spread in hospital and community.

Objective: To detect the prevalence of MBL production in imipenem-resistant *Pseudomonas* isolated from different clinical samples and their antibiotic sensitivity profile.

Methods: *Pseudomonas* isolates from various clinical samples were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines. Imipenem-resistant *Pseudomonas* isolates were further processed for MBL detection by combined disc synergy test (CDST) and double disc synergy test (DDST) with imipenem.

Result: Among 7493 processed clinical specimens, 202 *Pseudomonas* spp. were isolated. Of these, a total of 73 *Pseudomonas* spp. was found resistant to imipenem. 69 (94.52%) imipenem-resistant *Pseudomonas* isolates were positive for MBL by CDST-imipenem-EDTA (CDST-IPM) method, whereas 65 (89.04%) were positive by DDST-IPM method, respectively. MBL positive *Pseudomonas* was most prevalent in pus (36.23%). MBL-producing *Pseudomonas* was most susceptible to piperacillin-tazobactam combination (43.48%). All MBL-producing *Pseudomonas* isolates were resistant to ceftriaxone.

Conclusion: MBL-producing *Pseudomonas* was highly prevalent in Kota region, which is one of the major causes of resistance to imipenem and other antimicrobial agents. There is a requirement of regular surveillance and strict antibiotic policy to fight against these multi-drug resistant pathogens.

Key words: Carbapenem resistance, Combined disc synergy test-imipenem-EDTA, DDST-imipenem-EDTA, Metallo-beta-lactamase, *Pseudomonas aeruginosa*

INTRODUCTION

The aerobic pseudomonads are rod-shaped, Gram-negative, oxidase-positive bacteria, motile using one or more polar flagella.[1] *Pseudomonas aeruginosa* is a virulent agent having a tendency to develop resistance to the majority of the antibiotics available for treatment. It is a leading cause of life-threatening nosocomial infection. Its intrinsic resistance to many antimicrobial agents and development of multidrug resistance impose severe therapeutic problem for clinicians.[2]

The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria are widely accepted as a major problem that has been observed over the last decade.[3] Among the β-lactams, carbapenems are potent agents for the serious treatment of Gram-negative bacterial infections. These antibiotics are well-suited to this use because of their broad spectrum activity and resistance to hydrolysis by most β-lactamases, including the extended-spectrum

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β-lactamases (ESBL).[4] Carbapenem resistance has been observed frequently in non-fermenting bacilli *P. aeruginosa* and *Acinetobacter* spp.[5] Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin-binding proteins, and carbapenem-hydrolyzing enzymes-carbapenemase.[6]

β-lactamases are classified into four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs. Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl-enzyme through an active site serine, whereas class B β-lactamases are metallo enzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis.[7] Due to this zinc dependency, chelators such as EDTA inhibit metallo-beta-lactamase (MBL) activity.[8]

Acquired MBL has recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β-lactams including carbapenems with the exception of aztreonam. Because their genes are carried on highly mobile elements, allowing easy dissemination. Such strains are not susceptible to therapeutic serine β-lactamase inhibitors (such as clavulanate and sulfones).[9]

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Government Medical College, Kota, Rajasthan, from November 2014 to October 2015.

Pseudomonas spp. isolated from various clinical specimens, such as urine, pus, respiratory secretions, body fluids, and cerebrospinal fluid (CSF), of patients attending Outpatient Department (OPD) or Inpatient Department (IPD) of MBS Hospital and associated group of hospitals were processed for the study.

Organisms grown were identified by their:

a. Motility testing by hanging drop method
b. Colony characteristics on solid media
c. Gram's staining of the isolated colonies
d. Battery of biochemical reactions including: Oxidase test, triple sugar iron (TSI) test, oxidation fermentation test, urease test, indole production, citrate test, and decarboxylation of amino acids.

