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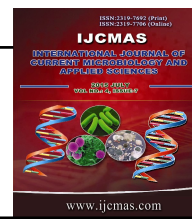

Original Research Article

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

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A B S T R A C T

Metallo-beta-lactamases (MBL) producing *Pseudomonas aeruginosa* and *Acinetobacter* spp are responsible for several nosocomial outbreaks in tertiary care centers across the world. It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. A total of 179 isolates of *P. aeruginosa* from various specimens between September 2010 and September 2011 were subjected to susceptibility testing against various antibiotics by disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Imipenem, meropenem and ceftazidime resistant isolates were selected for the detection of MBL production by disc potentiation test. Enhancement of inhibition zone around imipenem, meropenem and ceftazidime discs impregnated with EDTA as compared to those without EDTA confirmed MBL production. Out of 179 Pseudomonas aeruginosa isolates, 52 (29.05%) were found MBLs producers. MBL producing Ps. Aeruginosa were 100% sensitive to colistin and polimyxin B. followed by amikacin (34.62%), doxycycline hydrochloride and ciprofloxacin (9.62%), cefoperazone (5.77%) and ceftriaxone (3.85%), respectively. Our findings showed that there is a need to do surveillance to detect MBL producers, judiciously use carbapenems to prevent their spread and use effective antibiotics after sensitivity testing for treatment.

Keywords

MBL producers, *Pseudomonas aeruginosa, Acinetobacter* spp., Nosocomial

Introduction

Development of antibiotic resistance is a major concern in the management of bacterial infections. The gram negative bacteria are showing slow but steady upward trends of resistance as compared to gram positive bacteria. The main problem with these gram negative bacterial infections is their effective treatment. The beta lactam group of antibiotics that includes penicillin, cephalosporin, monobactams and carbapenems form the main stay of therapy. The production of beta lactamases by gram negative bacteria is the major defense mechanism against these beta lactam

antibiotics. The bacteria have responded by producing newer beta lactamases like extended spectrum beta lactamases (ESBLs), plasmid mediated Amp-C enzymes, and carbapenem hydrolyzing beta lactamases (MBL) (Medeiros, 1997; Gniadkowski, 2001).

The beta-lactamases are the major defense system of gram-negative bacteria against beta-lactam antibiotics. The beta-lactamases can be broadly divided into enzymes with a serine residue at the active site, similar to bacterial penicillin-binding proteins and metallo enzymes with zinc ion as a cofactor (Joris *et al.,* 1988; Garau *et al.,* 2004; Hall and Barlo, 2004).

Metallo-beta-lactamases have recently become more prominent among the beta-lactam-hydrolyzing enzymes. It is anticipated that plasmid-mediated resistance to carbapenems will continue to increase (Bush *et al.,* 1995).

Metallo-β-lactamases have potent hydrolyzing activity not only against carbapenem but also against other β-lactam antibiotics such as penicillins and cephalosporins. The genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. The genes responsible for MBL production may be chromosomally mediated and therefore poses a threat of spread of resistance by gene transfer among the Gram-negative bacteria (Bush *et al.,* 1995; Bush, 1998; Bennett, 1999).

MBLs require zinc for their catalytic activity and are inhibited or blocked by metal chelators, such as EDTA and thiol- based compounds (2-mercaptopropionic acid MPA). MBLs are not inhibited by the commercially available inhibitors like clavulanic acid, sulbactam and tazobactam.

Carbapenems are often used as antibiotics of last resort for treating infections due to multi-drug resistant Gram-negative bacilli. Therefore, detection of MBL-producing Gram negative bacilli is crucial for the optimal treatment of patients and to control the spread of resistance (Bush *et al.,* 1995).

Consequently, the rapid detection of carbapenemase production is necessary to initiate effective infection control measures to prevent their dissemination.

