

Original Research Article

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## Metallo Beta Lactamase Producing *Pseudomonas aeruginosa* in a Tertiary Care Hospital

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### ABSTRACT

Acquired drug resistance is frequent in nosocomial isolates of *Pseudomonas aeruginosa*. Acquired metallo-beta-lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanism. The occurrence of an MBL positive isolate in a hospital environment 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. A total of 100 *Pseudomonas aeruginosa* isolated from various clinical specimens over a period of one year from January 2011 to December 2011 in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore were selected for the study. These isolates were subjected to antibiotic susceptibility testing and screened for MBL production using Imipenem- EDTA combined disc test. Out of the 100 *Pseudomonas aeruginosa* isolated, 22 (22%) were MBL producers. 36.4% were from pus, followed by blood (22.7%), urine (18.2%), CSF (13.6%), sputum and endotracheal aspirate (4.5%) respectively. 100(100%) of the isolates were resistant to Ceftazidime, Cefotaxime, Cefepime and Piperacillin. 16 (72.7%) were sensitive to Aztreonam, and 10(45.5%) to Imipenem. Multidrug resistance is common in *P.aeruginosa*. This study highlights the need to do surveillance to detect MBL producers, judicious use of antibiotics and implementation of appropriate infection control measures to control the spread of these strains in the hospital.

### Keywords

*Pseudomonas aeruginosa*, Kirby-Bauer disc diffusion technique, Metallo-beta-lactamase, Combined disc test, Multidrug Resistance, Imipenem.

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### Introduction

*Pseudomonas aeruginosa*, an opportunistic and worrisome nosocomial pathogen, is a Gram-negative, aerobic rod belonging to bacterial family Pseudomonadaceae (Winn

*et al.*, 2006; Ibukun *et al.*, 2007). 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 (Gamal *et al.*, 2007). Carbapenems are generally used as an option to treat serious infections caused by *P. aeruginosa*, since these drugs present a good spectrum of activity and are stable to hydrolysis by most  $\beta$ -lactamases, including the extended spectrum  $\beta$ -lactamases (ESBL). However, the use of carbapenems has been hampered by the emergence of strains that produce metallo- $\beta$ -lactamase (MBL), an enzyme that is able to hydrolyze and inactivate this class of antibiotics. Moreover, the spread of MBL-producing clones, even over distant regions, has been reported (Gales *et al.*, 2003).

Among  $\beta$ -lactamases, MBLs are unique in requiring the presence of zinc ion in the active site of the enzyme, and are, thus, inhibited by chelating agents such as EDTA. Since the early 90s, new genes coding for distinct MBLs have been described in clinical important pathogens like *Pseudomonas* spp, *Acinetobacter* spp. and even among members of the family *Enterobacteriaceae*. These genes are usually inserted in mobile elements facilitating the exchange of these resistance genes among several bacterial species (Luzzaro *et al.*, 2004). With the global increase in the

occurrence and types of MBLs, early detection is crucial; the benefits of which include timely implementation of strict infection control practices and treatment with alternative antimicrobials. 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.

### Materials and Methods

A total of 100 *Pseudomonas aeruginosa* isolated from various clinical samples like pus, urine, blood, sputum, endotracheal aspirate, CSF and other body fluids (pleural, ascitic, peritoneal fluid) over a period of one year from January 2011 to December 2011 from hospitals attached to Mysore Medical College and Research Institute, Mysore were included in the study.

The *Pseudomonas aeruginosa* isolated were subjected to antibiotic susceptibility testing by Kirby - Bauer disc diffusion technique according to CLSI guidelines. *P.aeruginosa* ATCC 27853 was used as control. The susceptibility testing was carried out against the following antibiotics.

Antimicrobial agent	Disc conc. $\mu$ g/disc
Cefotaxime	30
Ceftazidime	30
Cefepime	30
Ciprofloxacin	5
Gentamicin	10
Amikacin	30
Imipenem	10
Piperacillin	100
Piperacillin-Tazobactam	100/10
Aztreonam	30

All the antibiotic disks were procured commercially from Hi -Media laboratories Pvt. Ltd. Mumbai. The diameter of the zone

of inhibition was measured and interpreted according to CLSI guidelines.

