*Original Article*

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# Prevalance of Metallo Beta Lactamase Producing Pseudomonas Species in Clinical Specimens from S.S.G Hospital, Vadodara

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**Abstract**

*Background: Pseudomonas* species are the commonest pathogens causing nosocomial infections. *Pseudomonas* is basically resistant to many antibiotics and they are known to produce Extended Spectrum Beta Lactamase and Metalo beta lactamase.

*AIM:* To detect Metalo beta lactamase producing *Pseudomonas* spp. from clinical samples in tertiary care hospital.

*Material and Methods:* The study was agreed over a period of 6 months from January 2015 to June 2015. A total non repetitive of 329 *Pseudomonas* spp were isolated from unusual clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endo tracheal tube and secretions from the patients attending the hospital. Antimicrobial susceptibility test of all the isolates was performed by the disc-diffusion (Kirby Bauer disc diffusion method) according to CLSIs guidelines. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double- disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

*Result:* Of 329 samples, majority of the *Pseudomonas spp* were isolated from Pus/Wound 173 (52.58%) followed by Blood 103 (31.31%). The isolation rate was highest from pediatrics wards 112 (34.04%) and surgical wards 112 (34.04%). Total 329 samples, 24 (7.29%) isolates were MBL producer by CDST and DDST. Majority of the MBL producing *Pseudomonas spp.* were isolated from Pus/Wound 11 (45.83%) followed by Urine 10 (41.66%). The isolation rate was highest from Surgical wards 9 (37.5%) followed by the Pediatric wards 7 (29.16%).

*Conclusion:* Detetion of Metallo beta lactamase isolates of *Pseudomonas spp.* will help to implement rational use of antibiotics and strictly adhere to the concept of "reserve drugs" are important to identify because it poses therapeutic problems and serious concern for infection control management.

**Keywords:** Metalo Beta Lactamase (MBL); *Pseudomonas* Species; Nosocomial Infections.

**How to cite this article:**

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## Introduction

*Pseudomonas* species are the common pathogens causing nosocomial infections [1,2]. Infections caused by *Pseudomonas* are either exogenous or endogenous origin, depending on several factors such as use of immunosuppressant agents, injudious use of antimicrobial agents, prolonged surgical procedures and inadequate instrumentations. In recent years due to liberal and empirical use of antibiotics, non fermentative gram negative bacilli have emerged as an important health care associate pathogen. They have been incriminated in infections such as septicemia, pneumonia, urinary tract

infection and surgical site infection. *Pseudomonas* is essentially resistant to many antibiotics and they are known to produce extended spectrum beta lactamase and metallo-beta lactamase. Acquired drug resistance is frequent in nosocomial isolates of *Pseudomonas* spp [1,2]. Acquired Metallo β-Lactamase (MBL) in *Pseudomonas* spp. have recently emerged as one of the most troublesome resistance mechanism because of their capability to hydrolyze all beta-lactam antibiotic including penicillins, cephalosporins and carbapenams, with the exception of Aztreonam [1,2,3,4,5]. Now a days resistance to Aztreonam producing Metallo β-Lactamase (MBL) is also revealed. Currently, there are no recommendations available from CLSI (Clinical and Laboratory Standard Institute) for the detection of MBL. Several phenotypic methods are available for MBL detection. All these methods are based on the ability of metal chelators, such as EDTA and THIOI compounds to inhibit the activity of MBL. In present study, two phenotypic methods were used for the detection of MBL producing *Pseudomonas* species which includes the Imipenem-EDTA combined disc synergy test (CDST) and Imipenem- EDTA double- disc synergy test (DDST) [3,4,5,6].

### *Aim and Objectives*

*Aim*

- To determine MBL producing *Pseudomonas* spp. from clinical isolates in a tertiary care hospital setting.

*Objectives*

- To isolate and identify *Pseudomonas* from various clinical specimens (Blood, Body fluids, Sputum, Throat swab etc.)
- To determine antibiotic sensitivity of the isolates to various antibiotics by Kirby-Bauer disc diffusion method
- To screen for MBL producing isolates by detecting resistance to Imipenem (IPM).
- To confirm MBL production in MBL screen test positive by:

a. Imipenem EDTA combined disc synergy test

b. Imipenem EDTA double disc synergy test

- To study sensitivity and resistance pattern among isolates of *Pseudomonas* species from patients admitted in hospital.

