

Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients

Ami Varaiya, Nikhil Kulkarni, Manasi Kulkarni, Pallavi Bhalekar & Jyotsana Dogra

Department of Microbiology, S.L.Raheja Hospital, Mumbai, India

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Background & objectives: Metallo beta lactamase (MBL) producing *Pseudomonas aeruginosa* have been reported to be important cause of nosocomial infections. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial therapy. The present study was undertaken to determine the incidence of MBL producing *P. aeruginosa* in patients with diabetes and cancer admitted to the intensive care unit of a tertiary care hospital in western India and to assess the clinical outcome after antimicrobial treatment.

Methods: A total of 240 isolates of *P. aeruginosa* from various specimens between January and December 2005 were subjected to susceptibility testing against various antibiotics by disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Imipenem and meropenem resistant isolates were selected for the detection of MBL production by disc potentiation test. Enhancement of inhibition zone around imipenem and meropenem discs impregnated with EDTA as compared to those without EDTA confirmed MBL production.

Results: Of the 240 *P. aeruginosa* isolates, 60 (25%) were found to be carbapenem resistant and 50 (20.8%) were found to be MBL producers. Of the 50 MBL producing isolates, 38 (76%) were from diabetes patients and 12 (24%) from cancer patients. Overall, 36 per cent patients responded to gatifloxacin, 42 per cent responded to piperacillin/tazobactam while 14 per cent responded to combination of gatifloxacin and piperacillin/tazobactam. Due to this nosocomial pathogen, the average hospital stay was 32 days and was associated with 20 per cent mortality due to septicemia.

Interpretation & conclusions: Our findings showed that there is a need to do surveillance to detect MBL producers, judiciously use carbapenems to prevent their spread and use effective antibiotics, such as gatifloxacin and piperacillin-tazobactam, after sensitivity testing for treatment.

Key words Carbapenem resistance - metallo beta lactamase - *Pseudomonas aeruginosa*

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. Due to their broad spectrum of

activity and stability to hydrolysis by most beta lactamases, the carbapenems have been the drug of choice for treatment of infections caused by penicillin- or cephalosporin-resistant Gram-negative bacilli

especially, extended spectrum β -lactamase (ESBL) producing Gram-negative infections¹. The carbapenems available for use in India are imipenem and meropenem². However, carbapenem resistance has been observed frequently in non fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes-carbapenemase³. These carbapenemase are class B metallo β -lactamases (IMP, VIM) or class D-oxacillinases (OXA 23 to OXA 27) or class A - clavulanic acid inhibitory enzymes (SME, NMC, IMI, KPC)³.

Metallo beta lactamase (MBL) belongs to a group β -lactamase which requires divalent cations of zinc as cofactors for enzyme activity. These have potent hydrolyzing activity not only against carbapenem but also against other β -lactam antibiotics⁴. The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria⁵. The genes responsible for MBL production may be chromosomally or plasmid mediated and hence pose a threat of spread of resistance by gene transfer among the Gram-negative bacteria³.

Thus, MBL-producing *Pseudomonas aeruginosa* isolates have been reported to be important causes of nosocomial infections associated with clonal spread⁶. These constitute 20-42 per cent of all nosocomial isolates^{7,8}. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial chemotherapy⁶.

We undertook this study to determine incidence of MBL producing *P. aeruginosa* in diabetes and cancer patients admitted to the intensive care unit of a tertiary care hospital in western India over a period of one year from January to December 2005 and to observe the clinical outcome in these patients after treatment.

Material & Methods

Two hundred and forty isolates of *P.aeruginosa* were obtained during a one year period from January to December 2005 in the Department of Microbiology, S.L. Raheja Hospital, Mumbai, Maharashtra. The specimens processed were: respiratory secretions (61), tissue (58), swabs pus/ wound (55), urine (52), blood culture (10) and bile (4). With Universal safety precautions, samples were collected from patients in ICU, transported and processed in the laboratory

without delay. Blood cultures were processed using automated method with Versa Trek (Tri Vitron, India). Samples were cultured on brain heart infusion (BHI) blood agar and MacConkey's agar. Identification of organisms was done by the standard laboratory technique⁹. Antimicrobial sensitivity testing was performed on Mueller - Hinton (MH) agar plates with commercially available discs (Hi-media, Mumbai) by Kirby Bauer disc diffusion method and interpreted as per CLSI recommendations¹⁰. *P. aeruginosa* ATCC 27853 (β -lactamase negative) strain was used as control.

