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## RESEARCH ARTICLE

### Antibiotic Resistance Pattern and Frequency of PER-1, SHV-1 and AMPC Type B-Lactamase Genes in *Pseudomonas aeruginosa* Isolated from Clinical Samples

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#### Abstract:

##### Background:

The existence of Extended Spectrum B-lactamase (ESBL) genes plays an important role in spreading B-lactam antibiotic resistance in the producing strains of these enzymes. The resistance of gram-negative bacteria, such as *Pseudomonas aeruginosa*, to different antimicrobial agents, especially B-lactams, has increasingly been reported.

##### Objective:

This study was conducted to determine the prevalence of TEM-1 and VEB-1 beta-lactamases gene in *P. aeruginosa* isolates through Polymerase Chain Reaction (PCR) method.

##### Methods:

100 clinical isolates of *P. aeruginosa* were collected from different clinical samples. The antibiotic susceptibility was examined by the disc diffusion method. The presence of PER-1, SHV-1 and AMPC genes was detected by PCR method.

##### Results:

Out of the studied *P. aeruginosa* isolates, 7, 9 and 37 isolates were positive for PER-1, SHV-1 and AMPC B-lactamases resistance genes, respectively. Patients with urinary infection had the most resistant isolates. All isolates (100%) were sensitive to polymyxin B.

##### Conclusion:

Antibiotic resistance in isolates of *Pseudomonas* can be caused by B-lactamases resistance genes. Noticing the increasing rate of the ESBLs producing strains, using the appropriate treatment protocol based on the antibiogram pattern of the strains is highly recommended.

**Keywords:** *Pseudomonas aeruginosa*, B-Lactamase, PER-1 gene, SHV-1 gene, AMPC gene, Antibiotic resistance, Polymerase chain reaction.

#### Article History

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## 1. INTRODUCTION

*Pseudomonas aeruginosa* is a remarkably adaptive bacterial pathogen which can cause persistent infections in burn patients, immune-compromised patients, and individuals with the genetic disease cystic fibrosis. It is one of the most prevalent nosocomial pathogens, and the infections caused by *P. aeruginosa* can be very serious and life-threatening [1]. *P. aeruginosa* is responsible for 9% of all healthcare-associated

infections, and the resistance to *P. aeruginosa* is increasing [2, 3]. One of the most prominent attributes of these strains is their resistance to multiple clinically important antibiotics like the third generation of cephalosporins, imipenem and aztreonam. A great number of *P. aeruginosa* strains generate various classes of Extended Spectrum  $\beta$ -Lactamases (ESBLs) which allow the bacterium to tolerate against extended spectrum cephalosporins, such as cefotaxime, ceftriaxone and ceftazidime and they have been reported with a developing frequency [4].

Beta-lactam antimicrobial agents exhibit the most common treatment for bacterial infections and continue to be the

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prominent cause of resistance to b-lactam antibiotics among Gram-negative bacteria worldwide. The persistent exposure of bacterial strains to a multitude of b-lactams has induced dynamic and continuous production and mutation of b-lactamases in these bacteria, expanding their activity even against the newly developed b-lactam antibiotics [5, 6].

Resistance to ESBLs in *P. aeruginosa* is associated in most cases with the overproduction of AmpC [7]. However, a growing number of Ambler class A ESBLs, class B metallo-b-lactamases (MBLs), and class D extended spectrum oxacillinases (ES-OXAs) have been reported in *P. aeruginosa* clinical isolates [8]. Class A ESBLs reported in *P. aeruginosa* include PER, SHV, TEM, VEB, BEL, GES and CTX-M type [9]. PER-1 was first identified in a *P. aeruginosa* clinical isolate recovered from a Turkish patient in France, in 1991 [10]. Later, PER-1 was found in European and Asian countries [11].

AmpC contributes to the natural resistance of the microorganism toward labile and inducing molecules, such as aminopenicillins, first and second-generation cephalosporins [12]. More importantly, when overproduced as a result of mutations altering the peptidoglycan recycling process, AmpC becomes a major cause of resistance to widely used anti-pseudomonal penicillins (ticarcillin and piperacillin), monobactams (aztreonam), and third-generation (ceftazidime) and fourth-generation (cefepime) cephalosporins [13].

