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High Prevalence of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ Genes in the β-lactam-resistant Clinical Isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Background: Increased resistance to β-lactams is one of the most important considerations about *Pseudomonas aeruginosa*. This study aimed to investigate the prevalence of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes in β-lactam-resistant *P. aeruginosa*.

Materials and Methods: In this study, 100 clinical isolates were collected, and β-lactam-resistant strains were identified using the disk agar diffusion test. Bacterial DNA was extracted by the sodium dodecyl sulfate method, and the polymerase chain reaction was used to identify *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ β-lactamase genes.

Results: In total, 43 isolates were resistant to at least one of the β-lactams tested. Piperacillin-tazobactam and aztreonam were the most and least effective antibiotics, respectively. The resistance rate to a panel of β-lactams ranged from 12% to 37%. The highest rate of antibiotic resistance was related to wound and catheter specimens in burn and intensive care units. A significant relationship was observed between β-lactams resistance and the presence of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes. Among 43 resistant isolates, 100% and 83.72% carried the *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes, respectively.

Conclusion: High β-lactam resistance rates and prevalence of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ in this study indicate these enzymes' significant role in resistance to β-lactams, possibly due to overuse of these antibiotics and their easier access.

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Introduction

One of the most important problems of the present age is the emergence of *Pseudomonas aeruginosa*, which is resistant to several antibiotics and causes severe nosocomial infections [1]. This bacterium has several innate and acquired antibiotic-resistance mechanisms. It can infect the blood and urinary tract, burn wounds, skin and soft tissue, and ultimately can cause endocarditis, meningitis, and septicemia, especially in immunocompromised patients [1, 2]. Improper antibiotic use is one of the major challenges in developing bacterial resistance. As a result, *P. aeruginosa* is now resistant to the most commercially available antibiotics on the market, giving rise to the emergence of multidrug resistance (MDR) phenotype [3, 4].

The discovery of penicillin, as a β-lactam, in 1929 is known as a conjunctive point in the history of medicine [5]. Penicillins are still the most widely used and marketed class of antibiotics, proving their continued role in treating bacterial infections [6]. As with other antimicrobial classes, the widespread use of β-lactams has led to the emergence and spread of resistance among bacteria using different mechanisms [7]. Production of β-lactamases is the main mechanism of β-lactam resistance in gram-negative bacteria; however, resistance is often caused by a combination of mechanisms in the clinical setting [8]. Ambler divides β-lactamases into 4 distinct classes: A, B, C, and D [9]. Several enzymes are widely distributed in the most clinically important bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *Acinetobacter baumannii* [10].

The OXA enzymes from Ambler class D are among the most diverse β-lactamases with good activity against penicillins, cephalosporins, and carbapenems [11]. Many enzymes are chromosomal, but some class D cephalosporinases in *P. aeruginosa* and class D carbapenemases in *A. baumannii* and *Enterobacteriaceae* are carried by plasmids, indicating the clinical importance of this class [10]. Among the class D β-lactamases-degrading cephalosporins, the OXA-2 group (OXA-15, -32, -34, -36, -141, and -161) and the OXA-10 group (OXA-11, -13, -14, -16, -17, -19, -28, -129, -142, -145, -147, and -183) have a higher prevalence and are more clinically important [1]. These genes can be transferred between different gram-negative bacteria through integrons and plasmids [12]. OXA-2 β-lactamases are found in *P. aeruginosa* and were thought to be narrow-spectrum enzymes with a carbapenemase activity [13]. OXA-10 enzymes have also been reported in *P. aeruginosa*, *A. baumannii*, and *Enterobacteriaceae* and can increase the minimum inhibitory concentration (MIC) of the broad-spectrum cephalosporins, aztreonam, penicillins, and carbapenems [13]. Class D enzymes have significant role in antibiotic resistance of gram-negative bacteria in clinic, and we must study them more. Although many studies have been performed on these enzymes, there is an urgent need for further studies on how these enzymes produce resistance to clinically important β-lactams in important bacteria such as *P. aeruginosa*. Therefore, we decided to evaluate the prevalence of bla_{OXA-2} and bla_{OXA-10} genes in clinical isolates of *P. aeruginosa*.

