Comparison of Epidemiological and Antibiotic Susceptibility Pattern of Metallo-Beta-Lactamase-Positive and Metallo-Beta-Lactamase-Negative Strains of Pseudomonas Aeruginosa

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ABSTRACT

Background: The infections caused by metallo-beta-lactamases (MBLs) producing Pseudomonas aeruginosa are associated with higher rates of mortality, morbidity, and overall healthcare costs compared to non-MBL P. aeruginosa infections.

Purpose: To compare the epidemiologic factors and antibiograms of MBL-positive and MBL-negative P. aeruginosa isolates in a tertiary care hospital.

Methods: In an observational study, from January 2011 to December 2012, all non-duplicate P. aeruginosa isolates were subjected to an antimicrobial sensitivity test against 10 antibiotics of five different classes. All P. aeruginosa strains showing resistance to at least one of the carbapenems were subjected to the MBL-E test. Epidemiological features and antibiograms of MBL-positive and MBL-negative strains were compared and statistically analyzed.

Results: Out of 350 isolates (total sample = 5330) of P. aeruginosa, MBL was detected in 58 isolates by the E-test, resulting in a prevalence of 16.57%. Resistance to most of the antibiotics was significantly higher in the MBL-positive strains with 100% resistance to ciprofloxacin, tobramycin, and meropenem, followed by imipenem (93.10%) and gentamicin (89.66%). The prevalence of multidrug-resistant and pandrug-resistant strains was significantly higher among the MBL group as compared to that in the non-MBL group (55.17 vs. 7.88% (P < 0.0001) and 8.62 vs. 0.68% (P = 0.0006)), respectively.

Conclusions: MBL-positive P. aeruginosa strains showed very high resistance to various antibiotics, as compared to the non-MBL strains. Increasing prevalence of MBL-producing isolates in hospital settings makes it important to perform routine detection of MBL-positive P. aeruginosa strains by in vitro testing before antibiotic use, for the purposes of infection prevention, and control, and for minimizing the adverse outcomes of infections with MBL-producing strains.

Key words: Antibiogram, epidemiological factors, metallo-beta-lactamases, non-metallo-beta-lactamases, P. aeruginosa

INTRODUCTION

Treatment of infectious diseases is becoming more challenging with each passing year. This is especially true for infections caused by the opportunistic pathogen Pseudomonas aeruginosa (P. aeruginosa), due to its ability to rapidly develop resistance to multiple classes of antibiotics.\(^1\) P. aeruginosa is well-known for its persistence in the hospital environment, and hence, multidrug resistance mechanisms are often seen in such hospital isolates.\(^2\) Carbapenems are the antibiotics of choice for severe Pseudomonas infections, however, in recent years resistance to this novel antibiotic is increasing worldwide.\(^3\) The most common mechanism for carbapenem resistance is production of metallo-beta-lactamases (MBLs), which are broad-spectrum enzymes that hydrolyze most beta-lactam antibiotics, and are not inhibited by conventional beta-lactamase inhibitors like clavulanic acid or sulbactam.\(^4\) Since the first reporting of
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are associated populations in larger geographic
[1] [4] [5]
this period were subjected to antimicrobial sensitivity identified as the standard microbiological procedures. Isolates were catheter tips, and urine were processed according to departments sent to the Department of Microbiology, Routine clinical specimens from all medical and surgical
study (study registration No—01_M010_16928) and all the
Institutional Review Board approval was obtained for this
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to December, 2012, in the Department of Microbiology, MS Ramaiah Medical College, Bangalore, India. The

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The evolution, maintenance, and dissemination of MBL genes in P. aeruginosa populations in larger geographic healthcare regions is a dynamic process that requires ongoing study.[4] Although the import of resistance mechanisms on mobile genetic elements is always a concern, the most difficult challenge faced with P. aeruginosa is its ability to rapidly develop resistance during the course of treating an infection.[1] The lack of antibiotic policy in a hospital and sale of antibiotics over the counter, without prescription, leads to indiscriminate and injudicious use of antibiotics, further increasing the burden of antimicrobial resistant organisms. Therefore, there is a need to have a strong antibiotic policy, which is revised from time-to-time, with a stringent check over the sale of antibiotics, and incorporation of an antibiotic stewardship program. Hence, this study was conducted to detect the presence of MBLs in P. aeruginosa isolates obtained from the clinical sample, from a tertiary care hospital in South India, and to compare the antibiograms of MBL-producing and non-MBL-producing P. aeruginosa isolates, to guide clinicians in prescribing proper antibiotics and controlling hospital infection.

