Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients

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Received September 15, 2004

**Background & objectives:** Metallo betalactamase (MBL)-mediated resistance to carbapenems is an emerging threat in hospital isolates of *Pseudomonas aeruginosa*. Though there are several screening methods to detect this enzyme production, the National Committee for Clinical Laboratory Standards (NCCLS) does not have performance standards documented so far. There is not enough information from the Indian subcontinent regarding the prevalence and the screening methods for these enzymes. The present study was undertaken to detect MBL in nosocomial isolates of *P. aeruginosa* by two screening methods.

**Methods:** Fifty consecutive *P. aeruginosa* isolates obtained from hospitalized patients were subjected to susceptibility testing to antipseudomonal 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:** Sixteen per cent of the isolates tested were resistant to imipenem by disc diffusion method of which 87.5 per cent exhibited a zone size enhancement with EDTA impregnated imipenem and ceftazidime discs as well as a 4-fold reduction in MIC with imipenem EDTA. The imipenem susceptible isolates (84%) had normal MIC values and exhibited no zone diameter enhancement with EDTA impregnated antibiotic discs.

**Interpretation & conclusion:** MBL-mediated imipenem resistance in *P. aeruginosa* is a cause for concern in the therapy of critically ill patients. The two confirmatory methods i.e., zone diameter enhancement with EDTA impregnated imipenem and ceftazidime discs and 4-fold reduction in MIC with imipenem EDTA combination are equally effective for their detection.

**Key words** Carapenems - imipenem - metallo betalactamase - *Pseudomonas aeruginosa*

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by β-lactam-resistant bacteria. However, carbapenem resistance has been observed frequently in nonfermenting bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. The common form of resistance is mediated by lack of drug penetration (i.e., porin mutations and
efflux pumps) and/or carbapenem-hydrolyzing β-lactamases. Based on molecular studies, carbapenem-hydrolyzing enzymes are classified into four groups A, B, C and D. The metallo betalactamases (MBLs) belong to group B and are enzymes requiring divalent cations as cofactors for enzyme activity, being inhibited by the action of a metal ion chelator. The MBLs efficiently hydrolyze all β-lactams, except aztreonam in vitro.

P. aeruginosa is a common Gram-negative bacillus associated with hospital infections and 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 hospitalized patients, and to control the spread of resistance. There is not much information available on MBL producing P. aeruginosa isolates from India. We therefore undertook this study to detect the MBL in P. aeruginosa isolates obtained from hospitalized critically ill patients. Two different screening methods were used to find out their effectiveness in the detection of these isolates.

**Material & Methods**

Fifty consecutive non-repetitive isolates of P. aeruginosa from various specimens like urine (10), blood (2), respiratory specimen-BAL-broncho alveolar lavage (18) and exudates (20) were collected over a period of 1 month (June-July 2003). All the isolates were from critically ill patients from the intensive care unit of a tertiary care centre in south India.

The susceptibility to antipseudomonal drugs was done on Mueller Hinton agar by disc diffusion method in accordance with National Committee for Clinical Laboratory Standards (NCCLS) standards incorporating standard strain of P. aeruginosa (ATCC 27853). The antibiotics tested were gentamycin, amikacin, piperacillin, ciprofloxacin, ceftazidime, piperacillin-tazobactam, aztreonam (Hi-media Laboratories, Mumbai) and imipenem (BD Diagnostics, USA) EDTA, extra pure (Hi-media Laboratories, Mumbai) powder was used for screening MBL production.

MIC of imipenem for these isolates was done by agar dilution method in accordance with NCCLS standards. The pure form of the drug was obtained from Ranbaxy Laboratories, Mumbai.

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 discs, and the MIC reduction of minimum four-fold with imipenem EDTA combination. We used zone enhancement with EDTA impregnated imipenem and ceftazidime discs, and minimum of four-fold reduction in MIC of the isolates with imipenem-EDTA combination in this study.

(i) **Zone enhancement with EDTA impregnated imipenem and ceftazidime discs:** Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the NCCLS. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.2H₂O 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 EDTA solution was added to one of them to obtain a desired concentration of 750 µg. 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.

(ii) **MIC of imipenem EDTA combination:** A previous study reports the use of simple microdilution method for the determination of the MIC with a combination of imipenem and EDTA. However, we adopted the agar dilution method to determine the same.

