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RESEARCH ARTICLE

DETECTION OF MBL PRODUCING PSEUDOMONAS AERUGINOSA IN TERTIARY CARE HOSPITAL, PONDICHERRY

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ABSTRACT

Pseudomonas aeruginosa isolates are responsible for outbreaks of nosocomial infections in different parts of the world. Carbapenems are drug of choice for severe pseudomonas infections, but resistance to this antibiotic is increased worldwide. Carbapenem resistance is due to the production of metallo-beta lactamases (MBLs). The aim of the present study was to isolate MBL producing P. aeruginosa and determined the antibiotic susceptibility pattern. This study was conducted at a tertiary care hospital for 2 months with minimum of 50 P.aeruginosa isolates from various clinical samples. Antimicrobial sensitivity testing was performed by disk diffusion method. Results: Out of total 50 isolates of P. aeruginosa screened for MBL production by imipenem disk diffusion test, 11 isolates (20.4%) were imipenem resistant and rest 40 (79. 6%) were sensitive to imipenem. Conclusion:In the present study ,we isolated MBL producing P. aeruginosa and determined the antibiotic susceptibility pattern and compared different phenotypic methods currently in use . This would reduce therapeutic failure and thereby lowering the morbidity and mortality. It helps the clinician in prescribing proper antibiotic therapy.

INTRODUCTION

Pseudomonas aeruginosa is reported to be amongst the leading cause of nosocomial infection. Especially patients are at risk due to longer hospital stay, frequent use of broad spectrum antibiotics, invasive procedures, indwelling catheters and other co-morbidities. Carbapenems are drug of choice for severe pseudomonas infections (Cornaglia G *et al*,2007).Carbapenem resistance in Pseudomonas aeruginosa is mainly due to the production of metallo-beta-lactamases (MBLs).

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(Peleg AY *et al*,2005).

Emergence of MBL producing P.aeruginosa in tertiary care hospital is alarming and reflects excessive use of carbapenems. In recent years, MBL genes have spread from P. aeruginosa to members of Enterobacteriaceae (Wisplinghoff H *et al*,2004). Notably, high morbidity and mortality rates (range 27% to 48%) have been observed in critically ill patients (Kang CI *et al*,2004). Mortality rates are significantly higher in MBL producing P. aeruginosa (MBL-PA) compared to non-MBL-PA (Zavascki AP *et al*, 200

Five major enzyme types (IMP, VIM, SPM, GIM, and SIM) are implicated in most of the cases worldwide (Franklin C *et al*,2006). The first case was IMP-Type MBL described in P. aeruginosa strain in Japan in 1988 (Watanabe M *et al*,1991). Beta-lactam antibiotics comprise a large family of antimicrobial agents with a common feature of carrying beta-lactam ring in their molecules. Penicillin was the first antibiotic in this group introduced for clinical use in 1940(Koneman's Color Atlas and textbook of Diagnostic Microbiology,2005. Beta-lactamase enzymes which hydrolyse these antibiotics enzymatically appeared as a serious threat to clinical use of beta-lactams. These enzymes are diversified into several molecular as well as functional classes and they differ in avidity and spectrum of their substrates as well as inhibitors (Williams JD *et al* 1999, Walsh TR *et al*,2005).

After the global spread of extended-spectrum beta-lactamases (ESBL) and ampC beta-lactamases in gram negative bacteria, carbapenems and aztreonam established as reliable drug of choice (Helfand MS *et al*,2

There are few treatment options left for MBL-PA lesions. Since all anti-pseudomonal penicillins, cephalosporins, beta-lactamase inhibitors and frequently aminoglysides & quinolones are found resistant, the therapy solely depends on aztreonam and peptide antibiotics - polymyxin & Colistin despite their toxic profile.Finally, there are very few newer agents with activity against MBL producing organisms in the

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pipeline of development (Williams JD *et al* 1999). In the present study, we isolated MBL producing P. aeruginosa and determined the antibiotic susceptibility pattern and compared different phenotypic methods which are currently in use . This would reduce therapeutic failure and thereby lowering the morbidity and mortality. It helps the clinician in prescribing proper antibiotic therapy.

MATERIALS AND METHODS

This study was conducted at a tertiary care hospital in Pondicherry. It is a prospective, analytical study. Duration of the study is 2 months. **Sample size :** Minimum of 50 P.aeruginosa isolates.

Inclusion criteria

This study included all P.aeruginosa isolated from patients of all age groups and both sexes admitted in tertiary care hospital.

Exclusion criteria

Patients attending out-patient departments (OPD). Repeat isolates from the same patients. Samples were taken under complete aseptic conditions and included sputum, broncho-alveolar lavage (BAL), endotracheal aspirates, urine, blood, wound swabs and indwelling catheters.

