Distribution of Class A Extended-Spectrum β-Lactamases Among *Pseudomonas aeruginosa* Clinical Strains Isolated from Ardabil Hospitals

Fereshteh Hasanpour [1], Nima Ataei [1], Amirhossein Sahebkar [iD][2, 3, 4, 5] and Farzad Khademi [iD][1, *]

[1]Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
[2]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4]School of Medicine, The University of Western Australia, Perth, Australia
[5]Department of Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[*] *Corresponding author*: Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran. Email: k_farzad@yahoo.com

Abstract

Background: Currently, the emergence of extended-spectrum β-lactamase (ESBL)-producing bacteria is becoming a major threat to patients in the hospital and community. Such enzymes have been recently detected in *Pseudomonas aeruginosa*, but there is no epidemiological data on the prevalence of ESBL-producing clinical isolates in the hospitals of Ardabil City (Iran).

Objectives: This study aimed to determine the phenotypic and genotypic prevalence of class A ESBL-producing *P. aeruginosa* strains in Ardabil City.

Methods: A total of 120 clinical isolates of *P. aeruginosa* collected from Ardabil hospitals were used in this study. Phenotypic detection of class A ESBL-producing *P. aeruginosa* isolates was performed using a double-disk synergy test. In addition, the detection of class A ESBL-encoding genes, including *Pseudomonas* extended resistant (*PER*), Vietnamese extended-spectrum β-lactamase (*VEB*), temoniera (*TEM*), sulfhydryl variable (*SHV*), cefotaximase (*CTX-M*), guyana extended-spectrum β-lactamase (*GES*), and *Pseudomonas*-specific enzyme (*PSE*), was performed using the polymerase chain reaction (PCR).

Results: The prevalence of class A ESBL-producing *P. aeruginosa* strains was 8.3% (10 out of 120) based on the double-disk synergy test. However, 40% (48 out of 120) of these isolates were found to carry genes encoding class A ESBLs based on PCR. Among 48 class A ESBL-positive strains, the prevalence of *PSE*, *TEM*, *VEB*, *CTX-M*, and *PER* genes were 64.6% (31/48), 25% (12/48), 4.2% (2/48), 4.2% (2/48), and 2% (1/48), respectively. However, the frequency of other class A ESBL genes (*SHV* and *GES* genes) was 0%.

Conclusions: Our results confirmed the presence of class A ESBL-producing *P. aeruginosa* strains in the hospital environment of Ardabil. On the other hand, the use of molecular tests can be a more precise and reliable method than phenotypic ones to identify these resistant strains and prevent the emergence of antibiotic resistance and ensuing treatment failure.

Keywords: Pseudomonas aeruginosa, Antibiotic Resistance, ESBL

1. Background

Pseudomonas aeruginosa (a non-fermentative gram-negative bacillus) is an important opportunistic pathogen that causes severe infections in hospitalized, immunocompromised, and cystic fibrosis patients (1, 2). This organism is responsible for 8% of all nosocomial infections, especially in intensive care units (ICUs) (3). Of note, the emergence of antibiotic resistance has limited the treatment of *P. aeruginosa* infections (4). One of the most common antibiotics used for the treatment of *P. aeruginosa* infections is β-lactams, especially carbapenems (5). Nevertheless, the US Centers for Disease Control and Prevention (CDC) and WHO stated that carbapenem-resistant and multidrug-resistant (MDR) *P. aeruginosa* strains are becoming public health problems worldwide (6, 7). The prevalence of imipenem-resistant and MDR *P. aeruginosa* strains in Iran is 54% and 58%, respectively (8, 9).

Four mechanisms of resistance to β-lactam antibiotics have been identified in gram-negative bacteria, including (1) low affinity of penicillin-binding proteins (PBPs) to β-lactam antibiotics; (2) modification of the outer membrane porins; (3) drug efflux pump systems (Mex); and (4) production of β-lactamases (10). β-Lactamases, which were identified for the first time in *Escherichia coli*, are bacterial enzymes hydrolyzing β-lactam antibiotics and are

considered the most common factor accounting for the resistance of gram-negative bacteria to β-lactams ([10]). β-Lactamase enzymes are divided into four classes according to the Ambler classification scheme (β-lactamases A to D) ([5]). Class A β-lactamases hydrolyze penicillins and cephalosporins, class B β-lactamases hydrolyze carbapenems, class C β-lactamases hydrolyze cephalosporins and cephamycins, and class D β-lactamases hydrolyze penicillins, cephalosporins, and carbapenems ([10]). The frequency of different classes of β-lactamases has been assessed in *P. aeruginosa* strains isolated from clinical specimens in Ardabil, except for class A β-lactamases. Among class A β-lactamases, extended-spectrum β-lactamases (ESBLs) are clinically most important resistance enzymes, which can hydrolyze penicillins and the first-, second-, third-, and fourth-generations of cephalosporins and monobactam ([11]). ESBLs are inhibited by β-lactamase inhibitors and do not affect cephamycins or carbapenems ([11]).