The routine antibiotic sensitivity tests were put up for aminoglycosides [amikacin (30 μ g), gentamicin (10 μ g), netilmicin (30 μ g), tobramycin (10 μ g)], cephalosporin's [cefoperazone (75 μ g), cefepime (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), ceftizoxime (30 μ g)], fluoroquinolones [ciprofloxacin (5 μ g), gatifloxacin (5 μ g), lomefloxacin (10 μ g)], carbapenems [imipenem (10 μ g), meropenem (10 μ g)], chloramphenicol (30 μ g) and piperacillin/tazobactam (100/10 μ g).

MBL producing *P. aeruginosa* was suspected when the isolate was resistant to meropenem and imipenem.

Various methods have been recommended for screening MBL. These include the modified Hodge test, double disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA impregnated imipenem discs¹¹ and EDTA impregnated meropenem discs⁷. For MIC detection of imipenem, the E -test strip⁷ and microdilution (microtitre) plate method¹² is recommended.

We used disc potentiation test with EDTA impregnated imipenem and meropenem discs. However, MIC was not detected in this study as E-test strips were very expensive and a simple microdilution plate method is time consuming.

Disc potentiation test: A 0.5 M EDTA solution (pH 8.0) was prepared and was sterilized by autoclaving. Test organisms were inoculated onto plates of MH agar (Opacity adjusted to 0.5 McFarland opacity standards). Two 10 μ g imipenem discs and two 10 μ g meropenem discs were placed on inoculated plates and 5 μ l of EDTA solution was added to one imipenem and one meropenem disc. The zone of inhibition around imipenem and meropenem discs alone and those with EDTA was recorded and compared after 16-18 h incubation at 35°C. An increase in zone size of at least 7 mm around the imipenem-EDTA disc and meropenem-

EDTA discs was recorded as a positive result. The difference in the resistance pattern of MBL-positive and MBL-negative was considered to be statistically significant if the *P* value was <0.05.

Results

Of the 240 isolates of *P. aeruginosa*, 60 (25%) were found resistant to carbapenems (both imipenem and meropenem) and 50 (20.8%) were found to be MBL producers confirmed by disc potentiation method. The ATCC 27853 *P. aeruginosa* did not exhibit any zone size enhancement with EDTA impregnated imipenem discs.

Of the 50 MBL producing isolates, 30 (60%) were from diabetes patients and 20 (40%) from cancer patients [acute lymphoblastic leukaemia (5), chronic myeloid leukaemia (4), carcinoma of gallbladder (3), carcinoma of oesophagus (3), prostatic adenocarcinoma (1), carcinoma of sigmoid colon (1), non-Hodgkin's lymphoma (1), mesothelioma (1) and Hodgkin's disease (1)]. Of the 50 patients, 38 (76%) were males and 12 (24%) were females; the average age being 63 yr (50-75 yr).

Patients were treated with gatifloxacin, piperacillin/tazobactam, and combination of gatifloxacin and piperacillin/tazobactam. Antibiotic sensitivity pattern

of MBL positive and negative isolates is shown Table I. Antibiotic sensitivity pattern of MBL positive and negative isolates among cancer and diabetes patients is presented in Table II.

Table I. Antibiotic sensitivity (%) of MBL positive (n=50) and MBL negative (n=10) isolates

Antibiotics (µg)	MBL positive	MBL negative
Amikacin* (30)	0	10
Gentamicin (10)	0	0
Netilmicin* (30)	0	20
Tobramycin (10)	0	0
Cefoperazone (75)	0	0
Cefepime* (30)	0	30
Cetazidime* (30)	4	40
Ceftriaxone* (30)	0	20
Ceftizoxime (30)	0	0
Ciprofloxacin* (5)	2	40
Gatifloxacin (5)	36	30
Lomefloxacin* (10)	2	10
Chloramphenicol (30)	0	0
Pipracillin/tazobactam (100/10)	42	70
Imipenem (10)	0	0
Meropenem (10)	0	0

**P*<0.05

Table II. Antibiotic sensitivity (%) of MBL positive and MBL negative isolates in cancer and diabetes patients