## Materials And Methods

***Study Design:*** Cross- sectional

***Study Setting:*** Department of Microbiology

***Study Subject:*** The study was carried out over a period of 6 months from January 2015 to June 2015. A total non repetitive of 329 Pseudomonas spp were isolated from different clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endo tracheal tube and secretions from the patients attending the hospital. Antimicrobial susceptibility test of all the isolates was performed by the disc-diffusion (Kirby Baur disc diffusion method) according to CLSIs guidelines. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double- disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

***Phenotypic method for detection of Metallo- ß-Lactamases:*** *[7,8,9,10]*

*Preparation of 0.5 M EDTA Solution*

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.$2H_2O$ in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH and was sterilized by autoclaving. The solution has to be stored at -$20^0$C.

*Combined disk test (CDT): [11,12]*

The strains resistant to carbapenems were screened for MBL by CDT. Test was done for detection of metallo- ß- Lactamases in the imipenem resistant isolates. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. 10 µg imipenem disk and IMP (10 µg) + 5µl- 0.5 M EDTA (750 µg) was placed on the agar.. An increase of 7mm or more in zone diameter in the presence of EDTA compared to those with IMP, tested alone was considered to be a positive test for the presence of an MBL.

*Double disk synergy (DST) test: [7,8,13,14,15,16]*

Test was done for detection of metallo- ß-Lactamases in the imipenem resistant isolates. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. A 10 µg imipenem disk was placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no.

1) was kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA is added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to-edge apart from the imipenem disk. After incubating overnight at $37^0$C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production.

## Observation and Results

In this study, majority of *Pseudomonas spp.* were isolated from the age group of 1 to 10 years 91 (27.66%) and 21 to 30 years 65 (19.76%). *Pseudomonas spp* were isolated from male patients 219 (66.57%) as compared to female patients 110 (33.43%) (Table 1).

As can be seen from Table 2, majority of the *Pseudomonas spp.* were isolated from the wound swabs and pus samples 173 (52.58%) followed by the Blood 103 (31.31%). The isolation rate of *Pseudomonas spp.* was highest from the Surgical wards 112 (34.04%) and pediatric wards 112 (34.04%).

In the present study, of the 329 *Pseudomonas spp.* isolates, 24 (7.29%) isolates were MBL producer by CDST and DDST. While 305 (92.17%) isolates were Non- MBL producers (Table 3).

**Table 1:** Age and Sex distribution of isolated *Pseudomonas spp.* (n=329)

| Age in Years | Male | Female | Total |
|---|---|---|---|
| < 1 | 11 | 3 | 14 (4.26%) |
| 1 to 10 | 53 | 38 | 91 (27.66%) |
| 11 to 20 | 24 | 12 | 36 (10.94%) |
| 21 to 30 | 34 | 31 | 65 (19.76%) |
| 31 to 40 | 28 | 6 | 34 (10.33%) |
| 41 to 50 | 38 | 5 | 43 (13.07%) |
| 51 to 60 | 12 | 9 | 21 (6.38%) |
| 61 to 70 | 17 | 5 | 22 (6.69%) |
| 71 to 80 | 2 | 1 | 3 (0.91%) |
| Total | 219 (66.57%) | 110 (33.43%) | 329 |

**Table 2:** Isolation of *Pseudomonas* spp. from various clinical wards and various clinical samples.

| Clinical Samples | Blood | Body fluid | Pus/ Wound | Sputum | ET/ TT | Urine | Total |
|---|---|---|---|---|---|---|---|
| Surgical | 0 | 2 | 90 | 1 | 16 | 3 | 112 (34.04%) |
| Medical | 0 | 0 | 0 | 4 | 1 | 1 | 6 (1.82%) |
| Pediatrics | 102 | 0 | 0 | 0 | 0 | 10 | 112 (34.04%) |
| Orthopedic | 0 | 0 | 19 | 0 | 0 | 0 | 19 (5.77%) |
| Obs & Gynec | 0 | 0 | 2 | 0 | 0 | 2 | 4 (1.22%) |
| Burns | 0 | 0 | 60 | 0 | 0 | 0 | 60 (18.24%) |
| ENT/ Eye | 1 | 0 | 2 | 0 | 0 | 1 | 4 (1.22%) |
| Others (Ward 22,23) | 0 | 7 | 0 | 5 | 0 | 0 | 12 (3.65%) |
| Total | 103 (31.31%) | 9 (2.73%) | 173 (52.58%) | 10 (3.04%) | 17 (5.17%) | 17 (5.17%) | 329 |

