Original Article

Detection of vim- and ipm-type metallo-beta-lactamases in Pseudomonas aeruginosa clinical isolates

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Abstract

Background: *Pseudomonas aeruginosa* is the most important bacterium isolated from burn wounds, and its resistance to imipenem due to metallo-beta-lactamases is increasing. This study was designed to detect *vim1*, *vim2*, *ipm1* and *ipm2* metallo-beta-lactamases genes between *Pseudomonas aeruginosa* isolates isolated from Shahid Motahari Burns Hospital, Iran.

Methods: To that end, we isolated 483 nonduplicate consecutive isolates of *P. aeruginosa* from burn infections; and after biochemical confirmation, we examined the imipenem susceptibility via the Kirby-Bauer method. All the imipenem-resistant and imipenem-intermediate isolates were screened for *vim1*, *vim2*, *ipm1* and *ipm2* genes through the PCR method.

Results: From the 483 isolates, 272 (56%) and 63 (13%) isolates had resistant and intermediate zones in their imipenem antibiogram pattern, respectively. Fifty-four (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 in their PCR results, respectively.

Conclusion: MBL-mediated imipenem resistance in *P. aeruginosa* is a cause for concern in the treatment of infective burn patients. The rate of imipenem resistance due to MBL was increased dramatically and newer versions of MBL families were detected for the first time. These results suggest that an effective method should be provided to fight MBL production in clinical isolates.

Keywords: Burn wounds, Imipenem, Metallo-beta-lactamases, *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is one of the commonest causes of infection in burn units, and is responsible for numerous nosocomial infections.[1] Despite the use of potent antibiotics, invasive *P. aeruginosa* infection is associated with high mortality.[2] In the past decade, acquired multidrug resistance, relating to selective antibiotic pressure, has emerged in several countries; and in some cases, infections caused by multidrug resistant *P. aeruginosa* have been untreatable.[3] Standard therapy for *P. aeruginosa* infections includes broad-spectrum beta-lactamases-resistant penicillins, cephalosporins, carbapenems, and monobactams. Selected fluoroquinolones such as Ciprofloxacin and Levofloxacin previously offered a reasonable alternative for treating pseudomonal infections,[4–7] which have a high rate of morbidity and mortality; nevertheless, today beta-lactams are the most effective antibiotics against this microorganism. Several mechanisms can contribute to acquired beta-lactam resistance in *P. aeruginosa*, including beta-lactamase production, upregulation of efflux systems, and decreased outer membrane permeability.[8,9] Among beta-lactams, imipenem is the selective antibiotic against this bacterium, but *P. aeruginosa* can hydrolyze this antibiotic through metallo betalactamases.[10] Several kinds of metallo-beta-lactamases which belong to different families have been recognized.[11] In this study, we evaluated the existence of *vim1*, *vim2*, *ipm1* and *ipm2* metallo-beta-lactamases encoding genes between imipenem-resistant and intermediate *P. aeruginosa* strains isolated from burn wounds in Shahid Motahari Burns Hospital in Tehran-Iran.

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Materials and Methods

Collection of strains

P. aeruginosa used in this study were clinical isolates isolated from hospitalized burn patients in Shahid Motahari Burns Hospital, Tehran-Iran. A total of 483 nonduplicate consecutive isolates were collected from the Diagnostic Laboratory Department of the hospital between April 2008 and February 2009 and identified according to standard techniques.[12]

Imipenem susceptibility pattern

Imipenem susceptibility patterns of the 483 isolates were determined via the method of Bauer et al.[13] on Mueller-Hinton agar (Hi-Media, India) using imipenem 10 commercially available paper discs (MAST Co., England). *Pseudomonas aeruginosa* ATCC 27853 was used as standard strain. The results were interpreted according to the CLSI standard tables.

Extraction of total DNA

For molecular diagnosis, the total DNA content of each isolate was extracted via the boiling method. Briefly, 1 to 3 colony of

