



Prevalence of *SHV*, *TEM*, *CTX-M* and *OXA-48* β -Lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in Bandar-Abbas, Iran

Maryam Bahrami¹, Maryam Mohammadi-Sichani^{1*}, Vajihe Karbasizadeh²

¹Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

²Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

***Corresponding author:**

Maryam Mohammadi-Sichani,
Department of Microbiology,
Falavarjan Branch, Islamic
Azad University, Isfahan, Iran.
Tel: +989133092823;
Email:
mohamadi_m@iaufala.ac.ir

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Abstract

Background: *Pseudomonas aeruginosa* is one of the most important causes of opportunistic infections. Carbapenems are administrated for the treatment of resistant infections due to this bacterium. The aim of this study was to investigate the presence of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{OXA-48} beta-lactamase genes in clinical isolates of *P. aeruginosa* in Bandar Abbas, Iran.

Methods: In this descriptive study, 96 isolates of *P. aeruginosa* were obtained from clinical samples in Bandar Abbas. All isolates were identified by biochemical tests. The antibiotic susceptibility was examined by disk diffusion method. The presence of *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM} and *bla*_{OXA-48} genes was evaluated by polymerase chain reaction using a specific primer.

Results: In this study, 76 isolates of *P. aeruginosa* (79.2%) were resistant to at least one carbapenem. The highest bacterial resistance (100%) was obtained to nalidixic acid (30 μ g), followed by tetracycline (30 μ g) and amoxicillin (25 μ g). The frequencies of *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM} and *bla*_{OXA-48} genes were 23.95% (23 isolates), 23.08% (26 isolates), 57.29% (55 isolates) and 12.5% (12 isolate), respectively. Sixty isolates resistant to carbapenems (78.9%) carried at least one resistant gene (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{OXA-48}) and 16 isolates (21.1%) did not have any of these genes.

Conclusions: This study revealed the prevalence of carbapenem-resistant phenotype of *P. aeruginosa* clinical isolates and the frequency of extended-spectrum β -lactamase genes have increased.

Keywords: Extended-spectrum beta-lactamase, Carbapenems, Antibiotic resistance, *Pseudomonas aeruginosa*

Background

Pseudomonas aeruginosa is an aerobic gram-negative bacillus that is the cause of a range of opportunistic infections in humans. *P. aeruginosa* is one of the most important pathogenic bacteria in the development of nosocomial infections such as pneumonia associated with intensive care unit (ICU) ventilator, bacteremia, urinary tract infections, and infection in people with severe and septic burns (1, 2).

Infections caused by *P. aeruginosa* are often severe and life-threatening. *P. aeruginosa* infection is difficult to control and eliminate because it has intrinsic resistance to many antimicrobial agents (3). The rapid increase of extended-spectrum beta-lactamase (ESBL)-producing isolates of *P. aeruginosa* is a health risk. Many isolates of *P. aeruginosa* are susceptible to cephalosporins and carbapenems; however, this bacterium has acquired resistance to these antibiotics. *P. aeruginosa* resistance mechanisms include β -lactamase production, efflux pumps and changes in outer membrane proteins. Multi-drug resistant (MDR) strain of *P. aeruginosa* is able to produce a wide range of beta-lactamase enzymes. According to Ambler classification, beta-lactamases are

divided into four groups based on their structures (A-D). *P. aeruginosa* resistance to cephalosporins, monobactam and carbapenems may be acquired by ESBL enzymes (4,5). The prevalence of resistance to carbapenems among *P. aeruginosa* isolates is a common challenge facing the successful treatment of the life-threatening and permanent infections due to this bacterium (6,7).

Proper and up-to-date information about the antibiotic resistance of clinical isolates of *P. aeruginosa* is essential for the treatment of the infections caused by these strains. The aim of this study was to investigate the presence of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{OXA-48} beta-lactamase genes in the clinical isolates of *P. aeruginosa* in Bandar Abbas, Iran.

Materials and Methods

In this descriptive study, 96 *P. aeruginosa* strains isolated from wound patients hospitalized in Bandar Abbas Hospital from May to December 2017 were studied. Identification of these isolates was confirmed using Gram staining and biochemical tests including catalase and oxidase tests, fermentation in oxidation-fermentation

medium (+/-), K/K patterns on TSI medium, growth at 42°C and pigment production (8).