**Table 3:** Prevalence of MBL producer and MBL non-producer of *Pseudomonas spp.*

| Isolates | MBL producer N (%) | MBL non producer N (%) | Total N (%) |
|---|---|---|---|
| Pseudomonas spp | 24 (7.29%) | 305 (92.17%) | 329 (100%) |

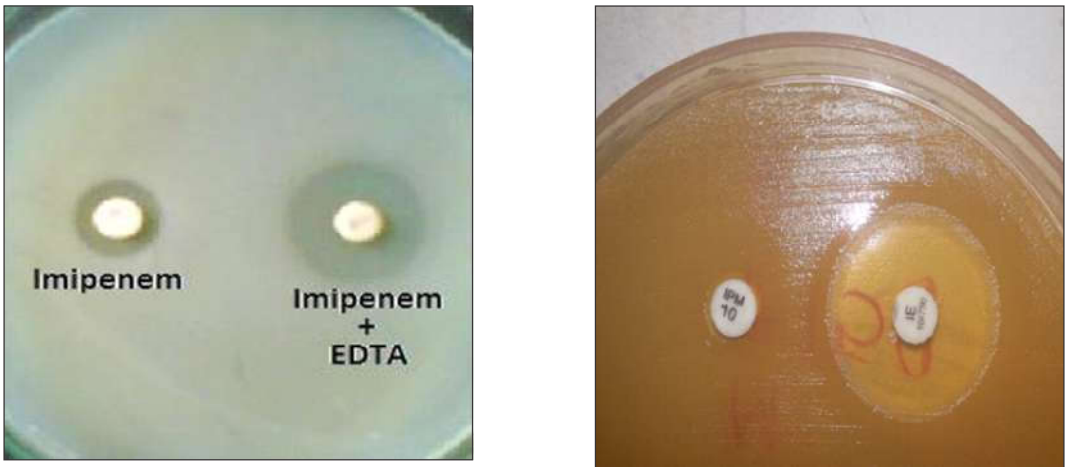


**Fig 1:** Combined Disk Test (CDT): positive strain shows a ≥ 7mm zone around the Imipenem+EDTA disk.

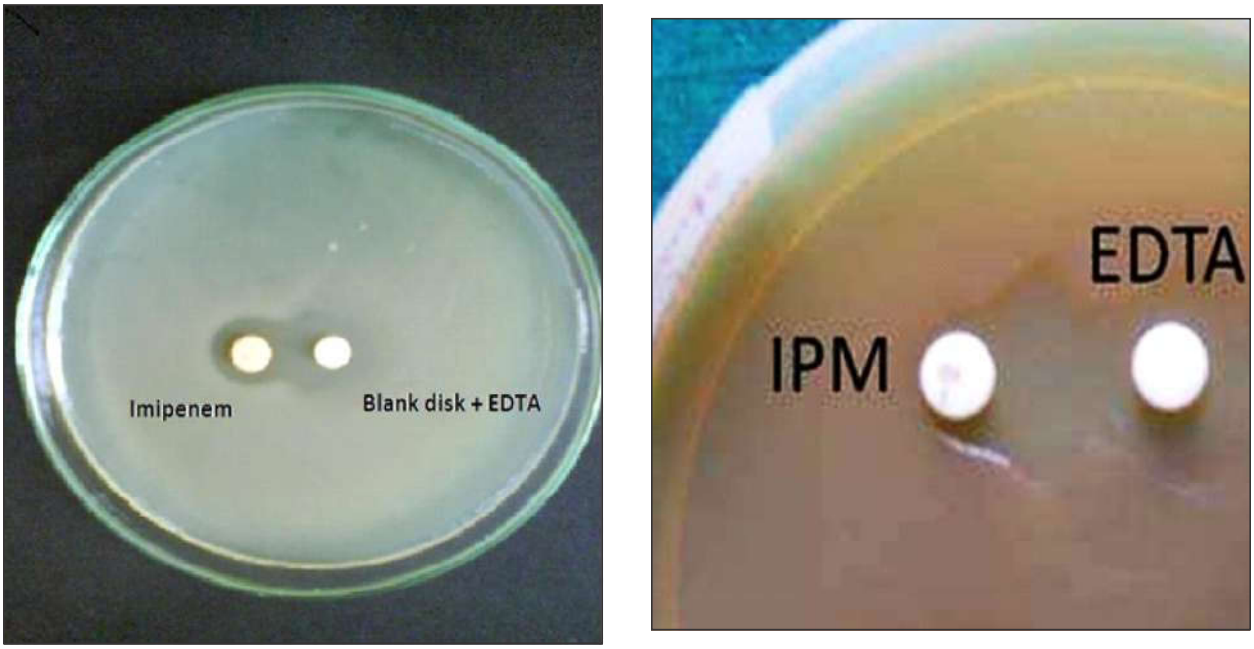


**Fig 2:** Double Disk Synergy Test (DDST)/ EDTA Disk Synergy Test: Positive strain shows a synergistic zone of inhibition between Imipenem and EDTA disc.