Materials and Methods

This study was carried out in the Department of Microbiology, Sadar Patel Medical College, Bikaner from September 2010 to September 2011 to detect Matello-beta-lactamase producing strains of *Pseudomonas aeruginosa* in various clinical specimens from attached Hospitals. Total 179 *Pseudomonas aeruginosa* were isolated from various clinical specimens such as urine, blood, wound swabs, pus, sputum and other respiratory tract specimens, body fluids, high vaginal swabs, ear swabs, stool etc. Received from patients attending various outpatient departments and admitted in wards at P.B.M. hospital and associate group of hospitals, were included in this study.

Inclusion criteria

All consecutive, non-duplicate isolates of *Pseudomonas aeruginosa* collected from various specimens of patients attending various outpatient departments and admitted in wards at P.B.M. hospital and associate group of hospitals.

Specimens included are urine, blood, wound, pus, sputum and other respiratory tract specimens, body fluids, high vaginal swab, stool and CSF etc.

Exclusion criteria

Isolation of three organism types with no predominating organism and repeated isolates from same patient were excluded from this study.

Processing of specimens

The clinical isolates were processed immediately as follows-

Microbiological methods (AVI Biopharma, 2007; Palmer *et al.,* 2007)

The samples were processed for the identification of organisms on the basis of conventional microbiological procedures and were screened for MBLs. All isolates were cultured on Mac Conkey Agar and Blood Agar and urinary isolates on Hichrome UTI media (obtained from Hi-Media, Mumbai, India) also and incubated at 37°C for 24 hrs. They were identified to species level by their characteristic appearances on the media, pigment production, Gram's stain, Oxidase test, Motility and the pattern of the biochemical reactions.

Antibiotic susceptibility testing

The isolates were tested for antimicrobial susceptibility on Mueller Hinton agar by Kirby Bauer disk diffusion method with an inoculum matched of 0.5 McFarland's turbidity standard as per CLSI recommendation. A lawn culture was made on the surface of medium by a sterile cotton swab with an inoculum matched with 0.5 McFarland turbidity standard, prepared by suspending few colonies of test strain in 0.9% sterile saline. Then the antimicrobial discs were applied on the inoculated agar surface with all sterile precautions, and the plates were incubated at 37°C overnight.

Pseudomonas aeruginosa ATCC 27853 was used to check the potency of the disc. Following antibiotic discs (obtained from Hi-Media, Mumbai, India) were used for antimicrobial sensitivity testing:

Amikacin (30 µg), Doxycycline hydrchloride (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Ceftriaxone (30 µg), Meropenem (10 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Cefoperazone (75 µg), Piperacillin (100 µg), Colistin (10 µg), Polymixin (300 µg) and Tobramycin (10 µg).

Metallo beta lactamases detection

Initial Screening Tests (Bhalerao *et al.,* 2010):

Inoculum was prepared by suspending few colonies of test strain in 0.9 % sterile saline and turbidity was adjusted to 0.5 McFarland turbidity tube. A lawn culture was made from the inoculum using a sterile cotton swab on the surface of Mueller-Hinton agar medium and Ceftazidime (30µg), Imipenem (10µg), and Meropenem (10µg) discs were applied with all sterile precautions. The plates were incubated for 18–24 hours at 37°C.

Interpretation: The isolates showing inhibition zone size of ≤16 mm with Imipenem (10 µg), Meropenem (10µg) and with Ceftazidime (30 µg) were identified as MBL producers and shortlisted for confirmation of MBL production.

Phenotypic confirmatory test with combination disks (Sofia Constantiniu *et al.,* 2004):

This test requires the use of a Imipenem, Meropenem and a Ceftazidime disk alone and in combination with a solution of EDTA (a 0.5-m EDTA solution was prepared by

dissolving 186.1 g of disodium EDTA $2H_2O$ in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH.

The mixture was sterilized by autoclaving). In this test, two 10-µg imipenem discs were placed at least 20 mm center to center on the plate of test organism and 10 µl of EDTA solution was added to one of the disc. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16–18 h of incubation at 37°C.