Materials and Methods

Samples and bacterial identification

A total of 100 non-repetitive *P. aeruginosa* isolates were collected from various clinical specimens, including sputum, urine, wound, catheter, blood, stool, and eye secretion. The specimens were collected from patients hospitalized in educational and therapeutic hospitals of Mazandaran Province, north of Iran, from 2018 to 2019. All patients in this study were hospitalized and received different antibiotics during hospitalization and before sampling. The isolates were identified by microbiological and biochemical tests [14]. Then, the bacteria were cultured in trypticase soy broth (TSB) (Merck, Germany) with 10% glycerol and were frozen at –20°C until use.

Antimicrobial susceptibility testing

The antibiotic resistance pattern of all clinical isolates was determined using the disk agar diffusion method. This test was conducted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [15] against 8 antibiotics: Piperacillin (10 µg), piperacillin-tazobactam (10-110 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), and doripenem (10 µg) (MAST, UK). The isolates were reported as resistant, intermediate resistant, or susceptible after 18 h incubation at 37°C. In antimicrobial susceptibility testing, *P. aeruginosa* ATCC 27853 was used as a control strain.

Genomic DNA extraction

The genomic DNAs of the *P. aeruginosa* clinical isolates were extracted by alkaline lysis method using sodium dodecyl sulfate (SDS) and NaOH [16]. We dissolved 0.5 g of SDS (Sigma, Germany) and 0.4 g of NaOH (Sigma) in 200 µL of sterile distilled water to prepare the extraction solution. Then, 4-6 pure colonies of the bacteria

were dissolved in 20 µL of this solution in a 1.5 mL sterile microtube. The microtubes were placed at 95°C for 10 min and then were centrifuged for 3 min at 13000×g. Finally, 180 µL of sterile distilled water was added to the microtubes, and the supernatant was considered the extracted DNA. We measured the supernatant's OD (optical density) using a NanoDrop (ND1000, USA). Also, we electrophoresed the product on 1.5% agarose gel (Wizbiosolutions, South Korea) to ensure the correct genomic DNA extraction. Finally, the extracted DNA was stored in a freezer at –20°C.

Detection of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes using PCR

We used the specific primers (Metabion, Germany), including OXA-2-forward-5'-AAGAAACGCTACTCGCCTGC-3' and reverse-5'-CCACTCAACCCATCCTACCC-3' for detection of *bla*$_{OXA-2}$ resistance gene with a product size of 485 bp, and OXA-10-forward-5'-TATCGCGTGTCTTTCGAGTA-3' and reverse-5'-TTAGCCACCAATGATGCC-3' for detection of *bla*$_{OXA-10}$ resistance gene with a product size of 774 bp by PCR [17]. The PCR reaction was performed in a final volume of 15 µL containing 7.5 µL of PCR master mix (Ampliqon, Denmark), 5 pmol of each primer, 5.5 µL of distilled water, and 1 µL (300 ng) of DNA. The initial denaturation step was performed for 5 min at 94°C. Then, 34 cycles, including denaturation at 94°C for 20 s, annealing step at 60°C for *bla*$_{OXA-10}$ and 63°C for *bla*$_{OXA-2}$ for 20 s, and extension at 72°C for 25 s were performed. Finally, the last extension step was used at 72°C for 10 min. The polymerase chain reaction (PCR) product was electrophoresed on 1% agarose gel (Wizbiosolutiotions) with 1.5 µL of Safe Stain (SinaClon, Iran).

Statistical analysis

The data obtained were imported into SPSS software, version 22. After examining the normality of the variables, the desired results were sorted out by descriptive tests, frequency, Mean±SD, and statistically analyzed using the Pearson chi-square test. The P<0.05 was considered statistically significant.

Results

Clinical data and bacterial isolates

The study included 100 non-recurrent *P. aeruginosa* isolates collected from 100 hospitalized patients. These isolates were collected from 5 educational and treatment hospitals in Mazandaran Province, Iran, as follows: Imam Khomeini (40 isolates), Razi (22 isolates), Bu-Ali Sina (17 isolates), Zare (11 isolates), and Fatemeh Al-Zahra (10 isolates). Of 100 clinical isolates, 60 were obtained from men and 40 from women. The mean age of patients was 46 years (women=47.85 and men=44.76). The bacterial strains were isolated from the following clinical specimens: Respiratory samples (37 isolates), wounds (20 isolates), urine (26 isolates), blood (5 isolates), stool (2 isolates), ocular discharge (2 isolates), and catheters (8 isolates). Patients were hospitalized in intensive care unit (ICU) (53), burn (6), internal (4), operating room and surgery (6), men (3), emergency (13), women (2), oncology (1), cardiac care unit (5), neurology (2), and pediatric (5).

