

Antimicrobial Resistance Patterns and Prevalence of *bla*PER-1 and *bla*VEB-1 Genes Among ESBL-producing *Pseudomonas aeruginosa* Isolates in West of Iran

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Background: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections worldwide. Resistance of *P. aeruginosa* strains to the broad-spectrum cephalosporins may be caused by extended-spectrum β -lactamases (ESBLs).

Objectives: The aim of this study was to determine the antimicrobial resistance patterns and prevalence of PER-1 and VEB-1 type genes among ESBL producing strains of *P. aeruginosa*.

Material and Methods: A total of 106 *P. aeruginosa* isolates were collected from two university hospitals in Hamadan, Iran, during a 7-month study (2009). The antimicrobial susceptibility of isolates was determined by disc diffusion method and interpreted according to the clinical and laboratory standards institute (CLSI) recommendations. Production of ESBL was determined by combined disk test and presence of PER-1 and VEB-1 type ESBL genes was identified by PCR.

Results: The resistance against broad-spectrum cephalosporins and monobactams were: cefepime (97%), cefotaxime (92.5%) ceftazidime (51%), and aztreonam (27%). Ciprofloxacin (91.5%), imipenem (84.9%) and meropenem (82.1%) were the most effective anti-pseudomonas agents in this study. The results revealed that 88.7% of the isolates were multidrug resistant, 58.25% of those were ESBL positive. Sixteen (26.6%), 9 (15%) and 3 (5%) strains among ESBL-producing strains contained *bla*PER-1, *bla*VEB and *bla*PER-1-*bla*VEB, respectively.

Conclusions: This study highlighted the need to establish antimicrobial resistance surveillance networks for *P. aeruginosa* to determine the appropriate empirical treatment regimens. The high prevalence of multidrug resistance and production of ESBLs in *P. aeruginosa* isolates confirms the necessity of protocols considering these issues in the hospitals.

Keywords: *Pseudomonas aeruginosa*; Antimicrobial Drug Resistance; Beta-lactamase

1. Background

Pseudomonas aeruginosa is an important cause of nosocomial infections, including pneumonia, burn infection, urinary tract infections, meningitis and bacteremia. The infections can be particularly develop to a severe form in immune deficient patients (1). Antibiotics have been used successfully for several decades, but resistance genes have emerged and disseminated particularly in the last few years (2).

Extended-spectrum β -lactamases (ESBLs) mediate resistance to various broad-spectrum cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, and aztreonam (3). These enzymes originally collected from *Klebsiella pneumoniae* and *Escherichia coli* and recently from *P. aeruginosa* (4-7). Most of the methods for detection of ESBLs

are used in bacterial species such as *Klebsiella* and *E. coli* lacking chromosomal β -lactamase activity (8, 9), However, the detection of ESBL production in *P. aeruginosa* has some difficulties, because this bacterium not only has an inducible *AmpC* enzyme but also has an efflux-mediated resistance and a higher degree of impermeability than *Enterobacteriaceae* (10, 11). The PER-1 and VEB-1 type ESBLs belong to class A of β -lactamases and is associated with high level of resistance to cephems, monobactams and ceftazidime (12, 13).

2. Objectives

The aim of this study was to determine the antibacterial resistance patterns and prevalence of PER-1 and VEB type ESBLs among *P. aeruginosa* isolated from patients in west of Iran.

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

This study highlights the need for an antimicrobial resistance surveillance network for *P. aeruginosa* to monitor the trends and new types of resistance mechanism emerging.

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3. Materials and Methods

3.1. Bacterial Isolates

A total of 106 isolates of *P. aeruginosa* recovered from various clinical specimens (Table 1) in Beasat Teaching Hospital at the Hamadan University of Medical Sciences during a 7-month study in 2009. The identification were carried out by colonial morphology, positive oxidase test, pigment formation; growth test at 42°C on nutrient agar, Gram staining and motility test.

3.2. Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was determined by standard disk diffusion method (14). The following antibiotics were used: gentamicin (30 µg), aztreonam (30 µg), meropenem (10 µg), imipenem (10 µg), amikacin (30 µg), tobramycin (30 µg), piperacillin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefepime (30 µg) and cefotaxime (30 µg) (Himedia, India). *P. aeruginosa* ATCC 27853 was used as control. The results were interpreted according to the clinical and laboratory standards institute (CLSI) (15).