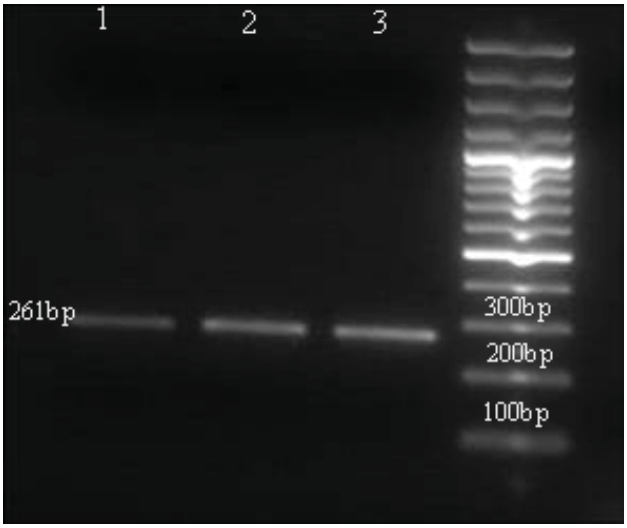

Figure 1. Agarose gel electrophoresis of *vim1* PCR products. Lines 1 and 2 show isolates positive for *vim1*. Line 3 shows the positive control PCR product. 100-1000bp ladder (CinnaGen, Iran).

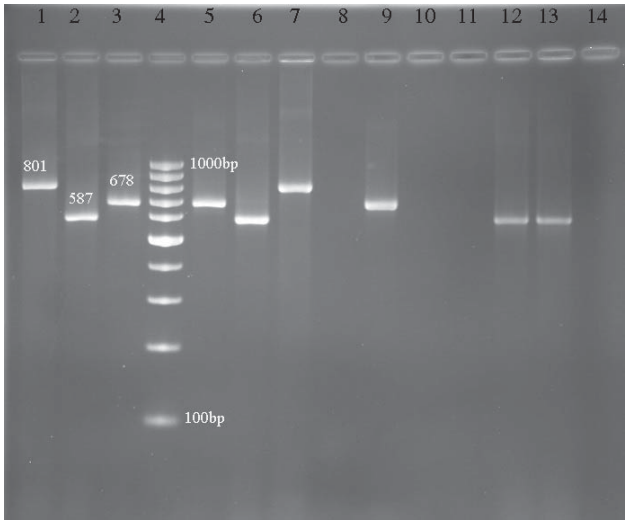

Figure 2. Agarose gel electrophoresis of *vim2*, *ipm1* and *ipm2* PCR products. Lines 1, 2 and 3 show *vim2*, *ipm1* and *ipm2* positive controls, respectively. Lines 5 and 9 show isolates positive for *ipm2*. Lines 6, 12 and 13 show isolates positive for *ipm1*. Line 7 shows an isolate positive for *vim2*. Line 14 shows the control negative. Lines 8, 10 and 11 are negative in their PCR results. 100-1000bp ladder (CinnaGen, Iran)

Table 1. Primer sequences and predicted lengths of PCR amplification products

Target gene	Primer	Oligonucleotide sequence (5›–3›)	Fragment size (pb)	Annealing temperature (°C)	Reference
vim1	*bla*VIM1	Forward: AGT GGT GAG TAT CCG ACA G Reverse: ATG AAA GTG CGT GGA GAC	261	55	Shibata *et al*.(2003)
vim2	*bla*VIM2	Forward: ATG TTC AAA CTT TTG AGT AAG Reverse: CTA CTC AAC GAC TGA GCG	801	51	Shibata *et al*.(2003)
ipm1	*bla*IMP1	Forward: ACC GCA GCA GAG TCT TTG CC Reverse: ACA ACC AGT TTT GCC TTA CC	587	51	Shibata *et al*.(2003)
ipm2	*bla*IMP2	Forward: GTT TTA TGT GTA TGC TTC C Reverse: AGC CTG TTC CCA TGT AC	678	51	Shibata *et al*.(2003)

each isolate was selected from a 16 h culture on the LB agar (Hi-Media, India) and was washed twice with sterile distilled water. The bacterial pellet was resuspended in 200µl sterile distilled water in a microtube and placed for 10 minutes in boiling water, following 3 minutes centrifugation in 10000rpm to isolate the cell debris as pellets. DNA containing supernatant was transferred to new sterile DNase free-RNase free microtubes.

PCR assays for the detection of metallo beta-lactamases encoding genes

Four pairs of specific primers introduced by Shibata et al.[14] were used in this study, which specifically detected *vim1, vim2, ipm1* and *ipm2* genes (Table 1). The mixture for the amplification of these genes consisted of 2.5µl of 10X PCR buffer (10mM Tris–HCl pH 9, 50mM KCl, and 0.1% Triton X-100), 1.25mM MgCl2, 0.2mM of each dNTPs, 1µM of each primer and 1.25U of *Taq* DNA polymerase, in a final volume of 25µl. The amplification condition consisted of an initial denaturation step at 94°C for 3 min, and 35 cycles of 94°C for 1 min, 51°C for 1 min, and 72°C for 1.5 min, and a final extension at 72°C for 7 min. A tube containing all PCR reaction mixture except template DNA was used as negative control. The bacterial strains used as controls included *bla*VIM1-containing *P. aeruginosa* VR-143/97 and *bla*VIM2-carrying *P. putida* NTU-91/99; and two separate plasmids one carrying *bla*IMP1 and the other one carrying *bla*IMP2, which were kindly presented by Kunikazu Yamane (Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Tokyo, Japan), were used as positive controls for *imp1* and *imp2* genes, respectively.

