

Prevalence of blaOxa10 Type Beta-lactamase Gene in Carbapenemase Producing *Pseudomonas aeruginosa* Strains Isolated From Patients in Isfahan

Zeynab Golshani^{1,*}, Ali Sharifzadeh²

¹Department of Microbiology, Young Researchers and elite Club, Falavarjan Branch, Islamic Azad University, Isfahan, IR Iran

²Department of Microbiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, IR Iran

*Corresponding author: Zeynab Golshani, Department of Microbiology, Young Researchers and elite Club, Falavarjan Branch, Islamic Azad University, Isfahan, Iran E-mail: zey_golshani@yahoo.com.

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Background: *Pseudomonas aeruginosa* is a leading cause of infections especially in patients with weakened immune systems. This pathogen is intrinsically resistant to many antibacterial agents.

Objectives: The aim of this study was to survey the antibiotic resistance pattern and frequency of bla OXA10 gene in Carbapenemase Producing *P. aeruginosa* isolated from patients in Isfahan hospitals.

Materials and Methods: Total of 100 clinical isolates of *P. aeruginosa* from different samples were collected. Antibiotic susceptibility for antimicrobial agents was carried out according to the clinical and laboratory standards institute (CLSI) guidelines, and the frequency of the gene encoding bla OXA10 was studied by polymerase chain reaction (PCR).

Results: The resistance rate of the isolated strains to certain antibiotics was as following: ciprofloxacin (56%), gentamicin (59%), tobramycin (61%), amikacin (65%), imipenem (55%), cefepime (55%), ceftazidime (57%), ceftriaxone (60%), cefotaxime (62%), oxacillin (100%), and piperacillin (48%), respectively. *P. aeruginosa* demonstrated the highest resistance rate to oxacillin (100%), and 55% of isolates were resistant to imipenem and cefepime, whereas 63% were multidrug-resistant (resistant to three or more classes of antibiotics). A MDR phenotype occurred frequently in *P. aeruginosa*. PCR was performed for all the resistant strains, where the frequency of blaOXA10 gene was 40 (64%).

Conclusions: Our results showed that the prevalence of MDR and extended-spectrum β lactamases producing *P. aeruginosa* in Isfahan was very high and proper infection control practices are essential to prevent the spreading of ESBL-producing *P. aeruginosa* in hospitals.

Keywords: *Pseudomonas aeruginosa*; Beta-Lactamase; Gene; Antibiotic; Resistance; Carbapenem

1. Background

Pseudomonas aeruginosa is an important pathogen that is frequently involved in infections of weakened immune system (1). *P. aeruginosa* is the most common pathogen isolated from hospitalized patients and is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. Attempts of treatment of *P. aeruginosa* from patients through intense antimicrobial therapy may lead to significant selection of resistance strains in care units of the hospitals (2).

β -lactamases are enzymes produced by bacteria that inactivate β -lactam antibiotics. Extended-spectrum β lactamases (ESBLs) have the ability to hydrolyze and cause resistance to various types of the modern β -lactam antibiotics. Many *P. aeruginosa* strains can produce different classes of ESBLs that enable organisms to resist against many cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime (3). ESBLs were discovered in Europe in

the early 1980s (4). Now, four classes are recognized, classes A, C, D, and class B or metallo- β -lactamases that need zinc for their action (3). These enzymes are encoded by chromosomal or plasmid-born genes, that are inactivated by the β -lactamases inhibitor such as clavulanate (5-7).

ESBL enzymes traditionally have been derived from TEM and SHV parent enzymes. The OXA-type ESBLs have been found mainly in *P. aeruginosa* isolates in Turkey and France (8). However, recently several classes such as A, B, and D ESBLs have been reported in *P. aeruginosa*. These beta-lactamases differ from the TEM and SHV enzymes. While most ESBLs have been found in *Escherichia coli*, *Klebsiella pneumoniae*, and other *Enterobacteriaceae*, the OXA-type ESBLs have been found mainly in *P. aeruginosa* isolates (9).

2. Objectives

This study was done to detect the frequency of OXA10

Implication for health policy/practice/research/medical education:

This study may help hospital care units understand survey of risk factors.

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Statistical Analysis SPSS version 11.5 was used to analyze the data, and a Fisher exact test was used for the categorical data. P value of ≤ 0.05 was considered significant.

4. Results

Total of 100 clinical isolates of *P. aeruginosa* were collected from different samples (February to June 2012), where 65% of the isolates were from urine, 3% were from ear mucus, 20% were from wounds, and 12% were from other samples. Using disk diffusion method, resistance rates are shown in Table 1. Our results reported the highest resistance rate to amikacin, oxacillin, cefotaxime, with 65%, 100%, and 62%, respectively; and lowest resistance rate to piperacillin, imipenem, cefepime with 48%, 55%, and 55%, respectively. In present study resistance to gentamicin 59%, tobramycin 61%, ceftazidime 57%, ceftriaxone 60%, and cefotaxime 62%, was observed. The 52 isolates were sensitive to piperacillin.