2. Objectives

This study aimed to determine the frequency of genes encoding class A ESBLs, including *Pseudomonas* extended resistant (*PER*), Vietnamese extended-spectrum β-lactamase (*VEB*), temoniera (*TEM*), sulfhydryl variable (*SHV*), cefotaximase (*CTX-M*), guyana extended-spectrum β-lactamase (*GES*), and *Pseudomonas*-specific enzyme (*PSE*), among *P. aeruginosa* clinical isolates obtained from patients referred to the hospitals in Ardabil City, Iran.

3. Methods

3.1. Pseudomonas aeruginosa Strains and Antimicrobial Susceptibility Testing

A total of 120 non-duplicate clinical isolates of *P. aeruginosa* were used in this study. These clinical isolates were collected between June 2019 and January 2022 from different wards of Imam Reza, Imam Khomeini, Bu-Ali, Alavi, Sabalan, Fatemi, and Ghaem hospitals in Ardabil City, northwest of Iran. *Pseudomonas aeruginosa* strains were confirmed using phenotypic tests, including pigment (blue/green), Gram stain, oxidase, catalase, triple sugar iron agar (red/red), oxidative-fermentative (OF), indole (-), methyl red (-), Voges-Proskauer (-), and citrate (+), as well as molecular identification of the *ITS* gene ([6]). Antimicrobial susceptibility testing of *P. aeruginosa* strains against different antibiotics was previously determined using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guideline ([12]).

3.2. Phenotypic Detection of ESBLs

A combined double-disk synergy test was used for phenotypic detection of class A ESBL-positive *P. aeruginosa* strains. Phenotypic detection of ESBLs in *P. aeruginosa* strains is difficult due to the presence of inducible AmpC β-lactamase, impermeability, and efflux-mediated resistance ([13]). For this purpose, a colony suspension of *P. aeruginosa*, equivalent to a 0.5 McFarland standard, was prepared in normal saline and inoculated on the Mueller-Hinton agar surface (Conda, Pronasida, Spain) ([14]). Antibiotic-containing disks (Padtan Teb, Iran) and phenylboronic acid (Sigma-Aldrich, Germany, i.e., ceftazidime (CAZ, 30 μg) vs. ceftazidime/phenylboronic acid and ceftazidime-clavulanate (CZA, 30/10 μg) vs. ceftazidime-clavulanate/phenylboronic acid) were placed at 2 cm apart (center to center; [Figure 1A]). The plate was incubated at 37°C for 16 - 18 hours. A 5 mm or larger increase in the diameter of the growth inhibition zone around the combination disks compared with the disks alone was considered ESBL-positive *P. aeruginosa*.

3.3. Detection of ESBL-Encoding Genes

All *P. aeruginosa* strains were investigated for detection of class A ESBL genes, including *PER*, *VEB*, *TEM*, *SHV*, *CTX-M*, *GES*, and *PSE*, using the polymerase chain reaction (PCR; Eppendorf Thermal Cycler, Germany) ([15]). Briefly, DNA extraction was performed using the boiling method, and the quality was confirmed by a spectrophotometer (NanoDrop 2000c, Thermo Scientific, USA) ([6]). The total volume of each PCR was 25 μL containing PCR Master Mix (20 μL; Ampliqon, Denmark), DNA template (3 μL), and forward and reverse primers (2 μL and 10 μmol/L). Gene amplification programs, along with specific primer sequences, are listed in [Table 1]. Further, 5 μL of each PCR product was electrophoresed on 1% agarose gel and observed under UV light ([Figure 1B]). The results were confirmed through DNA sequencing (Pishgam, Iran) ([6]). Clinical isolates of *Klebsiella pneumoniae* and *P. aeruginosa* ATCC 27853 carrying class A ESBL-encoding genes were used as positive control strains. Additionally, PCR Master Mix plus nuclease-free water was used as negative controls.