Antibiotic susceptibility pattern of the isolates

Table 1 lists the antibiotic susceptibility pattern of 100 *P. aeruginosa* clinical isolates against the 8 antibiotics tested. The highest resistance was observed against aztreonam, while piperacillin-tazobactam was the most effective antibiotic in this study. The resistance of the bacteria to all tested antibiotics was at least 12%, while 4% of the isolates were resistant to all tested antibiotics.

Moreover, Table 2 presents the antibiotic susceptibility pattern of *P. aeruginosa* clinical isolates regarding the clinical sample type. Accordingly, the highest resistance rate (more than 20%) was related to the bacteria isolated from the wound samples.

Identification of the antibiotic resistance genes by PCR

In the present study, 43% of *P. aeruginosa* clinical isolates carried the *bla*$_{OXA-2}$ gene (Figure 1), and 36% contained the *bla*$_{OXA-10}$ gene (Figure 2). Table 3 presents the number of β-lactam-resistant isolates containing the studied genes. This study observed a significant relationship between *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes and resistance to β-lactam antibiotics (P<0.05). However, all 43 clinical isolates resistant to at least one of the tested β-lactams also carried the *bla*$_{OXA-2}$ gene. Moreover, 7 isolates (16.27%) among these 43 resistant isolates had no *bla*$_{OXA-10}$ gene, while 3, 2, and 2 isolates were moderately resistant, resistant, and sensitive to piperacillin, respectively. Of these 7 isolates, 5, 1, and 1 isolate(s) were sensitive, resistant, and intermediate resistant against piperacillin-tazobactam, respectively. Also, among these isolates without the *bla*$_{OXA-10}$ gene, 4, 4, and 3 were resistant to imipenem, meropenem, and doripenem, respectively, while the rest were either sensitive or intermediate resistant. Also, two isolates lacking this gene were sensitive to ceftazidime and cefepime, and the other 5 isolates

Table 1. Antibiotic susceptibility pattern of 100 *P. aeruginos*a clinical isolates against 8 tested antibiotics

Antibiotics	Resistant	Intermediate Resistant	Susceptible
Piperacillin	26	21	53
Piperacillin-tazobactam	12	11	77
Aztreonam	39	33	28
Ceftazidime	27	2	71
Cefepime	31	5	64
Imipenem	31	8	61
Meropenem	37	5	58
Doripenem	28	11	61

were resistant to these two antibiotics. Both bla_{OXA-2} and bla_{OXA-10} genes in the clinical isolates of *P. aeruginosa* were significantly associated with resistance to all beta-lactams tested in this study ($P<0.05$).

Also, the relationship between the presence of bla_{OXA-2} and bla_{OXA-10} genes and the type of clinical sample in this study showed that the highest presence of these genes was observed in strains that were isolated from the catheter, wound, and blood samples (Table 4).

Discussion

P. aeruginosa is an opportunistic pathogen causing almost 10% of all nosocomial infections worldwide [18, 19]. The spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *P. aeruginosa* has an important effect on the rate of morbidity and mortality, length of hospital stay, and treatment costs of patients, especially in intensive care units [19]. Incidentally, in our study, most of the collected *P. aeruginosa* isolates (n=53) belonged to adult (49 isolates), burn (3 isolates), and neonatal (1 isolate) intensive care units. Increased antibiotic resistance of this bacterium, especially against carbapenems, aminoglycosides, and fluoroquinolones, has contributed to the emergence of MDR/XDR strains of *P. aeruginosa*, resulting in problems in treating infections [20]. By simultaneously inactivating transpeptidases and activating autolysin, β-lactams disrupt bacterial cell wall synthesis, ultimately leading to cell lysis and bacterial killing [21]. Penicillins (ticarcillin and piperacillin), and the combination of penicillin with

Table 2. Number and percentage of antibiotic-resistant *P. aeruginosa* clinical isolates considering the sample type