Antimicrobial Susceptibility Test

Antibiotic susceptibility of isolates was determined according to the Clinical & Laboratory Standards Institute (CLSI) by the Kirby-Bauer testing (9). The antibiotic discs tested included chloramphenicol (30 μ g), tobramycin (10 μ g), co-trimoxazole (25 μ g), nalidixic acid (30 μ g), amikacin (30 μ g), piperacillin (100 μ g), ciprofloxacin (5 μ g), levofloxacin (5 μ g), tetracycline (30 μ g), amoxicillin (AMX: 25 μ g); ceftazidime (30 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), cefepime (30 μ g), imipenem (10 μ g); meropenem (10 μ g) and aztreonam (30 μ g). After measurement of the diameter of inhibition zone, the results were categorized as sensitive (S), intermediate (I) and resistant (R) based on the standard zone of inhibition. The strains *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used to control the antibiogram (10,11).

PCR Assay

All isolates of *P. aeruginosa* were evaluated for the presence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{OXA-48} by PCR assay. The primers used in this study are listed in Table 1 (12,13).

The amplification was carried out in a thermocycler (Eppendorf, Germany) in a 25 μ L reaction solution containing 2.5 μ L 10x PCR buffer, 0.6 μ L 50 mM MgCl₂, 0.4 μ L dNTP Mix (10 mM each), 0.5 μ L 20 pmol/ μ L each primer, 1 μ L template DNA, 1 μ L Taq DNA polymerase (5 U/ μ L) and 18.5 μ L nuclease-free water. The reaction solution was prepared for each primer (Table 2).

Electrophoresis of PCR product was performed at 80 v, 380 mA in 1.0% agarose gel and by DNA Green Viewer

staining.

The Fisher exact test (SPSS version 17.0) was used to examine the significance of association between presence of different β -lactamase genes and antibiotic resistance of isolates. The significance level (*P*) was < 0.05.

Results

In this study, 96 *P. aeruginosa* isolates were obtained from 44 male and 52 female patients. The antibiotic susceptibility tests showed that all isolates were completely resistant to nalidixic acid, tetracycline and amoxicillin. In addition, the sensitivities of *P. aeruginosa* isolates to aztreonam and amikacin were obtained 76.4% and 73.95%, respectively. The frequencies of sensitive, intermediate and resistant isolates of *P. aeruginosa* against the tested antibiotics is presented in Table 3. More than 70% of isolates were resistant to new cephalosporins such as ceftazidime, cefotaxime, ceftriaxone and cefepime significantly. In addition, 77% of *P. aeruginosa* isolates were resistant to imipenem and 47% showed resistance to meropenem.

Polymerase chain reaction (PCR) was performed for all isolates of *P. aeruginosa*. The results showed that 54 (56.3%), 25 (26.0%), 23 (23.9%) and 11 (11.5%) clinical isolates of *P. aeruginosa* expressed *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{OXA-48} respectively. Furthermore, 74 (77.1%) of *P. aeruginosa* isolates carried at least one of these genes and 22 (22.9%) of the isolates did not have any resistant gene. The frequency of ESBL genes in the clinical isolates of *P. aeruginosa* according to the type of gene is shown in Table 4. Statistical analysis showed a significant association between the presence of *bla*_{TEM} (*P*<0.01) and *bla*_{SHV} (*P*<0.05) genes and resistance to carbapenems such as imipenem and meropenem. Similarly, a significant association was observed between the presence of *bla*_{TEM} (*P*<0.01), *bla*_{SHV} (*P*<0.05) and *bla*_{CTX-M} (*P*<0.05) genes and resistance to cephalosporins. Statistical analysis revealed a significant association between the presence of *bla*_{TEM} (*P*<0.01), *bla*_{OXA-48} (*P*<0.05) and *bla*_{CTX-M} (*P*<0.05) genes and resistance to aztreonam.

Discussion

The ESBL-producing isolates of *P. aeruginosa* are one of the rapidly emerging antibiotic resistance-related problems in clinics. The clinical isolates of *P. aeruginosa* especially carbapenems have also acquired antibiotic resistance. In the current study, the highest rate of antibiotic

Table 1. Primers Used for Detection of Extended-Spectrum Beta-lactamase -Producing *Pseudomonas aeruginosa*

Primers	Nucleotide Sequences	Product Size (bp)
TEM-F	5'ATGAGTATTCAACATTTCCG-3'	867
TEM-R	5'CTGACAGTTACCAATGCTTA-3'	
SHV-F	5'GATGAACGCTTTCCCATGATG-3'	214
SHV-R	5'CGCTGTTATCGCTCATGGTAA-3'	
CTX-M-F	5'TTTGCGATGTGCAGTACCAGTAA-3'	590
CTX-M-R	5'CGATATCGTTGGTGGTGCCATA-3'	
OXA-F	5'CATCAAGTTCAACCAACCG-3'	438
OXA-R	5'CATGTTTGGTGGTCTTGT-3'	