4. Results

In this study, 120 clinical isolates of *P. aeruginosa* were obtained from 59 males and 61 females. The age of the patients was between 6 and 89 years. Strains were collected from Imam Khomeini (47.5%), Alavi (35.9%), Imam Reza (7.5%), Bu-Ali (3.3%), Sabalan (3.3%), Fatemi (1.6%), and Ghaem (0.9%) hospitals. In addition, these clinical specimens were isolated from urine (48.3%), sputum (25%),
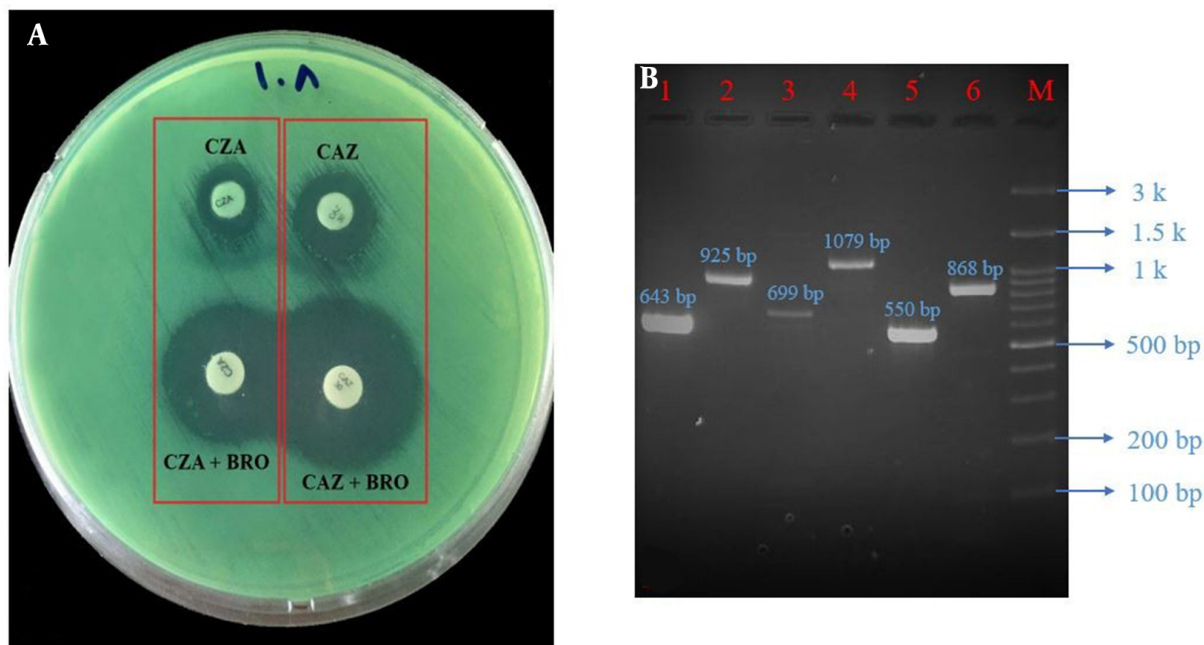
Figure 1. A, Phenotypic; and B, Genotypic detection of ESBL-producing *Pseudomonas aeruginosa*; Lane 1, Vietnamese extended-spectrum β-lactamase (*VEB*; 643 bp); lane 2, *Pseudomonas* extended resistant (*PER*; 925 bp); lane 3, *Pseudomonas*-specific enzyme (*PSE*; 699 bp); lane 4, temoniera (*TEM*; 1079 bp); lane 5, cefotaximase (*CTX-M*; 550 bp); lane 6, sulfhydryl variable (*SHV*; 868 bp); lane M, ladder (100 bp). Note that the *SHV* gene band is related to the positive control strain of *Klebsiella pneumoniae*. Abbreviations: CAZ, ceftazidime; CAZ/BRO, ceftazidime/phenylboronic acid; CZA, ceftazidime-clavulanate; CZA/BRO, ceftazidime-clavulanate/phenylboronic acid.

Table 1. A List of the Primers and Polymerase Chain Reaction Programs Used in the Present Study

Gene	Oligonucleotide Sequence (5' to 3')	Thermal Cycling Condition for Amplification	Amplicon Size (bp)	Reference
VEB	F: CGACTTCCATTTCCCGATGC; R: GGACTCTGCAACAAATACGC	1 cycle: Initial denaturation at 95°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min	643	(15)
PER	F: AATTTGGGCTTAGGGCAGAA; R: ATGAATGTCATTATAAAAGC	1 cycle: Initial denaturation at 95°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min	925	(15)
PSE	F: AATGGCAATCAGCGCTTC; R: GCGCGACTGTGATGTATA	1 cycle: Initial denaturation at 95°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min	699	(15)
GES	F: ATGCGCTTCATTCACGCAC; R: CTATTTGTCCGTGCTCAGG	1 cycle: Initial denaturation at 95°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min	864	(15)
SHV	F: TGGTTATGCGTTATATTCGCC; R: GGTTAGCGTTGCCAGTGCT	1 cycle: Initial denaturation at 95°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min	868	(15)
TEM	F: ATAAAATTCTTGAAGACGAAA; R: GACAGTTACCAATGCTTAATCA	1 cycle: Initial denaturation at 94°C for 5 min; 34 cycles: Denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min	1079	(15)
CTX-M	F: CGCTTTGCGATGTGCAG; R: ACCGCGATATCGTTGGT	1 cycle: Initial denaturation at 94°C for 5 min; 34 cycles: Denaturation at 94°C for 45 s, annealing at 51°C for 40 s, and extension at 72°C for 1 min	550	(15)