Antibiotics	Samples No. (%)						
	Urine (n=26)	Respiratory (n=37)	Wound (n=20)	The Catheter (n=8)	Blood (n=5)	Stool (n=2)	Eye (n=2)
Piperacillin	6(23.07)	7(18.91)	8(40)	2(25)	2(40)	0	1(50)
Piperacillin-tazobactam	2(7.69)	3(8.1)	4(20)	2(25)	1(20)	0	0
Aztreonam	7(26.92)	12(32.43)	12(60)	5(62.5)	2(40)	0	1(50)
Ceftazidime	6(23.07)	8(21.62)	7(35)	5(62.5)	0	0	1(50)
Cefepime	4(15.38)	9(24.32)	12(60)	5(62.5)	1(20)	0	1(50)
Imipenem	5(19.23)	10(27.02)	10(50)	3(37.5)	2(40)	0	1(50)
Meropenem	4(15.38)	13(35.13)	12(60)	5(62.5)	2(40)	0	1(50)
Doripenem	4(15.38)	7(18.91)	9(45)	5(62.5)	2(40)	0	1(50)

Table 3. Relationship between the presence of *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ genes in clinical isolates of *P. aeruginosa* and resistance to β-lactams

Antibiotics and Antibiotic Resistance Pattern, No.		No. (%)			
		bla$_{OXA-2}$		*bla*$_{OXA-10}$	
		Positive	Negative	Positive	Negative
Piperacillin	R, 26	26(100)	-	23(88.46)	3(11.53)
	I, 21	13(61.9)	8(38.09)	10(47.61)	11(52.38)
	S, 53	4(7.54)	49(92.45)	2(3.77)	51(96.22)
Piperacillin-tazobactam	R, 12	12(100)	-	11(91.66)	1(8.33)
	I, 11	11(100)	-	9(81.81)	2(18.18)
	S, 77	20(25.97)	57(74.02)	15(19.48)	62(80.51)
Aztreonam	R, 39	39(100)	-	32(82.05)	7(17.94)
	I, 33	4(12.12)	29(87.87)	3(9.09)	30(90.9)
	S, 28	-	28(100)	-	28(100)
Ceftazidime	R, 27	27(100)	-	21(77.77)	6(22.22)
	I, 2	2(100)	-	2(100)	-
	S, 71	14(19.71)	57(80.28)	12(16.9)	59(83.09)
Cefepime	R, 31	31(100)	-	25(80.64)	6(19.35)
	I, 5	5(100)	-	5(100)	-
	S, 64	7(10.93)	57(89.06)	5(7.81)	59(92.18)
Imipenem	R, 31	23(74.19)	8(25.8)	17(54.83)	14(45.16)
	I, 8	4(50)	4(50)	4(50)	4(50)
	S, 61	16(26.22)	45(73.77)	14(22.95)	47(77.04)
Meropenem	R, 37	30(81.08)	7(18.91)	24(64.86)	13(35.13)
	I, 5	3(60)	2(40)	2(40)	3(60)
	S, 58	10(17.24)	48(82.75)	9(15.51)	49(84.48)
Doripenem	R, 28	25(89.28)	3(10.71)	21(75)	7(25)
	I, 11	6(54.54)	5(45.45)	3(27.27)	8(72.72)
	S, 61	12(19.67)	49(80.32)	11(18.03)	50(81.96)

Abbreviations: R: Resistant; I: Intermediate resistant; S: Susceptible.

β-lactam inhibitors (piperacillin-tazobactam), as well as monobactams (aztreonam), and carbapenems (imipenem, meropenem, and doripenem) are effective on *P. aeruginosa* [22]. Chromosomal and plasmid-encoded β-lactamase production are common mechanisms in resistance to β-lactams in *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* [21]. Many β-lactamases are known as extended-spectrum β-lactamases (ESBLs), while OXA-type ESBLs are significantly increasing and have been reported mainly in *P. aeruginosa* [23]. On the other hand, most OXA-type ESBLs are derivatives of OXA-2 and OXA-10, while these enzymes can develop

Table 4. Relationship between the presence of bla_{OXA-2} and bla_{OXA-10} genes in *P. aeruginosa* isolates and the type of clinical sample

PCR Results	bla_{OXA-2}			bla_{OXA-10}		
	No. (%)		P	No. (%)		P
	Positive	Negative		Positive	Negative	
Urine (n=26)	8 (30.76)	18 (69.23)	NS	6 (23.07)	20 (76.92)	NS
Respiratory (n=37)	12 (32.43)	25 (67.56)	NS	8 (21.62)	29 (78.37)	NS
Wound (n=20)	13 (65)	7 (35)	NS	12 (60)	8 (40)	NS
The catheter (n=8)	7 (87.5)	1 (12.5)	0.03	7 (87.5)	1 (12.5)	0.03
Blood (n=5)	2 (40)	3 (60)	NS	2 (40)	3 (60)	NS
Stool (n=2)	-	2 (100)	NS	-	2 (100)	NS
Eye (n=2)	1 (50)	1 (50)	NS	-	2 (100)	NS

