Phenotypic and genotypic characterization of metallo-β-lactamase producing Pseudomonas aeruginosa isolated from burn patients

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen associated with many nosocomial infections. This study aimed to detect blaIMP and blaVIM genes and their common subtypes, including blaIMP1, blaIMP2, blaVIM1, and blaVIM2, among imipenem-resistant Pseudomonas aeruginosa strains. In this study, 117 P. aeruginosa strains were isolated from clinical samples of burn wound patients in Velayat hospital, Rasht, Iran, between 2018 and 2019. These isolates were tested for antimicrobial susceptibilities by disk diffusion and Metallo-β-Lactamase (MβL) activity. The polymerase chain reaction (PCR) test was applied to detect MβLs encoding genes in MβL-producing strains. The resistance rates were as follows: Tobramycin (59%), Gentamicin (57%), Piperacillin (52%), Ciprofloxacin (51%), Ceftazidime (32%), and Amikacin (26%). Among 27 (23%) imipenem-resistant isolates, 13 (48%) produced the MβL enzyme. PCR results of imipenem-resistant isolates showed that five and four isolates contained the blaVIM (4 blaVIM1, 2 blaVIM2) and blaIMP (4 blaIMP1, 2 blaIMP2) genes, respectively. In addition some of isolates had more than one gene. In this study, 48% of imipenem-resistant strains produced the MβL enzyme. Therefore, systematic surveillance to detect MβL-producing bacteria and rational prescription and use of carbapenems could be helpful to prevent the spread of carbapenem resistance.

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Keywords: Burn patients, Metallo-β-lactamases, Pseudomonas aeruginosa

Abbreviations: MβL, Metallo-β-Lactamase; MDR, Multidrug drug resistance

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I. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen associated with a wide range of nosocomial infections [1]. These strains cause infection in hospitalized patients especially burn units. In this regard, it is estimated that about 75% of burn patients’ mortality is related to infections [2].

The emergence of antibiotic-resistant bacteria, such as P. aeruginosa, is a serious concern in health care [3,4]. Carbapenems, especially imipenem, are used to treat these infections, regarding the worldwide increase in the spread of resistant strains [5].

Alteration of penicillin-binding protein, efflux system, reduced the outer membrane permeability, and enzymes that hydrolyzed carbapenem (carbapenemases) are the major ways of resistance mechanism [6]. These carbapenemases are Class A clavulanic acid inhibiting enzyme, Class B Metallo-β-lactamase (MβL), and Class D oxacillinase [7].

The genes encoding the MβLs are usually located on mobile genetic elements that also carry the aminoglycoside and other resistance genes [8]. The transfer of these genes between strains leads to the emergence of multidrug drug resistance.
(MDR) bacteria [9]. The definition most frequently used for MDR bacteria is ‘resistant to three or more antimicrobial classes’ [10].

The reports from all world regions have so far described six major families of MBL, namely blaIMP, blaVIM, blaSPM, blaGIM, blaAIM, and blaFIM. The blaVIM and blaIMP types are the clinically remarkable carbapenemases with 14 and 23 different subtypes, respectively [11].

This study aimed to detect blaIMP and blaVIM genes and common subtypes of them, including blaIMP-1, blaIMP-2, blaVIM-1, and blaVIM-2, among imipenem-resistant Pseudomonas aeruginosa strains isolated from patients with burn wounds in velayat Hospital in Rasht (Iran).

2. Materials and methods

2.1. Sampling and characterization of the study population

In this study, 117 P. aeruginosa strains isolated from clinical samples of burn wound patients in Velayat Burn Hospital, Rasht, Iran, between 2018 and 2019 were transferred to the Laboratory of Microbiology and Immunology of Infectious Diseases, Rasht. Microbiological characteristics were done by standard microbiological procedures including oxidase test, culture on the Triple Sugar Iron (TSI), Simmons citrate agar, and movement examination in the Sulfur, Indole, Motility (SIM) medium (Merck Co, Germany).

2.2. Antimicrobial susceptibility testing

The antimicrobial susceptibility test was evaluated using the Kirby Bauer disk diffusion method and interpreted based on the Clinical Laboratory Standard Institute (CLSI). The antibiotic disks included Imipenem (10 μg), Ceftazidime (30 μg), Piperacillin (100 μg), Gentamicin (10 μg), Amikacin (30 μg), Ciprofloxacin (5 μg), and Tobramycin (10 μg) (MAST Co., England). In this study, P. aeruginosa ATCC 27853 was used as a control strain.

2.3. Confirmation of MBL producing strains

Phenotypic detection of MBL strains was performed on imipenem-resistant isolates according to Yong et al. [12]. Briefly, the agar was inoculated with a 0.5 McFarland microbial suspension in the medium. After 15 minutes, the imipenem alone and imipenem EDTA were placed 2.5 cm apart in the medium. The presence of a distorted inhibition zone around IMP-EDTA compared to IMP after overnight incubation was interpreted as a positive test result. After 16-18 hours of incubating the plates at 37°C, the zone diameter surrounding the disks was measured. A zone ≥7mm in the presence of 750 μg of EDTA compared to an imipenem disk alone was considered a positive test for recognizing the MBL-producing resistant bacteria.

2.4. Molecular analysis of MBL encoding genes

The imipenem-resistant isolates of P. aeruginosa were selected. Next, their total DNA was extracted by the boiling method [13,14] and stored at -20°C until PCR amplification. Polymerase chain reaction (PCR) was carried out to detect blaIMP and blaVIM genes and common subtypes of blaIMP-1, blaIMP-2, blaVIM-1, and blaVIM-2 with specific primers (Table 1). The gels were stained with KBC stain (CinnaGen, Iran), and the PCR products were visualized with UV light [10].