Abbreviations: *PER*, *Pseudomonas* extended resistant; *VEB*, Vietnamese extended-spectrum β-lactamase; *TEM*, temoniera; *SHV*, sulfhydryl variable; *CTX-M*, cefotaximase; *GES*, guyana extended-spectrum β-lactamase; *PSE*, *Pseudomonas*-specific enzyme

blood (15.9%), wound (10%), and cerebrospinal fluid (0.8%). However, the infection type of patients referred to hospitals was not clear. According to the results of the double-disk synergy test, 8.3% (10 out of 120) of clinical isolates of *P. aeruginosa* were selected as class A ESBL-producing strains. The class A ESBL-positive *P. aeruginosa* strains were found in 5 male and 5 female patients. These bacteria were isolated from urine (n = 5), sputum (n = 4), and wound (n = 1) specimens. In addition, *P. aeruginosa* strains carrying class A ESBLs in phonotypic tests were obtained from patients referred to Imam Reza (n = 2), Imam Khomeini (n = 4), and Alavi (n = 4) hospitals. The characteristics of 10 ESBL-positive *P. aeruginosa* strains are listed in Table 2.

On the other hand, of the 120 *P. aeruginosa* strains, 48 (40%) carried genes encoding class A ESBLs based on the PCR. Among 48 class A ESBL-positive strains, the prevalence of *PSE*, *TEM*, *VEB*, *CTX-M*, and *PER* genes were 64.6% (31/48), 25% (12/48), 4.2% (2/48), 4.2% (2/48), and 2% (1/48), respectively. However, the frequency of genes encoding other class A ESBLs (*SHV* and *GES* genes) was 0%. The accession numbers for amplified genes (i.e., OQ024009 to OQ024015) were retrieved from the GenBank database.

5. Discussion

The following antibiotic-resistant microorganisms are considered serious health concerns worldwide: *Enterobacteriaceae*, *Acinetobacter*, *Campylobacter*, *Candida*, *Enterococcus*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Mycobacterium*, *Clostridium*, *Neisseria*, and *Pseudomonas* (7, 16). Furthermore, concerns regarding the spread of ESBL-producing bacteria are significantly increasing because treatment outcome in patients infected with such pathogens is less satisfactory (11). ESBLs have been abundantly reported in *E. coli* and *K. pneumonia* and have recently been detected in *P. aeruginosa* (11). Therefore, it is necessary to prevent the spread of ESBL-producing bacteria, especially in hospital environments. There are various phenotypic and genotypic tests to detect ESBLs in *Enterobacteriaceae* clinical isolates with little or no chromosomal β-lactamase activity (11). Based on the ESBL screening phenotypic test, 8.3% of *P. aeruginosa* strains isolated from Ardabil hospitals were identified as ESBL-producing strains. Similar results to our study have been reported by Nazari Alam et al. (12.7%) (17), Tavajjohi and Moniri (9.2%) (18), and Laudy et al. (12.2%) (19). However, other studies from Iran and other countries have reported higher prevalence rates, including Vahdani et al. (18%) (20), Akhi et al. (39.3%) (21), Mirsalehian et al. (39.4%) (22), Jabalameli et al. (42.8%) (23), Tawfik et al. (16%) (24), Shaikh et al. (25.1%) (25), and Chen et al. (87.5%) (26).

Cefotaximase, TEM, and SHV enzymes are considered the most prevalent ESBLs in bacterial pathogens globally (11). Various studies have reported the prevalence of these enzymes in clinical isolates of *P. aeruginosa* worldwide. In this regard, Bokaeian et al. found that TEM was present in 100% of isolates and SHV in 6.6% of them (27). Imani Foolad et al. reported SHV in 37.5% and TEM in 12.5% of isolates (28), while Nazari Alam et al. found SHV in 52.4% and TEM in 33.3% of isolates (17). Dong et al. did not detect any SHV or TEM in their isolates (29), and Jiang et al. found TEM in 17% of their isolates (13).

