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STUDY OF METALLOBETALACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN A TERTIARY CARE HOSPITAL FROM WESTERN INDIA

Microbiology

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ABSTRACT

Introduction : *Pseudomonas aeruginosa* is one of the most common pathogen causing nosocomial infections. Emergence of Metallo-betalactamase producing *P.aeruginosa* (MBL –PA) in hospital is alarming and poses not only a therapeutic problem, but also a serious concern for infection control management. Hence the present study was conducted to find out the prevalence of MBL-PA isolated from clinical specimens in a tertiary care hospital from Weastern Maharashtra.

Material and methods : A total of 48 strains of *P. aeruginosa* were recovered from various clinical specimens and screened for imipenem resistance by Kirby Bauer disk diffusion method and imipenem-EDTA combined disk test according to CLSI guidelines.

Results : We found 18.75% MBL-PA ; with highest incidence in surgery, burns unit and ICU specially in high risk patients.

Conclusion : We found high prevalence of MBL-PA which appears to be worrisome. Rapid detection of MBL producing isolates is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

KEYWORDS

P. aeruginosa , Metallobetalactamase, high risk patients

Introduction :

Pseudomonas aeruginosa, an opportunistic and worrisome nosocomial pathogen, is a gram negative, aerobic rod, belonging to bacterial family Pseudomonadaceae[1].This microorganism is an important cause of nosocomial infections, including pneumonia, wound infections, bacteremia, and urinary tract infection[2] especially patients at risk due to longer hospital stay, frequent use of broad spectrum antibiotics, invasive procedures, indwelling catheters and other co-morbidities.[3]

Infections caused by *P. aeruginosa* are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs[4].Metallo-β-lactamase (MBL) mediated resistance to carbapenem is an emerging threat in Pseudomonas isolates. Acquired MBL in *Pseudomonas* spp. has recently emerged as one of the most worrisome resistance mechanism[5]. MBLs are broad-spectrum enzymes that hydrolyse most of the beta lactam antibiotics except monobactams and are not inhibited by conventional beta-lactamase inhibitors like clavulanic acid or sulbactam. The detection of MBL depends on the principle that MBLs are affected by removal of zinc from their active site. Due to increasing diversity, the rapid spread of these enzymes, and the fact that they are often encoded on mobile genetic elements (integrons, transposons, plasmids) together with other resistance genes, MBL-producers belong to the group of clinically relevant multidrug-resistant bacteria[6].

In recent years, MBL genes have spread from *P. aeruginosa* to members of Enterobacteriaceae[7]. Notably, high morbidity and mortality rates (range 27% to48%) have been observed in critically ill patients[8]. Mortality rates are significantly higher in MBL producing *P. aeruginosa* (MBL-PA) compared to non-MBLPA[9].

Emergence of MBL producing *P.aeruginosa* in tertiary care hospital is alarming and reflects excessive use of carbapenems[7]and poses not only a therapeutic problem, but is also a serious concern for infection control management. Hence the present study was conducted to detect the prevalence of MBL producing *P. aeruginosa* in a tertiary care hospital and to formulate antibiotic policy and plan a proper hospital infection control strategy to prevent the spread of these strains.

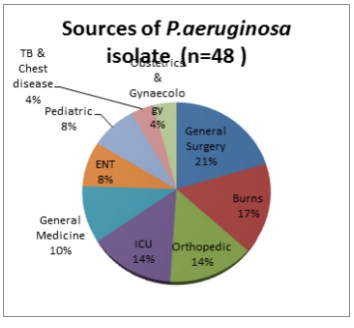
Material and methods:

A total of 48 *P. aeruginosa* isolated from various clinical specimens like pus ,urine, blood, sputum, endotracheal aspirate, CSF and other body fluids over a period of six months from September 2011 to February 2012 in the Department of Microbiology, Government Medical College and hospital, Aurangabad, Maharashrta were selected for the study. The bacterial strains were isolated and identified as per the standard guidelines[10]. Antimicrobial susceptibility for all *Pseudomonas* isolates was determined by Kirby-Bauer disc diffusion method[11] as per CLSIs guideline[12]. *P.aeruginosa* ATCC 27853 was used as control. The susceptibility testing was carried out against the following antibiotics: Piperacillin 100 µg, Piperacillin/Tazobactum 100/10 µg, Ceftazidime, Cefepime, Aztreonam and Amikacin each 30 µg, Gentamicin, Tobramycin, Imipenem and Colistin each 10 µg, ciprofloxacin 5µg and Polymyxin B300 units. The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines[12].In all imipenem resistant isolates of *P.aeruginosa* MBL detection was done by Imipenem-EDTA combined-disc synergy test (CDST).