All the non-duplicate *Pseudomonas* isolated during study were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method for cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), ciprofloxacin (5 μg), piperacillin (100 μg) and piperacillin-tazobactam (100/10 μg). All discs were procured commercially from Hi-media laboratory limited, India. The zone diameter was measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates resistant to imipenem were considered screening positive. Then, the isolates were further tested for MBLs production by combined disc synergy test (CDST) using imipenem and EDTA (CDST-IPM) and double disc synergy test (DDST) using IPM and EDTA (DDST-IPM).

CDST-IPM

The IPM CDST was performed as described by Yong *et al*. The test organisms was inoculated on Mueller-Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 g of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two imipenem (10 μg) discs were placed on the surface of an agar plate at distance of 25 mm and 10 μl EDTA solution added to one of them to obtain the desired concentration of 750 μg. The zones of inhibition of imipenem and IPM discs were compared after 16 to 18 hrs of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the imipenem and IPM disc was ≥7 μm than the imipenem alone, it was considered MBL positive (Figures 1-3).[10]

DDST-IPM

Test strains were adjusted to the McFarland 0.5 standard and used to inoculate Mueller-Hinton agar plates. Depending on the test, a 10 μg imipenem disc or a 30 μg ceftazidime disc was placed on the plate, and a blank filter paper disc was placed at a distance of 10 mm (edge to edge). To the blank disc 10 μl of a 0.5 M EDTA (750 μg) solution was added. After overnight incubation, the presence of even a small synergistic inhibition zone was interpreted as positive (Figures 1-3).[11]

RESULTS

During the study period, a total of 7493 clinical specimens were processed out of which 202 *Pseudomonas* spp. were recovered. Of these, a total of 73 *Pseudomonas* spp. were found resistant to imipenem. Imipenem-resistant *Pseudomonas* spp. was most commonly isolated from pus (38.35%) followed by urine (30.14%), respiratory secretions (12.33%), body fluid (12.33%), and CSF (6.85%), respectively (Table 1, Figure 4). Maximum prevalence of imipenem-resistant *Pseudomonas* was observed in 21-40 years age group (30.14%). IPD:OPD ratio was 1.81:1.

Maximum number of imipenem-resistant *Pseudomonas* isolates were susceptible to piperacillin-tazobactam combination (43.83%) followed by ciprofloxacin (32.88%), amikacin (23.29%), gentamicin (23.29%), piperacillin (9.59%), cefepime (5.48%), cefotaxime (4.11%), ceftazidime (4.11%), and ceftriaxone (1.37%), respectively (Table 2, Figure 6).

Out of 73 imipenem-resistant *Pseudomonas* isolates, 69 (94.52%) were positive for MBL by CDST-IPM method, whereas 65 (89.04%) were positive by DDST-IPM method, respectively (Table 3). MBL positive *Pseudomonas* was most prevalent in pus (36.23%) followed by urine (31.89%), respiratory secretions (11.59%), body fluid (13.04%), and CSF (7.25%), respectively (Table 1, Figure 5). MBL positive *Pseudomonas* was most prevalent in 21-40 years age group (31.88%). IPD:OPD ratio for MBL-producing *Pseudomonas* was 2:1.

MBL-producing *Pseudomonas* was most susceptible to the piperacillin-tazobactam combination (43.48%) followed by ciprofloxacin (33.33%), gentamicin (21.74%), amikacin (20.29%), piperacillin (8.70%), ceftazidime (2.90%), cefepime (1.45%), and cefotaxime (1.45%), respectively.