Antibiotics (µg)	Cancer patients (22)		Diabetes patients (38)	
	MBL positive (n=20)	MBL negative (n=2)	MBL positive (n= 30)	MBL negative (n= 8)
Amikacin* (30)	0	50	0	0
Gentamicin (10)	0	0	0	0
Netilmicin* (30)	0	100	0	0
Tobramycin (10)	0	0	0	0
Cefoperazone (75)	0	0	0	0
Cefepime* (30)	0	50	0	37
Cetazidime* (30)	0	100	6.6	37
Ceftriaxone* (30)	0	50	0	12.5
Ceftizoxime (30)	0	0	0	0
Ciprofloxacin* (5)	5	100	0	12.5
Gatifloxacin (5)	45	50	23.3	37
Lomefloxacin* (10)	0	50	0	0
Chloramphenicol (30)	0	0	0	0
Pipracillin/tazobactam (100/10)	35	100	46.6	37.5
Imipenem (10)	0	0	0	0
Meropenem (10)	0	0	0	0

**P*<0.05

Statistically significant difference was found in the resistance pattern of MBL positive and negative isolates for amikacin, netilmicin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin and lomefloxacin ($P < 0.05$).

The average hospital stay of patients with MBL producers was 32 days (range 4 days-2 months). Of the 50 patients, 10 had *P. aeruginosa* as the sole isolate from blood culture. All these patients died due to *Pseudomonas* septicaemia. Thus, the mortality was 20 per cent. Among the 40 patients with MBL producing *P. aeruginosa* who survived, 10 needed re-admissions to the hospital because of deterioration in their clinical condition due to progressive disease. All these patients were cancer patients.

Discussion

P. aeruginosa is a pathogen associated with numerous nosocomial infections in immunocompromised patients¹³. Carbapenems are the drugs of choice for multidrug resistant *P. aeruginosa* and ESBL producing organisms. However, resistance to carbapenems due to reduced uptake of drug leads to imipenem/meropenem resistant isolates¹⁴.

In various studies across the world, varying resistance (4-60%) has been seen towards imipenem and meropenem^{15,16}. We found 25 per cent resistance to imipenem and meropenem. *P. aeruginosa* producing MBL was first reported from Japan in 1991¹⁷. In 2002 from India, Navneeth *et al*¹⁸ first reported MBL production in *P. aeruginosa* to be 12 per cent. Since then, the incidence of MBL production in *P. aeruginosa* has been reported to be 10-30 per cent from various clinical specimens across the country⁸. We found 20.8 per cent MBL production in *P. aeruginosa* of which 30 per cent were obtained from respiratory specimens in our study. Another study conducted by Shashikala *et al*¹⁴ reported 20.7 per cent carbapenem resistant *P. aeruginosa* isolates from endotracheal aspirates showing indwelling devices as major risk factors for the development of resistance.

Amongst the MBL positive isolates from diabetes and cancer patients admitted to ICU in this study, maximum sensitivity was observed for piperacillin/tazobactam followed by gatifloxacin. Amongst the MBL negative isolates maximum sensitivity was observed for piperacillin/tazobactam followed by ciprofloxacin, ceftazidime, gatifloxacin, cefepime, netilmicin, ceftriaxone, amikacin, and lomefloxacin. In the study conducted by Taneja *et al*⁸, piperacillin and amikacin had

the best *in vitro* susceptibility. In our study, 18 patients responded to gatifloxacin, 24 to piperacillin/tazobactam while 7 patients responded to combination of gatifloxacin and piperacillin/tazobactam. Amongst the 20 cancer patients in whom MBL producers were isolated, 9 showed sensitivity to gatifloxacin and 7 showed sensitivity to piperacillin/tazobactam. Amongst the 30 diabetes patients in whom MBL producers were isolated, 7 showed sensitivity to gatifloxacin and 14 showed sensitivity to piperacillin/tazobactam.

P. aeruginosa are responsible for 3-7 per cent bloodstream infections and high mortality rates (27-48%) in critically ill patients¹⁸. We observed 20 per cent mortality due to *P. aeruginosa* septicaemia in our patients.

In conclusion, our findings showed that there is a need to do surveillance to detect MBL producers, judiciously use carbapenems to prevent their spread and use effective antibiotics, such as gatifloxacin and piperacillin-tazobactam, after sensitivity testing for treatment.

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Reprint requests: Dr Ami Varaiya, Consulting Microbiologist, Department of Laboratory Medicine
S.L. Raheja Hospital, Mahim West, Mumbai 400 016, India
e-mail: amivaraiya@yahoo.com