In the current study, the prevalence of *TEM*, *CTX-M*, and *SHV* genes were 10% (12/120), 1.6% (2/120), and 0%, respectively. Among *P. aeruginosa* and *Acinetobacter* species, PER- and oxacillinase (OXA)-type enzymes are more prevalent (11). In studies conducted by Lee et al. in Korea (15), Tawfik et al. in Saudi Arabia (24), and Chen et al. in China (26), as well as other studies in Iran, including Akhi et al. in Tabriz (21), Jabalameli et al. in Tehran (23), Alikhani et al. in Hamadan (30), and Amirkamali et al. in Qazvin (31), the prevalence of PER-type ESBLs were 0%, 0%, 13.8%, 27.5%, 50%, 26.6%, 0%, respectively. Nevertheless, the frequency of the *PER* gene was 0.8% (1/120) in the present study. Another most common class A ESBLs among *P. aeruginosa* strains is PSE-type enzymes (15). In this study, the most prevalent gene encoding class A ESBLs was *PSE* (25.8%; 31/120). Lee et al. reported the prevalence of PSE-type ESBLs at 6.3% in *P. aeruginosa* strains (15).

The frequency of VEB-type (1.6%; 2/120) and GES-type (0%) ESBLs in *P. aeruginosa* strains was lower in the current study than that reported by Tawfik et al. in Saudi Arabia (68% and 20%, respectively) (24). However, Lee et al. in Korea reported that none of the *P. aeruginosa* strains harbored genes encoding VEB- and GES-type ESBLs (15). Differences between the results of phenotypic and genotypic tests for ESBL screening in this study can be attributed to the presence of inducible chromosomal β-lactamases, efflux pumps, and higher impermeability in *P. aeruginosa* compared with *Enterobacteriaceae* (13). In studies conducted by Khademi et al. on the same clinical isolates of *P. aeruginosa* in Ardabil, the prevalence and upregulation/downregulation of AmpC β-lactamase enzyme, efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM), and OprD porin among carbapenem-resistant and MDR strains were evaluated (data unpublished). The prevalence of inducible AmpC β-lactamase enzyme in phenotypic and genotypic tests was 52.5% and 100%, respectively. Among them, 33.3% of isolates showed a high expression level of the *ampC* gene based on real-time PCR. In addition, the role of 4 efflux pumps of *P. aeruginosa* in the emergence of drug-resistant clinical isolates was obvious. Overexpression of the *mexA*, *mexC*, *mexE*, and

Table 2. Characteristics of Extended-Spectrum β-Lactamase-Positive *Pseudomonas aeruginosa* Strains Isolated from Ardabil Hospitals

Strain Number	Specimen	Virulence Gene Profiles <u>a</u>	Antibiotic Resistance Profiles [a]	
			Phenotypic Resistance	Genotypic Resistance
23	Wound	*algD, plcN, lasB, plcH, exoS, toxA, exoU*	MDR, MBL, AmpC, ESBL	*gyrA, parC, IMP, qacE△1, oprD, oxa-2, oxa-10, intI-1*
35	Urine	*algD, plcN, lasB, plcH, toxA, exoU*	MDR, MBL, AmpC, ESBL	*gyrA, parC, IMP, qacE△1, fabV, cepA, oprD, oxa-10*
50	Urine	*algD, plcN, lasB, plcH, toxA, exoU*	MDR, MBL, AmpC, ESBL	*IMP, qacE△1, oprD, oxa-2, oxa-10, intI-1*
59	Sputum	*algD, plcN, lasB, plcH, exoS, toxA, exoU*	MDR, MBL, AmpC, ESBL	*gyrA, parC, qacE△1, fabV, cepA, oprD, oxa-2, oxa-10, oxa-23, intI-1*
61	Sputum	*plcN, lasB, plcH, exoS, toxA*	MDR, AmpC, ESBL	*gyrA, parC, qacE△1, qacE, qacG, fabV, cepA, oprD, intI-1*
65	Sputum	*algD, plcN, lasB, plcH, exoS, toxA, exoU*	MDR, MBL, AmpC, ESBL	*gyrA, parC, IMP, qacE△1, fabV, cepA, oprD, oxa-2, oxa-10, oxa-23, intI-1, PSE*
73	Urine	*algD, plcN, lasB, plcH, toxA, exoU, pilB*	AmpC, ESBL	*qacE△1, qacE, fabV, cepA, oprD, oxa-23, intI-1, PSE*
79	Urine	*algD, plcN, lasB, plcH, toxA, exoU, pilB*	MDR, MBL, AmpC, ESBL	*qacE△1, qacE, qacG, fabV, cepA, oprD, oxa-2, oxa-23, intI-1*
100	Urine	*algD, plcN, lasB, plcH, exoS, toxA, exoU, pilB*	MDR, ESBL	*oprD, oxa-2, oxa-10, oxa-23, intI-1*
108	Sputum	*algD, plcN, lasB, plcH, exoS, toxA, pilB*	AmpC, ESBL	*oxa-2, oxa-10, PSE*