EDTA (1 ml) solution was added to 1 ml of the imipenem solution spanning similar concentrations as done for MIC to imipenem alone. Each 2 ml of EDTA and imipenem in graded concentrations was added to 18 ml 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.
Results & Discussion

Of the 50 isolates of *P. aeruginosa*, 8 (16%) showed resistance to imipenem by the disc diffusion method. These isolates also had high MIC values to imipenem ranging from 8-128 µg/ml. Seven of these 8 isolates exhibited a significant zone size enhancement with the EDTA impregnated discs when compared with the plain antibiotic discs. Four isolates had zone size enhancement with both ceftazidime EDTA (caEDTA) and imipenem EDTA (imEDTA) discs. The zone size enhancement was 5-28 mm for caEDTA and 7-27 mm for imEDTA. Two isolates exhibited a zone size increase only with caEDTA disc and one only with imEDTA disc. A 8-128 fold reduction of MIC with imipenem EDTA combination was observed in all the 7 isolates which had a significant zone diameter increase with the EDTA impregnated antibiotic discs. The ATCC 27853 *P. aeruginosa* neither exhibited a zone size enhancement nor a fall in MIC.

The remaining 42 isolates were susceptible to imipenem with their MIC levels within the acceptable range and showed a zone size increase of only 0-5 mm with the EDTA impregnated antibiotic discs. Twenty three (46%) of the 50 isolates were resistant to the third generation cephalosporins tested.

All the 8 isolates which exhibited high imipenem MIC values were also resistant to fluoroquinolones and aminoglycosides.

There are reports on MBL production in *P. aeruginosa* from various countries like Brazil, Korea, Singapore, and France. Metallo-beta-lactamase was first reported as a zinc dependent enzyme in *Bacillus cereus* in mid 1960s. A few decades later, imipenem-hydrolyzing metallo enzymes were found in *Aeromonas hydrophila* and *Bacteroides fragilis*. All these enzymes were produced by chromosomal genes and at first were recovered only from single clinical isolates. In 1991, Japan reported the first plasmid-mediated metallo beta-lactamase in *P. aeruginosa*. This was soon followed by another report of transferable metalloenzyme in *B. fragilis*. Apart from *P. aeruginosa*, other bacteria like *Serratia, Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, E. cloacae, Citrobacter freundii, Proteus vulgaris, P. putida, Acinetobacter and Alcaligenes xylosoxidans* were also shown to produce MBL.

There are frequent reports of MBL production in *P. aeruginosa* from the Asian and the Pacific countries, namely Hong Kong, Taiwan, and Japan. In this study, we observed 16 per cent resistance to carbapenem among the *P. aeruginosa* screened and 87.5 per cent of this was MBL mediated as per the screening results. Another study from south India reported 12 per cent MBL-mediated imipenem resistance in *P. aeruginosa*.

Though there are several screening methods recommended for the detection of MBL production, no single test when used alone is specific for these enzymes. The modified Hodge test which is used to screen the carbapenemase production in Gram-negative bacilli can also produce false negative results. 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 in this study. Similar observations have been made with use of EDTA by other workers. However, we found that the ceftazidime EDTA disc could pick up additional isolates of MBL producer as compared to the imipenem EDTA disc.

A MIC reduction of 8-128 fold with imipenem EDTA combination was observed in our study as compared to a 4-512-fold reduction reported by Migliavacca *et al*. One isolate which showed a high MIC to imipenem did not exhibit zone size potentiation with the EDTA impregnated antibiotics discs suggesting other mechanisms of resistance such as permeability of the outer membrane and/or active efflux probably associated with overproduction of endogenous class C beta-lactamase.

MBL production is a significant problem in hospital isolates of *P. aeruginosa*. With increasing isolation of ESBL producing isolates in the hospital setting necessitating the use of carbapenems, the problem of MBL production is also increasing. The
development of simple screening tests, designed to detect acquired MBL production will be a crucial step towards large scale monitoring of these emerging resistant determinants. The two methods used for screening of MBL production namely, the use of EDTA impregnated antibiotic discs and the MIC to imipenem EDTA combination both proved equally effective for the detection of MBL-producing isolates.

References


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