



Detection of Metallo-Beta-Lactamase-Encoding Genes Among Clinical Isolates of *Pseudomonas aeruginosa* in Qom Province

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Abstract

Background: Carbapenem-resistant *Pseudomonas aeruginosa* has emerged as a serious problem in many hospitals and intensive care units. The purpose of this study was to determine the antibiotic susceptibility profile and the distribution of metallo-beta-lactamase genes among *P. aeruginosa* isolates from Qom province of Iran.

Methods: In this study, 200 *P. aeruginosa* isolates were collected from several clinical samples in hospitals affiliated to Qom University of Medical Sciences, Qom province, Iran. The antibiotic susceptibility of the isolates was tested by the Kirby-Bauer disc diffusion test and the distribution of MBL genes among carbapenem-resistant isolates was determined by polymerase chain reaction (PCR) method.

Results: Among carbapenem non-susceptible isolates of *P. aeruginosa*, 38% (76 isolates) were multidrug resistant, 26% (52 isolates) were pan-drug-resistant and 5% (10 isolates) were extensive drug resistant. 52% of the isolates were resistant to carbapenems. Among carbapenem non-susceptible isolates of *P. aeruginosa*, 40 isolates (38.4%) exhibited MBL production. Of 40 MBL-producing isolates, 38 isolates (36.5%) contained the bla_{IMP} gene and two (1.9%) isolates contained the bla_{VIM} gene.

Conclusions: Outbreaks of MBL-producing *P. aeruginosa* isolates will be a serious problem in hospitals in the future and rapid identification of these isolates is necessary to control further dissemination of MBL resistance genes. This is the first report of the rates of MDR, XDR, PDR, and MBL-producing *P. aeruginosa* isolated from hospitals affiliated to Qom University of Medical Sciences in Qom province of Iran.

Keywords: Metallo-Beta-Lactamases, Polymerase Chain Reaction, *Pseudomonas aeruginosa*

1. Background

Pseudomonas aeruginosa is a Gram-negative, glucose non-fermentative bacillus that is often involved in nosocomial infection outbreaks (1). This opportunistic pathogen can cause a variety of infections including pneumonia, septicemia, meningitis, urinary tract infections, and burn wound infections (2, 3). The antibiotic therapy of nosocomial infection caused by *P. aeruginosa* is often complicated because the drug resistance of this organism has increased recently (4). The terms pan-drug-resistant (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) are defined as resistance to all antibiotics in all different categories of antimicrobial agents, resistance to at least one antibiotic in all except one or two antimicrobial categories, and resistance to at least one antibiotic in ≥ 3 antimicrobial categories, respectively. The antimicrobial categories contain aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, monobactams, and polymyxins (5). Carbapenems are effective

agents for the treatment of important Gram-negative bacilli infections, especially *P. aeruginosa* (3). Among various mechanisms involved in resistance to carbapenems in *P. aeruginosa*, the production of metallo-beta-lactamases (MBLs) is a very important mechanism through which all carbapenems are hydrolyzed (4, 6, 7). Furthermore, the metallo-beta-lactamase genes located on integrons may elucidate their wide distribution between strains (8). At present, different groups of MBLs have been identified in *P. aeruginosa* but the IMP (Imipenemase) and VIM (Verona integron-encoded metallo-beta-lactamase) groups are the most widespread groups of MBLs (9-). This study was done to detect the antimicrobial susceptibility profile and the rate of MDR, XDR, and PDR among *P. aeruginosa* isolated from multiple hospitals of Qom province and to determine the distribution of MBL genes among carbapenem non-susceptible isolates.

2. Methods

2.1. Bacterial Isolation and Identification

In a study that was done between November 2013 and October 2014, 200 non-duplicated *P. aeruginosa* isolates were collected from different specimens at Shahid Beheshti, Nekoei, Kamkar, and Valiasr Hospitals (affiliated to Qom University of Medical Sciences, Iran). The clinical specimens included tracheal aspirate, urine, burn wound, blood, surgical wound, and stool. The samples were immediately inoculated in MacConkey agars. The identification of the isolates was carried out using standard microbiological and biochemical methods including Gram-staining, colony morphology, glucose oxidation, citrate utilization, oxidase test, and pigment production (13).

2.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility profile of isolates was tested by standard disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (14). The antibiotic discs contained ceftriaxone (30 µg), ceftazidime (30 µg), ceftizoxime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), Amikacin (30 µg), ciprofloxacin (5 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), Aztreonam (30 µg), colistin (10 µg), and polymyxin B (300 units) (MAST, United Kingdom). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains in the tests. The isolates that were intermediate-resistant to antibiotics were considered as resistance. The minimum inhibitory concentrations (MIC) of all imipenem non-susceptible isolates for imipenem was obtained by microbroth dilution method according to the recommendations of the standard protocol of CLSI (14).