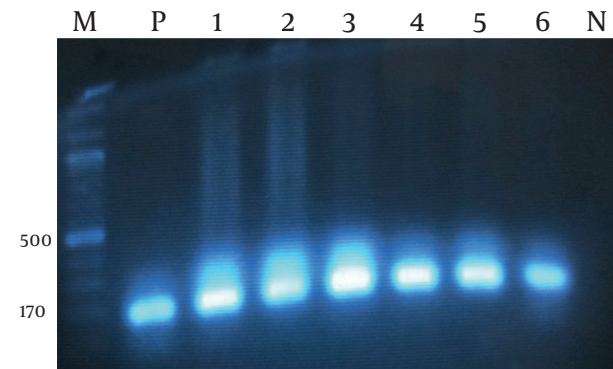
Also PCR was performed for all the resistant strains, where the frequency of bla OXA10 gene was 64% among 63 strain with multidrug resistance (Figure 2), which have been a significant increase compared to previous studies. There was 13 (36%) isolates that were resistant to three antibiotics (cefotaxime, ceftriaxone, ceftazidime), but did not carry the blaOxa10 gene. All of isolates were resistant to Oxacillin, while 40 (64%) were carriers of this gene. It could be said that in the present study, most of the urine samples with multiple resistance were producers of bla OXA10 B-lactamase.

Table 1. Pattern of Resistance to 11 Antimicrobial Agents Among *P. aeruginosa* Isolates

Antibiotics	Resistance, No (%)	Intermediate, No (%)	Sensitive, No (%)
CIP ^a	56	0	44
CTX ^a	62	0	38
CAZ ^a	57	0	43
TOB ^a	61	0	39
AN ^a	65	0	35
GM ^a	59	0	41
IPM ^a	55	6	39
FEP ^a	55	0	45
OX ^a	100	0	0
PIP ^a	48	0	52
CRO ^a	60	0	40

^a Abbreviation: CIP, Ciprofloxacin; CTX, Cefotaxime; CAZ: Ceftazidime, TOB, Tobramycin; AN, Amikacin; GM, Gentamicin; IPM, Imipenem; FEP, Cefepime; OX, Oxacillin; PIP, Piperacillin; CRO, Ceftriaxone.

Figure 2. Agarose gel Electrophoresis of PCR Product of Class D Beta-lactamase blaOXA-10 Detected From *P. aeruginosa* Isolates.



M = Molecular weight marker (100-bp), P = positive control, 1, 3 - 6 = strains producing OXA10 gene (170 bp), N = negative control

5. Discussion

The prevalence of multidrug-resistant *P. aeruginosa* producing ESBLs strains is increasing Worldwide (13). Plasmid or chromosomal mediated antibiotic resistance is common in *P. aeruginosa* isolated from patients in different hospitals in Iran (14). In our study, amikacin resistance value was higher than the values reported in the study conducted by Amutha et al (15). In the study of Prashant et al. (16), the highest resistance rate was to ceftazidime (53%) and the lowest resistance rate was to imipenem (12%). Thus, our results are different from the results in India. Strateva et al. (17), reported resistance rate to ticarcillin 53% and clavulanic acid 22%. Lee et al. (18), studied 252 isolates where 53 (21.0%) isolates carried OXA-type enzymes. Among those, blaOXA-10 was most prevalent, followed by OXA-4, OXA-2, OXA-30, and OXA-17. Six isolates (2.4%) carried two different b-lactamases and one carried three enzymes (18).

Bert et al. reported that four isolates produced ESBLs according to the double disc synergy test. PCR detecting sequences for enzymes of the OXA-10 group was positive in 68 (26.3%) isolates; where 31 isolates carried blaOXA-10, 1 isolate carried blaOXA-14, and 36 isolates carried a new variant intermediate between blaOXA-13 and blaOXA-19. The blaOXA-2 gene was identified in 13 (5%) isolates (17). Lee et al. (18), found that in 64 (25.4%) isolates, the frequency of structural genes including OXA-10 was 13.1%, OXA-4 was 4.3%, OXA-30 was 2.0%, OXA-2 was 2.3%, and OXA-17 was 0.4%; where their distribution varied between provinces. Suk et al. (19), reported that 2.9% of isolates were multidrug-resistant (MDR), where PCR amplification with specifically designed primers and direct sequencing of the

PCR products showed that the blaOXA-10, blaVIM-2, blaOXA-2, blaOXA-17, blaPER-1, blaSHV-12, and blaIMP-1 genes were carried by 34.3%, 26.9%, 3.0%, 3.0%, 1.5%, 1.5%, and 1.5% of 67 MDR *P. aeruginosa* isolates, respectively (20). The most prevalent beta-lactamase genes were blaVIM-2 and blaOXA-10 in the study of Suk et al. (19). Prevalence of blaOXA10 in the study of Shahcheraghi in Tehran was 92% in burnt patients (21).

Finally, the prevalence of MDR *P. aeruginosa* encoding blaOxa10 in Iran (64%) was five-fold higher compared with from Korea (13%), and also higher than from France (26%) and India. The prevalence of this gene in Tehran (92%) is higher than in our study, where this is probably due to the high prevalence of these resistant genes in burnt patients. According to results of present study, it can be concluded that bla OXA-10 gene has a high frequency in the *P. aeruginosa* strains isolated from hospitalized patients in Iran compared to another countries such as France in 2002, India in 2011, Korea in 2005, and China in 2006.

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Authors' Contribution

None declared.

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