Original Article

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Microbiology Section

Metallo-Betalactamase Producing Clinical Isolates of *Pseudomonas aeruginosa* from Intensive Care Unit Patients of a Tertiary Care Hospital

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is an important nosocomial pathogen in the health care settings. Increasing resistance to carbapenems mediated by metallo-betalactamase (MBL) and other mechanisms is a cause for concern because they adversely affect clinical outcomes and add to treatment costs. This study was undertaken to determine the prevalence of MBL production in carbapenem-resistant isolates and to study the factors influencing the clinical outcomes of infections.

Materials and Methods: One hundred tweleve consecutive *P. aeruginosa* isolates obtained from hospitalised patients were subjected to susceptibility testing to anti-pseudomonal drugs by disc diffusion, and minimum inhibitory concentration (MIC) of imipenem was determined. The production of MBL was detected by 4-fold reduction in MIC with imipenem-ethylene diamine tetraacetic acid (EDTA) and the zone size enhancement with EDTA impregnated imipenem and ceftazidime discs.

Results: By disc diffusion method, 31.2% of the *P. aeruginosa* isolates tested were found resistant to imipenem. 15 resistant isolates, showed a significant enhancement in zone size with the EDTA impregnated discs as well as a 4-fold reduction in MIC with imipenem EDTA. Thus 42.8% isolates of imipenem resistant *Pseudomonas aeruginosa* were found to be MBL producers. Overall prevalence of MBL production was 13.4% among all the *Pseudomonas aeruginosa* isolates.

Conclusion: MBL-mediated imipenem resistance in *P. aeruginosa* is a cause for concern in the therapy of critically ill patients. Intensity of selection pressure for usage of broad spectrum antibiotics is high in ICUs. Therefore, a strict antibiotic policy should be followed in intensive care areas to prevent further spread of MBLs. Detection of MBLs by Disk potentiation test should be routinely performed in all microbiology laboratories for all imipenem-resistant isolates, which will help to reduce morbidity and mortality in these patients.

Keywords: Carbapenems, Metallo-betalactamase, *Pseudomonas aeruginosa*

INTRODUCTION

Carbapenem resistance has been observed frequently in non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacer spp*. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin-binding proteins and carbapenem hydrolyzing enzymes-carbapenemase. These carbapenemase are class B metallo-β-lactamases (MBLs; IMP, VIM) or class D oxacillinases (OXA 23 to OXA 27) or class A clavulanic acid inhibitory enzymes (SME, NMC, IMI, KPC) [1]. Metallo-β-lactamase belongs to class B, which requires divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolysing activity not only against carbapenem but also against other β-lactam antibiotics [2]. The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. Thus, MBL-producing *P. aeruginosa* strains have been reported to be important causes of nosocomial infections associated with clonal spread [1].

P. aeruginosa is often difficult to eradicate due to its resistant drug profile. Therefore, detection of MBL-producing Gram-negative bacilli especially *P. aeruginosa* is crucial for the optimal treatment of patients particularly in critically ill and hospitalised patients, and to control the spread of resistance [3].

Hence, the present study was conducted to detect the MBL in *P. aeruginosa* isolates obtained from hospitalised critically ill patients.

MATERIALS AND METHODS

Hundred and tweleve consecutive non-repetitive isolates of *P. aeruginosa* from various specimens of the adult patients admitted to the intensive care unit were studied for MBL production. All the clinical details were noted.

Risk factors	Imipenem Resistant Isolates (35)	
	MBL + (15)	MBL – (20)
Previous antibiotic use	15	20
Hospital stay > 8 days	15	20
Catheterization	15	19
Mechanical ventilation	12	17
Endotracheal intubation	12	16
Intravenous line	15	20

[Table/Fig-1]: Risk factors present in imipenem resistant isolates

The susceptibility to antipseudomonal antibiotics was performed on Mueller Hinton agar by disc diffusion method in accordance with Clinical Laboratory Standards Institutes (CLSI) guidelines [4]. *P. aeruginosa* ATCC 27853 was used as a control strain.

The antibiotics tested were gentamicin, amikacin, piperacillin, ciprofloxacin, ceftazidime, piperacillin-tazobactam, aztreonam and imipenem.

MIC of imipenem for these isolates was done by agar dilution method in accordance with (CLSI) guidelines [5].

MBL detection was done by Zone enhancement with EDTA impregnated imipenem and ceftazidime discs [6] and minimum of four-fold reduction in MIC of the isolates with imipenem-EDTA combination [7].

1) Zone enhancement with EDTA impregnated imipenem and ceftazidime discs:

Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI [4]. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. $2H_2O$ in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two 10 µg imipenem discs and two 30 µg ceftazidime discs were placed on the surface of an agar plate and 10 µl of 0.5 M EDTA solution containing 750 µg was added to one of them to obtain a desired concentration of 750 µg per disc. The inhibition zones of imipenem, ceftazidime and imipenem-EDTA and ceftazidime- EDTA discs were compared after 16-18 h of incubation in air at 35°C.

