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Prevalence of Virulence Genes and Drug Resistance Profiles of *Pseudomonas aeruginosa* Isolated from Clinical Specimens

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Abstract

Background: Drug resistance and virulence genes are two key factors for the colonization of *Pseudomonas aeruginosa* in settings with high antibiotic pressure, such as hospitals, and the development of hospital-acquired infections.
Objectives: The objective of this study was to investigate the prevalence of drug resistance and virulence gene profiles in clinical isolates of *P. aeruginosa* in Ardabil, Iran.
Methods: A total of 84 *P. aeruginosa* isolates were collected from clinical specimens of Ardabil hospitals and confirmed using laboratory standard tests. The disk diffusion method was used for antibiotic susceptibility testing and polymerase chain reaction (PCR) for the identification of *P. aeruginosa* virulence genes.
Results: The highest and the lowest antibiotic resistance rates of *P. aeruginosa* strains were against ticarcillin-clavulanate (94%) and doripenem (33.3%), respectively. In addition, the frequency of multidrug-resistant (MDR) *P. aeruginosa* was 55.9%. The prevalence of virulence factor genes was as follows: *algD* 84.5%, *lasB* 86.9%, *plcH* 86.9%, *plcN* 86.9%, *exoU* 56%, *exoS* 51.2%, *toxA* 81%, *nan1* 13.1%, and *pilB* 33.3%. A significant association was observed between resistance to some antibiotics and the prevalence of virulence genes in *P. aeruginosa*.
Conclusions: Our results revealed a high prevalence of antibiotic resistance, especially MDR, and virulence-associated genes in clinical isolates of *P. aeruginosa* in Ardabil hospitals. Owing to the low resistance rates against doripenem, gentamicin, and tobramycin, these antibiotics are recommended for the treatment of infections caused by highly resistant and virulent *P. aeruginosa* strains.

Keywords: *Pseudomonas aeruginosa*, Antibiotic Resistance, Virulence Gene

1. Background

Pseudomonas aeruginosa is a small Gram-negative bacillus that is commonly arranged in pairs. It is an obligately aerobic, non-fermentative, and oxidase-positive bacterium (1-3). This opportunistic microorganism is ubiquitous, especially in hospitals, and is rarely related to infections in the natural host. Nevertheless, *P. aeruginosa* is a causative agent for 8% of all hospital-acquired infections, including pulmonary infections (tracheobronchitis and necrotizing bronchopneumonia), skin and soft-tissue infections (burn wounds and surgical site infections), urinary tract infections, bacteremia, and endocarditis (1, 4). Acute and chronic infections of *P. aeruginosa* are more common in hospitalized patients in the intensive care unit (ICU), ventilator-dependent and immunocompromised patients, and those receiving broad-spectrum antibiotics (1, 4). On the other hand, there are community-acquired infections associated with *P. aeruginosa*, including ulcerative keratitis, otitis externa, skin, and soft-tissue infections (diabetic foot infections) (5).

Simple growth requirements, broad environmental distribution, antimicrobial resistance, and virulence factors account for the widespread *P. aeruginosa* infections (1, 6). *Pseudomonas aeruginosa* drug resistance and the presence of virulence factors have been shown in numerous studies. However, there are no data on the prevalence of *P. aeruginosa* antibiotic resistance and virulence factors profile in Ardabil city of Iran. Antibiotic resistance in *P. aeruginosa* strains has an intrinsic, acquired, or adaptive char-

acteristic (1). Based on the Centers for Disease Control and Prevention (CDC) report, multidrug-resistant (MDR) *P. aeruginosa* strains are a significant threat to public health, and are associated with 13% of hospital-acquired infections (4). On the other hand, drug-resistant and virulent strains of *P. aeruginosa* possess many virulence factors such as adhesins (flagella, pili, lipopolysaccharide (LPS), and alginate), secreted toxins (exotoxin A), enzymes (elastases, phospholipases, and exoenzymes), and secretion systems, which contribute to the development of severe infections that are hard to treat (1, 7).