Table.1 Distribution of MBL producing *Pseudomonas aeruginosa*

S. No	Organism	Total numbers	MBL producers	Percentage (%)
1	*Pseudomonas aeruginosa*	179	52	29.05 %

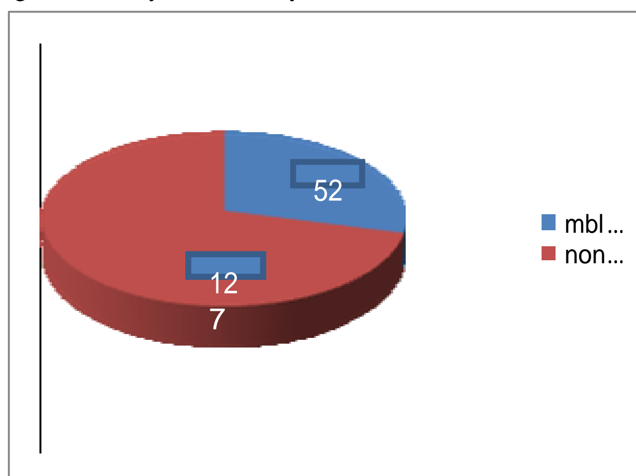
Table.2 Distribution of MBL producing and non MBL producing *Pseudomonas aeruginosa* isolates from various clinical specimens

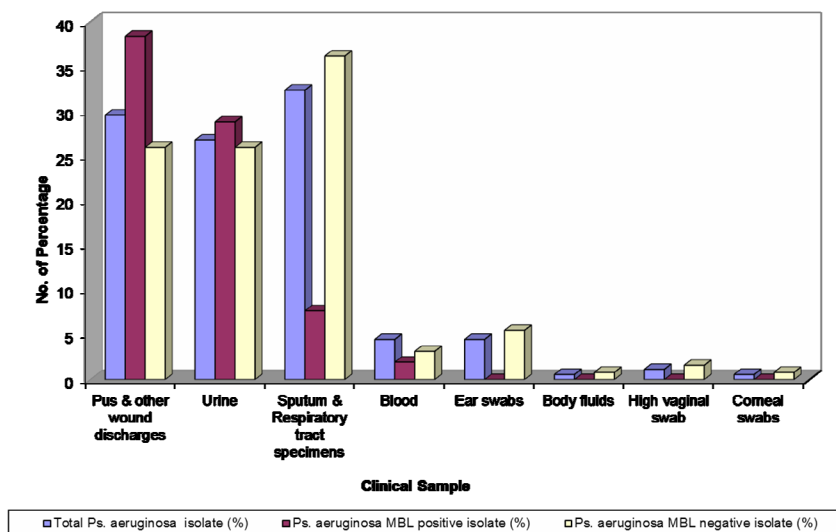
S.N.	Clinical sample	Total *Pseudomonas aeruginosa* isolate (%)	*Pseudomonas aeruginosa* MBL positive isolate (%)	*Pseudomonas aeruginosa* MBL negative isolate (%)
1.	Pus & other wound discharges	53 (29.61%)	20 (38.46%)	33 (25.98%)
2.	Urine	48 (26.82%)	15 (28.85%)	33 (25.98%)
3.	Sputum & Respiratory tract specimens	58 (32.40%)	12 (23.08%)	46 (36.22%)
4.	Blood	08 (4.47%)	04 (7.69%)	04 (3.15%)
5.	Ear swabs	08 (4.47%)	01 (1.92%)	07 (5.51%)
6.	Body fluids	01 (0.56%)	0 (0.00%)	01 (0.79%)
7.	High vaginal swab	02 (1.12%)	0 (0.00%)	02 (1.57%)
8.	Corneal swabs	01 (0.56%)	0 (0.00%)	01 (0.79%)
9.	TOTAL	179	52	127