2) MIC of imipenem-EDTA combination by agar dilution method [4,7]:

EDTA (1ml) solution was added to 1ml of the imipenem solution spanning similar concentrations as done for MIC to imipenem alone. Each 2ml of EDTA and imipenem in graded concentrations was added to18ml of molten Mueller Hinton agar and poured on plates that were allowed to set. A fixed inoculum of the test strains was spot inoculated on these plates before incubation. The reading was taken after18-24 h of incubation. The highest dilution inhibiting the growth of the organisms was taken as the MIC.

RESULTS

Out of 112 strains of *Pseudomonas aeruginosa* isolated from patients from intensive care unit, 35 (31.2%) were found resistant to imipenem by the disc diffusion method. These isolates also exhibited high MIC values ranging from 8-128 µg/ml on agar dilution.

15 out of 35 imipenem resistant isolates showed a significant enhancement in zone size with the EDTA impregnated discs. Eight isolates had zone size enhancement with both, imipenem EDTA and ceftazidime EDTA discs. Four isolates had zone size enhancement with only imipenem-EDTA disc and three isolates had zone size enhancement with only ceftazidime-EDTA disc. All the 15 isolates exhibited 8-128 fold reduction in MIC with imipenem-EDTA combination. Thus 42.8% (15 out of 35) isolates of imipenem resistant *Pseudomonas aeruginosa* were found to be MBL producers. Overall prevalence of MBL production was 13.4% (15 out of 112) among all the *Pseudomonas aeruginosa* isolates.

All the MBL positive isolates were resistant to all the antibiotics tested except aztreonam. Out of 15 MBL producers, 14 isolates (93.3%) were sensitive to aztreonam and only one isolate was found resistant to aztreonam.

Various risk factors were studied in imipenem resistant isolates. These are tabulated in [Table/Fig-1].

DISCUSSION

The first plasmid-mediated MBL was reported in *Pseudomonas aeruginosa* in Japan in 1991. Since then, many such MBL producing strains were reported from various countries including India [1,6-8]

The emergence of these MBLs in gram-negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins. Moreover, the treatment alternatives are unavailable or expensive or toxic with poor outcome [8].

Therefore, rapid detection of MBL production is necessary to modify therapy & to initiate effective infection control to prevent their dissemination.

Various methods have been recommended for detecting MBL. These include the modified Hodge test [9], double disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs [10]. EDTA impregnated imipenem discs [6], and the MIC reduction of minimum four-fold with imipenem EDTA combination [7]

Though, there are several screening methods, no single test when used alone is specific for these enzymes.

In the present study, the use of EDTA impregnated imipenem and ceftazidime discs resulted in a significant increase in the zone size for the MBL producers when compared to the non producers. Similar observations have been made with use of EDTA by other workers [6].

MIC detection is the gold standard to detect MBL production. In the present study, MIC reduction of 8-128 folds with imipenem-EDTA combination was observed as compared to a 4-512 fold reduction reported by Migliavacca et al., [7]. There was 100% concurrence between results of disc potentiation test & MIC for MBL production.

Thus the disc potentiation test is simple, reliable, less cumbersome and cheap.

In India, the prevalence of MBL production varies from 12% to 27% among clinical isolates of *P.aeruginosa* [1,11-13].Among carbapenem resistant isolates of *P.aeruginosa*, the prevalence of MBL production varies from 26.9% to 87.5% [12,14,15]. In the present study, overall prevalence of MBL producing strains was found to be 14.2% among the clinical strains of *P.aeruginosa* and 40.8% prevalence was recorded among the imipenem resistant strains of *P.aeruginosa*.

Possible risk factors for acquisition of MBLs are documented as prolonged hospitalisation, prior antimicrobial therapy; treatment in ICU and haematology [16]. In the present study, multiple risk factors like hospital stay > 8d, catheterization, IV line and previous antibiotic use were present in all MBL-positive isolates. [Table/Fig-1]. All these were also the major risk factors for imipenem resistance.

CONCLUSION

Emergence of MBL producing *P.aeruginosa* in ICUs is alarming and reflects excessive use of carbapenems. There is urgent requirement of strict statuary guidelines implanting intervention for limiting inappropriate uses of antibiotics. Ignorance of rational antibiotics prescribing principles, lack of awareness of the problem of the alarming rise in the multi resistance & pharmaceutical promotion are possible combining factors leading to unnecessary antimicrobial usage. Inadequate infection control is further compounding the problem.

The early detection of MBL producing *P. aeruginosa* will help in appropriate antimicrobial therapy and avoid the development & dissemination of these multidrug resistance strains. All isolates of *P. aeruginosa* resistant to imipenem should be screened for MBL production. Disk potentiation test should be introduced in every clinical microbiology laboratory in order to aid infection control.

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