The most common *P. aeruginosa* virulence factors that are assessed in the current study include: (1) pili, encoded by the *pilB* gene; (2) alginate, encoded by the *algD* gene, which is an exopolysaccharide capsule with an important role in bacterial survival against host's immune response and drugs; (3) sialidase, encoded by the *nan1* gene, which is involved in the adherence to the respiratory tract; (4) exotoxin A, encoded by the *toxA* gene, which disrupts protein synthesis in eukaryotic cells; (5) las B elastase, a zinc metalloprotease encoded by the *lasB* gene, which destroys the collagen and elastin proteins in lung tissue; (6) hemolytic phospholipase C, encoded by the *plcH* gene; (7) non-hemolytic phospholipase C, encoded by the *plcN* gene; (8) exoenzyme S, encoded by the *exoS* gene, which is involved in colonization, invasion, and bacterial dissemination; and (9) exoenzyme U, encoded by the *exoU* gene, which is involved in tissue destruction and inflammatory response (1, 8).

2. Objectives

Awareness of the distribution of virulence genes and antibiotic resistance in clinical isolates is important for understanding *P. aeruginosa* infections epidemiology. Therefore, this study aimed to assess the *P. aeruginosa* drug resistance pattern and the most prevalent *P. aeruginosa* virulence gene profiles.

3. Methods

3.1. Pseudomonas aeruginosa Strains

Eighty-four clinical isolates of *P. aeruginosa* were collected from five hospitals (Imam Reza, Imam Khomeini, Bu-Ali, Alavi, Sabalan, Fatemi, and Ghaem) in Ardabil, Iran, between June 2019 and February 2021. *Pseudomonas aeruginosa* isolates were obtained from various specimens including wound, blood, urine, sputum, and cerebrospinal fluid (CSF) and then identified using standard microbiology tests such as phenotypic methods, i.e., pigment production, Gram staining, colony morphology, and oxidase and the genotypic method using PCR with specific primers of species (9). Isolated bacteria were then stored in cryovials containing tryptic soy broth plus 20% glycerol at -20°C until drug susceptibility testing, genomic DNA extraction, and PCR assay.

3.2. Drug Susceptibility Testing

Pseudomonas aeruginosa susceptibility patterns were determined using the Kirby-Bauer disk diffusion method against three antibiotic groups recommended by the Clinical and Laboratory Standards Institute (CLSI) guideline (10). They were group A antibiotics including piperacillin-tazobactam (100/10 μg), ceftazidime (30 μg), gentamicin (10 μg), and tobramycin (10 μg), group B antibiotics including cefepime (30 μg), aztreonam (30 μg), imipenem (10 μg), meropenem (10 μg), doripenem (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), and levofloxacin (5 μg), and group O antibiotics including piperacillin (100 μg), ticarcillin-clavulanate (75/10 μg), netilmicin (30 μg), norfloxacin (10 μg), lomefloxacin (10 μg), and ofloxacin (5 μg) (Padtan Teb, Iran, Cypress Diagnostics, Belgium). For this purpose, a bacterial standard suspension (0.5 McFarland turbidity standard) equivalent to 1.5×10^8 CFU/mL was prepared and then poured on Mueller-Hinton agar medium (Conda, Pronasida, Spain). Antibiotic disks with specific concentrations were placed onto the lawns of bacteria and then incubated for 16 - 18 hours at 37°C. Inhibition zones around each disk were measured based on the CLSI guidelines and reported as susceptible, intermediate, or resistant. *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of disks, media, inoculum preparation, and zones' measurement in drug susceptibility tests.

3.3. Genomic DNA Extraction and PCR Assay

Bacterial genomic DNA from each confirmed *P. aeruginosa* strain was extracted using the boiling method and used for amplifying the selected virulence genes by an Eppendorf Thermal Cycler (Hamburg, Germany). Table 1 shows the oligonucleotide primers (SinaClon, Iran) along with programs used in the PCR assay. Each reaction of PCR assay was performed in a volume of 25 μL containing 20 μL of master mix (Tris-HCl pH 8.5, (NH4)2SO4, 3 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM dNTPs, 0.2 units/μL Ampliqon Taq DNA polymerase) (Ampliqon, Denmark), 1 μL of each of the forward and reverse primers (10 μmol/L), and 3 μL of template DNA. The presence of *P. aeruginosa* virulence genes in PCR products was detected using 1% agarose gel electrophoresis, with the TBE 0.5x buffer (Tris-Borate-EDTA) at 100 V for one hour, and then confirmed by sequencing.