On the basis of the result of the E-test, P. aeruginosa isolates were divided into two groups MBL-producing and non-MBL-producing strains, and retrospectively their epidemiological features and antibiograms were compared. All findings were entered in an MS excel data sheet and on completion of the study data were statistically analyzed. The data was expressed as mean ± SD and percentages.

RESULTS

During the study period, a total of 350 isolates of P. aeruginosa was collected. The mean age of all the patients, from whom isolates were collected, was 43.68 ± 19.41 years. The male to female ratio of the study sample was 1.85 (n = 227:123). Out of 350 isolates of P. aeruginosa, MBL was detected in 58 isolates by E-test, while the remaining 292 isolates were MBL-negative. Thus, the prevalence of MBL-producing strains among the clinical isolates of P. aeruginosa collected in our study was 16.57% (95% CI: 12.67–20.47). There was no statistical difference between the mean age of

MBL-producing P. aeruginosa in Japan, in 1991, infection with MBL-producing organisms has become a major problem in various parts of the world.[4] The infections caused by MBL-producing P. aeruginosa are associated with higher rates of mortality, morbidity, need for surgical intervention, length of hospital stay and chronic care, and overall healthcare costs compared to non-MBL-producing P. aeruginosa infections.[1,2,4,5]

The antibiotic susceptibility of all P. aeruginosa isolates was determined against the following antibiotics: Ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), netilmicin (30 µg), ciprofloxacin (5 µg), aztreonam (30 µg), meropenem (10 µg), and imipenem (10 µg). All the disks were procured commercially from the same source (Hi-media laboratories limited, India). The diameter of the zone of inhibition was measured and interpreted according to the CLSI guidelines. The different classes of antibiotics, evaluated in this study, were defined as cephalosporins, aminoglycosides, fluoroquinolones, monobactams, and carbapenems. Isolates resistant to all the antimicrobials of at least three classes used in this study were defined as multidrug-resistant (MDR) strains. Isolates resistant to all the antimicrobials of all the classes used in this study were defined as pandrug-resistant (PDR) isolates. All P. aeruginosa strains that were found to be resistant to at least one of the carbapenems (imipenem or/and meropenem) using the disk diffusion test were screened for MBL activity. MBL-E test (Imipenem/ethylene diamine tetra acetic acid E-test: IMP/EDTA Epsilometer-test) (Hi-media laboratories limited, India) was applied to all positively screened isolates, to confirm the presence of the MBL phenotype. The E-test was performed following the CLSI recommendations and manufacturer’s instructions. The MIC ratio of IMP/IMP-EDTA of ≥8 was interpreted as being indicative of MBL production.

The evolution, maintenance, and dissemination of MBL production involves transfer of resistance genes to other P. aeruginosa isolates by mobile genetic elements like plasmids and transposons, and the MBLs are encoded by mobile genetic elements like plasmids and transposons. The presence of MBLs in P. aeruginosa is an ongoing study. Although the import of resistance mechanisms on mobile genetic elements is always a concern, the most difficult challenge faced with P. aeruginosa is its ability to rapidly develop resistance during the course of treating an infection. The lack of antibiotic policy in a hospital and sale of antibiotics over the counter, without prescription, leads to indiscriminate and injudicious use of antibiotics, further increasing the burden of antimicrobial resistant organisms. Therefore, there is a need to have a strong antibiotic policy, which is revised from time-to-time, with a stringent check over the sale of antibiotics, and incorporation of an antibiotic stewardship program. Hence, this study was conducted to detect the presence of MBLs in P. aeruginosa isolates obtained from the clinical sample, from a tertiary care hospital in South India, and to compare the antibiograms of MBL-producing and non-MBL-producing P. aeruginosa isolates, to guide clinicians in prescribing proper antibiotics and controlling hospital infection.