Identification of Pseudomonas aeruginosa isolates by oxidase reaction, colony morphology on Blood agar and Mac Conkey's agar, pigment production, citrate utilisation, sugar fermentation, TSI reaction and polymyxin B sensitivity testing. Antimicrobial sensitivity testing was performed on Muller-Hinton agar plates by Kirby-Bauer disk diffusion method. The following antibiotics (Hi-Media, India) were tested by the disk diffusion method: piperacillin/ tazobactam (100 µg/ 10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), amikacin (30 µg), ciprofloxacin (5 µg) and imipenem (10 µg), gentamicin (10 µg), netilmicin (30 µg) , polymyxin-B (300 units) and colistin (10 µg). P. aeruginosa ATCC 27853 was used for quality control.

Different phenotypic tests for detection of MBL production in P. aeruginosa have also been compared. The statistical analysis was done by taking percentage and simple ratios. The data were expressed as mean ±SD and the percentage.

Detection of Metallo-beta- lactamases (MBLs) by two methods

1. **Imipenem (IMP)-EDTA combined disc test**

   The combined disc method was performed as described by Yong *et al* . A lawn culture of the test isolate will be prepared. After allowing it to dry for 5 minutes, two imipenem discs one with 0.5 M EDTA and the other plain were placed on the surface of the agar plate approximately 30mm apart. The plates were incubated overnight at 37 ^{o}C. An increase in the zone diameter of ≥ 7 mm around imipenem+EDTA disc in comparison to imipenem disc alone indicated production of MBL.

2. **Imipenem-EDTA double disc synergy test (DDST**)

   The IMP-EDTA double disk synergy test was performed as described by Lee *et al*.(24) Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An imipenem (10 µg) disc was placed 20 mm centre to centre from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.

RESULTS

The study was performed during May, 2014 to June, 2014 at tertiary care hospital, from Pondicherry. This study was carried out to find out the incidence MBL production in isolates of P. aeruginosa , their antibiotic susceptibility pattern and associated mortality in affected patients in our hospital. Different phenotypic tests for detection of MBL production in P. aeruginosa have also been compared. The results were analyzed and presented in following tables & figures

Sources of P. aeruginosa isolates

A total of 50 non-duplicate isolates of P. aeruginosa from both medical and surgical wards were included in this study.

Table-1 Distribution of P. aeruginosa in hospital wards

Sources of *P. aeruginosa* isolates	Number of isolates (n=50)	Percentage
General Surgery	21	42.8 %
Orthopedic	7	14.3%
ENT	6	12.2%
ICU	4	8.1%
General Medicine	4	8.1%
TB & Chest disease	3	6.1%
Obstetrics & Gynaecology	2	4.0%
Pediatric Surgery	2	4.0%
Urology	1	2.0%

Among all inpatients number of cases of *P. aeruginosa* infection was highest in surgical ward, followed by other wards.

Table-2 Distribution of *P. aeruginosa* in clinical samples

Sources of *P. aeruginosa* isolates	Number of isolates (n=50)	Percentage
Pus swab	27	55.1%
Sputum	8	16.3%
Pus aspirate	6	12.2%
ET secretion	4	8.1%
Urine	2	4.0%
Bronchial aspirate	2	4.0%
Vaginal swab	1	2.0%

P. aeruginosa strains were isolated most commonly from pus & exudates, followed by other samples.

Table-3 Gender distribution of patients infected with Ps. aeruginosa.

Patients with *P. aeruginosa* infection	Total cases (n=50)	Percentage
Male	42	83.7%
female	8	16.3%

Among the 50 patients harboring P. aeruginosa infection, 42 (83.7%) were male and only 8 (16.3%) were female.

Table 4 Imipenim resistance in P.aeruginosa strains

	Total cases(n=50)	Percentage
IMIPENEM SENSITIVE	11	22.4%
IMIPENEM RESISTANT	39	77.6

All 11 imipenem resistant P. aeruginosa strains were recovered from male patients.

Out of total 50 isolates of *P. aeruginosa* screened for MBL production by imipenem disk diffusion test, 11 isolates (22.4%) were imipenem resistant and rest 39 (77. 6%) were sensitive to imipenem.

Comparison of phenotypic tests for detection of MBL production

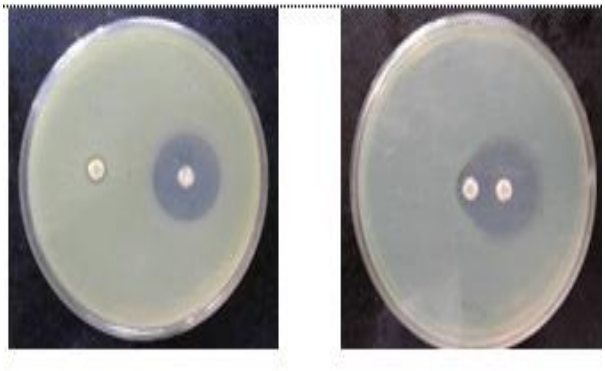
All 11 *P. aeruginosa* strains showing resistance to imipenem 10 µg disk in screening test, were further tested by disk synergy test & combined disk test.