Imipenem-EDTA combined-disc test (CDST): The test organisms were inoculated on Mueller Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 gm of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaOH. The Mixture then sterilize by autoclaving. Two imipenem (10ug) discs were placed on the surface of an agar plate at distance of 30 mm and 4 µl EDTA solution was added to one of them to obtain a desired concentration of 750 µg. The inhibition zones of imipenem and imipenem-EDTA discs can compared after 16 to 18 hours of incubation in air at 37˚C. In the combined disc test, if the increase in inhibition zone with the imipenem-EDTA disc is ≥7 mm than the imipenem alone, it is considered as MBL positive[13].

Results : A total of 48 non-duplicate isolates of *P. aeruginosa* from both medical and surgical wards were included in this study. Sources of *P. aeruginosa* isolates are depicted in **Diagram 1**.

Diagram 1. Sources of *P. aeruginosa* isolates

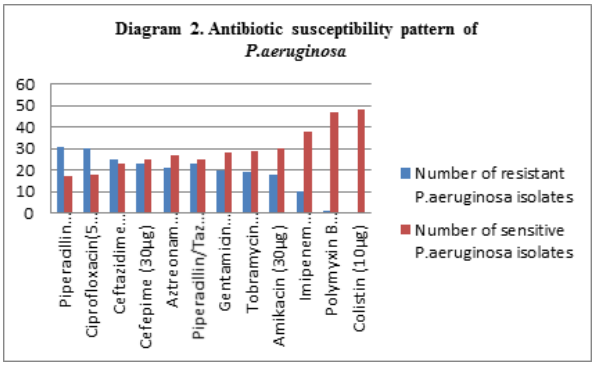


Pus swab and aspirate were most common source (45.83%), followed by respiratory specimens(20.8%) and urine(20.8%). Among other samples, *P. aeruginosa* was recovered from 4 blood samples and 2 vaginal swabs.

Among the 48 patients harboring *P. aeruginosa* infection, 37(77.08%) were male and only 11(22.91%) were female.

Antibiotic sensitivity testing of *P. aeruginosa*, in present study, revealed maximum resistance to piperacillin 31 (64%), followed by ciprofloxacin 30(62.5%), ceftazidime 25 (52.8%), cefepime 23 (47.91%), piperacillin/tazobactum 23(47.91%) , aztreonam 21(43.75%), gentamicin 20(41.6%), tobramycin 19(39.58%), and amikacin 18(37.5%). Low resistance were seen to imipenem 10(18.75%), polymyxin B 1(2.08%) and no resistance to colistin (Diagram 2).

Diagram 2. Antibiotic resistance pattern of *P. aeruginosa* in clinical isolates.



Out of total 48 isolates of *P. aeruginosa* screened for MBL production by imipenem disk diffusion test, 10isolates (18.75%) were imipenem resistant (Image 1) and the remaining 38 (79.16 %) were sensitive to imipenem.

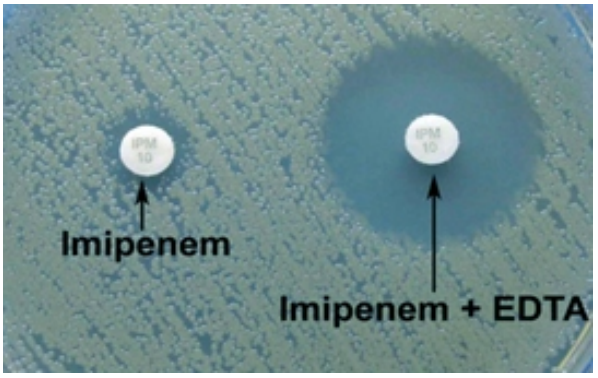


Image 1. Isolate showing imipenem resistance

In present study, imipenem, and Gentamicin resistance amongst MBL-PA were 100%, tobramycin, amikacin, ceftazidime, piperacillin/tazobactum and ciprofloxacin resistance amongst MBL-PA were 90%, 80%,90%, 80% and 90% respectively. Least resistance was reported to polymyxin B and no resistance to colistin as depicted in **table 1**.