Table 1: Sample-wise distribution of imipenem-resistant and MBL-producing *Pseudomonas*

Type of sample	Number of imipenem-resistant *Pseudomonas* (*n*=73) (%)	Number of MBL positive *Pseudomonas* (*n*=69) (%)
Pus	28 (38.35)	25 (36.23)
Urine	22 (30.14)	22 (31.89)
Respiratory secretions	9 (12.33)	8 (11.59)
Body fluids	9 (12.33)	9 (13.04)
CSF	5 (6.85)	5 (7.25)
Total	73 (100)	69 (100)

MBL: Metallo-beta-lactamase, CSF: Cerebrospinal fluid

Table 2: Antibiotic susceptibility pattern of MBL-producing *Pseudomonas*

Antibiotic (concentration µg/disc) (HIMEDIA)	Sensitivity of imipenem-resistant *Pseudomonas* (*n*=73) (%)	Sensitivity of MBL positive *Pseudomonas* (*n*=69) (%)
Amikacin (30)	17 (23.29)	14 (20.29)
Gentamicin (10)	17 (23.29)	15 (21.74)
Ceftazidime (30)	3 (4.11)	2 (2.90)
Cefotaxime (30)	3 (4.11)	1 (1.45)
Ceftriaxone (30)	1 (1.37)	0 (0.00)
Cefepime (30)	4 (5.48)	1 (1.45)
Ciprofloxacin (5)	24 (32.88)	23 (33.33)
Piperacillin (100)	7 (9.59)	6 (8.70)
Piperacillin-Tazobactam (100/10)	32 (43.83)	30 (43.48)

MBL: Metallo-beta-lactamase

Table 3: Prevalence of MBL in imipenem-resistant *Pseudomonas* spp.

Number of imipenem-resistant *Pseudomonas* (%)	MBL positive *Pseudomonas* (%)	
	By CDST-IPM method (*n*=73)	By DDST-IPM method (*n*=73)
73 (100)	69 (94.52)	65 (89.04)

MBL: Metallo-beta-lactamase, CDST-IPM: Combined disc synergy test- imipenem-EDTA, DDST-IPM: Double disc synergy test-imipenem-EDTA

Figure 1: Metallo-beta-lactamase positive by both combined disc synergy test-imipenem-EDTA (IPM) and double disc synergy tests-IPM method

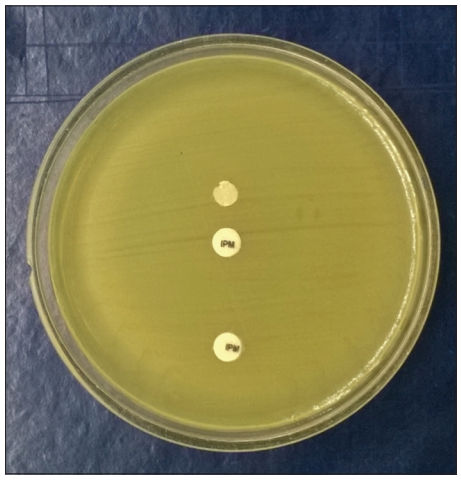

Figure 2: Metallo-beta-lactamase negative by both combined disc synergy test-imipenem-EDTA (IPM) and double disc synergy test-IPM method

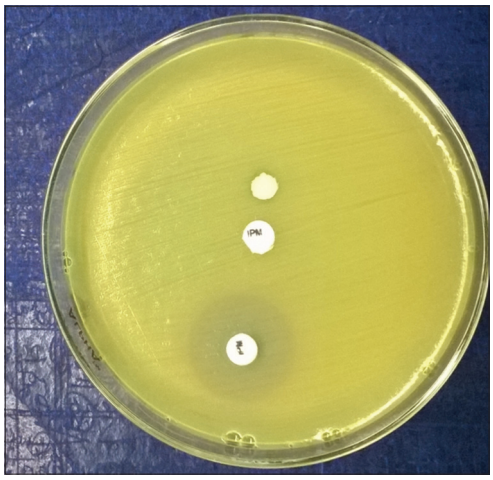

Figure 3: Metallo-beta-lactamase positive by combined disc synergy test-imipenem-EDTA (IPM) and negative by double disc synergy test-IPM method

All MBL-producing *Pseudomonas* isolates were resistant to ceftriaxone (Figure 7).