2.3. Detection of MBL Producing Isolates and MBL Genes

The detection of MBL production in carbapenem-resistant isolates of *P. aeruginosa* was carried out by double disk synergy test (DDST) (15). DNA of carbapenem resistant isolates was extracted by boiling method (15). The detection of bla_{IMP} and bla_{VIM} genes in *P. aeruginosa* isolates was achieved by PCR with specific primers (Table 1) (16). The amplification steps were as follows: Initial denaturation for 3 minutes at 94°C; 35 cycles with denaturation for 1 minute at 94°C, annealing for 1 minute at 53°C, extension for 1 minute at 72°C, and final extension for 7 minutes at 72°C. Gel electrophoresis was performed in 1.2% agarose gel at 85 V. *P. aeruginosa* ATCC 27853 was used as the negative control strain and *P. aeruginosa* harboring bla_{IMP} and bla_{VIM} genes (obtained from Dr. Hashemi, Iran) were used as the positive control.

Table 1. The Sequence of Primers Used for Detection of MBL-Encoding Genes

Primers	Sequence (5'-3')	Product Size, bp
IMP-F	ACCGCAGCAGAGTCTTTGCC	587
IMP-R	ACAACCAGTTTTGCCTTACC	
VIM-F	ATGTTCAAACCTTTGAGTAAG	801
VIM-R	CTACTCAACGACTGAGCG	

2.4. Statistical Analysis

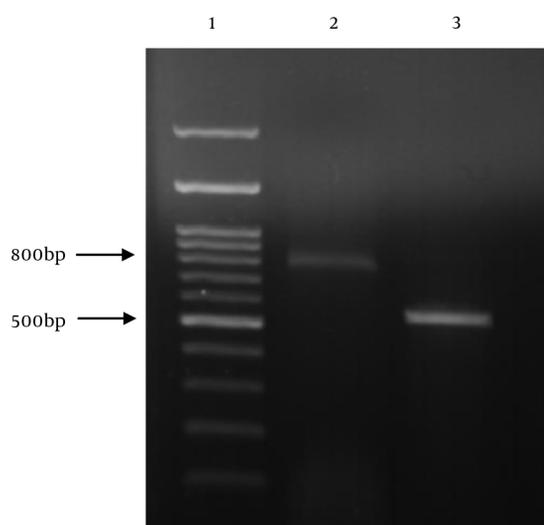
Differences in variables were analyzed using Chi-Square test and P-values of less than 0.05 were considered statistically significant. All the available data were analyzed by a computer program (SPSS).

3. Results

In total, 200 *P. aeruginosa* isolates were collected from tracheal aspirate (78 isolates, 39%), urine (52 isolates, 26%), burn wound (42 isolates, 21%), blood (13 isolates, 6.5%), surgical wound (12 isolates, 6%), and stool (three isolates, 1.5%). 73 patients (73%) were male and 26 (27%) were female. The age range of the patients was 20 - 86 years. *P. aeruginosa* isolates were recovered from several hospital settings as follows: intensive care unit (ICU) (39%), burn unit (21%), trauma and emergency wards (16.5%), general surgery wards (14%), and internal wards (9.5%). Among the *P. aeruginosa* isolates, 76 isolates (38%) were multidrug-resistant (MDR), 52 isolates (26%) were pan-drug-resistant (PDR), and 10 isolates (5%) were extensive drug resistant (XDR) (Table 2). On the other hand, among the 200 *P. aeruginosa* isolates, 104 isolates (52%) were resistant to imipenem and meropenem. The majority of isolates (94.4%) showed MIC ≥ 32 mg/L for imipenem. The results of the DDST method showed that among 104 carbapenem non-susceptible isolates of *P. aeruginosa*, 40 isolates (38.4%) produced metallo-beta-lactamases. The *P. aeruginosa* isolates producing metallo-beta-lactamases were mainly obtained from tracheal aspirate (63%), burn wound (28%), and urine (9%). Of the 104 carbapenem non-susceptible isolates, 38 (36.5%) isolates carried the bla_{IMP} gene and two (1.9%) isolates carried the bla_{VIM} gene (Figure 1). Of the 40 MBL-producing isolates, 21 isolates (52.5%) were PDR and 19 (47.5%) isolates were MDR. The Pearson Chi-Square analysis showed that the rates of resistance to imipenem, meropenem, ceftazidime, cefotaxime, gentamicin, amikacin, ciprofloxacin, and piperacillin-tazobactam were significantly higher in isolates with bla_{IMP} gene than in isolates without the gene (P-values ≤ 0.001). The rates of resistance to piperacillin and colistin were statistically higher in isolates with bla_{IMP} gene than in isolates without the gene (P-values ≤ 0.05). The rates of resistance to tested antibiotics were not significantly different between isolates in which bla_{VIM} gene was detected and isolates in which it was not detected.