Table 2. Program Used for the PCR of Selected Genes

Primer	Initial Denaturing	35 Cycles			Final Extension
		Denaturing	Annealing	Extension	
TEM	95°C/5 min	94°C/45 s	58°C/45 s	72°C/45 s	72°C/5 min
SHV	95°C/5 min	94°C/30 s	61°C/30 s	72°C/30 s	72°C/5 min
CTX-M	95°C/5 min	94°C/45 s	60°C/45 s	72°C/45 s	72°C/5 min
OXA-48	95°C/5 min	94°C/45 s	55°C/45 s	72°C/45 s	72°C/5 min

Table 3. Results of Antibiotic Resistance of 96 Clinical Isolates of *Pseudomonas aeruginosa*

Antibiotic	<i>Pseudomonas aeruginosa</i> Isolates		
	Resistant	Intermediate	Susceptible
Chloramphenicol	83 (86.0)	10 (10.4)	3 (3.1)
Tobramycin	55 (57.3)	17 (17.7)	24 (25.0)
Amikacin	25 (26.0)	-	71 (74.0)
Co-trimoxazole	95 (99.0)	1 (1.0)	-
Nalidixic Acid	96 (100)	-	-
Amoxicillin	96 (100)	-	-
Piperacillin	69 (71.9)	4 (4.2)	23 (24.0)
Ciprofloxacin	20 (20.9)	32 (33.3)	44 (45.8)
Levofloxacin	47 (49.0)	-	49 (51.0)
Tetracycline	96 (100)	-	-
Ceftazidime	74 (77.1)	-	22 (23.9)
Cefotaxime	72 (75.0)	-	24 (25.0)
Ceftriaxone	76 (79.2)	6 (6.3)	14 (14.6)
Cefepime	65 (67.7)	-	31 (32.3)
Aztreonam	23 (24.0)	-	73 (76.0)
Imipenem	74 (77.1)	-	22 (23.9)
Meropenem	45 (46.9)	3 (3.1)	48 (50.0)

susceptibility was observed against aztreonam (76.0%) followed by amikacin (74.0%). The antibiotic resistance rate against imipenem and meropenem were 77.1% and 46.9%, respectively. Furthermore, the antibiotic resistance rate against various cephalosporins ranged from 67.7% to 79.2%. Salimi et al also reported that 73.3% of *P. aeruginosa* isolates were resistant to imipenem, and antibiotic resistance rates against ceftazidime and cefepime were 70.0% and 75.0%, respectively (14). Adjei et al reported that the antibiotic resistance rates against aztreonam, imipenem, meropenem and ceftazidime were 94%, 88%, 82% and 76%, respectively (15). However, the results of other studies suggest a low antibiotic resistance rate against carbapenems and cephalosporins (16-19).

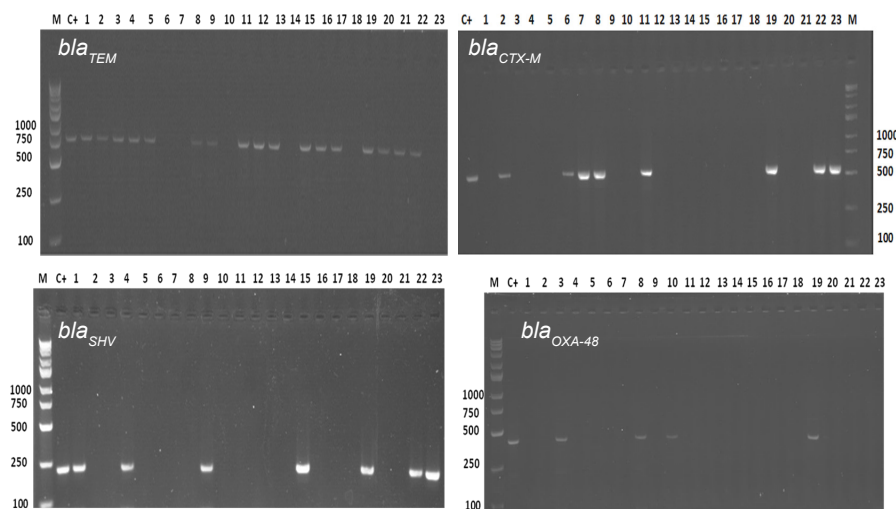
The research of Komijani et al revealed that the highest

Table 4. Distribution of Extended-Spectrum Beta-lactamase Genes of Clinical Isolates of *Pseudomonas aeruginosa* from Bandar Abbas Hospital

