Detection of metallo-β-lactamase–producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran

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**Abstract**

The antimicrobial activities of 6 common antimicrobial agents including carbapenems were tested against 100 clinical *Pseudomonas aeruginosa* isolates using a disk diffusion method. All imipenem (IPM)-resistant isolates were screened for metallo-β-lactamase (MBL) production by Etest assay and were subsequently subjected to polymerase chain reaction (PCR) analysis with the *bla*IMP and *bla*VIM genes. Of 41 IPM-nonsusceptible isolates detected, 8 (19.51%) appeared to produce MBL, as determined by Etest. Using PCR assay, these isolates were positive for *bla*VIM genes, whereas none were positive for *bla*IMP genes.

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The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by β-lactam–resistant bacteria (Kahan et al., 1983). Because of their broad spectrum of activity and stability to hydrolysis by most β-lactamases, the carbapenems have been the drugs of choice for treatment of infections caused by penicillin- or cephalosporin-resistant Gram-negative bacteria (Bradley et al., 1999). Carbapenems, including meropenem (MEM) and imipenem (IPM), are recognized as among the most potent agents with activity against *Pseudomonas aeruginosa*, which exhibits resistance to a variety of antimicrobials including β-lactams.

The common forms of resistance to carbapenems in *P. aeruginosa* are low degree of outer membrane permeability due to loss of porin D2 (Pai et al., 2001), reduced levels of drug accumulation due to active efflux mediated by resistance nodulation cell division (RND) type efflux system (Walsh et al., 2002), and, occasionally, production of carbapenem-hydrolyzing β-lactamas, which can be either chromosomally encoded or plasmid mediated (Lagatolla et al., 2006).

Based on molecular studies, 2 classes of carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site and metallo-β-lactamases (MBLs), requiring divalent cations as cofactors for enzyme activity (Walsh et al., 2005). MBLs belong to class B of Ambler (1980) or to group 3 of Bush (1998). Five distinct types of MBLs are known in *P. aeruginosa*: IMP, VIM, SPM, GIM (Castanheira et al., 2004), and SIM (Lee et al., 2005). The 3 main clusters of the VIM MBL among 11 variants of this type have been identified so far, represented by VIM-1, VIM-2, and VIM-7 (Henrichfreise et al., 2005).

The genes of both IMP- and VIM-type MBLs (*bla*IMP and *bla*VIM, respectively) are often encoded on mobile gene cassettes inserted into class I integrons (Yatsuyanagi et al., 2004). Because of frequent infections caused by *P. aeruginosa* in our burn unit, the present study was undertaken to detect the MBL-producing clinical isolates phenotypically and to determine the IMP- and VIM-type MBL prevalence in these isolates.

A total of 100 clinical isolates of *P. aeruginosa* collected at the Central Laboratory, Taleghani Hospital, Ahwaz, Iran, between October 2005 and July 2006 were investigated. Each isolate was obtained from a different patient. The patients were 71 females and 29 males, and their age ranged from 43 to 68 years with a mean of 47.6 years. The isolates...
were reidentified and confirmed by using standard culture and biochemical tests (Forbes et al., 2002).

The antimicrobial susceptibility testing was performed using an agar disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (document M100-S12, CLSI, 2002). Mueller–Hinton agar and Mueller–Hinton broth (High media, India) were used for susceptibility testing. The antimicrobial agents used in this study were as follows: ceftazidime (CAZ, 30 μg), ticarcillin (TC, 75 μg), piperacillin (PRL, 100 μg), cefepime (FEP, 30 μg), IPM (10 μg), and MEM (10 μg). All the antibiotics were purchased from Mast, UK. *P. aeruginosa* standard strain (ATCC 27853) was used as a reference strain for quality control in susceptibility testing. Phenotypic screening activity for MBL production was carried out by Etest MBL assay (AB Biodisk, Solna, Sweden), under the conditions recommended by the test manufacturer. *P. aeruginosa* VR-14397 was used as positive control for MBL detection.

DNA template preparation was performed using a simple boiling method. A few colonies were removed from an overnight culture and suspended in 500 μL of sterile distilled water and boiled for 10 min. After centrifugation at 12,000 × g for 3 min, the supernatant was used as a source of template for amplification.