Table 1 :Antibiotic susceptibility pattern of imipenem resistant *P.aeruginosa* strains

Antibiotic	Resistant	Intermediate	Sensitive
Gentamicin	10 (100%)	0	0
Tobramycin	9 (90%)	0	1 (10%)
Amikacin	8 (80%)	0	2 (20%)
Ceftazidime	9 (90%)	1 (10%)	0
Piperacillin/Tazobactum	8(80%)	2 (20%)	0
Ciprofloxacin	9 (90%)	0	1 (10%)
Imipenem	10 (100%)	0	0
Polymyxin B	1 (10%)	0	9 (90%)
Colistin	0 (0%)	0	10 (100%)

Among 10 patients with MBL-PA infection, four (40%) were diabetic, two (20%) were alcoholic , five(50%) patients had history of prior antibiotic use and four (40%) were having history of prolonged hospitalization and device insertion. **(Table 2)**

Table 2: Comparison of risk factors among imipenem sensitive and resistant *P. aeruginosa*

Isolates	Imipenem resistant strains (n=10) (%)	Imipenem sensitive strains (n=38)
Diabetes	4 (40%)	7 (18.4%)
Alcoholism	2 (20%)	8 (21.0%)
History of prior antibiotic use	5 (50%)	9 (23.6%)
Prolonged hospital stay(>7 days)	4 (40%)	7 (18.4%)
Insertion of device	4 (40%)	11(28.9%)

Discussion:

P. aeruginosa is a pervasive pathogen in hospital acquired infections, especially among critically ill patients and the leading cause of mortality in hospitals. In this study we elucidate prevalence of MBL-PA infection and risk factors in our hospital.

Among all samples studied the highest number of cases with *P. aeruginosa* infections were from the surgical ward (20.8%), followed by burns ward(16.6%), orthopaedic (14.5%), ICU (14.5%) general medicine(10.4 %), ENT and paediatrics (8.3%), pulmonary medicine (4.1%) and obs and gynaecology wards (4.1%). Among seven orthopaedic patients, five developed fracture site infections. Ulcerative lesions were predominant in surgery cases. Out of 10 general surgery patients, 6 had ulcerative lesion *viz.* diabetic ulcer (n=3) traumatic ulcer (n=2) and varicose ulcer (n=1). Similarly all medicine, ICU & chest medicine patients had either primary lung disease or developed respiratory co-morbidity. These findings are in keeping with other studies where *P. aeruginosa* was found frequently to cause respiratory & suppurative skin infections (Jayakarni K *et al*[3]. Zavascki AP *et al*[9],)

Distribution of *P. aeruginosa* in various clinical samples were studied and results were compared with other studies. In our study, Pus swab and aspirate were most common source (45.83%) of *P. aeruginosa* as described *by* Shenoy S et al[14], Rashid A et al[15] and Deeba B et al[19]. followed by respiratory specimens including endotrachal aspirate and sputum(20.8%) which were in proportion with study reported by Aggarwal R et al[16] and Varaiya et al[17]. A large proportion of *P. aeruginosa* i.e.20% were also recovered from urine sample specially from catheterized patients. Other studies such as Rashid A et al[15] , Varaiya et al[17] and Attal RO et al[18] reported recovery of P. aeruginosa in 29.25%, 21.6% and 22.9% respectively. Among other samples, *P. aeruginosa* was recovered from 4 blood samples i.e. in 8.33% cases and 2 vaginal swabs. Though most of the studies [14,16,19] report recovery of *P. aeruginosa* in 18.9%, 18.9% and 20.8% respectively ; in our study only 8.33% *P. aeruginosa* were recovered from blood sample which are in proportion with Varaiya et al[17] which reported recovery of *P. aeruginosa in* only 4.18% blood samples and the reason may be attributed to short duration of study.