NS: Non-statistically significant.

resistance to ceftazidime, cefotaxime, ceftriaxone, and other beta-lactams [24]. In our study, 43 isolates were resistant to at least one of the β-lactams studied. However, all of them (100%) carried the bla_{OXA-2} gene, and 36 (83.72%) had the bla_{OXA-10} gene. There was a statistically significant relationship (P<0.05) between the presence of these two genes and resistance to all beta-lactams tested in this study. Even in the case of piperacillin-tazobactam-resistant isolates, 100% and 91.66% of them contained bla_{OXA-2} and bla_{OXA-10} genes, respectively, which could indicate the ineffectiveness of β-lactamase inhibitors such as tazobactam on these enzymes [24]. Similar efficacy was observed for aztreonam, whereas for carbapenems, despite the statistical significance of the presence of these genes in resistant strains, they appeared to be less effective than other β-lactams tested. In a study conducted in Shiraz City, Iran, the prevalence of the bla_{OXA-10} gene in ESBL-positive *P. aeruginosa* isolates was 33.3%, which shows a significant difference from our study [24]. The type of antibiotics used can be the reason for this difference between the two regions. However, the prevalence of TEM and SHV genes in the mentioned study was 90.4% and 52.4%, respectively, indicating the role of other β-lactamase genes in developing resistance to β-lactams in their isolates. In other studies conducted in 2010 and 2011 on burn wound isolates of *P. aeruginosa* in Tehran City, Iran, the prevalence of the bla_{OXA-10} gene was 76.42% and 70%, respectively [25, 26]. However, like our study, the study of Mirsalehian et al. [25] found a statistically significant relationship between the presence of this gene and resistance to aztreonam, ceftazidime, and cefepime, but not with carbapenems in contrast with our study.

In studies conducted in two neighboring countries (Turkey and Saudi Arabia), the prevalence of the bla_{OXA-10} gene has been reported to be 11% and 56%, respectively [27, 28]. The study of Saudi Arabia on *P. aeruginosa* burn isolates showed a 40% correlation between the presence of the bla_{OXA-10} gene and resistance to ceftazidime, while in our study, 77.77% of the resistant isolates and both intermediate resistant isolates were carrying this gene. Because the OXA β-lactamases can develop resistance to carboxypenicillins, ureidopenicillins, ceftazidime, cefotaxime, cefepime, and aztreonam [28], we also found a statistically significant relationship with the presence of both bla_{OXA-2} and bla_{OXA-10} genes and resistance to piperacillin, ceftazidime, cefepime, and aztreonam. In another study published in Taiwan, the prevalence of bla_{OXA-10} gene-carrying isolates of *P. aeruginosa* was reported to be 19.04%. In comparison, 14.28% of their isolates carried the *OXA-142* gene, a derivative of the bla_{OXA-10} gene [29].

In a study conducted in Iran, 92 (87.61%) and 5 (4.76%) out of 105 isolates collected from Ilam City [24], which were phenotypically positive, were carrying bla_{OXA-10} and bla_{OXA-2} genes, respectively, which differed from the results of our study. Also, among 120 isolates collected from Kerman City, Iran hospitals, 46 isolates were positive in terms of phenotypic test, and among them, 29 (63%) and 1 (2.1%) isolate(s) contained bla_{OXA-10} and bla_{OXA-2} genes, respectively [24]. This difference in results indicates the significant role of antibiotic consumption in the country since 2010, while in recent years, the use of beta-lactams to treat infections caused by *P. aeruginosa* has increased significantly. Another study published in Iran investigated the presence