Table 1. List of Oligonucleotide Primers and PCR Programs Used in the Present Study

Gene	Oligonucleotide Sequence (5′ to 3′)	Thermal Cycling Condition for Amplification	Amplicon Size (bp)	References
algD	F: CGTCTGCCGCGAGATCGGCT; R: GACCTCGACGGTCTTGCGGA	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 63°C for 1.5 min; extension at 72°C for 1 min (30 cycles)	313	(11)
lasB	F: GGAATGAACGAAGCGTTCTCCGAC; R: TTGGCGTCGACGAACACCTCG	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 63°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	284	(11)
plcH	F: GCACGTGGTCATCCTGATGC; R: TCCGTAGGCGTCGACGTAC	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 60°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	608	(11)
plcN	F: TCCGTTATCGCAACCAGCCCTACG; R: TCGCTGTCGAGCAGGTCGAAC	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 63°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	481	(11)
exoU	F: GGGAATACTTTCCGGGAAGTT; R: CGATCTCGCTGCTAATGTGTT	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 56°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	428	(12)
exoS	F: CGTCGTGTTCAAGCAGATGGTGCTG; R: CCGAACCGCTTCACCAGGC	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 60°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	444	(11)
toxA	F: CTGCGCGGGTCTATGTGCC; R: GATGCTGGACGGGTCGAG	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 63°C for 1.5 min, extension at 72°C for 1 min (30 cycles)	270	(11)
nan1	F: AGGATGAATACTTATTTTGAT; R: TCACTAAATCCATCTCTGACCCGATA	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 49°C for 1 min, extension at 72°C for 1 min (30 cycles)	1316	(13)
pilB	F: ATGAACGACAGCATCCAACT; R: GGGTGTTGACGCGAAAGTCGAT	Initial denaturation at 95°C for 5 min (1 cycle); Denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min (30 cycles)	826	(12)

3.4. Statistical Analysis

The prevalence of virulence genes and antibiotic resistance profile based on gender, sample source, and hospital ward was determined and then compared between different groups using the chi-square test. A P value of < 0.05 was considered statistically significant. All analyses were performed using SPSS software (version 16).

4. Results

4.1. Characteristics of the Patients and Specimens

A total of 84 *P. aeruginosa* strains were isolated from different clinical specimens in this cross-sectional study. The detailed information of the patients, specimens, and hospital wards were as follows: (1) 47.6% (n = 40) of *P. aeruginosa* strains were obtained from males and 52.4% (n = 44) from females. The mean age of the patients was 56.6 ± 18.6 years (6 - 89); (2) 46.5% (n = 39) of *P. aeruginosa* strains were obtained from Alavi hospital, 32.1% (n = 27) from Imam Khomeini hospital, 10.7% (n = 9) from Imam Reza hospital, 7.1% (n = 6) from Bu-Ali hospital, and 3.6% (n = 3) from Sabalan, Fatemi and Ghaem hospitals; (3) 51.2% of *P. aeruginosa* strains were isolated from urine specimens (n = 43), 17.9% from blood (n = 15), 19% from sputum (n = 16), 10.7% from wound (n = 9), and 1.2% (n = 1) from CSF; (4) The highest rate of *P. aeruginosa* strains isolated belonged to the ICU (32.1%, n = 27), followed by internal (31%, n = 26), emergency (16.6%, n = 14), neurology (17.9%, n = 15), and pediatric (2.4%, n = 2) wards.