3.3. Phenotypic Detection of Beta-Lactamase

The isolates were tested for the ESBLs production by using combine disk test (CDT) as CLSI recommendations. CDT were performed on ceftazidime, cefotaxime, cefepime and aztreonam resistant strains by placing disks of ceftazidime, and cefotaxime (30 µg each) at a 20 mm distance from a disk containing ceftazidime-clavulanic acid (30/10µg), cefotaxime-clavulanic acid (30/10µg) and cefepime-clavulanic acid (30/10 µg) (16). ESBL production was inferred when the cephalosporin inhibitory zones were expanded by the clavulanate.

3.4. PCR Amplification

PCR amplifications were done using specific primers for the β-lactamases PER-1, and VEB-1 genes, as described

previously (17). PCR was performed for all ESBL-producers which their resistance to cephalosporins and phenotypic was identified by confirmatory tests. DNA was extracted by the boiling method as previously described (17). The DNA amplification program consisted of an initial denaturation (94 °C, 5 minutes) followed by 35 cycles of denaturation (94 °C, 60 seconds), annealing (50 °C for PER-1 and 55 °C for VEB-1, 60 seconds), extension (72 °C, 45 seconds) and a single final extension for 5 minutes at 72 °C. Reaction mixtures for PCR contained 1.5mM MgCl₂, 0.5 mM of each primer, 0.2 mM of dNTPs, 1 U of Taq polymerase, 1X PCR buffer and 2 µL of DNA. Primers PER-F (5'-AATTTGGGCTTAGGGCAGAA-3') and PER-R (5'-ATGAATGTCATTATAAAAAGC-3') were used for blaPER-1; and VEB-F (5'-CGACTTCCATTCCCGATGC-3') and VEB-R (5'-GGACTCTGCAACAAAT AC GC-3') were used for blaVEB-1 amplification.

4. Results

4.1. Antimicrobial Susceptibility Test

Table 2 shows the antimicrobial susceptibility pattern of *P. aeruginosa* strains. Ciprofloxacin (91.5%), imipenem.

Table 1. Distribution of *P.seudomonas aeruginosa* by Site of Isolation

Source	Isolates, No. (%)
Burn wounds	55 (51.9)
Trashes	20 (18.9)
Urine	12 (11.3)
Blood	7 (6.6)
Feces	6 (5.7)
Sputum	5 (4.7)
CSF ^a	1 (0.9)
Total	106 (100)

^a Abbreviation: CFS, Cerebral spinal fluid

Table 2. Antimicrobial Susceptibility Pattern of *P. aeruginosa* Strains

Antimicrobials	Resistance, No. (%)	Sensitive, No. (%)	Intermediate, No. (%)
Amikacin	32 (30.2)	70 (66)	4 (3.8)
Ceftazidime	54 (51)	40 (37.7)	12 (11.3)
Aztreonam	29 (27.4)	70 (66)	7 (6.6)
Cefepime	103 (97.2)	1 (0.9)	2 (1.9)
cefotaxime	53 (50)	10 (9.4)	43 (40.6)
Ciprofloxacin	5 (4.7)	97 (91.5)	4 (3.8)
Gentamicin	39 (36.8)	55 (51.9)	12 (11.3)
Imipenem	8 (7.5)	90 (84.9)	8 (7.5)
Meropenem	14 (13.2)	87 (82.1)	5 (4.7)
Ofloxacin	31 (29.2)	72 (67.9)	3 (2.8)
Piperacillin	99 (93.4)	7 (6.6)	0
Tobramycin	39 (36.8)	67 (63.2)	0

(84.9%) and meropenem (82.1%) were the most active antimicrobial agents followed by ofloxacin (67.9% susceptibility). Aztreonam, a monobactam, and amikacin showed antibiotic activity against 66% of the strains. Susceptibility to the cephalosporins was reduced to 37.7% for ceftazidime, followed by cefotaxime (9.4%) and Cefepime (0.9%).