Abbreviations: *algD*, GDP-mannose 6-dehydrogenase; *plcN*, non-hemolytic phospholipase C; *plcH*, hemolytic phospholipase C; *LasB*, elastase; *exoS*, exoenzyme S; *exoU*, exoenzyme U; *toxA*, exotoxin A; *pilB*, type 4 fimbrial biogenesis protein; *gyrA*, DNA gyrase subunit A; *parC*, topoisomerase IV subunit A; *intI1*, class 1 integrase; MBL, metallo-β-lactamase; AmpC, AmpC cephalosporinase; MDR: multidrug-resistant; ESBL, extended-spectrum β-lactamase; *IMP*, imipenemase; *OXA*, oxacillinase; *qacE△1*, *qacE*, *qacG* and *cepA*, efflux pumps genes; *fabV*, triclosan resistance gene; *oprD*, imipenem outer membrane porin; *PSE*, *Pseudomonas*-specific enzyme
[a] Virulence gene and antibiotic resistance profiles are based on previous studies.

mexY genes was seen in 36 (75%), 40 (83.3%), 5 (10.4%), and 20 (41.6%) carbapenem-resistant and MDR *P. aeruginosa* clinical isolates. Furthermore, *oprD* downregulation was observed in 79.1% of these *P. aeruginosa* isolates. The sequencing method was used to identify many TEM and SHV family variants. Temoniera and SHV family variants were not determined in our study, and this was the main limitation of the current study.

5.1. Conclusions

Our results confirmed that *PSE*, *TEM*, *VEB*, *CTX-M*, and *PER* were the predominant genes encoding class A ESBLs in *P. aeruginosa* strains in Ardabil City. On the other hand, we concluded that the use of molecular tests could be a more precise and reliable method than phenotypic ones to identify these resistant strains and prevent the emergence of antibiotic resistance and ensuing treatment failure.

Footnotes

Authors' Contribution: Study concept and design: F. K.; acquisition of data: F. H. and N. A.; analysis and interpretation of data: F. K.; drafting of the manuscript: F. K.; revision

of the manuscript: A. S.; all authors read and approved the final manuscript.

Conflict of Interests: The authors declare that there are no conflicts of interest.

Ethical Approval: IR.ARUMS.MEDICINE.REC.1401.035

References

1. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs*. 2007;**67**(3):351–68. [PubMed ID: 17335295]. https://doi.org/10.2165/00003495-200767030-00003.
2. Namaki M, Habibzadeh S, Vaez H, Arzanlou M, Safarirad S, Bazghandi SA, et al. Prevalence of resistance genes to biocides in antibiotic-resistant Pseudomonas aeruginosa clinical isolates. *Mol Biol Rep*. 2022;**49**(3):2149–55. [PubMed ID: 34854015]. https://doi.org/10.1007/s11033-021-07032-2.
3. Bazghandi SA, Arzanlou M, Peeridogaheh H, Vaez H, Sahebkar A, Khademi F. Prevalence of virulence genes and drug resistance profiles of Pseudomonas aeruginosa isolated from clinical specimens. *Jundishapur J Microbiol*. 2021;**14**(8). https://doi.org/10.5812/jjm.118452.

4. Khademi F, Maarofi K, Arzanlou M, Peeri-Dogaheh H, Sahebkar A. Which missense mutations associated with DNA gyrase and topoisomerase IV are involved in Pseudomonas aeruginosa clinical isolates resistance to ciprofloxacin in Ardabil? *Gene Rep*. 2021;**24**. https://doi.org/10.1016/j.genrep.2021.101211.

5. Safarirad S, Arzanlou M, Mohammadshahi J, Vaez H, Sahebkar A, Khademi F. Prevalence and characteristics of metallo-beta-lactamase-positive and high-risk clone st235 Pseudomonas aeruginosa at Ardabil hospitals. *Jundishapur J Microbiol*. 2021;**14**(3). https://doi.org/10.5812/jjm.115819.

