

A STUDY OF METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN CLINICAL SAMPLES OF S.S.G. HOSPITAL

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ABSTRACT

Introduction: Pseudomonas spp. is common pathogen causing nosocomial infection. Acquired drug resistance is frequent in nosocomial isolates of Pseudomonas spp. Acquired metallo- β -lactamases (MBL) in pseudomonas spp. have recently emerged as one of the most worrisome resistance mechanism because of their capacity to hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam.

Aim: To detect metallo- β -lactamase producing isolates of Pseudomonas aeruginosa from various clinical samples from patients admitted in our hospital.

Material and methods : In this study we studied the prevalence, following standard methods of isolation and identification techniques of these bacteria from clinical materials

Source : Samples of patients from different wards of S.S.G.Hospital are proceeded in Microbiology department, Medical College and S.S.G.Hospital Baroda.

Results: Of total study of 150 isolates of Pseudomonas aeruginosa, 8 isolates are resistance to Imipenem. Of 8 samples, all are producing Metallo-Beta-Lactamase enzyme.

Conclusion : Infection cause by MBL (metallo- β -lactamase) positive isolates of Pseudomonas aeruginosa is important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management.

Keywords: metallo-beta-lactamase, producing pseudomonas aeruginosa, drug resistance

INTRODUCTION

Pseudomonas spp. is common pathogen causing nosocomial infection. Acquired drug resistance is frequent in nosocomial isolates of Pseudomonas spp.^{1,2}. Acquired metallo- β -lactamases (MBL) in pseudomonas spp. have recently emerged as one of the most worrisome resistance mechanism because of their capacity to hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. Throughout the world bacteria like *Serratia marcescens*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Acinetobacter* spp. And *Alcaligenes xylosoxidans* are generally noted as MBL producer^{3,4,5}.

Characteristics of Metallo- β -lactamase (MBL):

1. Metallo- β -lactamase requires zinc for their catalytic activity.

2. Their activity is inhibited by Metal chelators, such as EDTA and THIOL compounds.
3. Metallo- β -lactamase hydrolyze all beta-lactam antibiotics including carbapenems, with the exception of aztreonam (monobactam).
4. MBL producing strains are not susceptible to serine beta lactamase inhibitors, (e.g. clavulanate)^{3,4,5}.

MBLs, like all β -lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes. In some countries, *P. aeruginosa* possessing MBLs constitute nearly 20% of all nosocomial isolates, whereas in other countries the number is still comparatively small. Structure of Metallo-Beta-Lactamase consists the protein domain fold characteristic of metallo- β lactamase consist of four layered beta sandwich with two mixed beta sheets flanked with Alfa helices^{6,7}.

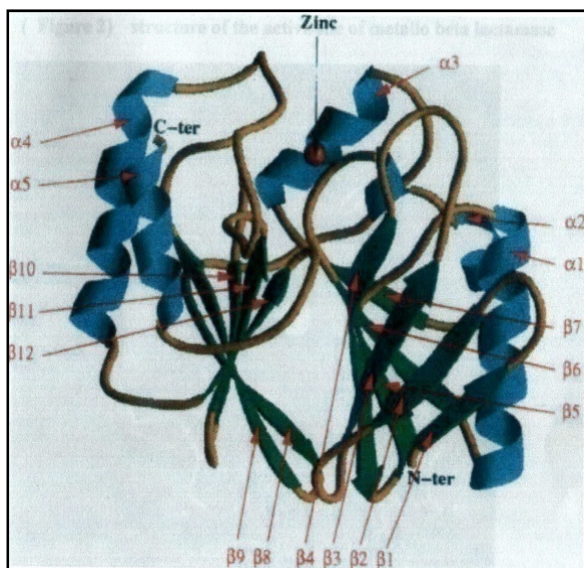


Figure 1: Mechanism of action of Metallo-beta-lactamsae

In MBL the zinc ion has dual role in catalysis. Firstly the zinc ion bound water molecule is activated to perform a nucleophilic attack on the peptide carbonyl of the β lactam antibiotics . Secondly the zinc ion binds and polarizes this carbonyl group. A negatively charged group Glu 143 participates in the activation of water molecule. After the water molecule attack , a tetrahedral intermediate is formed , followed by the back delivery of the peptide nitrogen and the cleavage of the peptide bond . Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM , among them IMP and VIM are most predominant^{6,7} .