DISCUSSION

Imipenem-resistant *P. aeruginosa* is a current and significant concern, especially because of the limited therapeutic options for this pathogen. MBL enzymes may play a critical role in imipenem resistance in *P. aeruginosa*.[4] Multiple beta-lactamase-producing *P. aeruginosa* can cause major therapeutic failure and poses a significant clinical challenge if remain undetected. Therefore, early identification of the infections due to these microorganisms is necessary as the appropriate treatment might reduce the spread of these resistant strains as well as the morbidity and mortality in hospitalized patients. This emphasizes the need for the detection of isolates that produce these enzymes to avoid therapeutic failures and nosocomial outbreaks.[12]

Since there are no standard guidelines for detection of MBL, different studies have reported the use of different methods. PCR analysis is the gold standard method for the detection of MBL production, but it is not feasible in the routine microbiology laboratory.[13] A few studies have reported that "CDST-IPM method" as the most sensitive method for detection of MBL production in Gram-negative bacilli.[13,14] Hence, this method was used in this study in association with "IPM DDST" for detection of the prevalence of MBL production in imipenem-resistant non-fermentative Gram-negative bacilli.

In the present study, imipenem-resistant *Pseudomonas* was most commonly isolated from pus (38.35%) which is comparable with the study of Attal *et al.*[15] (43.7%). In the study of Anita Nandi *et al.*[2] (59.08%) and Dr. Khakhkhar *et al.*,[6] 61.53% of the imipenem-resistant *Pseudomonas* was isolated from pus in much higher percentage. However, in the study of Roberto Morais *et al.*[16] and Smita Sood *et al.*,[17] imipenem-resistant *Pseudomonas* was most commonly isolated from respiratory secretions, 52.7%, and 39.13%, respectively, whereas in the present study, 12.33% of the imipenem-resistant *Pseudomonas* was isolated from respiratory secretions.

There is wide variation in antibiotic sensitivity pattern in all studies, which may be due to the selective pressure of antibiotic used in the particular area. Sensitivity pattern of imipenem-resistant *Pseudomonas* in the present study is comparable with the study of Khakhar *et al.*[16] for amikacin (26.92%) and with the study of Maria *et al.*[4] for Piperacillin-tazobactam (47.80%) and gentamicin (34.80%) to some extent.

P. aeruginosa-producing MBL was first reported in India in 2002.[18] In the present study, 94.52% and 89.04% of the *Pseudomonas* were MBL producer by CDST-IPM and DDST-IPM method, respectively, which is comparable to

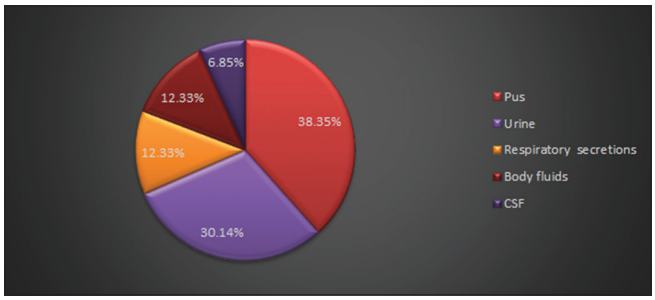

Figure 4: Sample-wise distribution of imipenem-resistant *Pseudomonas*

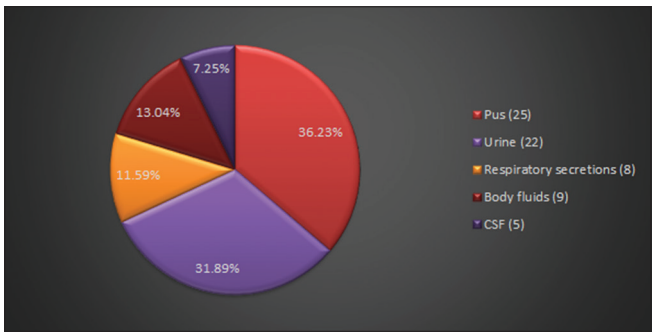

Figure 5: Sample-wise distribution of metallo-beta-lactamase positive *Pseudomonas*