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the patients infected with MBL-positive \(P. aeruginosa\) isolates and of those with MBL-negative \(P. aeruginosa\) isolates (43.97 ± 18.02 and 43.62 ± 19.70 years, respectively, \(P = 0.9026\)). Table 1 shows the demographic comparison of patients infected with MBL-producing and non-MBL-producing \(P. aeruginosa\) isolates. Out of 58 MBL-positive \(P. aeruginosa\), 42 isolates (72.41%) were recovered from male patients and 16 isolates (27.59%) from female patients. Among 292 non-MBL-producing \(P. aeruginosa\) isolates, 185 isolates (63.36%) were recovered from males and 107 isolates (36.64%) from females. The age-group distribution pattern for MBL-positive and MBL-negative \(P. aeruginosa\) isolates were statistically similar (\(P = 0.6890\)). For both the groups, the isolates were collected most commonly from the 41-60-year age group (41.38 and 38.01%, respectively) followed by the 21-40-year age group (36.21 and 26.37%, respectively). The sample-wise distribution of MBL-positive and MBL-negative \(P. aeruginosa\) isolates has been summarized in Table 2. For both MBL-producing and non-MBL-producing \(P. aeruginosa\), the most common sample source was pus (48.28 and 51.03%, respectively) followed by urine (13.79 and 20.21%, respectively).

Table 3 shows and compares the result of an antibiogram, for routinely tested antimicrobial agents of MBL-producing and non-MBL-producing \(P. aeruginosa\) isolates. Resistance to all the antibiotics used in the study, except ceftazidime and netilmicin, was significantly more common in the MBL-producing strains, compared to that in the non-MBL-producing strains. The MBL-producing isolates showed 100% resistance to ciprofloxacin, tobramycin, and meropenem, followed by imipenem (93.10%) and gentamicin (89.66%), while resistance to these antibiotics by the non-MBL-producing isolates was 39.73, 52.74, 6.8%, 8.22, and 32.53%, respectively. The prevalence of multidrug-resistant and pandrug-resistant isolates was significantly higher among the MBL group as compared to that in the non-MBL group (55.17 vs. 7.88% \(P < 0.0001\) and 8.62% vs. 0.68% \(P = 0.0006\), respectively [Table 4].

### DISCUSSION

The occurrence of MBL-producing \(P. aeruginosa\) isolates in a hospital setting poses a therapeutic problem as well as a serious concern for infection control management, due to the rapid spread of these multidrug-resistant isolates. In our present study, the prevalence of MBL-producing isolates was 16.57%, while in a previous study from our hospital (2002), the MBL-production in \(P. aeruginosa\) was recorded as 12%. Thus, the prevalence of MBL-producing \(P. aeruginosa\) strains

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**Table 1: Comparison of the demographic details of MBL-positive and MBL-negative \(P. aeruginosa\) isolates**

| Characteristics                  | MBL-positive | MBL-negative | Statistical analysis |
|----------------------------------|--------------|--------------|---------------------|
| Mean age (years)                 | 43.97±18.02  | 43.62±19.70  | \(P=0.9026^*\)      |
| Sex distribution                 |              |              |                     |
| Male                             | 42 (72.41%)  | 18 (30.56%)  | \(P=0.2423^{**}\)  |
| Female                           | 16 (27.59%)  | 107 (36.64%) |                     |
| Age-group distribution (years)   |              |              |                     |
| 1-20                             | 6 (10.34%)   | 46 (15.75%)  | \(P=0.6890^*\)      |
| 21-40                            | 21 (36.21%)  | 77 (26.37%)  |                     |
| 41-60                            | 24 (41.38%)  | 111 (38.01%) |                     |
| 61-80                            | 5 (8.62%)    | 51 (17.47%)  |                     |
| >80                              | 2 (3.45%)    | 7 (3.49%)    |                     |