6. Khademi F, Ashrafi S, Neyestani Z, Vaez H, Sahebkar A. Prevalence of class I, II and III integrons in multidrug-resistant and carbapenem-resistant Pseudomonas aeruginosa clinical isolates. *Gene Rep*. 2021;**25**. https://doi.org/10.1016/j.genrep.2021.101407.

7. World Health Organization. *WHO publishes list of bacteria for which new antibiotics are urgently needed*. Geneva, Switzerland: World Health Organization; 2017, [cited 24 October 2017]. Available from: http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/.

8. Vaez H, Salehi-Abargouei A, Khademi F. Systematic review and meta-analysis of imipenem-resistant Pseudomonas aeruginosa prevalence in Iran. *Germs*. 2017;**7**(2):86–97. [PubMed ID: 28626739]. [PubMed Central ID: PMC5466827]. https://doi.org/10.18683/germs.2017.1113.

9. Vaez H, Salehi-Abargouei A, Ghalehnoo ZR, Khademi F. Multidrug resistant Pseudomonas aeruginosa in Iran: A systematic review and metaanalysis. *J Glob Infect Dis*. 2018;**10**(4):212–7. [PubMed ID: 30581263]. [PubMed Central ID: PMC6276320]. https://doi.org/10.4103/jgid.jgid_113_17.

10. Bonomo RA. beta-lactamases: A focus on current challenges. *Cold Spring Harb Perspect Med*. 2017;**7**(1). [PubMed ID: 27742735]. [PubMed Central ID: PMC5204326]. https://doi.org/10.1101/cshperspect.a025239.

11. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. *Curr Opin Pharmacol*. 2007;**7**(5):459–69. [PubMed ID: 17875405]. [PubMed Central ID: PMC2235939]. https://doi.org/10.1016/j.coph.2007.08.003.

12. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. 31st ed. Wayne, USA: Clinical and Laboratory Standards Institute; 2021.

13. Jiang X, Zhang Z, Li M, Zhou D, Ruan F, Lu Y. Detection of extended-spectrum beta-lactamases in clinical isolates of Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 2006;**50**(9):2990–5. [PubMed ID: 16940093]. [PubMed Central ID: PMC1563526]. https://doi.org/10.1128/AAC.01511-05.

14. Glupczynski Y, Bogaerts P, Deplano A, Berhin C, Huang TD, Van Eldere J, et al. Detection and characterization of class A extended-spectrum-beta-lactamase-producing Pseudomonas aeruginosa isolates in Belgian hospitals. *J Antimicrob Chemother*. 2010;**65**(5):866–71. [PubMed ID: 20200037]. https://doi.org/10.1093/jac/dkq048.

15. Lee S, Park YJ, Kim M, Lee HK, Han K, Kang CS, et al. Prevalence of Ambler class A and D beta-lactamases among clinical isolates of Pseudomonas aeruginosa in Korea. *J Antimicrob Chemother*. 2005;**56**(1):122–7. [PubMed ID: 15890715]. https://doi.org/10.1093/jac/dki160.

16. The Centers for Disease Control and Prevention (CDC). *Antibiotic resistance threats in the United States*. Atlanta, USA: The Centers for Disease Control and Prevention (CDC); 2019.

17. Nazari Alam A, Sarvari J, Motamedifar M, Khoshkharam H, Yousefi M, Moniri R, et al. The occurrence of blaTEM, blaSHV and blaOXA genotypes in Extended-Spectrum β-Lactamase (ESBL)-producing Pseudomonas aeruginosa strains in Southwest of Iran. *Gene Rep*. 2018;**13**:19–23. https://doi.org/10.1016/j.genrep.2018.08.002.

18. Tavajjohi Z, Moniri R. Detection of ESBLs and MDR in Pseudomonas aeruginosa in a tertiary-care teaching hospital. *Arch Clin Infect Dis*. 2011;**6**(1):18–23.

19. Laudy AE, Rog P, Smolinska-Krol K, Cmiel M, Sloczynska A, Patzer J, et al. Prevalence of ESBL-producing Pseudomonas aeruginosa isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. *PLoS One*. 2017;**12**(6). e0180121. [PubMed ID: 28658322]. [PubMed Central ID: PMC5489192]. https://doi.org/10.1371/journal.pone.0180121.

20. Vahdani M, Azimi L, Asghari B, Bazmi F, Rastegar Lari A. Phenotypic screening of extended-spectrum ss-lactamase and metallo-ss-lactamase in multidrug-resistant Pseudomonas aeruginosa from infected burns. *Ann Burns Fire Disasters*. 2012;**25**(2):78–81. [PubMed ID: 23233825]. [PubMed Central ID: PMC3506211].