The purpose of this study was to investigate the frequency of PER-1, SHV-1 and AMPC type B-Lactamase genes in isolated *P. aeruginosa* strains from clinical samples in Tabriz hospitals, Iran

## 2. MATERIALS AND METHODS

### 2.1. Bacterial Isolates

In this descriptive study, 100 isolates of *P. aeruginosa* were isolated from clinical specimens (burn, urinary tract infection, ulcers, lung secretions and eyes) in Asadabadi hospital in Tabriz in 2018. The standard tests, including oxidase, catalase, oxidation/fermentation (OF), arginine dehydrogenase, pigment production in the Muller Hinton Agar medium and growth ability at 42°C were performed to confirm the *P. aeruginosa* isolates. Then, obtained isolates stored in the BHI medium containing 18% glycerol for further experiments. Ethical approval to perform the study was obtained from the institutional review board of Tabriz University of Medical Sciences. Written informed consent was obtained from all patients included in the study.

### 2.2. Antimicrobial Susceptibility Testing

Antibiotic resistance of isolated *P. aeruginosa* was investigated using a disc diffusion (Kirby-Bauer) method based on CLSI (Clinical and Laboratory Standard Institute) standards. The antimicrobial discs were as following: Polymyxin B, Gentamicin, Tobramycin, Amikacin, Ceftazidime, Cipro-floxacin, Piperacillin, Piperacillin-Tazobactam and Tricaricin. *P. aeruginosa* ATCC 27853 was used as the control strain for susceptibility testing [14,15].

### 2.3. Molecular Detection

Extraction kit (Jena Bioscience) was used to extract DNA from isolated *P. aeruginosa*. The quantity and quality of extracted DNA were investigated nanodrop instrument and electrophoresis on 1% agarose gel, respectively. The sequence of primers for the detection of PER-1, SHV-1 and AMPC genes was selected from references and synthesized with Sina Clone Company (Table 1), [16 - 18]. The polymerase chain reaction (PCR) was carried out to detect the PER-1, SHV-1 and AMPC genes. For this purpose, the Amplicon kit (Pishgam Co.) was used according to the manufacturer's protocol. The master mix of PCR reaction with a final volume of 25µl was prepared (2µl of MgCl<sub>2</sub>, 2.5µl PCR buffer, 0.5µl dNTPs, 17.3µl distilled water, 1µl forward primer, 1µl reverse primer, 0.1µl Taq polymerase). Finally, 1µl of DNA template was added to each micro tubes. The temperature and cycle's program was as following: 1 cycle initial denaturation (95°C for 3 min), 35 cycle denaturation (93°C for 45 sec), 35 cycle annealing (30 sec), 35 cycle extension (72°C for 25 seconds) and 1 cycle final extension (72°C for 1 min). The PCR products were electrophoresed by agarose gel 1% containing ethidium bromide for one hour. The products were examined by a gel doc instrument [15].

## 3. RESULTS

In this study, 100 samples of *P. aeruginosa* were isolated from different clinical specimens. Antibiogram tests showed that the highest resistance to the antibiotic was related to Piperacillin (88%), Ceftazidime (76%) and Amikacin (71%). The results of antibiogram tests are shown in Table 2.

The obtained results showed that among 100 tested isolates, 18 (18%) cases had PER-1 gene, 21 (21%) isolates had SHV-1 gene, and 61 cases (61%) had AMPC gene. The results showed that 17 (17%) isolated strains had two resistance genes at the same time. Also, 1 (1%) isolated strain had three resistance genes at the same time.

Results showed that the highest and lowest isolated positive samples related to patients with urinary tract infection and patients with eye infection, respectively. The frequency of PER-1, SHV-1 and AMPC genes based on the type of clinical specimen is shown in Table 3.

## 4. DISCUSSION

The identification of antibiotic-resistant strains in hospitals seems necessary to prevent the release of genes resistant [19]. Today, due to the high prevalence of antibiotic-resistant bacteria, controlling infectious diseases often fail [20]. This may be due to a lack of sufficient information about dominant flora of hospitals and the origin of antibiotic resistance in hospitalized patients. Therefore, the study of antibiotic resistance in hospital strains gives us a clear view of the challenges [21, 22]. Determination of antibiotic resistance of *P. aeruginosa* in the present study showed that resistance to ciprofloxacin is increasing, while a high sensitivity was observed to Polymyxin B.