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**Table 2: Comparison of sample-wise distribution of MBL-positive and MBL-negative \(P. aeruginosa\) isolates**

| Types of sample | Number of isolates (percentage) |
|-----------------|---------------------------------|
| MBL-Positive (58) | MBL-Negative (292) |
| PuS             | 28 (48.28)   | 12 (21.21) |
| Urine           | 8 (13.79)    | 59 (20.21) |
| ET Secretion    | 7 (12.07)    | 25 (8.56)  |
| Tissue          | 6 (10.34)    | 18 (6.16)  |
| Sputum          | 3 (5.17)     | 17 (5.82)  |
| Blood           | 2 (3.45)     | 13 (4.5)   |
| Miscellaneous   | 4 (6.82)     | 11 (3.77)  |

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**Table 3: Comparison of antibiogram of MBL-positive and MBL-negative \(P. aeruginosa\) isolates**

| Antibiotics | Number of resistant isolates (percentage) | Statistical analysis |
|-------------|------------------------------------------|---------------------|
| MBL-Positive (58) | MBL-Negative (292) |
| Amikacin    | 24 (41.38) | 49 (16.78) | \(P=0.001^*\) |
| Aztreonam   | 31 (53.45) | 91 (31.6) | \(P=0.002^*\) |
| Cefepime    | 24 (41.38) | 39 (13.56) | \(P=0.001^*\) |
| Cefazidime  | 17 (28.95) | 79 (27.05) | \(P=0.847^*\) |
| Ciprofloxacin | 58 (100) | 116 (39.73) | \(P=0.001^*\) |
| Gentamicin  | 52 (89.66) | 95 (32.5) | \(P=0.001^*\) |
| Imipenem    | 54 (93.10) | 24 (8.22) | \(P=0.001^*\) |
| Meropenem   | 58 (100)  | 20 (6.85) | \(P=0.001^*\) |
| Netilmicin  | 31 (55.17) | 125 (42.84) | \(P=0.1130\) |
| Tobramycin  | 58 (100)  | 154 (52.74) | \(P=0.001^*\) |

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**Table 4: Prevalence of MDR and PDR isolates among MBL-positive and MBL-negative \(P. aeruginosa\)**

| Groups | Number of isolates (%) | Statistical analysis |
|--------|------------------------|----------------------|
| MDR isolates | 32 (55.37%) | 23 (7.88%) | \(P=0.0001\) |
| PDR isolates | 5 (8.62%) | 2 (0.68%) | \(P=0.006\) |

*Chi-squared test with Yate's correction, two-tailed P value, MBL: Metallo-beta-lactamases, ET: Endotracheal tube
in our hospital seemed to have increased by about 5% in a decade. However, in a similar study, Vitkauskienė et al. reported the prevalence of MBL-producing \textit{P. aeruginosa} as 15.8\% in 2003, which increased to 61.9\% in 2008, resulting in a 46\% rise in only a five-year period.\textsuperscript{[8]} These results clearly demonstrate the increasing burden of MBL strains in the same hospital setup, although the rate of increment varies widely. Interestingly, in our setup the increase in the MBL-positive strain from 2002 to 2012 has not been very drastic (12 to 16.57\%) in contrast to the study by Vitkauskienė et al.\textsuperscript{[9]} In India, the incidence of MBL production in \textit{P. aeruginosa} has been reported to be 10.30\% from various clinical specimens across the country.\textsuperscript{[10]} A similar prevalence rate of MBL-producing strains of \textit{P. aeruginosa} has been observed in studies conducted by Hemalatha V et al.\textsuperscript{[11]} in Chennai, India (14\%), Saderi H et al.\textsuperscript{[12]} in Brazil (19.7\%) and Mihaní et al.\textsuperscript{[13]} in Iran (19.51\%).