Agarose gel electrophoresis of PCR products

Horizontal electrophoresis system containing 1% agarose gel in the TAE buffer was used. Voltage was set at 5V per each centimeter distance between the two electrodes. To determine molecular weight, 100 – 1000bp ladder (CinnaGen, Iran) was used and ethidium bromide staining (0.5µg/ml) was performed for 20 minutes. Additionally, 254 nanometer UV waves through the Gel Doc Instrument (Biometra, Germany) were employed for gel imaging.

Statistical analysis

The results are expressed as absolute frequencies and percentages. For the statistical analyses, the statistical software SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL) was utilized. The curves were plotted using Excel software version 2003 (Microsoft Corporation, USA).

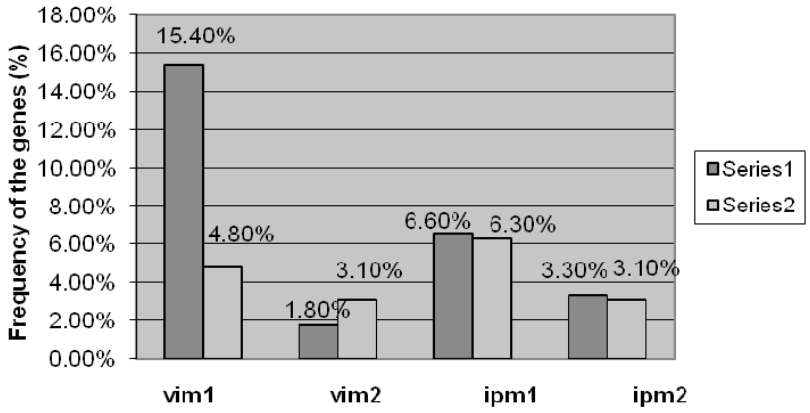

Figure 3. The frequency of different MBL encoding genes between imipenem resistant and intermediate isolates of *P. aeruginosa*. Series 1 and 2 show the frequency of MBL encoding genes between imipenem resistant and intermediate isolates, respectively.

Results

In total, 483 *Pseudomonas aeruginosa* nonduplicate consecutive isolates were collected from the burn wound infections of the patients in Shahid Motahari Burns Hospital, Tehran, Iran during 2008. From this total, 360 and 123 were isolated from men and women, respectively. From the 483 isolates, 272 (56%) were resistant to imipenem with a growth zone < 13mm, 63 (13%) isolates had an intermediate pattern with a growth zone of between 13 – 15 mm, and 148 (31%) were susceptible to imipenem with a growth zone > 15 mm.

The isolates with resistant and intermediate patterns to imipenem were subjected to PCR for *vim1*, *vim2*, *ipm1* and *ipm2* genes (Figures 1 and 2). 54 (16.1%), 7 (2.1%), 22 (6.6%), and 11 (3.3%) of these isolates had *vim1*, *vim2*, *ipm1* and *ipm2* genes in their PCR results, respectively.

Fourteen (4.2%) isolates had both *vim1* and *ipm1* genes, 4 (1.2%) isolates had both *vim1* and *ipm2* genes, 4 (1.2%) isolates had both *ipm1* and *ipm2* genes, only one (0.3%) isolate had *vim2* and *ipm1* genes, and only one (0.3%) isolate had both *vim2* and *ipm2* genes simultaneously. There was one isolate which carried *vim1*, *ipm1* and *ipm2* genes and another isolate which carried *vim2*, *ipm1* and *ipm2* simultaneously.

From the 483 isolates, 272 (56%) and 63 (13%) isolates had resistant and intermediate patterns in their imipenem antibiogram, respectively. The rates of the incidence of *vim1*, *vim2*, *ipm1* and *ipm2* genes between the imipenem resistant isolates were 15.4%, 1.8%, 6.6%, and 3.3%, respectively; whereas between the intermediate isolates, these rates were 4.8%, 3.1%, 6.3%, and 3.1%, respectively (Figure 3).