3. Results

During the study period (i.e., 2018 to 2019), 117 P. aeruginosa strains were isolated from patients hospitalized in Velayat Burns Center (Rasht, Iran), of which 75 (64%) and 45 (36%) were male and female, respectively.

According to the disk diffusion test results, the resistance rate was Tobramycin 59%, Gentamicin 57%, Piperacillin 52%, Ciprofloxacin 51%, Ceftazidime 32%, and Amikacin 26%. In addition, 27 (24%) of all isolates were resistant to Imipenem (Fig. 1).

MBL enzyme-producing phenotypic test was done for the 27 imipenem resistant isolates, of which 13 (48%) isolates produced MBL enzyme according to combination disk diffusion test (CDDT) and 14 (52%) isolates were negative for this test.

The imipenem-resistant isolates were selected, and the PCR method was applied to detect blaIMP, blaVIM, blaVIM1, blaVIM2, blaIPM1, and blaIPM2 genes. PCR results showed that five and four isolates carried the blaVIM and blaIMP genes, respectively of which three isolates have both genes. Specific PCR identified four blaVIM1, two blaVIM2, four blaIPM1, and two blaIPM2.

| Terminal | Forward Primer | Reverse Primer | Gene | Size (bp) |
|----------|----------------|----------------|------|-----------|
| VIM      | GAT GGT TGG TCG CAA | AGC ACC AGC | VIM 1 | 261 |
| IMP      | GGA ATA GAG TCG CTC | TCT GCC CCA | IMP 1 | 390 |
| VIM1     | AGT GGT GAG TAT CGG | ACA G | IMP 2 | 390 |
| VIM2     | ATG TTC AAA CTT TTG | AGT AAG | IMP 2 | 390 |
| IMP1     | ACC GCA GGA GAG TGC | TGC CCG | IMP 2 | 390 |
| IMP2     | ACC ACC AGT TTT GCC TTA | CCA TTC CCA | IMP 2 | 390 |

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4. Discussion

*P. aeruginosa* is an opportunistic pathogen associated with many infections [15], especially in immunocompromised and burn patients [1]. This study aimed to detect blaIMP and blaVIM genes and common subtypes of blaIMP-1, blaIMP-2, blaVIM-1, and blaVIM-2 among imipenem-resistant *P. aeruginosa* strains isolated from patients with burn wounds in Velayat Hospital in Rasht, Iran.

In the present study, 117 *P. aeruginosa* isolated from burn wounds were assessed for antibiotic resistance pattern and imipenem-resistant strains were selected for more phenotypic and genotypic investigation.

The results of this study show the highest and lowest levels of resistance to Tobramycin (59%) and Imipenem (24%), respectively. Also, 38% of the isolates were multi-drug-resistant (MDR). Resistance rates to Amikacin (26%), Cefazidime (32%), Ciprofloxacin (51%), and Piperacillin (52%) in this study were lower compared to those reported by Owlia et al. and Japoni et al. studies [16,17].

In recent years, a larger number of MβL-producing bacteria have been isolated from nosocomial outbreaks, making a considerable concern due to their limited therapeutic options. In the present study, we detected that 27 (24%) of *P. aeruginosa* strains were resistant to imipenem, of which 13 (48%) isolates were MβL phenotypically productive. Plotto et al. [11] showed that 54/56 (96.4%) of isolates were resistant to imipenem, and among imipenem-resistant *P. aeruginosa* isolates, 30% produced MβL. Khosravi et al. [10] assessed 100 *P. aeruginosa* burn isolates and demonstrated that 41% of the isolates were resistant to imipenem and 8% were MβL-positive.

The frequency of MβL encoding genes between imipenem resistant isolates in this study was 4 (14%) blaIMP, 5 (18%) blaVIM, 4 (14%) blaVIM1, 2 (7%) blaVIM2, 4 (14%) blaIMP1, and 2 (7%) blaIMP2, wherein some isolates had more than one gene. Shahcheraghi et al. [18] examined 68 clinical isolates of imipenem-resistant *P. aeruginosa* by PCR for blaIMP, blaVIM-1, and blaSPM metallo-beta-lactam genes. In the this study, 16 isolates were produced metallo-beta-lactamase, that all of the isolates were positive for the blaVIM-1, and none of them had blaIMP genes. In comparison with our study, the prevalence of blaVIM and IMP genes were higher than above study. The result of other study that performed in Shahid Motahari Burns Hospital of Tehran, 54 (16.1%), 7 (2.1%), 22 (6.6%), and 11 (3.3%) of the resistant and intermediate isolates had VIM1, VIM2, IPM1, and IPM2 genes, respectively [13].

Based on the results obtained in the mentioned studies, although it can be concluded that the percentage of MβL genes were different based on the results obtained in the mentioned studies, the differences were not large as well as the prevalence of these resistance genes were almost in the high range. However, the type of isolated genes is different in most studies. However the blaVIM was the most isolated gene. Differences in the prevalence indicated the variable distribution of different types of MβL genes in different geographies.

In conclusion, in this study found almost a high prevalence of blaVIM and blaIMP genes among imipenem-resistant isolates. Therefore, identifying imipenem-resistant strains and checking for producing beta-lactamase enzyme could be helpful to prevent and control the spread of *P. aeruginosa* infection.

**Ethical declarations**

This study was approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1398.092) and is in compliance with the declaration of Helsinki.
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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Moslem Karampoor: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Fatheme Akhlaghi: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Mohammad Reza Mobayen: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Farhad Afrasiabi: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Ramin Khodayary: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Meisam Moradzadeh: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. Iraj Nikokar: Data curation, Formal analysis, Writing – original draft. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.
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