21. Akhi MT, Khalili Y, Ghottaslou R, Aghazadeh M, Seroush Bar Hagh MH, Yousefi S. Prevalence of PER-1- type extended-spectrum beta-lactamaes in clinical strains of Pseudomonas aeruginosa isolated from Tabriz, Iran. *Iran J Basic Med Sci*. 2012;**15**(1):678–82. [PubMed ID: 23493931]. [PubMed Central ID: PMC3586864].

22. Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli F, Goli H, Kalantari N. Detection of VEB-1, OXA-10 and PER-1 genotypes in extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients. *Burns*. 2010;**36**(1):70–4. [PubMed ID: 19524369]. https://doi.org/10.1016/j.burns.2009.01.015.

23. Jabalameli F, Mirsalehian A, Sotoudeh N, Jabalameli L, Aligholi M, Khoramian B, et al. Multiple-locus variable number of tandem repeats (VNTR) fingerprinting (MLVF) and antibacterial resistance profiles of extended spectrum beta lactamase (ESBL) producing Pseudomonas aeruginosa among burnt patients in Tehran. *Burns*. 2011;**37**(7):1202–7. [PubMed ID: 21703769]. https://doi.org/10.1016/j.burns.2011.05.012.

24. Tawfik AF, Shibl AM, Aljohi MA, Altammami MA, Al-Agamy MH. Distribution of Ambler class A, B and D beta-lactamases among Pseudomonas aeruginosa isolates. *Burns*. 2012;**38**(6):855–60. [PubMed ID: 22348803]. https://doi.org/10.1016/j.burns.2012.01.005.

25. Shaikh S, Fatima J, Shakil S, Danish Rizvi SM, Kamal MA. Prevalence of multidrug resistant and extended spectrum beta-lactamase producing Pseudomonas aeruginosa in a tertiary care hospital. *Saudi J Biol Sci*. 2015;**22**(1):62–4. [PubMed ID: 25561885]. [PubMed Central ID: PMC4281620]. https://doi.org/10.1016/j.sjbs.2014.06.001.

26. Chen Z, Niu H, Chen G, Li M, Li M, Zhou Y. Prevalence of ESBLs-producing Pseudomonas aeruginosa isolates from different wards in a Chinese teaching hospital. *Int J Clin Exp Med*. 2015;**8**(10):19400–5. [PubMed ID: 26770582]. [PubMed Central ID: PMC4694482].

27. Bokaeian M, Shahraki Zahedani S, Soltanian Bajgiran M, Ansari Moghaddam A. Frequency of PER, VEB, SHV, TEM and CTX-M genes in resistant strains of Pseudomonas aeruginosa producing extended spectrum beta-lactamases. *Jundishapur J Microbiol*. 2015;**8**(1). e13783. [PubMed ID: 25789123]. [PubMed Central ID: PMC4350043]. https://doi.org/10.5812/jjm.13783.

28. Imani Foolad A, Rostami Z, Shapouri R. [Antimicrobial resistance and ESBL prevalence in Pseudomonas aeruginosa strains isolated from clinical specimen by phenotypic and genotypic methods]. *J Ardabil Univ Med Sci*. 2010;**10**(3):189–98. Persian.

29. Dong F, Xu XW, Song WQ, Lu P, Yang YH, Shen XZ. [Analysis of resistant genes of beta-lactam antibiotics from Pseudomonas aeruginosa in pediatric patients]. *Zhonghua Yi Xue Za Zhi*. 2008;**88**(42):3012–5. Chinese. [PubMed ID: 19080083].

30. Alikhani MY, Karimi Tabar Z, Mihani F, Kalantar E, Karami P, Sadeghi M, et al. Antimicrobial resistance patterns and prevalence of blaPER-1 and blaVEB-1 genes among ESBL-producing Pseudomonas aeruginosa isolates in West of Iran. *Jundishapur J Microbiol*. 2014;**7**(1). e8888. [PubMed ID: 25147662]. [PubMed Central ID: PMC4138671]. https://doi.org/10.5812/jjm.8888.

31. Amirkamali S, Naserpour-Farivar T, Azarhoosh K, Peymani A. Distribution of the bla OXA , bla VEB-1 , and bla GES-1 genes and resistance patterns of ESBL-producing Pseudomonas aeruginosa isolated from hospitals in Tehran and Qazvin, Iran. *Rev Soc Bras Med Trop*. 2017;**50**(3):315–20. [PubMed ID: 28700048]. https://doi.org/10.1590/0037-8682-0478-2016.