## Detection of MBL

### Imipenem EDTA combined disc test

Metallo  $\beta$ -lactamase detection was done by combined disc diffusion test, using two Imipenem (10 $\mu$ g) discs and 10  $\mu$ L of 0.5M EDTA solution. The test organism was inoculated on to Mueller- Hinton agar plate as recommended by the CLSI. Two imipenem (10  $\mu$ g) discs were placed at a distance of 20 mm centre to centre on the plate. 10  $\mu$ L of 0.5 M EDTA (750  $\mu$ g) solution was added to one of imipenem disc and incubated overnight at 37<sup>o</sup>C. Enhancement of the zone of inhibition of Imipenem –EDTA disc compared to that of Imipenem disc alone by  $\geq 7$  mm was considered positive for MBL production

## Results and Discussion

Antibiotic when first introduced was considered as a magic bullet. Unfortunately the genes expressing resistance to antimicrobials have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration.

*Pseudomonas aeruginosa* is known to cause a variety of infections like urinary tract infections, wound infections, septicemia and device infections is now emerging as MBL producer and is spreading it in horizontal way to members of Enterobacteriaceae (Bhalero *et al.*, 2010).

The overall prevalence of MBL producers are found to vary greatly in different geographical areas and in different institutes.

In the present study, an attempt has been made to know the prevalence of MBL

producing *Pseudomonas aeruginosa* isolated from various clinical samples and their antibiotic susceptibility pattern.

The antibiotic sensitivity pattern in the present study is in accordance with the study done by Saderi *et al.*, (2008); Dwivedi *et al.*, (2009), Ibukun *et al.*, (2008).

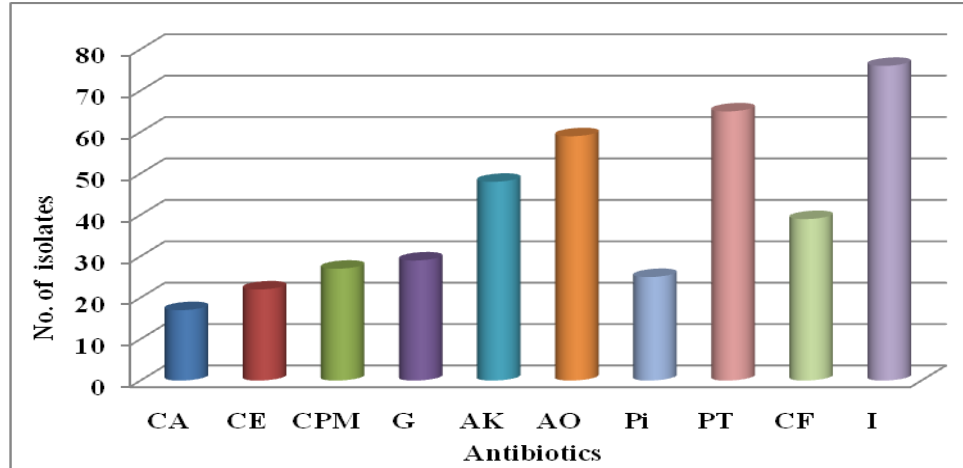
Out of 100 isolates of *Pseudomonas aeruginosa* screened, 22(22 %) were MBL producers. This is comparable to the studies of Varaiya *et al.*, and John *et al.*, But in India the published reports indicate the prevalence of MBLs to range from 7-65% (Manoharan *et al.*, 2010).

In my study maximum number of MBL producers were from pus samples, which is in accordance with studies done by Basak *et al.*, (2010) and Bhalero *et al.*, (2008).

In a study by Uma *et al.*, (2008) MBL producing *Pseudomonas aeruginosa* were commonly isolated from urine (20%) and blood (11.43%). 22 (100%) of the MBL producers were resistant to Ceftazidime, Cefotaxime, Cefepime and Piperacillin. This is comparable with the studies done by Varaiya *et al.*, (2008); Deeba *et al.*, (2011); Basak *et al.*, Uma *et al.*, and Irfan *et al.*, (2011), 18 (81.8 %) of the MBL producers were resistant to Piperacillin- Tazobactam. This is in accordance with the study done by Deeba *et al.*, (2011).