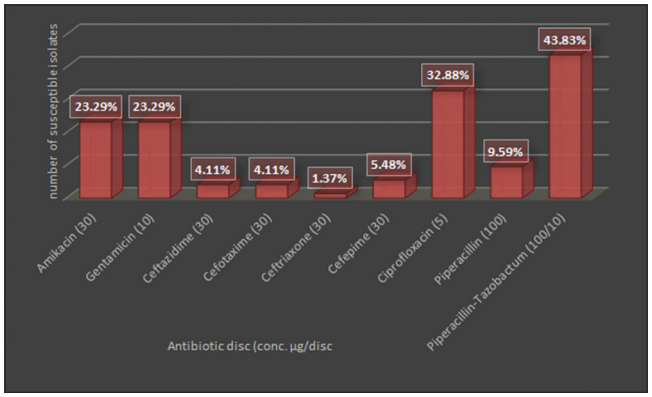

Figure 6: Antibiotic susceptibility pattern of imipenem-resistant *Pseudomonas*

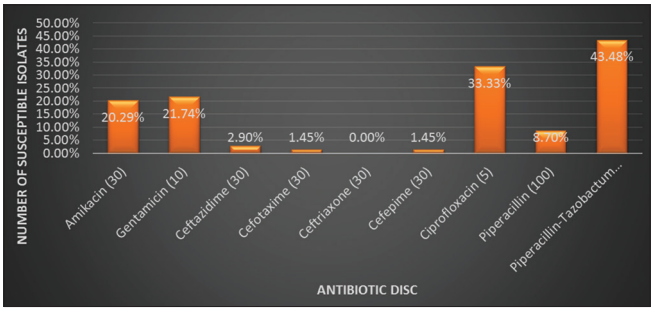

Figure 7: Antibiotic susceptibility pattern of metallo-beta-lactamase-producing *Pseudomonas*

study of Sood *et al*.[17] (100%), Irfan *et al*.[19] (100%), Attal *et al*.[15] (88.89%), and Fam *et al*.[3] (87.5%).

In present study, MBL-producing *Pseudomonas* was most commonly isolated from pus (36.23%), which is comparable with the study of Attal *et al*.[15] (43.7%) but also in study of Dr. Khakhkhar *et al*.[6] and Anita Nandi *et al*.[2] MBL-producing *Pseudomonas* was much more commonly isolated from pus (66.67% and 62.75%, respectively), while in study of Smita Sood *et al*.[17] and Sangeetha *et al*.[20] MBL-producing *Pseudomonas* was most commonly isolated from respiratory secretions (39.13% and 35.29%, respectively) and in study of Dr. Wankhede *et al*.[21] MBL-producing *Pseudomonas* was most commonly isolated from urine (44.12%).

There is wide variation in antibiotic susceptibility pattern of MBL-producing *Pseudomonas* in different studies. In present study, MBL-producing *Pseudomonas* was most susceptible to piperacillin-tazobactam combination (43.48%). In a study of Maria *et al*.[4] and Ami Varaiya *et al*.,[5] 76.2% and 84% of the MBL-producing *Pseudomonas* were sensitive to this combination which was much higher than the present study. Sensitivity pattern of present study for amikacin was comparable with study of Roberto Morais *et al*.[16] (22.2%) and Dr. Vipul M Khakhkhar *et al*.[6] (20%).

CONCLUSION

MBL-producing *Pseudomonas* was highly prevalent in Kota region, which is one of the major causes of resistance to imipenem and other antimicrobial agents. In the absence of novel agents in the future, the spread of MBL producers may lead to the therapeutic dead end. Early detection may avoid the spread of these multi-drug resistant isolates and may help to maintain first- and second-line therapies and reduction of morbidity and mortality rates. There is a requirement of regular surveillance and strict antibiotic policy to fight against these multi-drug resistant pathogens.

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