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Prevalence of bla_{IMP} and bla_{VIM} gene carriage in metallo-β–lactamase-producing burn isolates of *Pseudomonas aeruginosa* in Tehran

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Background/aim: To study the prevalence of bla_{VIM} and bla_{IMP} genes in metallo-β-lactamase (MBL)–producing burn isolates of *Pseudomonas aeruginosa* in relation with AmpC and extended-spectrum β-lactamase (ESBL) production.

Materials and methods: Thirty-two carbapenem-resistant MBL-producing *P. aeruginosa* burn isolates from Shahid Motahari Burn Hospital in Tehran were employed. Antibiotic susceptibility was determined to 13 antibiotics including imipenem and meropenem by disk diffusion. AmpC and ESBL production was detected by the AmpC disk test and combined disk diffusion assay, respectively. bla_{IMP} and bla_{VIM} gene carriage was shown by polymerase chain reaction and type-specific primers.

Results: AmpC production was observed in 81% and ESBL production was detected in 12.5% of the isolates. bla_{IMP} carriage was observed in 56.25% and bla_{VIM} gene in 46.8% of the isolates. Surprisingly, 43.5% of the isolates carried both bla_{IMP} and bla_{VIM} genes.

Conclusion: We think that this is the first report on the cocarriage of bla_{IMP} and bla_{VIM} in *P. aeruginosa*. There was also a strong association between MBL gene carriage and AmpC β-lactamase production.

Key words: *Pseudomonas aeruginosa*, metallo-β-lactamase, MBL, bla_{IMP}, bla_{VIM}, AmpC, ESBL

1. Introduction

Pseudomonas aeruginosa is one of the most important hospital-acquired pathogens in burned patients. Nosocomial isolates of *P. aeruginosa* are often resistant to many classes of antibiotics and treatment of infections is frequently complicated due to the emergence of multidrug-resistant (MDR) strains. Carbapenems have been used to treat difficult gram-negative infections due to their broad spectrum of activity and resistance to hydrolysis by most β-lactamases. However, prevalence of carbapenem-resistant *P. aeruginosa* has increased worldwide (1,2). Carbapenem resistance in *P. aeruginosa* may be mediated by decreased outer membrane permeability, upregulation of multidrug efflux pumps, interplay between impermeability, and production of β-lactamases or carbapenemases such as metallo-β-lactamases (MBLs) (3–5). In addition, production of AmpC and extended-spectrum β-lactamases (ESBLs) can complicate the results of antibiotic therapy in MDR isolates. MBL-producing *P. aeruginosa* was first reported from Japan in 1991 and has since been detected worldwide (6). MBLs hydrolyze all β-lactam antibiotics with the exception of aztreonam, are inhibited by metal chelators such as ethylenediaminetetraacetic acid (EDTA) and thiol compounds, and are resistant to serine β-lactamase inhibitors such as clavulanate and tazobactam. MBL-encoding genes are divided into 6 groups: IMP, VIM, SIM, SPM, GIM, and AIM (2,7,8). These genes are usually integron-mediated and can be carried by transferable plasmids, or may be chromosomal (8). Clinical isolates harboring the bla_{IMP} and bla_{VIM} genes have been increasingly reported, mostly in Europe and Asia (6,9). Considering the rapid rate of dissemination of MBL-producing bacteria, early detection may be critical. We studied the production of AmpC and ESBL in imipenem-resistant MBL-producing burn isolates of *P. aeruginosa*, as well as the prevalence of bla_{VIM} and bla_{IMP} gene carriage in these isolates.

2. Materials and methods

2.1. Bacterial isolates

Thirty-two imipenem-resistant MBL-producing *P. aeruginosa* isolates from burn wounds were chosen from a microbial collection from patients admitted to Shahid Motahari Burn Hospital in Tehran from July to November 2011. The antibiotic susceptibility and MBL production

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of the isolates were determined by disk diffusion and the double disk synergy test (DDS), as previously reported in the literature (10). The organisms were stored at – 20 °C in a brain heart infusion broth (Oxoid, UK) containing 10% dimethyl sulfoxide until use. *P. aeruginosa* ATCC27853 was used as the antibiotic-susceptible strain. *Acinetobacter baumannii* AC54/97 carrying bla_{IMP}, *P. aeruginosa* PO510 harboring bla_{VIM-1}, and *P. aeruginosa* COL-1 encoding bla_{VIM-2} (kindly provided by Dr Shahcherachi, Pasteur Institute, Tehran, Iran) were used as positive controls in polymerase chain reaction (PCR) experiments.