MATERIALS AND METHODS

Collection of samples:

Different samples, Wound swab, blood, sputum, pleural fluid, pus, urine and ocular discharge are collected from different wards 116 (77%) from surgical ward, 9 (6%) from pediatrics ward, 6 (4%) from orthopedics ward, 3(2%) from gynec ward, 4 (3%) from medical ward, 2 (1%) from ENT ward, 3 (2%) from Ophthal ward and 7 (5%) from Tb – chest ward.

Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Baur disc diffusion method) according to CLSIs guidelines. The following antibiotics were tested by disc diffusion method, ceftazidime (30 ug), piperacillin (100ug), piperacillin-tazobactam (100/10ug), imipenem (10 ug), amikacin (30ug), tobramycin (10 ug) aztreonam (30ug) ciprofloxacin (5 ug). All imipenem resistant isolates were tested for MBL by Imipenem - EDTA double-disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

1. Imipenem- EDTA combined disc test (CDT).

The Imipenem- EDTA combined disc test (CDT) was performed as described by yong et al. The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by theCLSI. 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 (10ug) discs were placed on the surface of an agar plate at distance of 25 mm and 4 ul EDTA solution was added to one of them to obtain a desired concentration of 750 ug.The inhibition zones of imipenem and imipenem- EDTA discs were compared after 16 to 18 h of incubation in air at 37 c. In the combined disc test, if the increase in inhibition zone with the imipenem and imipenem- EDTA disc was ≥ 7 mm than the imipenem alone, it was considered MBL positive⁸⁻¹⁰.

2. Imipenem - EDTA double- disc synergy test <ddst>.

The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by theCLSI. An imipenem (10ug) disc was placed 20 mm center to center from a blank disc containing 4 ul of ,0.5 M EDTA (750 ug). Positive result when enhancement of zone of inhibition between imipenem and EDTA disc ≥ 5 mm⁸⁻¹⁰.

3 Modified Hodge Test:

The Modified Hodge Test (MHT) detects carbapenemase production in isolates of *Ps. aeruginosa*. Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922) towards a carbapenem disk. The result is a characteristic cloverleaf-like indentation.Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 ml of broth or saline. Streak a lawn of the dilution of *E.coli* ATCC 25922 to a Mueller Hinton agar plate and allow to dry 3–5 minutes. In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug. Incubate overnight at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in ambient air for 16–24 hours¹¹.

RESULTS

Prevalence of MBL producing *pseudomonas aeruginosa* in this study is 5%.

The data suggests that , prevalence of MBLproducing *Ps. aeruginosa* from total 150 sample is 5% and MBL non-producers are 95%.

Table 1: Total number of MBL producer *Ps. aeruginosa* in present study are as follow:-

Total MBL isolates of <i>Ps.aeruginosa</i>	150
MBL producer	08 (5%)
MBL non-producer	142 (95%)

Table 2: Ward wise prevalence of MBL producing *Ps. aeruginosa*

Ward	No. of Sample (%)
Surgical ward	6 (75)
Ortho ward	1 (12.5)
Medical ward	1 (12.5)

Isolates were mostly encountered in surgery wards , less encountered in medical and orthopedics ward.

Table 3: Disease wise prevalence of MBL producing *Ps.aeruginosa*

	No. of Sample (%)
Burns	2 (25.0)
Ulcer	2 (25.0)
Penetrating wound	1 (12.5)
Pleuraleffusion	1 (12.5)
Abscess	2 (25.0)

Isolates were mostly encountered in patient of burns, ulcer and abscess, less encountered in patient of pleural effusion and patient of trochanteric fracture.