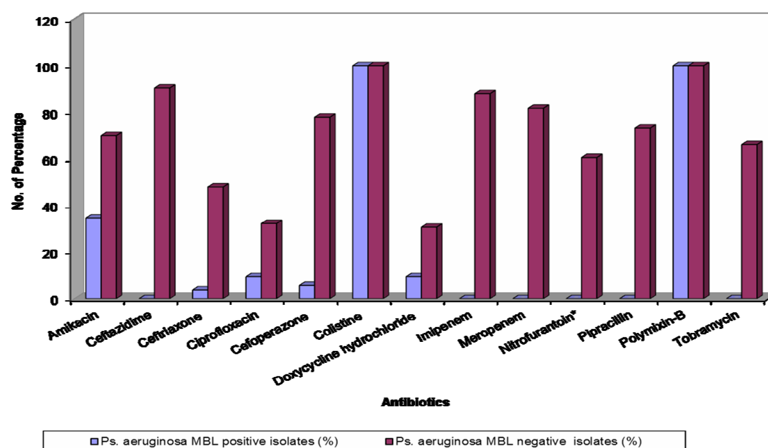
Table.3 Antimicrobial susceptibility pattern of MBL producing and non-MBL producing *Pseudomonas aeruginosa*

S. No.	Antibiotics	*Pseudomonas aeruginosa* MBL positive isolates (%)	*Pseudomonas aeruginosa* MBL negative isolates (%)
1.	Amikacin	18 (34.62%)	89 (70.08%)
2.	Ceftazidime	00 (0.00%)	115 (90.55%)
3.	Ceftriaxone	02 (03.85%)	61 (48.03%)
4.	Ciprofloxacin	05 (09.62%)	41 (32.28%)
5.	Cefoperazone	03 (05.77%)	99 (77.95%)
6.	Colistine	52 (100%)	127 (100%)
7.	Doxycycline hydrochloride	05 (09.62%)	39 (30.71%)
8.	Imipenem	00 (0.00%)	112 (88.19%)
9.	Meropenem	00 (0.00%)	104 (81.89%)
10.	Nitrofurantoin*	00 (0.00%)	20 (60.60%)
11.	Pipracillin	00 (0.00%)	93 (73.22%)
12.	Polymixin-B	52 (100%)	127 (100%)
13.	Tobramycin	00 (0.00%)	84 (66.14%)

* Nitrofurantoin was tested against urinary isolates only







Interpretation: When there was an increase of ≥ 7 mm in inhibition zone diameter around combination disk of Imipenem and EDTA solution versus the inhibition zone diameter around Imipenem disk alone, was considered as confirmed MBL producer.

In the similar manner Ceftazidime and Meropenem antibiotic discs were used with and without EDTA for detection of MBL producing organisms.

MBLs have been identified from clinical isolates worldwide, with an increasing frequency over the past few years and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections. The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. The accurate identification and reporting of MBL-producing *Pseudomonas* aeruginosa will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

Acinetobacter spp. is also notorious, both for its ability to acquire antibiotic resistance and for the ability of some strains, to cause nosocomial outbreaks. Therefore, early laboratory detection is of great clinical importance.
MBL producing isolates are associated with a higher morbidity and mortality. The occurrence of an MBL positive isolate poses not only a therapeutic problem but is 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 MBL gene transfer, with other pathogens in the hospitals (Deeba Bashir *et al.,* 2011).

In the present study MBL production was noticed in 29.05% isolates of *Pseudomonas aeruginosa*. This high incidence of MBL production was also reported by M.J.C. Noyal et al (2009) where MBL production was observed in 31.07% *Pseudomonas aeruginosa*, De *et al.* (2010) observed MBL production in 28.57% *Pseudomonas*.

In the present study MBL producing *Pseudomonas aeruginosa* were recovered most frequently from pus and other wound discharges 38.46% followed by urine 28.85%, sputum and respiratory tract specimens 23.08%, similar observations were noticed by Pitout *et al.* (2005) where MBL producing *Pseudomonas aeruginosa* were most frequently from urine 43% followed by wounds (purulent) 21%, respiratory tract specimens 20%.

The antibiotic sensitivity pattern of MBL producing *Pseudomonas aeruginosa* was as follow; 100% sensitivity for Polymixin B & Colistin that was similar to Sarkar *et al.* (2006) and Deeba Bashir *et al.* (2011). MBL producer *Pseudomonas aeruginosa* were 34.62% sensitive to Amikacin and 3.85% sensitive to Ceftriaxone that was similar to Horieh Saderi *et al.* (2008).

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