**Table 4:** Prevalence of MBL producing *Pseudomonas spp.* Fromvarious clinical samples and various clinical wards.

| Clinical Samples | Blood | Body fluid | Pus/ Wound | Sputum | ET/ TT | Urine | Total |
|---|---|---|---|---|---|---|---|
| Surgical | 0 | 0 | 7 | 0 | 1 | 1 | 9 (37.5%) |
| Medical | 0 | 0 | 0 | 0 | 0 | 0 | 0 (0%) |
| Pediatrics | 1 | 0 | 0 | 0 | 0 | 6 | 7 (29.16%) |
| Orthopedic | 0 | 0 | 1 | 0 | 0 | 0 | 1 (4.17%) |
| Obs & Gynec | 0 | 0 | 0 | 0 | 0 | 2 | 2 (8.33%) |
| Burns | 0 | 0 | 3 | 0 | 0 | 0 | 3 (12.5%) |
| ENT/ Eye | 0 | 0 | 0 | 0 | 0 | 1 | 1 (4.17%) |
| Others (Ward 22,23) | 0 | 0 | 0 | 1 | 0 | 0 | 1 (4.17%) |
| Total | 1 (4.17%) | 0 (0%) | 11 (45.83%) | 1 (4.17%) | 1 (4.17%) | 10 (41.66%) | 24 |

As can be seen from Table 4, majority of the MBL producing *Pseudomonas spp.* were isolated from Pus and wound swabs 11 (45.83%) followed by urine samples 10 (41.66%). The isolation rate was highest from surgical wards 9 (37.5%) followed by the pediatric wards 7 (29.16%).

**Table 5:** Antibiogram pattern of isolates of Pseudomonas spp. to different antibiotics are as follow:

| Name of Drugs | Sensitive | Resistance |
|---|---|---|
| Piperacillin | 278 (84.50%) | 51 (15.5%) |
| Piperacillin + Tazobactam (100µg/ 10 µg) | 312 (94.83%) | 17 (5.17%) |
| Amikacin (30 µg) | 195 (59.27%) | 134 (40.73%) |
| Cefoperazone (75 µg) | 282 (85.71%) | 47 (14.29%) |
| Levofloxacin (5 µg) | 278 (84.50%) | 51 (15.5%) |
| Ceftazidime (30 µg) | 260 (79.03%) | 69 (20.97%) |
| Gentamicin (10 µg) | 197 (59.88%) | 132 (40.12%) |
| Imipenem (10 µg) | 305 (92.71%) | 24 (7.29%) |

All Imipenem-resistant strains in our study showed high resistance to other antibiotics as well**.** High resistance was also observed to Amikacin 134 (40.73%), Gentamicin 132 (40.12%) and also Ceftazidime 69 (20.97%) and 51 (15.5%) isolates were also resistant to Piperacillin and Levofloxacin (Table 5).

## Discussion

*Pseudomonas spp.* are the most frequent nosocomial pathogen and the infections due to these are often difficult to treat because of 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. For many years, these MBL producing isolates were restricted to Japan, but now it has disseminated worldwide. [8] In India, MBL producing *P.aeruginosa* was first reported in 2002. [8] *Pseudomonas spp*., a virulent opportunistic pathogens which is one of the major causes of hospital acquired infection has the unique ability to infect all body systems. [17] It almost exclusively infects hospitalized patients with lowered host resistance and is the most frequent pathogens isolated from nosocomial infections in ICU.

In the present study, total 329 isolates from *Pseudomonas* spp. from different clinical samples were studied for their susceptibility or resistance to the antibiotics by (Kirby Baeur disc diffusion method) according to CLSIs guidelines.

Isolation of Pseudomonas spp from various clinical wards and various clinical samples.