P. aeruginosa infection was predominantly found among males (77.08%, when compared to females (22.91%). The mean age of patients with *P. aeruginosa* infection was 41.2 ± 18.9 years while patients with MBL-PA infection had 43.6 ± 21.2 year mean age. The preponderance of males can be explained by greater number of cases from surgery & orthopaedic wards which usually have more admissions of male patients in our hospital. Our findings are similar to study reported by Tsakris *et al*[21], which detected 93.3% patients of MBL-PA were male and considered male gender as an independent high risk association.

Antibiotic sensitivity testing of *P. aeruginosa*, in present study, revealed maximum resistance to piperacillin (64%), followed by ciprofloxacin (62.5%), ceftazidime (52.8%), cefepime (47.91%), piperacillin/tazobactum (47.91%) , aztreonam (43.75%), gentamicin

(41.6%), tobramycin (39.58%), and amikacin (37.5%). Low resistance were seen to imipenem (18.75%), polymyxin B (2.08%) and no resistance to colistin .

The overall prevalence of MBL producers are found to vary greatly in different geographical areas and in different institutes. MBL production in present study (18.75%) is comparable with Varaiya et al[17] (20.8%),Attal et al[18](11.4%)and Deeba B et al[19](11.66%). Several other workers Sederi H et al [22] and John S et al[23] reported high incidence of MBL production such as 39.06% and 27.7% respectively.
Diverse resistance pattern were described by different workers (Sharma M *et al*[24]) .Tsakris *et al*[21], reported 100% resistance to ceftazidime, cefepime, carbapenems, amikacin, netilmycin & ciprofloxacin in VIM-2 type MBL-PA which had only 44% & 47% resistance to gentamicin and piperacillin tazobactam respectively. In a recent Indian study[25], imipenem, gentamicin, ciprofloxacin, netilmycin, piperacillin and amikacin resistance amongst MBL-PA were 77.5%, 77%,72.1%, 67.3%, 57.7% and 56.1% respectively. While another Indian study[26] pointed out 100% resistance to all aminoglycosides, beta-lactam & quinolones. Our study shows maximum resistance to most of the commonly used antipseudomonal antibiotic.(as described in table 4). Though Polymyxin resistance is uncommon among *P. aeruginosa* and several studies2[6] reported MDR strains where polymyxin was uniformly sensitive ; in our study 1 MBL–PA strain was found to be resistant to polymixin B. All the strains were sensitive to colistin. This antibiotic resistance pattern may be attributed to the misuse or overuse of commonly prescribed antibiotics at our hospital.

The high numbers of MBL producers in the present study are isolated from pus, respiratory secretions and urine (specially in device insertion such as urinary catheter or endotracheal tube) reveals that such organisms might have been acquired by the patients from the hospital environment. This signifies that the transmission could have been person to person, so the necessity of proper hand washing by the health personnel and the visitors, while attending the patients is necessary.

Among 10 patients with MBL-PA infection, four (40%) were diabetic, two (20%) were alcoholic , five(50%) patients had history of prior antibiotic use and four (40%) were having history of prolonged hospitalization and device insertion. De et al.[27] in his study reported prolong hospitalisation > eight days, mechanical ventilation and endotracheal intubation were common risk factors of MBL-PA in ICU patients. In a multivariate analysis Zavascki et al[28] have shown that prior exposure of a β-lactam or fluoroquinolone, neurological disease, urinary tract infection, renal failure and ICU stay were significant risk factors for MBL-PA infections. Among drugs, prior uses of fluoroquinolones or carbapenems were associated with resistance to imipenem resistance. This is in accordance with our observation. We found 50% patients had prior exposure to antibiotic use, especially ciprofloxacin, norfloxacin metronidazole and cefotaxime and cefepime.

The main limitation of this study was the short duration of the study period and small sample size. Because of the short duration of the study our observations might have been affected by seasonal trends of patient admission in different wards of hospital. Still our study can guide the physician about the emerging strains of MBL-PA and the recent trend of antibiotic resistance pattern so as to take proper infection control measures and avoid misuse of available antibiotics.

Results:
MBL-PA is an emerging threat and cause of concern for physician particularly in high risk patients with prolonged history of hospitalization, history of prior antibiotic usage and device associated infections. Hence, detection of MBLs among *P. aeruginosa* is crucial for the optimal treatment of patients and to control spread of resistance.

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