

Original Research Article

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## A Study on Metallo-beta-lactamase Mediated Resistance in Clinical Isolates of *Pseudomonas aeruginosa*

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### ABSTRACT

The emergence of MBL as a mode of carbapenem resistance has proved a global threat due to its broad spectrum of activity and rapid rate of dissemination. MBL producing *Pseudomonas aeruginosa* strains are responsible for several nosocomial outbreaks in tertiary care centers across the world. This study was undertaken to determine the prevalence of MBL among clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital and to determine their antibiotic susceptibility pattern. 143 clinical isolates of *Pseudomonas aeruginosa* were obtained over a period of 1 year from December 2012 to November 2013 at Sree Gokulam Medical College and Research Foundation, Venjaramoodu, Trivandrum. Their antibiotic susceptibility pattern was determined by Kirby Bauer disc diffusion method as per CLSI guidelines. The carbapenem resistant strains were screened for MBL production by Imipenem-EDTA Combined disc test. MBL E-test was used for confirmation of MBL production 47 (32.87%) out of 143 isolates showed resistance to carbapenems. Among these, 20 were positive for MBL production by the combined disc test. Of these, 19 isolates were positive for MBL production by E-test. Prevalence of MBL among *Pseudomonas aeruginosa* was thus found to be 13.3%. The MBL producing strains showed almost complete resistance to beta-lactams (except aztreonam), aminoglycosides and fluoroquinolones. Colistin was found to be 100% sensitive and hence may prove a possible therapeutic option.

### Keywords

Metallo-beta-lactamase,  
*Pseudomonas aeruginosa*,  
combined disc test,  
E-test,  
carbapenemase.

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### Introduction

Metallo-beta-lactamases (MBL) are carbapenemases that confer resistance to all beta lactam antibiotics except monobactams (Mandell, Douglas and Bennett's principles and practice of infectious diseases, 2010). They are not inhibited by clavulanic acid, tazobactam, or sulbactam; hence beta-lactam

combinations with the currently available beta-lactamase inhibitors are not useful (Bonomo *et al.*, 2006). Apart from their broad spectrum of activity, another factor causing concern is that many of the MBL genes may be located on plasmids with genes encoding other antibiotic resistance

determinants, i.e., aminoglycoside resistance genes. These MBL-positive strains are usually resistant to beta-lactams, aminoglycosides, and fluoroquinolones (Walsh *et al.*, 2005). Even with over 15 years of on-going research on MBL inhibitors, we still have not produced anything remotely approaching phase-1 clinical trials. All these factors combined make MBLs the most molecularly diverse carbapenemases and the greatest clinical threat (Walsh, 2010).

The first MBL was described in 1991 in Japan, (Watanabe *et al.*, 1991) and in India it was first reported in 2002 (Navaneeth *et al.*, 2002). The MBL producers that are most clinically significant are primarily those where the gene encoding the enzyme is transferable and include *P.aeruginosa* and *Acinetobacter spp.* and to a lesser extent enterobacterial species (Walsh *et al.*, 2005).

With global increase in the occurrence and types of MBLs early detection is essential for implementing appropriate antibiotic therapy as well as infection control practices. Keeping this in view, the present study was undertaken to determine the prevalence of metallo-beta-lactamase producing strains of *Pseudomonas aeruginosa* in our hospital, and their antibiotic sensitivity pattern, so that appropriate infection control strategy and antibiotic policy can be formulated to prevent their spread.

## Materials and Methods

143 isolates of *Pseudomonas aeruginosa* were obtained during a one year period from December 2012 to November 2013 in the Microbiology Laboratory of Sree Gokulam Medical College and Research Foundation, Venjaramoodu, Trivandrum. The isolates were subjected for antibiotic susceptibility

testing by employing Kirby Bauer disc diffusion techniques according to CLSI guidelines (CLSI, 2014). The isolates showing resistance to carbapenems (imipenem or meropenem) were subjected to screening test for MBL production:

### Imipenem and Imipenem-EDTA combined disc test (CDT)

The IMP-EDTA combined disk test was performed as described by Yong *et al.*, 2002. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI (2014). Two 10 µg Imipenem disks (Himedia) were placed on the plate, and appropriate amounts of 10 µL of 0.5M EDTA solution were added to one of them to obtain the desired concentration (750µg). The inhibition zones of the Imipenem and Imipenem-EDTA discs were compared after 16 to 18 hours of incubation in air at 35°C. If the increase in inhibition zone with the Imipenem and EDTA disc was  $\geq 7$  mm than the Imipenem disc alone, it was considered as MBL positive. (Fig 1)

### Confirmatory Test for MBL: E-test

Isolates positive for MBL by CDT were confirmed by MBL E-test. The E Test MBL strip (Biomérieux) contains a double sided seven-dilution range of Imipenem (4 to 256 µg/mL) and Imipenem (1 to 64 µg/mL) in combination with a fixed concentration of EDTA. The E-test was done according to manufacturer's instructions (Fig 2). The test was considered positive if it satisfied any of the following criteria

1. MIC ratio of IP (Imipenem)/IPI (Imipenem-EDTA) of  $>8$  or  $>3 \log_2$
2. Appearance of phantom zone
3. Deformation of the elliptical zone of inhibition.

## Results and Discussion

Among the 143 isolates of *Pseudomonas aeruginosa*, 79 (55.2%) were from pus samples, 30 (21%) from urine samples, 11(7.7%) from sputum samples, 10 (7%) from suction tip, 7 (4.9%) from ear discharge, 3 (2.1%) from blood and 3 (2.1%) from tissue samples.

47 (32.87%) isolates of *Pseudomonas aeruginosa* showed carbapenem resistance (resistance to Imipenem or Meropenem or both) by disc diffusion method. Among these 47 isolates, 20 of them were MBL screening positive by CDT. The mean difference in the zone sizes of Imipenem and Imipenem-EDTA disc among the MBL positive isolates was found to be 11.6 mm in the combined disc test.

The 20 isolates which screened positive for MBL were subjected to E-test for confirmation. 19 of these 20 isolate showed positive results by E-test, which accounts for 13.3% of the total sample size.

The predominant source of MBL positive isolates were obtained from catheterized urine in which 58.3% (7 out of 12) samples tested positive. Among the other samples, the figures were 16.7% (3 out of 18) in mid stream urine, 14.3% (1 out of 7) in ear discharge, 9.1% (1 out of 11) in sputum, and 8.9% (7 out of 79) in pus.

All the MBL producing isolates of *P. aeruginosa* were multidrug resistant with sensitivity pattern different from that of the MBL non-producers. Statistically significant difference was found in the resistance pattern of MBL positive and negative isolates for cephalosporins, aminoglycosides, fluoroquinolones and carbapenems ( $P < 0.05$ ). All the isolates were found to be sensitive to colistin. (Table 1)

Carbapenems are the currently recommended therapeutic option for infections due to multi-drug resistant pathogens such as *P. aeruginosa*. Spread of MBL mediated resistance among these pathogens and transfer to other gram-negative bacteria will significantly restrict treatment options (Nordmann *et al.*, 2002), (Goel *et al.*, 2013). The occurrence of an MBL positive isolate poses not only a therapeutic problem but is also a serious concern for infection control management. As a result of being difficult to detect, such organisms pose significant risks particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer, with other pathogens in the hospital (Agrawal *et al.*, 2008).

Various studies from across India have reported carbapenem resistance among *Pseudomonas aeruginosa* isolates to be between 12% and 77% (Goel *et al.*, 2013), (Behera *et al.*, 2008), (Hemalatha *et al.*, 2005), (John *et al.*, 2011), (Rit *et al.*, 2013), (Irfan *et al.*, 2008), (Sarkar *et al.*, 2006), (Attal *et al.*, 2010), (Oh *et al.*, 2003), (Hodiwala *et al.*, 2013), (Faghri *et al.*, 2014). In the current study carbapenem resistance among *Pseudomonas aeruginosa* was found to be 32.9% (47/143). Among the 47 carbapenem resistant *P. aeruginosa* strains, 19 were MBL producers. Carbapenem resistance in the remaining 28 may be explained by other mechanisms such as oxa-carbapenemases, efflux pump mechanism or decreased membrane permeability.