In present study isolation of *P.seudomonas spp*. was found to be maximum from wound swabs and pus cultures 173 (52.58%) followed by blood cultures 103 (31.31%). Findings of Horieh Saderi et al. [6] (89.85%), Bashir et al. [9] (46.3%), D.E. Premalatha et al. [18] (58%) and B. Anuradha et al. [19] (39.39%) have also reported a maximum isolation from wound swabs and pus samples which corroborates well with our study. A review of surveillance data collected by the CDC National Nosocomial Infections Surveillance System from 1986 to 1998 shows that P. aeruginosa was identified as the fifth most frequently isolated nosocomial pathogen, accounting for 9% of all hospital-acquired infections in the United States. P. aeruginosa was also the second leading cause of Nosocomial pneumonia (14 to 16%), third most common cause of urinary tract infections (7 to 11%), fourth most frequently isolated pathogen in surgical site infections (8%), and seventh leading contributor to bloodstream infections (2 to 6%).

The isolation of *Pseudomonas spp*. in our study was maximum from the Surgical wards 112 (34.04%) and pediatric wards 112 (34.04%). Rajat Rakesh et al. [20] have stated a similar finding 48% from surgical wards followed by Pediatric wards 23%.

In present study, 7.29% isolates of *P.seudomonas* spp. were found to be MBL producers phenotypically. Most of the authors [6,8,21] have mentioned similar figures. The occurrence of an MBL-positive isolates in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management [6]. In our study majority of the MBL producer Pseudomonas spp. were isolated from pus and wound samples 11 (45.83%) followed by the Urine samples 10 (41.66%). Bashir et al. [9] reported that the predominant source of MBL positive strains was urinary tract (27.3%) followed by wound swabs and pus (27.3%) which correlated with our study. Anil Rajput et al [22] have reported 42.9% MBL producing isolates from Wound swabs and pus samples followed by urine samples 21.4%.

**Table 6:** Prevalence of MBL producers among *Pseudomonas spp.*

| Years of study | Place of study | Authors | MBL Producers |
|---|---|---|---|
| 2005 | Chennai | Hemalatha et al. [1] | 87.50% |
| 2008 | Mumbai | Varaiya et al. [3] | 20.80% |
| 2008 | Pakistan | S Irfan et al. [23] | 100% |
| 2008 | Iran | Horieh Saderi et al. [6] | 53.20% |
| 2009 | Puducherry | Noyel et al. [8] | 50.00% |
| 2010 | Mumbai | Anuradha S De et al. [24] | 28.57% |
| 2011 | Kashmir | Bashir et al. [9] | 11.66% |
| 2011 | Tamil Nadu | John and Balagurunathan [25] | 14.80% |
| 2011 | Ahmadabad | Anil Rajput [22] | 12.00% |
| 2011 | Pondicherry | Umadevi S et al. [26] | 74.50% |
| 2012 | Maharashtra | Simit H kumar [27] | 32.04% |
| 2013 | Kolkata | Rit K et al. [21] | 41% |
| Present study | Vadodara | | 7.29% |

*Antibiotic resistance pattern of MBL producing Pseudomonas aeruginosa isolates*

All Imipenem-resistant strains in our study showed high resistance to other antibiotics as well. High resistance was also observed to Amikacin 134 (40.73%), Gentamicin 132 (40.12%) and also Ceftazidime 69 (20.97%). 51 (15.5%) isolates were also resistant to Piperacillin and Levofloxacin. This high level of resistance to Aztreonam is very alarming because it is the drug of choice for MBL producing *P.seudomonas* aeruginosa. John and Balagurunathan [25] reported 56.7% resistance to Amikacin, 100% resistance to Piperacillin, Gentamicin and Ciprofloxacin. Kumar SH et al. [27] reported that all MBL –positive isolates were resistant all antibiotics with only 6.06% of the isolates showing susceptibility of Piperacillin/ Tazobactum. Irfan et al. [23] found resistance to antibiotics including third generation cephalosporin, Aminoglycoside and Quinolone. Bashir et al. [9] in his study found that the MBL producers were 100% resistant to ceftazidime, gentamicin and Tobramycin but 100% sensitive to polymyxin B. Rit et al. [21] stated that MBL producing isolates were multi drug resistant except for Colistin (100%) and for Polymyxin B (90%).

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

The prevalence of detect Metallo-beta-lactamase producing *P.seudomonas* spp. in our setup was 7.29%.

## Conclusion

MBL (metallo-β-lactamase) positive isolates of *P.seudomonas* spp. are important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management. There is also a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

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