Table 4: Ward wise incidence of *Ps. aeruginosa*

	No. of Sample (%)
Surgery	116 (77.3)
Pediatrics	9 (6.0)
Ortho	6 (4.0)
OG	3 (2.0)
Medicine	4 (2.7)
ENT	2 (1.3)
Ophthal	3 (2.0)
Tb and chest	7 (4.7)

Total isolates of *Ps.aeruginosa* were mostly encountered in Surgical ward , then Pediatrics and Tb-chest ward and few from orthopedics, gynec, medicine, ENT, Ophthalmology .

DISCUSSION

P. aeruginosa is one of the most frequent Nosocomial pathogen and the infections due to these are often difficult to treat due to antibiotic resistance. Acquired drug resistance is frequent in nosocomial isolates of *Pseudomonas* spp. Acquired metallo- β -lactamases (MBL) in *pseudomonas* spp. have recently emerged as one of the most worrisome resistance mechanism because of their capacity to hydrolyze all beta-lactam

antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. Nosocomial infection producing MBL (metallo- β -lactamase) positive isolates of *Pseudomonas aeruginosa* is important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management.

In the present study, total 150 isolates from different clinical samples were studied for their susceptibility or resistance to the antibiotics; ceftazidime (30 ug), piperacillin (100ug), piperacillin-tazobactam (100/10ug), imipenem (10 ug), amikacin (30ug), tobramycin (10 ug) aztreonam (30ug) ciprofloxacin (5 ug).

Of total isolates , 116 (77%) from surgical ward, 9 (6%) from pediatrics ward, 6 (4%) from orthopedics ward, 3(2%) from gynec ward, 4 (3%) from medical ward, 2 (1%) from ENT ward, 3 (2%) from Ophthal ward and 7 (5%) from Tb – chest ward.

Majority of isolates are isolate were obtained from sample of wound swab. Lowest number of isolates were obtained from pleural fluid, ocular discharge, ascitic fluid and blood. Majority of isolates of *Ps.aeruginosa* were isolated from patients of ulcer and burns. Less numbers were isolated from patients of URTI, Ascitis, gynec patient, nephritis and ocular discharge.

Metallo-beta-lactamase enzyme is an emerging threat and cause of concern for physician. The metal ion active site appear to decrease their susceptibility to beta lactamase inhibitors and enable them to hydrolyze broad spectrum including carbapenems. The Metallo-beta-lactamase are plasmid mediated , so the resistance can be spread among hospital pathogen and will cause problems in treating infections.

In present study, attempt was made to detect Metallo-beta-lactamase producing *Ps.aeruginosa*. Of 150 isolates of *Ps.aeruginosa*, 8 (5%) were resistance to imipenem . All 8 isolates were found to be MBL producers. Of 8 isolates of MBL, 6 (75%) were isolated from surgical ward patients suffering from burns, ulcer and abscess; 1(12.5%) from medical ward patient suffering from pleural effusion and 1 (12.%) from orthopedic patient of trochanteric fracture. The prevalence of detect Metallo-beta-lactamase producing *Ps.aeruginosa* in our setup if 5%.

CONCLUSION

- *Ps.aeruginosa* aer mostly isolated from surgical ward in compare to other wards.
- *Ps.aeruginosa* are mostly infect person suffering from ulcer, burns and cellulitis.
- Majority of isolates of *Ps.aeruginosa* are sensitive to imipenem and aztreonam ; majority of isolates are resistant to ciprofloxacin , amikacin , piperacillin, piperacillin+tazobactam and tobramycin.

- All metallo-beta-lactamase producing *Ps.aeruginosa* are resistant to all groups of antibiotics include in this study except aztreonam.
- Majority of isolates of *Ps.aeruginosa* producing metallo-beta-lactamase were isolated from patient of burns, ulcer and abscess.

Nosocomial infection producing MBL (metallo- β -lactamase) positive isolates of *Pseudomonas aeruginosa* is important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management

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