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Determination of extended spectrum beta-lactamases, metallo-beta-lactamases and AmpC-beta-lactamases among carbapenem resistant *Pseudomonas aeruginosa* isolated from burn patients

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an important cause of morbidity and mortality in patients with burns.
Method: A total of 214 nonduplicated burn wound isolates of *P. aeruginosa* were recovered from burn patients. Identification of carbapenem resistant isolates and their antimicrobial susceptibility pattern was carried out using the phenotypic methods. The presence of genes encoding extended spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) enzymes were determined by PCR. The genetic relationships between carbapenem resistant isolates were determined by Random Amplified Polymorphic DNA (RAPD)-PCR.
Results: Of 214 investigated *P. aeruginosa* isolates, 100 (46.7%) were carbapenem resistant. All carbapenem resistant *P. aeruginosa* were resistant to imipenem, meropenem, ertapenem, carbenicillin, aztreonam, gentamicin and ciprofloxacin but susceptible to polymyxin B. Among 100 carbapenem resistant *P. aeruginosa* isolates, 3%, 65% and 52% were identified as ESBLs, carbapenemase and AmpC overproduction positive isolates respectively. The most prevalent ESBLs and MBLs genes included $bla_{\text{OXA-10}}$ (97%), bla_{TEM} (61%), bla_{VIM} (55%), bla_{PER} (13%), bla_{IMP} (3%) and bla_{AIM} (1%). RAPD analysis yielded 13 distinct profiles among 92 isolates. A dominant RAPD type was designated as A that consisting of 80 isolates.
Conclusion: This is the first report of Adelaide IMipenmase (AIM) MBLs producing *P. aeruginosa* from Iran and also of the high prevalence of AmpC overproduction isolates. According to the results of current study, *P. aeruginosa* isolates producing OXA-10, TEM, VIM, PER and IMP beta-lactamases are frequent and the population structures of these isolates are highly similar.

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1. Introduction

Patients with thermal injuries are usually at a high risk of nosocomial infection. *Pseudomonas aeruginosa* is known as an important cause of morbidity and mortality in patients with burns [1].

Carbapenems are the most important therapeutic options that effect against serious infections caused by *P. aeruginosa* strains producing extended spectrum beta-lactamases (ESBLs) and chromosomal cephalosporinases (AmpC-beta-lactamases) [2]. However, the emergence of carbapenem resistance among *P. aeruginosa* is an increasing problem in many parts of the world [3,4]. Resistance against carbapenem by *P. aeruginosa* may be occurred through different mechanisms including: loss of the outer membrane porin OprD protein, reduced levels of drug accumulation due to efflux-pumps over-expression and increased production of AmpC beta-lactamases. Also, *P. aeruginosa* may obtain genes encoding carbapenemase enzymes such as metallo-beta-lactamases (MBLs) or *Klebsiella pneumoniae* carbapenemase (KPC) [2,5,6]. The MBL enzymes are able to hydrolyze carbapenems efficiently and thus they are considered the most clinically significant mechanism of carbapenem resistance in *P. aeruginosa* isolates [6–9].

Multi-drug resistant *P. aeruginosa* has emerged as an important pathogen in Iran compared to other countries, which confronted clinicians with serious challenges for treating infected patients. There are several reports on the prevalence of bla_{VIM} and bla_{IMP} among carbapenem resistant *P. aeruginosa* strains isolates from burn patients in Iran but the characteristics of other mechanisms of resistance to carbapenems such as AmpC producer (AmpC overproducer) and KPC are unknown [4,10,11]. This study was designed to determine the existence of different genes encoding beta-lactamase among carbapenem resistant *P. aeruginosa* isolated from burn patients in Tehran, Iran in a period of two years.

2. Materials and methods

2.1. Bacterial strains

A collection of 214 isolates of *P. aeruginosa* were recovered from burn patients who were admitted to Shahid Motahari Hospital of Tehran University of Medical Sciences during 2011 and 2012. All the strains were isolated from burn wounds and only one isolate per patient (according to patient's names and ID) was included in the study. Bacterial identification was performed by standard biochemical tests [12]. Briefly was based on colony morphology, Gram staining, ability to produce oxidase and catalase, no acid production in the slant or butt of Kligler iron agar, oxidative-fermentative (OF) test, pigment production and growth in 42 °C. Isolates identified as carbapenem resistant *P. aeruginosa* were investigated in accordance to the aims of study.

2.2. Antimicrobial susceptibility testing

Carbapenem resistant isolates and their antimicrobial susceptibility patterns were investigated using the disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [13]. The following antibiotics (MAST, UK) were tested: Imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), polymyxin B (300 Unit), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), cefoxitin (30 μg), gentamicin (10 μg), ceftriaxone (30 μg), carbenicillin (30 μg), aztreonam (30 μg) and ciprofloxacin (5 μg). ESBLs producing strains were detected using the combined double-disk test [4,13]. Organisms were also screened for carbapenemase production by the modified Hodge test [13].

The minimum inhibitory concentrations (MICs) of imipenem (IMI), meropenem (MEM) and ceftazidime (CAZ) were determined by the microbroth dilution method. Susceptibility breakpoints were defined according to CLSI recommendations. *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *K. pneumoniae* 700603 were used as controls.

AmpC overproduction was confirmed according to the method of Rodríguez-Martínez et al. [8]. The isolates were considering as AmpC overproduction when there was at least a twofold dilution difference between the MICs of CAZ, IMI and MEM and the MICs of CAZ, IMI and MEM plus cloxacillin.

2.3. DNA extraction and PCR detection of β-lactamases genes

The whole genomic DNA from cultured strains was prepared using genomic DNA Extraction Kit (tip 100; Bioneer, Korea) according to the manufacturer's instructions. The bla_{TEM}, bla_{SHV}, bla_{PER}, bla_{PSE}, $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}10}$, $bla_{OXA\text{-}48}$, bla_{KPC}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{AIM}, bla_{SPM}, bla_{NDM} and bla_{SIM} genes were detected by PCR method using specific oligonucleotide primers listed in Table 1. The PCR mixtures (25 μl) contained 3 μl of DNA, 12.5 μl of PCR master mix (Sinaclon, Iran), and 0.5 μM of each primer; Reaction conditions for all the primers were as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 47 °C to 61 °C (Table 1) for 1 min and extension at 72 °C for 1 min; followed by a final extension step of 72 °C for 5 min. Amplicons were revealed by electrophoresis on a 1.2% agarose gel and a subsequent exposure to UV light in the presence of KBC power load dye (GelRed Nucleic Acid Gel Stain, 10,000× in water, Kawsar Biotech Co., Tehran, Iran).

2.4. RAPD PCR amplification

The primer used for Random Amplified Polymorphic DNA (RAPD) PCR were RAPD-208 (5′-AGCGGGCCAA-3′) and 272 (5′-ACGGCCGACC-3′) [20,21]. The RAPD PCR amplification was carried out in T-100 (BioRad) by using PCR Master Kit (Sinaclon Inc., Tehran, Iran) according to manufacture guideline. PCR conditions were as follows: initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 38.5 °C for 1 min, extension at 72 °C for 2 min. The final extension step was continued for another 10 min at 72 °C. PCR products were then separated by electrophoresis in 1.5% agarose gels with 0.5× TBE buffer. DNA bands were observed by staining with KBC power load dye and photographed under UV illumination. RAPD patterns were analyzed visually and any pattern differing by one or more bands was classified as a distinct RAPD type.