MBL production was first reported in India in 2002 by Navaneeth *et al.*, and since then, several studies have been conducted across the country to determine its prevalence. The prevalence of MBL among *Pseudomonas aeruginosa* was found to 13.3% in the

present study, which is comparable to studies by Navaneeth *et al.*, (2002), Hemalatha *et al.*, (2005), Attal *et al.*, (2010), Deeba Bashir *et al.*, (2011) and Rajput *et al.*, (2012).

The predominant source of MBL positive strains in the current study was the urinary tract, mainly catheterized urine samples, which is similar to the findings of Bashir *et al.*, (2011), Rossolini *et al.*, (2008) and Hirakata *et al.*, (2003). This calls for careful measures in disposing urinary samples from labs as well as from patient's bedside, especially in the ICUs.

The antibiotic sensitivity pattern of MBL producers in our study showed statistically significant difference with respect to cephalosporins, aminoglycosides,

fluoroquinolones and carbapenems, as compared to the MBL non-producers. MBL genes are carried on mobile genetic elements which also codes for resistance genes to aminoglycosides and fluoroquinolones (Walsh *et al.*, 2005). This explains for the concurrent high resistance rates to these two classes of antibiotics along with MBL production.

Colistin showed 100% sensitivity among MBL producing *Pseudomonas* hence providing a probable treatment option. Other authors have also recorded similar findings. (Rit *et al.*, 2013) (Gupta, 2008), (Kaleem *et al.*, 2010). However judicious use of this antibiotic is necessary since a few studies have reported colistin resistance among MBL producing strains (Franco *et al.*, 2010) (Varaiya *et al.*, 2008) (Rao *et al.*, 2014).

**Table.1** Comparison of Antibiotic sensitivity pattern of MBL positive and MBL negative isolates

	% Sensitive	
	MBL positive(n=19)	MBL negative(n=124)
Piperacillin*	0	82.3% (102)
Piperacillin-Tazobactam*	0	94.4% (117)
Ceftazidime*	0	84.7% (105)
Cefepime*	0	87.9% (109)
Gentamicin*	0	77.4% (96)
Tobramycin*	0	79% (98)
Amikacin*	15.8% (3)	82.3% (102)
Ciprofloxacin*	15.8% (3)	76.6% (95)
Ofloxacin*	15.8% (3)	76.6% (95)
Imipenem*	15.8% (3)	96% (119)
Meropenem*	0	77.4% (96)
Aztreonam*	26.3% (5)	93.5% (116)
Colistin	100% (19)	100% (124)