4.2. Antibiotic Resistance Profile

The distribution of antibiotic resistance profiles is shown in Table 2. The highest and the lowest antibiotic resistance rates of *P. aeruginosa* were against ticarcillin-clavulanate (94%) and doripenem (33.3%), respectively. In addition, the prevalence of MDR isolates was 55.9% (n =

47). *Pseudomonas aeruginosa* isolates resistant to at least one antibiotic in the three classes of antibiotics were considered as MDR strains (9). The presence of statistically significant associations between the prevalence of antibiotic resistance and virulence genes was as follows: piperacillin vs. *exoU* and *pilB*, piperacillin-tazobactam vs. *exoU* and *pilB*, ticarcillin-clavulanate vs. *exoU*, ceftazidime vs. *exoU* and *pilB*, cefepime vs. *exoU* and *pilB*, aztreonam vs. *plcH*, *plcN*, and *pilB*, doripenem vs. *exoU*, *nan1*, and *pilB*, imipenem vs. *exoU*, *exoS*, *nan1*, and *pilB*, meropenem vs. *exoU* and *pilB*, gentamicin vs. *exoU* and *pilB*, tobramycin vs. *exoU* and *pilB*, amikacin vs. *exoU* and *pilB*, netilmicin vs. *exoU* and *pilB*, ciprofloxacin vs. *exoU*, *exoS*, and *pilB*, levofloxacin vs. *lasB*, *exoU*, and *exoS*, norfloxacin vs. *exoU* and *exoS*, lomefloxacin vs. *exoU*, and ofloxacin vs. *exoS* (P value < 0.05).

4.3. Virulence Genes Profile

The frequency of virulence genes of *P. aeruginosa* was assessed using the PCR method and confirmed by sequencing (Figure 1). Nucleotide sequences were submitted to the GenBank database and the accession numbers were received (OK146924 to OK146931). The total prevalence of virulence genes of *P. aeruginosa* was as follows: (1) *algD* 84.5%, (2) *lasB* 86.9%, (3) *plcH* 86.9%, (4) *plcN* 86.9%, (5) *exoU* 56%, (6) *exoS* 51.2%, (7) *toxA* 81%, (8) *nan1* 13.1%, and (9) *pilB* 33.3%. Based on the findings of this study, there was no statistically significant association between the presence of virulence genes and sample type, except for the *plcH* and *plcN* genes (P = 0.02). Additionally, there was no significant difference in the prevalence of virulence genes between different genders and hospital wards (Table 3). Noteworthy, only one strain of *P. aeruginosa* isolated from the urine specimen of a (male) child hospitalized in the pediatric ward simultaneously contained all evaluated virulence genes (nine genes). As shown in Table 3, the highest prevalence rate of *algD*, *lasB*, *plcH*, *plcN*, *exoU*, *exoS*, *toxA*, *nan1*, and *pilB* genes was seen in urine specimens. Furthermore, isolates containing these genes were mostly recovered from patients hospitalized in the ICU and internal wards.

5. Discussion

The emergence of bacterial antibiotic resistance is a growing threat to public health all over the world, especially in developing countries (14). According to predictions, antibiotic-resistant infections may lead to 10 million deaths per year by 2050 (14). Antibiotic resistance in *P. aeruginosa* is an important issue, particularly in patients with cystic fibrosis as well as hospitalized and immunocompromised patients (5). In 2017, the World Health Organization (WHO) listed carbapenem-resistant *P. aeruginosa* strains among important pathogens for which there is a need for new antibiotics (15). On the other hand, infections caused by MDR *P. aeruginosa* are also a global public health issue (16). In the present study, the frequency of MDR *P. aeruginosa* was 55.9%, which is close to the average prevalence reported from Iran (58%) (17). Carbapenems along with fluoroquinolones are two effective treatments against severe infections caused by MDR *P. aeruginosa* in hospital settings (18, 19).