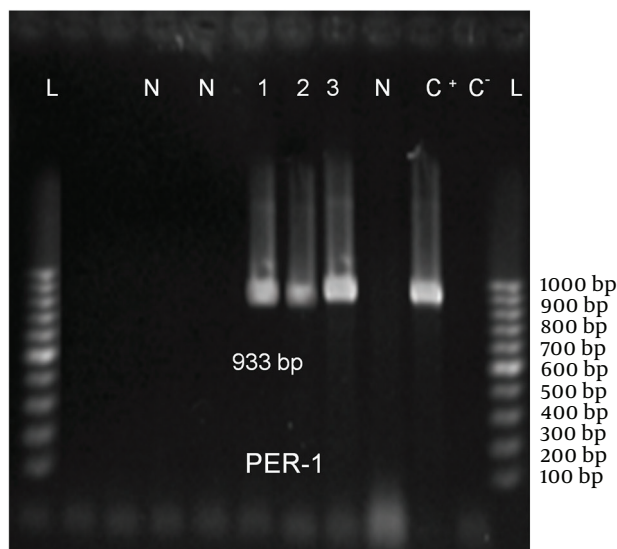
ESBL Production: Among 106 isolates, 94 (88.7%) were multidrug resistant and 60(58.3%) were putative ESBL producers using phenotypic confirmatory tests (Table 3). Sixteen (26.6%), 9 (15%) and 3 (5%) strains among 60 ESBL-producing strains had *blaPER-1* (Figure 1), *blaVEB-1* (Figure 2), and *blaPER-1- blaVEB* related genes, respectively (Table 4).

Table 3. Susceptibility Pattern to Antimicrobial Agents in ESBLs and non ESBLs-producing *P. aeruginosa* Strains

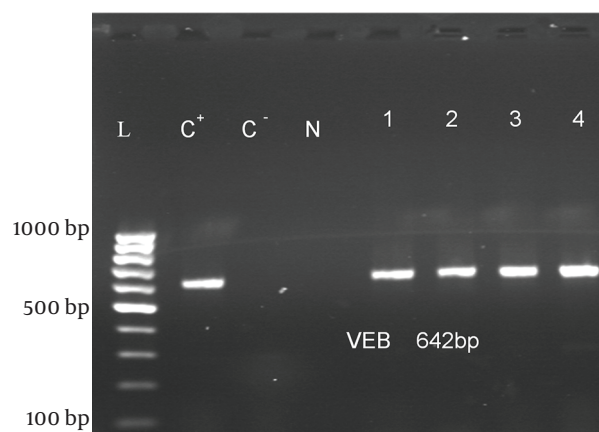
Antimicrobials	ESBL Positive (n=60)			ESBL Negative (n=46)		
	Resistant, No. (%)	Susceptible, No. (%)	Intermediate, No. (%)	Resistant, No. (%)	Susceptible, No. (%)	Intermediate, No. (%)
Amikacin	29 (48.3)	30 (50)	1 (1.7)	4 (9.3)	37 (86)	2 (4.7)
Ceftazidime	40 (66.7)	18 (30)	2 (3.3)	13 (28.3)	24 (55.2)	6 (13)
Aztreonam	25 (41.7)	32 (53.3)	3 (5)	3 (6.97)	39 (90.69)	1 (2.32)
Cefepime	58 (96.7)	0	2 (3.3)	42 (97.67)	1 (2.32)	0
cefotaxime	41 (68.3)	6 (10)	13 (21.7)	12 (27.90)	3 (6.97)	28 (65.11)
Ciprofloxacin	5 (8.3)	50 (83.3)	5 (8.3)	0	42 (97.67)	1 (2.32)
Gentamicin	32 (53.3)	28 (46.7)	0	7 (16.27)	36 (83.72)	0
Imipenem	7 (11.7)	45 (75)	8 (13.3)	1 (2.32)	41 (95.34)	1 (2.32)
Meropenem	13 (21.7)	44 (73.3)	3 (5)	2 (4.65)	39 (90.69)	2 (4.65)
Ofloxacin	30 (50)	29 (48.3)	1 (1.7)	4 (9.30)	37 (86.04)	2 (4.65)
Piperacillin	54 (90)	6 (10)	0	41 (95.34)	2 (4.65)	0
Tobramycin	31 (51.6)	28 (46.7)	1 (1.7)	4 (9.30)	39 (90.69)	0