Discussion

P. aeruginosa is an opportunistic pathogen which causes serious diseases in immunodeficient patients such as burn patients. Different antibiotics are used for the treatment of its infections, including betalactams, aminoglycosides, and quinolones. Be that as it may, this microorganism can achieve different ways to protect itself against these antibiotics.[15,16] One of the most important ways to become resistant against imipenem is metallo-beta-lactamases (MBL) production, of which the vim and ipm MBL families are very prevalent between Gram negative rods.[17] The presence of vim-type and ipm-type MBLs between the isolates of different bacteria such as *Acinetobacter baumannii*, *Serratia marcescens*, *Morganella morgannii*, *Enterobacter cloacae, Klebsiella pneumoniae*, *Citrobacter freundii*, and *Aeromonas hydrophila* has been shown by different researchers.[18–21] The present study was designed to evaluate the rate of *vim1*, *vim2*, *ipm1*, and *ipm2* MBLs genes between the *P. aeruginosa* isolates of Motahari Hospital Burns patients.

In the present study, we demonstrated that from 483 *P. aeruginosa* isolates, 272 (56%) and 63 (13%) had resistant or intermediate patterns to imipenem. Shahcheraghi et al. assessed 350 *P. aeruginosa* clinical isolates collected from two Iranian general hospitals (Imam Khomeini Hospital and Tehran Children Center) and showed that only 5% of the isolates were resistant to imipenem.[22] However, Saderi et al. evaluated 128 *P. aeruginosa* clinical isolates collected from Shahid Motahari Burns Hospital and demonstrated that 38.28% of the isolates were resistant to imipenem.[23]

Shahid Motahari Burns Hospital is a specific center for burns patients and unfortunately, imipenem therapy is the choice treatment for burn infections caused by Gram negative bacteria. On the other hand, some metallo-beta-lactamases encoding genes are located on transposable genetic elements and can transfer between bacterial strains and isolates.[24] So the difference between our results and Shahcheraghi's results may be because of the difference between the antibiotic therapy regimens in our respective hospitals. According to our study and in comparison with Saderi's study, the resistance rate of *P. aeruginosa* is rising in Shahid Motahari Burns Hospital and preventive strategies such as more precise antibiotic selection for infection treatment and less physical contact between patients should be taken against this phenomenon. Rasmussen and Bush[25] stated that because of an increase in the carbapenem usage, the increase of MBL-producing organisms would be inevitable. Lee et al. showed that after nine years of the usage of carbapenems in Korea, the imipenem-resistance rate of *P. aeruginosa* had rapidly risen from 6% in 1996 to 19% in 2001.[26]

Our study showed that 54 (16.1%), 7 (2.1%), 22 (6.6%), and 11 (3.3%) of the isolates had *vim1*, *vim2*, *ipm1*, and *ipm2* genes

in their PCR results, respectively. Shibata et al. screened 180 *P. aeruginosa* MBL producers for vim-type and imp-type genes through the PCR method and showed that 35%, 0.5%, and 64.5% of the isolates carried *vim2*, *ipm1*, and *ipm2* genes, respectively. None of the isolates carried *vim1* gene.[14] Laupland et al. evaluated the presence of *vim2* gene between 98 MBL producing *P. aeruginosa* isolates in Calgary Health Region in Canada between May 2002 and April 2004 and showed that 92% of them were vim2 positive.[27] Lee et al. assessed the prevalence of vim2 and imp1 MBLs between 415 *P. aeruginosa* clinical isolates in Korea and demonstrated that 45 isolates were MBL producers, of which 7 (1.7%) and 0 (0%) of isolates were vim2 and imp1 producers, respectively.[28] It seems that the spread patterns of different MBLs between countries are different and their relationships with geographical areas, hygienic conditions and chromosomal structure of bacterial strains should, therefore, be evaluated.

ipm types of MBLs were rare in Iran until the time of the commencement of our study, which recorded the first isolation of *ipm1* and *ipm2* carrying *P. aeruginosa* isolates from Shahid Motahari Burns Hospital. Because of the sudden high prevalence of *ipm1* (22 out of 335) and *ipm2* (11 out of 335) carrying isolates, it is necessary to assess the distribution and transportation pattern of these pathogens between the patients of this hospital.

MBL-mediated imipenem resistance in *P. aeruginosa* is a cause for concern in the treatment of infected burns patients. The rate of imipenem resistance due to MBLs was increased dramatically and newer versions of MBL families were detected for the first time. These results suggest that an effective method should be provided to fight MBL production in clinical isolates.

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