Table 2. Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Isolated from Four University Hospitals in Qom, Iran

Antimicrobial Agent	Susceptible, %	Intermediate, %	Resistant, %
Ceftizoxim	7	11	82
Cefotaxim	7	28	65
Imipenem	48	7	45
Meropenem	48	10	42
Ceftazidime	41	9	50
Ceftriaxone	22	28	50
Ciprofloxacin	41	13	46
Gentamicin	37	17	46
Aztreonam	34	23	43
Piperacillin	59	6	35
Piperacillin-tazobactam	49	23	28
Amikacin	63	9	28
Polymyxin B	97	-	3
Colistin	88	-	12

**Figure 1.** PCR amplification of IMP-type and VIM-type MBLs of *P. aeruginosa* isolates. Lane 1: DNA size marker; Lane 2: bla_{VIM} positive isolate; Lane 3: bla_{IMP} positive isolate

4. Discussion

P. aeruginosa is one of the most important Gram-negative opportunistic pathogens, which is recognized as an agent of nosocomial infections. Our study showed that 39% of the *P. aeruginosa* isolates were collected from patients in ICUs, indicating that invasive devices play an important role in *P. aeruginosa* infections (17-20). The rate

of resistance to carbapenems in *P. aeruginosa* isolates in this study was 52%, which is higher than the rates of resistant to carbapenems (32% and 12.4% among *P. aeruginosa* isolates) in previous studies reported from Iran (21, 22). These results indicate that carbapenem-resistant *P. aeruginosa* has increased recently (17, 23). In the present study, the distribution of MDR, PDR, and XDR was found to be 38%, 26%, and 5%, and this finding is the first report of the distribution of PDR and XDR isolates in *P. aeruginosa* from Iran. In the present study, higher rates of resistance to third-generation cephalosporins including ceftizoxime (93%), cefotaxime (93%), ceftazidime (59%), and ceftriaxone (78%) were observed, implying that third-generation cephalosporins are no longer secure to treat *P. aeruginosa* in our hospitals. Therefore, finding an appropriate therapy for infections caused by multidrug-resistant *P. aeruginosa* is a newly difficult situation. Our results were consistent with the observations in previous studies from Iran indicating that the majority of *P. aeruginosa* isolates showed MIC \geq 32 mg/L for imipenem (21). In our study, we tested MBLs production by using the DDST method and demonstrated that 38.4% (n = 40) of the carbapenem non-susceptible *P. aeruginosa* isolates were MBL positive. This result is consistent with those of previous reports from Iran and Brazil (21, 24, 25). In the present study, 61.6% of the carbapenem non-susceptible *P. aeruginosa* were MBL negative, which suggests that other factors such as deficiency in porin or reduced expression of outer membranes proteins might be associated with carbapenem resistance. In the present study, the distribution of IMP-type MBLs among carbapenem-resistant isolates of *P. aeruginosa* was 36.5%. Other studies demonstrated that 5.77% of *P. aeruginosa* isolates from the northwest of Iran (2010) (21) and 24% from the center of Iran (2013) (26) were IMP-type positive. These results indicate that the distribution of IMP-type MBLs among the isolates of *P. aeruginosa* in Iran in recent years has increased significantly. In our study, 1.9% of the imipenem non-susceptible isolates of *P. aeruginosa* carried VIM-type MBLs. Bagheri Bejestani et al. reported that bla_{VIM} gene was not detected among the studied isolates in the center of Iran (27). However, several previous reports revealed that the genes of bla_{IMP} and bla_{VIM} -type MBL are often encoded on mobile genetic cassettes, which can easily spread among isolates.

4.1. Conclusions

This study showed that susceptibility to carbapenems among isolates of *P. aeruginosa* reduced in Iran and the IMP-type MBLs were the most prevalent MBLs among imipenem non-susceptible *P. aeruginosa* isolates in this area. MBL-producing *P. aeruginosa* in recent years has become a serious problem. The rapid identification of MBL-producing isolates is very important and the use of specific methods is necessary to control the further transmission of infection by these organisms.

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