2.2. Antibacterial susceptibility

Antibiotic susceptibility of the isolates was determined by disk diffusion according to the Clinical Laboratory Standard Institute recommendations (CLSI, 2011) (11). The antibiotics (Mast, UK) were: ceftazidime (30 µg), aztreonam (30 µg), carbenicillin (100 µg), piperacillin (100 µg), ticarcillin (75 µg), cotrimoxazole (25 µg), amikacin (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), meropenem (10 µg), imipenem (10 µg), and piperacillin/tazobactam (110 µg).

2.3. ESBL production

All isolates were screened for ESBL production by the phenotypic confirmatory test (12). Disks containing ceftazidime (30 µg) alone and in combination with clavulanic acid (10 µg) were placed on bacterial lawns before incubation at 37 °C overnight. An increase of 5 mm in the inhibition zone around the combination disk was considered as ESBL production.

2.4. AmpC β-lactamase production

AmpC β-lactamase production was detected by the AmpC disk test (13). Briefly, a blank disk moistened with sterile saline was inoculated with a few colonies of the test strain. The disk was then placed next to a cefoxitin disk (30 µg) on the surface of a Mueller Hinton agar plate inoculated with a lawn of *Escherichia coli* ATCC 25922. The plate was incubated overnight at 37 °C. An indentation of the cefoxitin inhibition zone adjacent to the disk containing the test strain indicated AmpC β-lactamase production.

2.5. Detection of bla_{VIM} and bla_{IMP} genes

Bacterial DNA was extracted using a boiling method (14). PCR amplification of bla_{VIM} was carried out using the following primers: 5'-GTTTGGTCGCATATCGCAAC-3' (forward) and 5'-CTACTCGGCGACTGAGCGAT-3' (reverse) (alpha DNA, Canada), with an amplicon size of 645 bp (15). Primers used for bla_{IMP} were: 5'-GAAGGCGTTTATGTTCATAC-3' (forward) and 5'-GTATGTTTCAAGAGTGATGC-3' (reverse) (Bioneer, Korea), with an amplicon size of 587 bp (6). PCR mixtures (25 µL) contained 1 µL of DNA template, 1.5 mM $MgCl_2$, 0.4 mM of each dNTP, 1 pM of each primer, and 1 U of Taq DNA polymerase (CinnaGen, Iran). PCR amplifications were performed in a thermal cycler (Peqlab, Germany) using the following program for bla_{VIM}: initial denaturation at 95 °C for 4 min, followed by 35 cycles of 1 min at 94 °C, 1 min of annealing at 56 °C, and 45 s of extension at 72 °C, with a final extension of 7 min at 72 °C. For bla_{IMP}, amplification was carried out with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 1 min at 95 °C, 1 min of annealing at 54 °C, and 90 s of extension at 72 °C, with a final extension of 10 min at 72 °C. PCR products were run on 1% agarose gels, stained with Red Safe dye (Intron Bio, Korea), and visualized using an image analysis system (UVItec, St John's Innovation Centre, UK).

Duplex PCR for amplification of bla_{IMP} and bla_{VIM} genes was carried out using the same primers as in the simple PCR. Reaction mixtures (25 µL) contained 5 µL of DNA template, 1.5 mM MgCl, 0.4 mM of each dNTP, 5 pM of each primer, and 3 U of Taq polymerase. PCR amplifications were performed using the following program: 5 min at 95 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, with a final extension of 10 min at 72 °C. PCR products were analyzed by electrophoresis in 2% agarose gels, stained, and visualized as before.

3. Results

All 32 MBL-producing test bacteria were resistant to all antibiotics except for the following: 1 isolate was sensitive to aztreonam and amikacin, 1 to ceftazidime, 5 to piperacillin, and 10 to piperacillin/tazobactam. AmpC production was shown in 26 (81%) and ESBL production was observed in 4 (12.5%) isolates. All ESBL producers were also positive for AmpC production.