Table 5 Comparison of disk synergy test & combined disk test for detection of MBL producing strains

Test method	Positive	Percentage
Imipenem + EDTA combined disk test	11	100%
Disk synergy test	8	72.7%

Among the two phenotypic tests evaluated in this study, combined disk test results showed better correlation (100%) with imipenem disk diffusion screening test compared to disk synergy test (72.7%).

Combined disk test using imipenem & imipenem+EDTA
Imipenem-EDTA double disc synergy test



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 risk factors & prevalence of MBL-PA infection in our hospital and its impact in terms of mortality.

Out of 50 non-duplicate *P. aeruginosa* strains recovered during this study period, most cases were from surgical inpatients where 21 (42.8%) strains were isolated from general surgery followed by other wards. In contrast, less number of isolates were recovered from medicine war

The commonest specimen was pus swab (55.1%, n=27) and pus aspirates (12.2%, n=6) followed by other specimens. In general, majority of pus samples were obtained from general surgery 18 (54.5%), orthopaedics 6 (18.8%) and ENT inpatients 5 (15.1). Ulcerative lesions were predominant in surgery cases. 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 (Zavascki AP *et al*,2006)

The first case was IMP-Type MBL described in *P. aeruginosa* strain in Japan in 1988. Subsequently it was also detected among other gram negative species. IMP-1 was found prevalent in Japan along with three minor IMP variants *i.e*., IMP-3, IMP-6 and IMP-10(*Iyobe S et al,2000* ,Yano H *et al*,2001) *P. aeruginosa* strains carrying IMP gene was also reported outside Japan i.e., IMP-1from Korea & Brazil, IMP-4 from Australia, IMP-7 from Canada and Malaysia, however local emergence of these MBL types were proposed instead of dissemination from Japanese strains. Outbreak was also reported by *P. aeruginosa* carrying IMP-7 in Canadian hospitals(Yano H *et al*,2001).

Despite frequent observation of blood stream infection by *P. aeruginosa* by various authors Wisplinghoff H *et al* , Marra AR *et al*., no strain of *P. aeruginosa* was recovered from blood culture in this study. This may be attributed to the small duration of our study. Similarly, *P. aeruginosa* with carbapenem resistance was often detected in urinary infection (Tsakris A *et al*). But only two isolate of *P. aeruginosa* we recovered from urine.

P. aeruginosa infection was predominantly found among males (83.7%, n=50) when compared to females (16.3%,n=50). The mean age of patients with *P. aeruginosa* infection was 43.3 ± 18.9 years while patients with MBL-PA infection had 44.6 ± 21.2 year mean age. In this study, the mean age of patients is much lower than the mean age commonly reported by (Marra *et al*). 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. Other authors also had similar findings (Tsakris *et al*), detected 93.3% patients of MBL-PA were male and considered male gender as an independent high risk association.

The imipenem disk diffusion screening divided total study isolates into 2 groups – 11 isolates (22.4%) of imipenem resistant and 39 (77. 6%) isolates of imipenem sensitive *P. aeruginosa.* This test was employed as a screening

Polymyxin resistance is uncommon among *P. aeruginosa* and several studies reported MDR strains where polymyxin was uniformly sensitive(Williams JD *et al* 1999, Nouer SA *et al* 2005). Diverse resistance pattern were described by different workers (Sharma M *et al* 2010) .Tsakris *et al*, 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, 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 (Gibb AP *et al* 2002). While another Indian study pointed out 100% resistance to all aminoglycosides, beta-lactam & quinolones (John S *et al* 2011).Our study shows lesser resistance to most of the non-beta lactam agents compared to others and it might be attributed to rational antibiotic usage.

All 11 imipenem resistant *P. aeruginosa* isolates were tested for MBL production by disk synergy test (DST) and combined

disk test (CDT) using imipenem & EDTA. Positive result was detected in all strains in case of CDT (100%) and 8 strains in DST (72%). Hence, CDT showed better correlation with imipenem disk diffusion.

The prevalence of MBL production among *P. aeruginosa* in our hospital was 22.4% which is in accordance with other Indian studies (Gibb AP *et al* 2002, John S *et al* 2011). EDTA based resistance reduction tests are most commonly practiced phenotypic tests for MBL. Behera *et al* reported equal efficacy of both combined disc test and E test. Some workers reported CDT to be satisfactory for screening in spite of its low specificity because of easy procedure & simple interpretation (Yong D *et al*,2002 , Samuelsen O *et al*, 2008) while others found DST was superior to & more reliable than CDT & modified Hodge test.(Gibb AP *et al* ,2002) .However, our findings are consistent with the first observation.

CONCLUSION

MBL production was main resistance mechanism in carbapenem resistant *P. aeruginosa* in our institution. We found imipenem resistance detected by disk diffusion test which correlates well with combined disk test method. Multidrug resistance significantly associated with MBL production in *P. aeruginosa*. The early detection of MBL producing *P.aeruginosa* may help in appropriate antimicrobial therapy and avoid the development & dissemination of these multidrug resistance strains

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