The present study has tried to compare the demographic factors associated with the development of infections caused by MBL-producing and non-MBL-producing isolates of \textit{P. aeruginosa} in the general population. We observed no significant difference in the age-distribution pattern for both the groups with infection being most prevalent in the 40-60-year age group followed by 20-40-year age group. Similar observations have been reported by Anupurba S et al.\textsuperscript{[14]} We observed a significantly higher rate of infection caused by both MBL and non-MBL strains, although there was no difference in the sex-distribution pattern between the two groups. This observation was also in accordance with the results of Anupurba S et al. suggesting that males are more predisposed to acquire \textit{P. aeruginosa} infection.\textsuperscript{[14]}

Also, no difference was observed between the two groups in terms of most frequent sample sources.

Our study has illustrated that the MBL-producing strains are an important cause of resistance to commonly used antimicrobial agents. MBL-producing isolates showed the highest resistance to the fluoroquinolone (ciprofloxacin 100\%), aminoglycosides (tobramycin 100\% and gentamicin 89.66\%), and carbapenems (meropenem 100\% and imipenem 93.10\%). Several studies have also highlighted greater resistance exhibited by MBL-positive strains toward almost all classes of antimicrobials, as compared to MBL-negative strains. Viskauskiene et al. have also reported that MBL-producing \textit{P. aeruginosa} strains are significantly more commonly resistant to ciprofloxacin, gentamicin, ceftazidime, cefepime, aztreonam, and piperacillin, as compared to MBL-negative strains.\textsuperscript{[8]} Similar findings were reported by Hemlatha et al.,\textsuperscript{[11]} Navneeth et al.,\textsuperscript{[8]} and Varaiya et al.\textsuperscript{[15]} Thus, MBL strains are developing resistance to not only carbapenems, but are also resistant to other commonly used drugs, thus rendering them ineffective. This suggests a possible existence of co-resistance to quinolones, aminoglycosides, and other classes of antibiotics on the gene responsible for MBL production. Interestingly in our study, resistance to ceftazidime was pretty low (29.3\%) as compared to the finding of Arunagiri et al., who has reported 100\% resistance to ceftazidime.\textsuperscript{[8]} This may be because of a lower pressure of selecting ceftazidime in treating pseudomonal infection and increasing the trend of using carbapenems, in our hospital.

The prevalence of multidrug-resistant isolates (resistant to three or more classes of antibiotics) was significantly higher in the MBL group (55.17\%) as compared to the non-MBL group (7.88\%) (\textit{P} < 0.0001). A similar result was found in the study conducted by Laupland et al., in which 89\% of the MBL-producing strains were resistant to three classes of antibiotics, in addition to imipenem, whereas, resistance to antibiotics was uncommon (7\%) in the non-MBL-producing isolates (\textit{P} < 0.0001).\textsuperscript{[8]} In our study, 8.62\% of the MBL-producing strains were found to be resistant to all the classes of antimicrobials used in the study, whereas, only 0.68\% of the non-MBL-producing strains were pandrug-resistant (\textit{P} = 0.0006). Today the growing population of pan-resistant bacteria, including MBL-producing \textit{P. aeruginosa}, threatens to move us into what some consider the ‘post-antibiotic era’ of infectious disease.\textsuperscript{[11]} The significantly higher prevalence of multidrug- and pandrug-resistance among MBL-producing strains, as compared to that in non-MBL-producing strains supports the notion that clinical microbiological laboratories must be able to distinguish MBL-producing strains from those with other mechanisms responsible for carbapenem resistance. The identification of MBL production in \textit{P. aeruginosa} isolates also has clinical implications, because such isolates are more likely to cause invasive disease and are associated with a higher hospital case-fatality rate, compared with other imipenem-resistant isolates.\textsuperscript{[8,14]}

To conclude, the prevalence of MBL-producing \textit{P. aeruginosa} isolates in hospital settings are increasing day by day, leading to more treatment failure with commonly used antibiotics. Although there is no difference in the epidemiological factors associated with MBL-producing and non-MBL-producing \textit{P. aeruginosa}, they differ greatly in their susceptibility to various antibiotics. The routine detection and molecular characterization of MBL-producing \textit{P. aeruginosa} strains by \textit{in vitro} testing, before antibiotic use, is important for the purposes of infection prevention and control, and potentially for defining the risk for development of severe disease, and for minimizing the adverse outcomes associated with MBL-producing strains.
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