The Figure shows the PCR amplification products of bla_{IMP} and bla_{VIM} genes in MBL-producing *P. aeruginosa* burn isolates. Twenty-three isolates (71.9%) carried 1 or both MBL genes, of which 19 (82.6%) were also AmpC-positive. bla_{IMP} was observed in 18 (56.2%) and bla_{VIM} in 15 isolates (46.8%), 10 of which carried both genes (Table). ESBL production was only observed in bla_{IMP}-positive isolates, 1 of which also harbored bla_{VIM}. There was a strong association between MBL gene presence and AmpC production (Table). No relation was observed between

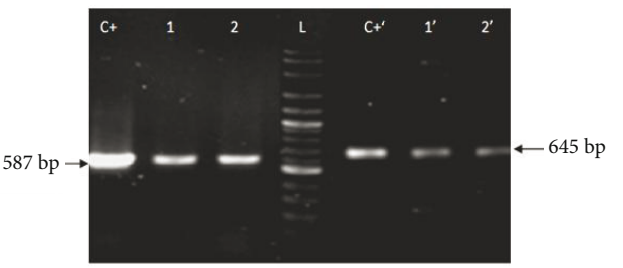

Figure. Amplification of bla_{IMP} and bla_{VIM} genes in MBL-producing *P. aeruginosa* burn isolates by single PCR. C+, bla_{IMP} control; C+′, bla_{VIM} control; lanes 1 and 2, bla_{IMP}; lanes 1′ and 2′, bla_{VIM}-harboring isolates; L, 100-bp DNA ladder.

Table. MBL gene carriage and AmpC and ESBL production in 32 MBL phenotype-positive clinical isolates of *P. aeruginosa*.

MBL gene type	No. (%) bacteria		
	MBL gene+	AmpC+	ESBL+
bla_{IMP}	18 (56.2)	13 (72.2)	0 (0.00)
bla_{VIM}	15 (46.8)	13 (86.7)	4 (26.7)
bla_{IMP} + bla_{VIM}	10 (43.5)	9 (90)	4 (26.7)
Total	23 (71.9)	19 (82.6)	4 (26.7)

MBL, metallo-β-lactamase; ESBL, extended-spectrum β-lactamase.

MBL gene carriage and ESBL production %. When duplex PCR was carried out on the 10 (43.5) isolates harboring both genes (as shown in single PCR experiments), only 1 showed 2 distinct bands. This could be due to the closeness of the PCR product sizes and technical problems. Nine of the MBL phenotypic positive isolates did not carry either bla_{IMP} or bla_{VIM}.

4. Discussion

MBL-mediated resistance to carbapenems has limited the use of these drugs for treatment of difficult gram-negative infections, including *P. aeruginosa*. In addition, production of AmpC and ESBLs can complicate the results of antibiotic therapy in MDR isolates. High levels of AmpC production not inhibited by clavulanic acid can be the cause of aztreonam resistance and can also lead to false ESBL results (16).

Among the MBL-encoding genes, bla_{VIM} has been shown to be the most prevalent worldwide, followed by bla_{IMP} (6–8,17). In Iranian reports, bla_{VIM} has also been reported as the most prevalent family of MBLs in *P. aeruginosa* (14,17–21). In fact, only a few local studies have reported bla_{IMP} gene carriage in *P. aeruginosa* and at much lower rates compared to bla_{VIM} (17,21).

We think that this is the first report showing cocarriage of bla_{VIM} and bla_{IMP} genes in *P. aeruginosa*. It is also the first report showing a high rate of bla_{IMP} carriage in *P. aeruginosa* burn isolates in Iran, as observed in 10 (31.2%) of our isolates. Coexistence of multiple MBL genes has been shown in *Enterobacteriaceae* and *Acinetobacter baumannii* (13,23). This phenomenon is quite alarming since MBL genes can be disseminated by mobile genetic elements such as integrons and transposons, mostly located on plasmids. Of the MBL phenotypic-positive isolates, 9 did not carry the MBL genes. Lagatolla et al. showed that bla_{VIM}-negative, carbapenem-resistant *P. aeruginosa* isolates were genotypically related to the bla_{VIM-1}-positive strains (24). It may also be possible that these isolates harbor other MBL genes that were not detected in this study. Another possibility may be the susceptibility of some bacterial strains to EDTA, which can affect bacterial membrane permeability, leading to false positive results for the MBL phenotype in the DDS method (25,26). The strong association found between MBL gene carriage and AmpC production is also alarming and would limit the choice of antibiotic therapy even more. Early detection of these organisms is necessary as appropriate treatment might reduce the spread of resistant strains and the mortality rate in hospitalized patients.

In conclusion, we found that there was a strong association between MBL gene presence and AmpC production. A majority of the isolates carried MBL genes, of which 43.5% harbored both bla_{IMP} and bla_{VIM}. This is the first report on cocarriage of bla_{IMP} *and* bla_{VIM} genes in *P. aeruginosa* burn isolates.

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