Table 4. Association of Antimicrobial Resistance Pattern and ESBL Genotypes

Antimicrobials	PER-1:16 (26.6%)			VEB: 9 (15%)		
	Resistant, No. (%)	Susceptible, No. (%)	Intermediate, No. (%)	Resistant, No. (%)	Susceptible, No. (%)	Intermediate, No. (%)
Amikacin	10 (62.5)	5 (31.25)	1 (6.25)	5 (55.5)	2 (22.2)	2 (22.2)
Ceftazidime	12 (75)	4 (25)	0	9 (100)	0	0
Aztreonam	10 (62.5)	4 (25)	2 (12.5)	9 (100)	0	0
Cefepime	16 (100)	0	0	9 (100)	0	0
cefotaxime	87.5 (14)	0	2 (12.5)	9 (100)	0	0
Ciprofloxacin	2 (12.5)	13 (81.25)	1 (6.25)	2 (22.2)	6 (66.6)	1 (11.11)
Gentamicin	10 (62.5)	6 (37.5)	0	6 (66.6)	3 (33.3)	0
Imipenem	5 (31.25)	5 (31.25)	6 (37.5)	1 (11.11)	6 (66.6)	2 (22.2)
Meropenem	7 (43.75)	8 (50)	1 (6.25)	6 (66.6)	2 (22.2)	1 (11.11)
Ofloxacin	11 (68.8)	5 (31.25)	0	8 (88.8)	1 (11.11)	0
Piperacillin	16 (100)	0	0	9 (100)	0	0
Tobramycin	10 (62.5)	6 (37.5)	0	6 (66.6)	3 (33.3)	0

Figure 1. PCR Amplification of *bla*PER-1 Gene

L: 100 bp DNA ladder, C⁻: negative control, C⁺: positive control, N: PER-1 negative strains, 1-3: PCR products from PER-1 positive strains.

Figure 2. PCR Amplification of *bla*VEB-1 Gene

L: 100 bp DNA ladder, C⁻: negative control, C⁺: positive control, N: negative strain, 1-4: PCR products from VEB-1 positive strains.

5. Discussion

P. aeruginosa has a high resistance to antibiotics and is a common cause of morbidity and mortality in hospitalized and immunocompromised patients (18). Treatment of *P. aeruginosa* infections is complicated by the inherited and acquired resistance to the most of commonly used antimicrobial agents (19). The results from this study showed the high resistance of *P. aeruginosa* to most of used antimicrobial agents. It was also demonstrated that the prevalence of antibiotic resistance of the isolates was very high in comparison to other studies and most of *P.*

aeruginosa isolates (88.7%) were multi-drug resistant (resistant to ≥ 3 different antibiotic classes) (20-25).

The prevalence of ESBL-producing *P. aeruginosa* isolates in this study was also higher than other investigations (5, 20, 26, 27). Among 60 ESBL-positive strains, 16 (26.6%), 9 (15%) and 3 (5%) contained *PER-1*, *VEB-1* and *PER-1-VEB-1* genes, respectively. These results indicated that the prevalence of *VEB-1* gene in our area, is higher than Turkey and Korea, but the prevalence of *VEB-1* and *PER-1* genes, is lower than in Thailand (94.44% *bla*VEB-1) and Italy (34.61% *bla*PER-1) (5, 13, 26). Data on the prevalence of ESBL-producing *P. aeruginosa* strains in our area is limited. In the study performed by Shahcheraghi and colleagues on *P. aeruginosa* isolates in Tehran, the rate of *bla*VEB and *bla*PER ESBLs were reported 24% and 17%, respectively, that was similar to our results (28). The high prevalence of *PER-1* and *VEB-1* indicated the high resistance to penicillins, ceftazidime and cefotaxime, as reported by other studies (26, 28).

This is the first report about the presence of these enzymes in *P. aeruginosa* isolates from west of Iran. It has shown that ESBL production in strains of *P. aeruginosa* can greatly complicate the clinical management of infection if advanced care is not taken. However, further studies are needed to determine other ESBL-types in *P. aeruginosa* strains in this area and their role in resistance to other antibiotic classes. The results of this study emphasizes on the need for a surveillance network to monitor the trends and emerge of new resistance mechanism in *P. aeruginosa* from different geographic regions. Therefore, the improvement in antibiotic prescription policies and infection control programs are of high necessity to prevent the spread of such resistant infectious agents.

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Authors' Contribution

All authors contributed in the research equally.

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