Resistance Genes	No. (%)
<i>bla</i> _{TEM}	28 (29.2)
<i>bla</i> _{SHV}	10 (10.4)
<i>bla</i> _{CTX-M}	4 (4.2)
<i>bla</i> _{OXA-48}	3 (3.1)
<i>bla</i> _{TEM} + <i>bla</i> _{SHV}	5 (5.2)
<i>bla</i> _{TEM} + <i>bla</i> _{CTX-M}	9 (9.4)
<i>bla</i> _{TEM} + <i>bla</i> _{OXA-48}	3 (3.1)
<i>bla</i> _{SHV} + <i>bla</i> _{CTX-M}	2 (2.1)
<i>bla</i> _{SHV} + <i>bla</i> _{OXA-48}	-
<i>bla</i> _{CTX-M} + <i>bla</i> _{OXA-48}	-
<i>bla</i> _{TEM} + <i>bla</i> _{SHV} + <i>bla</i> _{CTX-M}	4 (4.2)
<i>bla</i> _{TEM} + <i>bla</i> _{SHV} + <i>bla</i> _{OXA-48}	1 (1.0)
<i>bla</i> _{TEM} + <i>bla</i> _{CTX-M} + <i>bla</i> _{OXA-48}	1 (1.0)
<i>bla</i> _{SHV} + <i>bla</i> _{CTX-M} + <i>bla</i> _{OXA-48}	-
<i>bla</i> _{TEM} + <i>bla</i> _{SHV} + <i>bla</i> _{CTX-M} + <i>bla</i> _{OXA-48}	3 (3.1)
None of them	22 (22.9)
Total	96 (100)

level of antibiotic resistance in *P. aeruginosa* isolates was against ceftazidime (77.64%). They found 81.98% of *P. aeruginosa* isolates were ESBL-positive and the frequencies of the *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{OXA} genes were 60.86%, 29.81%, 24.22% and 14.28%, respectively (20). The antibiotic resistance and frequencies of ESBL genes among of *P. aeruginosa* isolates in Komijani et al research are similar to our results. Peymani et al reported that *P. aeruginosa* isolates (98.5%) were not susceptible to the studied extended spectrum cephalosporins, and 75 (28.6%) isolates were ESBL-producing. They reported that the *bla*_{TEM-1} (26.7%) was the most frequently seen gene followed by *bla*_{CTX-M-15} (17.3%), *bla*_{SHV-1} (6.7%), and *bla*_{SHV-12} (4%), either alone or in combination (21).

The prevalence of different ESBL genes varies in different

**Figure 1.** Agarose Gel Electrophoresis of the PCR Amplified Products of Extended-Spectrum Beta-lactamase Genes.

geographical locations. The *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA-48} and *bla*_{CTX-M} genotypes are prevalent in Asian countries. In this study, a total of 74 isolates (77.1%) carried at least one of the ESBL genes (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{OXA-48}), and 22 isolates (22.9%) carried no ESBL-producing genes. The most prevalent β -lactamase gene, according to the PCR results, was *bla*_{TEM} that was detected in 54 isolates (56.3%) and *bla*_{SHV} and *bla*_{CTX-M} were observed in 25 (26.05) and 23 (24.0%) isolates, respectively. The *bla*_{OXA-48} gene was detected in only 11 isolates (11.5%).

In the study by Bokaeian et al, the frequencies of *bla*_{TEM} and *bla*_{SHV} genes were 100% and 66%, respectively. Their results showed 6.89% of MDR isolates of *P. aeruginosa* were ESBL-positive (22). Ahmed et al in a research in the hospital of Makkah, Saudi Arabia observed that about 25.9% of *P. aeruginosa* isolates were ESBL-producing. Further, the frequencies of *bla*_{CTX-M}, *bla*_{OXA-10}, *bla*_{OXA-4} and *bla*_{GES} genes were 10.7%, 7.1%, 3.6% and 78.6%, respectively. The *bla*_{TEM} and *bla*_{SHV} were not observed in any isolates in Ahmed et al study. The frequency of the *bla*_{SHV} gene was reported 13.3% by Salimi et al. The study of Farzeali Shirehjini et al indicated that *P. aeruginosa* strains had high levels of resistance and 21.6% and 34.2% of the strains had *bla*_{CTX-M} and *bla*_{TEM} genes, respectively (23). The prevalence of *bla*_{TEM} gene in the study of Mohammad was 25% that is less than the corresponding prevalence in our study (24).

Conclusions

Carbapenems, including imipenem and meropenem, are the most important antibiotics used to treat infections caused by *P. aeruginosa*. Unfortunately, this study showed a high percentage of resistance to imipenem. Our results also indicated various ESBL genes such as *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{OXA-48} were expressed by *P. aeruginosa* clinical isolates. Therefore, unnecessary prescription and consumption of antibiotics should be avoided to prevent the emergence of resistant strains of *P. aeruginosa*.

Ethical Approval

None to be declared.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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