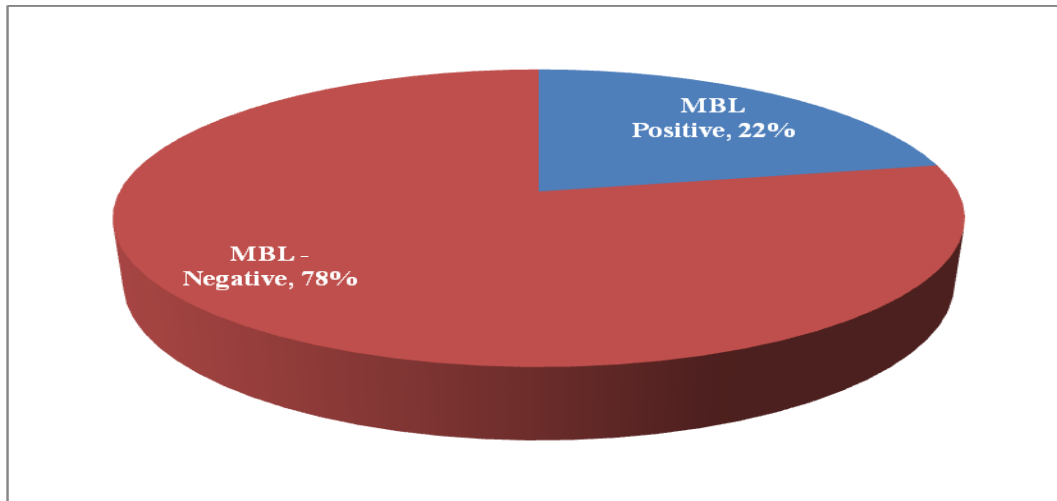
The unique problem with MBLs is their unrivalled broad –spectrum resistance profile. In addition, in many cases MBL genes may be located on plasmids with genes encoding other antibiotic determinants i.e., aminoglycoside resistance genes. These MBL positive strains are usually resistant to beta lactams, aminoglycosides and fluoroquinolones.

**Fig.1** Antibiotic susceptibility pattern of the isolates



CA-Ceftazidime, CE-Cefotaxime, CPM-Cefepime, G-Gentamicin, AK-Amikacin, AO-Aztreonam, Pi-Piperacillin, PT - Piperacillin-Tazobactam, CF-Ciprofloxacin, I- Imipenem.

**Fig.2** Percentage of MBL Producers



In the present study 16(72.7%) of the MBL producers were sensitive to Aztreonam. Uma *et al.*, (2008) also reported potent antimicrobial activity of aztreonam (79.17%) to MBL positive isolates.

In the present study 10 (45.5 %) of the MBL producers were imipenem sensitive. Only carbapenem resistant isolates were screened for MBL production in various studies like Behera *et al.*, Varaiya *et al.*, Irfan *et al.*, In the present study irrespective of the invitro

susceptibility to imipenem, MBL production was tested, because correlation between carriage of MBL genes and carbapenem resistance is often imperfect. This may be explained as either MBL genes are not always expressed or substantive resistance may require uptake of carbapenems as well as presence of MBLs. With the emergence of carbapenem sensitive MBL carrying organisms, the issue of which isolates to select for phenotypic MBL detection is controversial.

The present study underlines the unique problem of MBL mediated resistance, which has created a therapeutic challenge for Clinicians and Microbiologists. To overcome the problem of emergence and the spread of multidrug resistant *P.aeruginosa* a combined interaction and cooperation between Microbiologist, Clinicians and the infection control team is needed. We recommend a routine surveillance of antibiotic resistance in the hospital. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are curtailed and continuous education of infection control practices is maintained.

The control measures include judicious use of antibiotics, strict hand hygiene protocols and implementation of infection control team to prevent the spread of these strains in the hospital. Strict antibiotic policies and measures to limit the indiscriminate use of cephalosporins and carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple beta-lactam producing pathogens whose spread would leave no other option to treat Gram-negative nosocomial infections.

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