Polymerase chain reaction (PCR) amplification for the detection of *bla*IMP and *bla*VIM β-lactamase genes was carried out on a thermal cycler (BioRad, Italy) as described previously (Pitout et al., 2005), using the IMP and VIM type primers of MBLs as follows: IMP types were 5′-GAAGGCGTATGATGC-3′, which amplify a 587-base pair (bp) amplicon, and the primers for VIM types were 5′-GTTTGGTCGATCAGCAAC-3′ and 5′-AATGCCAG-CACCAGGATTAG-3′, which amplify a 382-bp amplicon.

Of the 100 clinical isolates, 75% were isolated from wound discharge, 14% from wound biopsy, 10% from blood, and the remaining 1% from urine. Forty-one of the total isolates showed resistance to IPM, in which the majority of these IPM-nonsusceptible *P. aeruginosa* strains was isolated from wounds (92.7%). Similarly, the isolates producing MBLs were more prevalent in wound samples (87.5%).

Based on the results from susceptibility testing, 38% of the isolates were multidrug resistant and showed resistance to all antimicrobial agents tested. Data analysis indicated that carbapenems (IPM and MEM) showed the higher antibacterial activity against *P. aeruginosa*. Fig. 1 represents the resistance pattern of *P. aeruginosa* to the antimicrobial agents used in the present study. Of 41 IPM-nonsusceptible isolates detected, 8 (19.51%) appeared to produce MBL, as determined by the screening test using Etest MBL strips. All the MBL-producing isolates were found to be resistant to all antimicrobial agents tested. PCR screening for the presence of *bla*IMP and *bla*VIM genes revealed that 8 (19.51%) were positive for *bla*VIM genes. These isolates were the same, which were detected by MBL Etest as MBL producers, whereas none of the isolates was positive for *bla*IMP genes. The sensitivity and specificity of PCR in detecting *bla*VIM MBL genes were determined as 100% in the present study.

MBL-producing *P. aeruginosa* is an emerging threat and a cause of concern for the physicians treating such infections.

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**Fig. 1.** Resistance pattern of clinical isolates of *P. aeruginosa* to different antimicrobial agents tested in the present study.
MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years (Pitout et al., 2005; Navaneeth et al., 2002; Toraman et al., 2004). The isolates in our work showed high resistance to β-lactam and moderate resistance to carbapenem antibiotics. The 8 detected MBL producers were among the isolates with high resistance to all antimicrobial agents we tested. In a recent study from Iran, the resistance patterns for CAZ, MEM, IPM, and FEP were reported as 84.3%, 31.2%, 32.9%, and 97.1%, respectively, from which they found approximately similar resistance in contrast to ours, except for PRL and TC in which their resistance rate was higher, probably because of broader application of these antibiotic in their burn unit. None of their isolates were MBL producer (Japoni et al., 2006). Similar values was reported previously from Turkey with no MBL producer (Gencer et al., 2002). According to reports from around the world, the rates of MBL producers among P. aeruginosa isolates were 0 of 106 (Sugino et al., 2001), 6 of 50 (Navaneeth et al., 2002), and 15 of 52 (Toraman et al., 2004).

The PCR analysis of the 8 MBL-producing P. aeruginosa isolates, using primers suitable for detection of blaIMP and blaVIM genes, which yielded from each of these a 382-bp product, determined the presence of blaVIM MBL. We could not find any of the MBL producers to be blaIMP positive. In contrast, in similar approach, Panagi et al. (2005) reported that all the 7 MBL-producing P. aeruginosa strains isolated from a nosocomial outbreak were blaIMP positive. In another report, 24 MBL-producing P. aeruginosa isolates were described as blaIMP positive (Senda et al., 1996). According to our findings, it seems that MBL-producing isolates of P. aeruginosa are not the main cause of IPM resistance among this species, and probably, other mechanisms of resistance are involved in resistance to this antibiotic because the majority of nonsusceptible IMP isolates (80.49%) was negative for both genes. Unfortunately, because of some limitations, such as lack of more sophisticated molecular facilities in our department and limited time to refer to other more equipped centers, we were not able to do genotypic comparisons of the isolates and MBL gene sequence as an essential step.

In conclusion, although the carbapenems showed the best activity among the antibiotics tested, resistance rates were high compared with other regions. Furthermore, other drugs with better activity, such as the polymyxins, were not tested because of low application of those in our burn unit because of their side effects. Our study seems to be the first documented description of MBL-producing P. aeruginosa from Iran.

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References