Fig.1 Imipenem-EDTA combined Disc Test

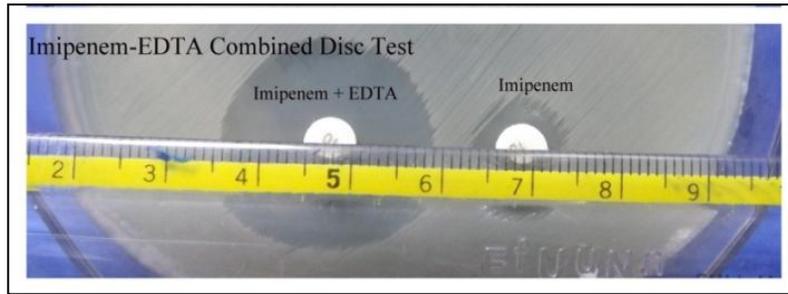
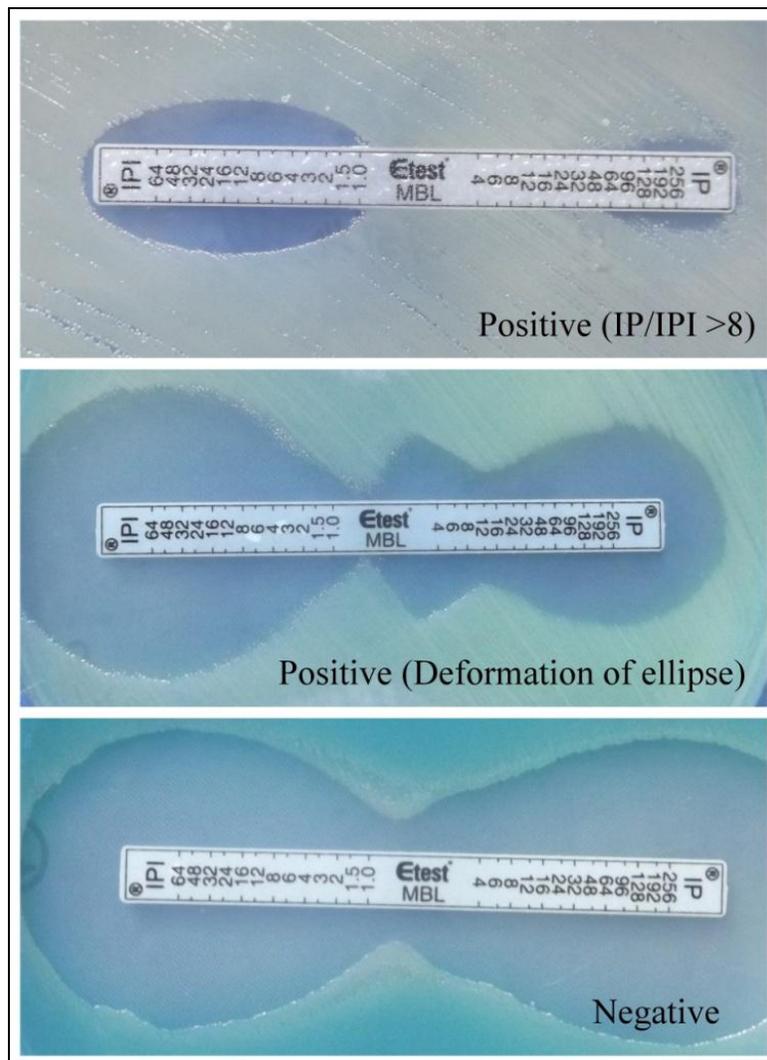


Fig.2 MBL E-test



The second most active drug against MBL *Pseudomonas aeruginosa* was Aztreonam, with sensitivity rate of 84.6%. Theoretically,

MBLs can hydrolyze all beta lactam antibiotics except aztreonam. 15.4% Aztreonam resistance among MBL

producers may be explained by the concurrent occurrence of other resistance mechanisms.

The MBL E-test was used as the confirmatory test in this study. Sensitivity of the E-test has had contradictory reports in literature (Behera *et al.*, 2008), (Yan *et al.*, 2004). False positive and false negative results by E-test have been reported previously for *Pseudomonas aeruginosa* in previous studies (Qu *et al.*, 2009), (Rossolini *et al.*, 2008), (Pagani *et al.*, 2005). Hence confirmation by PCR is required, which could not be done in this study.

In conclusion, this study documents the prevalence of MBL producers among clinical isolates of *Pseudomonas aeruginosa* at 13.3% in our hospital. Emergence of MBL-producing *Pseudomonas aeruginosa* in this hospital is alarming due to the looming possibility of hitting therapeutic dead-ends in the absence of novel therapeutic MBL inhibitors. The early detection of MBL-producing isolates would be important for the reduction of mortality rates of infected patients and also to avoid the intra hospital dissemination of such strains. CDT using imipenem can be used as a convenient screening method for detection of MBL production in gram negative bacilli in routine Microbiology laboratory where molecular methods are not feasible. In absence of therapeutic MBL inhibitors, colistin has shown to be effective against MBL producing *P.aeruginosa* infections. However, in the present scenario, its use should be judicious as strains with reduced susceptibility have emerged.

Continuous monitoring of MBL prevalence and formulation of appropriate antibiotic policy is the need of the hour for surveillance and control of MBL in the hospital.

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