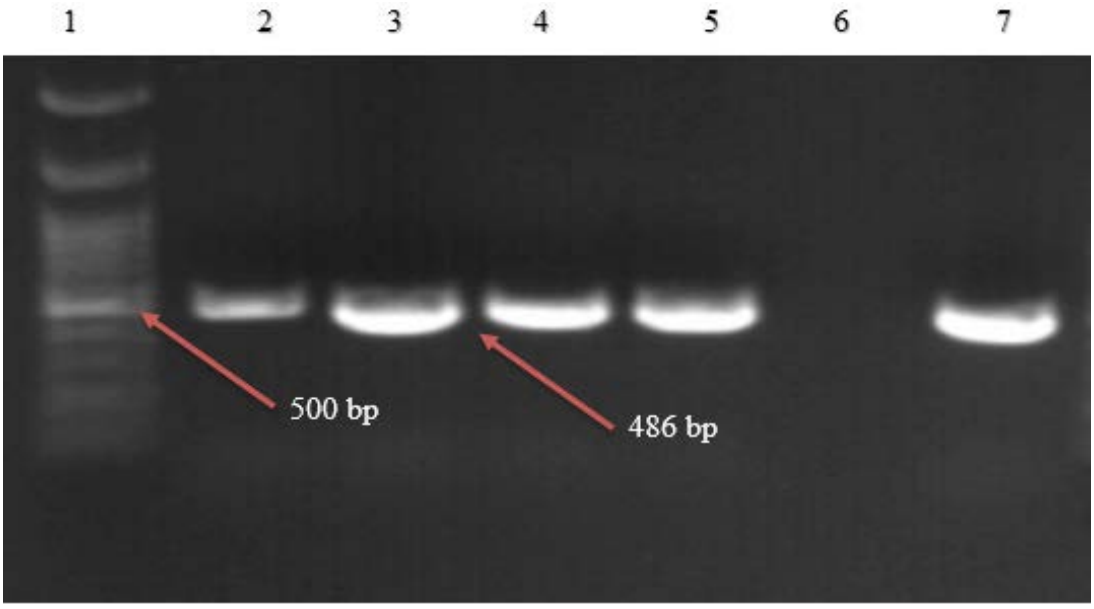

Figure 1. PCR product electrophoresis for *bla*$_{OXA-2}$ gene

Line 1: 100 bp plus DNA ladder; Lines 2, 3, 4, and 5: Clinical isolates carrying *bla*$_{OXA-2}$ gene in this study with 486 bp fragment length; Line 6: Negative control (master mix without primer and DNA); Line 7: Positive control strain.

of β-lactamase genes in 369 *P. aeruginosa* isolates collected from burn and public hospitals in Ahvaz, Isfahan, and Tehran cities, Iran. Among them, 236 (63.9%) were carbapenem-resistant isolates, and among carbapenem-resistant isolates, 32.2% of them carried the *bla*$_{OXA-10}$ gene [30]. In the mentioned study, as in our research, piperacillin-tazobactam was the most effective antibiotic against carbapenem-resistant strains carrying beta-lactamase genes.

Also, in another study in Egypt, out of 122 *P. aeruginosa* isolates, 41.7% of the isolates carried the *bla*$_{OXA-10}$ gene, while none had the *bla*$_{OXA-2}$ gene [31]. The lower rate of antibiotic resistance tested in the mentioned study compared to our study could be the reason for the lower