**Table 1. The sequence and characteristics of used primers.**

References	Annealing Temperature	Products	Sequence	Gene
16	46°C	933bp	ATGAATGTCATTATAAAAAGC	Bla <sup>PER-1</sup>
			AATTTGGGCTTAGGGCAGAA	
17	57°C	576bp	CTGCTCCACCAGGGCTAAAA	Bla <sup>SHV-1</sup>
			ATGAACGCGCAAATAGCGAC	
18	57°C	150bp	CTCGACCTCGCGACCTATAC	Bla <sup>AMPC</sup>
			GATGCTCGGGTTGGAATAGA	

**Table 2. Antibiotic resistance pattern of isolates of *P. aeruginosa* isolated from clinical samples.**

<i>P. aeruginosa</i>			Dose (µg)	Antibiotics
Semi-sensitive (%)	Resistant (%)	Sensitive (%)		
0	32	67	30	Polymyxin B
0	71	29	30	Amikacin
0	63	37	10	Tobramycin
0	69	31	5	Ciprofloxacin
0	65	35	10	Gentamicin
0	76	24	30	Ceftazidime
0	88	12	100	Piperacillin
0	69	31	100/10	Piperacillin/Tazobactam
0	63	37	75	Ticarcillin

**Table 3. Frequency of PER-1, SHV-1 and AMPC genes in isolates of *P. aeruginosa* isolated from clinical samples.**

Lung secretions	Eye	Wound	Urine	Burn	Gene
13%	7%	18%	31%	18%	PER-1
11%	11%	12%	23%	17%	SHV-1
42%	7%	12%	67%	53%	AMPC
8%	0%	2%	4%	4%	PER-1 and SHV-1
12%	0%	3%	5%	12%	PER-1 and AMPC
7%	0%	3%	6%	15%	SHV-1 and AMPC
0%	0%	0%	0%	0%	PER-1 and SHV-1 and AMPC

In a study conducted by Ranjbar *et al.* (2011) in Tehran hospitals, the resistance to amikacin and imipenem was reported 97.5% and 90%, respectively [23]. In a study by Rajaie *et al.* (2015) showed that the resistance to amikacin, tobramycin, ciprofloxacin, gentamicin and ceftazidime was 92%, 88%, 88%, 96% and 96%, respectively [24]. In the study by Fazeli *et al.* (2013) The rate of resistance to amikacin, gentamicin, ciprofloxacin was 60, 50, 65%, respectively [25]. In another study by Adabi *et al.* (2015) antibiotic resistant pattern to amikacin, ciprofloxacin, gentamicin and amikacin was reported 86%, 87%, 88% and 96%, respectively [26]. In the present study, 67% of the studied samples were sensitive to polymyxin B, which Rostampour *et al.* (2015) reported similar results [27]. Our results showed that most of the isolated *P. aeruginosa* was resistance to the ceftazidime, ciprofloxacin, amikacin, tobramycin, gentamicin, piperacillin, piperacillin-tazobactam and ticarcillin. Generally, with a glimpse of previous studies, it can be concluded that the resistance of *P. aeruginosa* strains to different antibiotics is relatively high. On the other hand, resistance patterns are constantly

changing, which should be considered. According to our results, the polymyxin B is currently the best choice for antibiotic therapy of *P. aeruginosa* infections.

In this study, the presence of PER-1, SHV-1, and AMPC antibiotic resistance genes was investigated by PCR method. The enzymes produced by these genes are known as extended spectrum  $\beta$ -lactamase, which inhibits the function of antibiotics. The PER-1 Beta-lactamase was first reported in 1991 and is able to hydrolyze penicillins and cephalosporins. The PER-1 enzyme has been found on bacterial chromosome and plasmid [28]. The SHV-1 enzyme is also an extended spectrum  $\beta$ -lactamase, which is able to destroy extended spectrum cephalosporins, such as cefotaxime, ceftriaxone and ceftazidime [29]. In the present study, all strains with SHV-1 enzyme showed a resistance to ceftazidime, which can be attributed to the presence of this enzyme. Another resistance mechanism in gram-negative bacteria is the production of chromosomal cephalosporins C, such as AMPC  $\beta$ -lactamases. Today, isolates that produce both AMPC and ESBLs are increasing, which has a high resistance to antimicrobial agents

[30]. These enzymes can hydrolyze penicillins, cephalosporins, cefamixins, and beta-lactamase inhibitors, and have only a low affinity for carbapenem and cephalosporin [31]. In the present study, high resistance to penicillin and cephalosporin antibiotics can be due to the expression of PER-1 and AMPC genes in the studied isolates.

## CONCLUSION

Due to the increased antibiotic resistance in clinical specimens, physicians must be careful in selection of effective antibiotics. In strains with SHV-1 beta-lactamase, the use of ceftazidime does not have an effect on infection control. Also, strains with AMPC gene are increasing, which could be a problem in the future. Also, polymyxin B is an effective antibiotic for treatment of *P. aeruginosa* infections in hospitals.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval in this study was obtained from the institutional review board of Tabriz University of Medical Sciences, Iran.

## HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

## CONSENT FOR PUBLICATION

Informed consent was obtained from all the participants prior to publication.

## AVAILABILITY OF DATA AND MATERIALS

All relevant data and materials are provided within the manuscript.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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