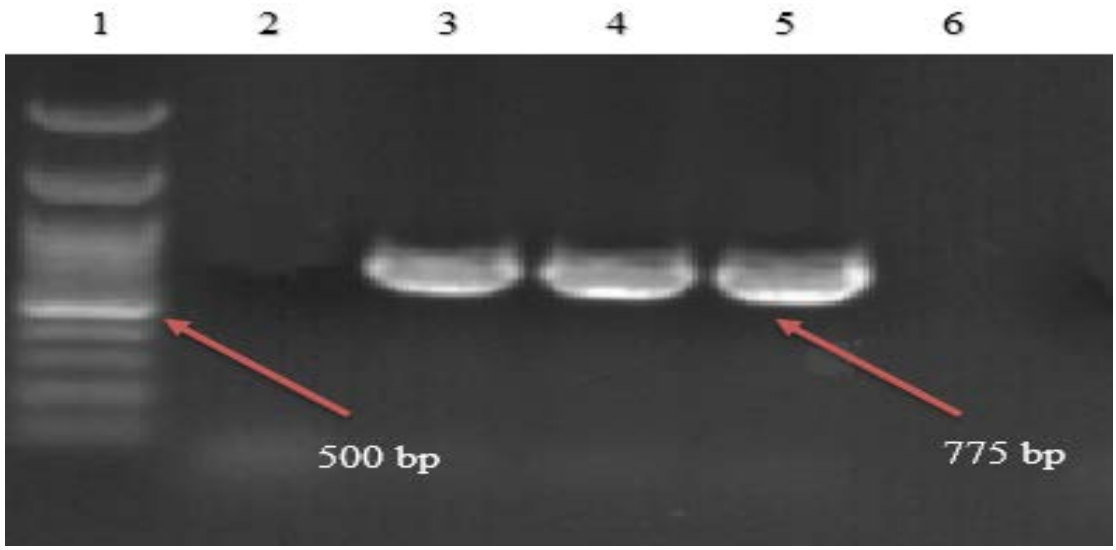

Figure 2. PCR product for *bla*$_{OXA-10}$ gene

Line 1: 100 bp plus DNA ladder; Line 2: Negative control (master mix without primer and DNA); Line 3: Positive control strain; Lines 4 and 5: Clinical isolates carrying the *bla*$_{OXA-10}$ gene in this study with a fragment length of 775 bp; Line 6: Strain without *bla*$_{OXA-10}$ gene.

prevalence of the genes. Also, in an Iranian study on 273 *P. aeruginosa* isolates collected from Qazvin hospitals, Qazvin City, Iran, from January 2014 to October 2015, none of the ESBL-positive isolates carried the bla_{OXA-10} and bla_{OXA-2} genes [32]. These results indicate that the spread of antibiotic resistance genes in different parts of the world and even within a country can be different and should be selected and managed based on the prevalence of antibiotic consumption policies. On the other hand, another study from Iran in 2017 showed that the bla_{OXA-10} gene was present in antibiotic resistance gene cassettes in 45.4% of isolates carrying class 1 integrons [33]. This result reflects that these genes can be transferred between bacteria through motile genetic elements, giving them antibiotic-resistance properties. Another study was performed in Poland on 900 clinical isolates of *P. aeruginosa*, while among 110 ESBL-positive isolates, 16 carried bla_{OXA-2} subtype genes, and 6 isolates contained bla_{OXA-10} subtype genes [34]. Also, in another study on 241 clinical isolates of *P. aeruginosa* collected in India from 2011 to 2012, 11 isolates (4.5%) carried one of the subtypes of the bla_{OXA-10} gene, while the bla_{OXA-2} gene was detected only in one isolate [12]. An Iranian study published in 2022 showed that 11.9% of their *P. aeruginosa* isolates contained $bla_{OXA-224}$ and $bla_{OXA-539}$, which belong to the OXA-2 group of beta-lactamases [35]. Another study conducted in Qatar exhibited that 72 isolates (96%) of 75 multidrug-resistant *P. aeruginosa* isolates contained a class D β-lactamase, from which 18 isolates (24%) were carrying the bla_{OXA-10} gene [36].

One of the most important limitations of this study was the lack of financial resources and the small sample size for a prevalence study.

Conclusion

P. aeruginosa has significant potential to develop or acquire antibiotic resistance in any geographical area by employing various mechanisms. It should be noted that among the factors that can reduce disease and mortality in invasive infections caused by this bacterium, the first important factor is the choice of an appropriate antibiotic. Therefore, timely identification and definitive treatment with a proper antibiotic can help prevent the spread of resistant strains and reduce the costs of long-term hospitalization and mortality in patients. Based on the results of this study and its comparison with similar studies, it is argued that, over time, the prevalence, diversity, and number of resistance genes carried by *P. aeruginosa* is increasing. The prevalence of OXA class resistance genes is geographically very diverse. Therefore, it is necessary to determine the prevalence in each country and geographical area separately. The diversity of these genes indicates a fundamental change in the treatment process and the use of antibiotics. The high prevalence of bla_{OXA-2} and bla_{OXA-10} genes in this study and similar studies suggest that using β-lactam family antibiotics requires more principled and well-planned measures.

Ethical Considerations

Compliance with ethical guidelines

This study was performed following the Declaration of Helsinki; however, the patients or a close relative delivered a printed informed agreement form, and categorizing data of each sample was kept secret. This study was approved by the Iran National Committee for Ethics in Biomedical Research (Code: IR.MAZUMS.REC.1398.075).

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Authors contribution's

Conceptualization, project administration, supervision, and validation: Hamid Reza Goli; Methodology: Hamid Reza Goli and Fatemeh Bazari Jamkhaneh; Formal analysis: Fatemeh Bazari Jamkhaneh and Mina Owrang; Software: Hamid Reza Goli and Mina Owrangh;Writing the original draft: Fatemeh Bazari Jamkhaneh; Investigation, data curation, data visualization, writing, review, and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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