In the current study, the prevalence of *P. aeruginosa* resistance to carbapenems was high (Table 2). Imipenem-resistant *P. aeruginosa* (66.7%) in this study was higher than those reported from Ahvaz (42.9%), Tabriz (49%), Urmia (30.8%), Zanjan (29.2%), Guilan (23.3%), Zahedan (17.2%), and Hamadan (7.5%), and lower than in Isfahan (76.1%) and Tehran (70.4%) (17). Additionally, the resistance rate to another carbapenem, i.e., meropenem, was high (42.9%) in Ardabil, which is comparable with that in Isfahan (93%), Tehran (78.8%), Ahvaz (44.1%), Urmia (39.4%), and Hamadan (13.2%) (17). Therefore, the use of carbapenems for the treatment of MDR *P. aeruginosa* infections is not recommended in Ardabil, except for doripenem (33.3%). Carbapenem-resistant *P. aeruginosa* strains indicated a high level of resistance to all β-lactam antibiotics, except for aztreonam (20). However, resistance to aztreonam was high in this study (42.9%). Similar results have been observed in other cities of Iran, including Ahvaz (91.3%), Tehran (83.7%), Isfahan (69%), Tabriz (60%), Urmia (56.3%), and Zanjan (37.5%), while the aztreonam-resistant rate reported from Zahedan was lower (14.7%) (17).

As seen in Table 2, resistance to other β-lactam antibiotics was high in Ardabil. *Pseudomonas aeruginosa* resistance to β-lactams can be attributed to β-lactamase enzymes, antibiotic efflux pumps, and reduced drug uptake (21). However, mechanisms of resistance to β-lactam antibiotics are not completely clear in local strains from Ardabil. As shown in Table 2, the resistance rate to fluoroquinolones was high in Ardabil city compared to other antibiotic classes. Fluoroquinolones, particularly ciprofloxacin, have remained as one of the most important antibiotics to treat a wide range of *P. aeruginosa* infections, including bacteremia, osteochondritis, ear and eye infections, external otitis, and chronic lung infections in cystic fibrosis patients (22). In this study, the prevalence of ciprofloxacin-resistant *P. aeruginosa* was lower in Ardabil (54.8%) than in Tehran (81.5%), Isfahan (78.7%), Guilan (66.3%), and Tabriz (65%), while it was higher than in Ahvaz (46.8%), Urmia (34.2%), Zanjan (32.5%), Hamadan (4.7%), and Zahedan (3.4%) (17).

Mutations in the ciprofloxacin target-encoding genes *gyrAB* and *parCE* and efflux pump overexpression are associated with resistance to fluoroquinolones (22). Amino

Table 2. Antibiotic Resistance Profiles Using Disk Diffusion Method

Antibiotic Category/Antibiotic Agent	Antibiotic Resistance Rate; No. (%)	
	Susceptible	Resistant [a]
Penicillins		
Piperacillin	36 (42.9)	48 (57.1)
β-lactam combination agents		
Piperacillin-tazobactam	45 (53.6)	39 (46.4)
Ticarcillin-clavulanate	5 (6)	79 (94)
Cephems		
Ceftazidime	45 (53.5)	39 (46.5)
Cefepime	42 (50)	42 (50)
Monobactams		
Aztreonam	48 (57.1)	36 (42.9)
Carbapenems		
Doripenem	56 (66.7)	28 (33.3)
Imipenem	28 (33.4)	56 (66.7)
Meropenem	48 (57.1)	36 (42.9)
Aminoglycosides		
Gentamicin	51 (60.7)	33 (39.3)
Tobramycin	52 (61.9)	32 (38.1)
Amikacin	43 (51.2)	41 (48.8)
Netilmicin	41 (48.8)	43 (51.2)
Fluoroquinolones		
Ciprofloxacin	38 (45.2)	46 (54.8)
Levofloxacin	40 (47.6)	44 (52.4)
Norfloxacin	40 (47.6)	44 (52.4)
Lomefloxacin	28 (33.3)	56 (66.7)
Ofloxacin	20 (23.8)	64 (76.2)

[a] *Pseudomonas aeruginosa* strains with intermediate sensitivity were considered as resistant isolates.

Table 3. Distribution of *Pseudomonas aeruginosa* Virulence Genes Based on the Gender, Sample Source, and Hospital Ward

Gene	Gender (%)		P Value	Sample Source (%)				P Value	Ward (%)					P Value
	Male	Female		Urine	Blood	Wound	Sputum		ICU	Emergency	Internal	Neurology	Pediatric	
algD	49.3	50.7	0.472	53.5	18.3	11.3	16.9	0.093	28.2	19.7	33.8	15.5	2.8	0.120
lasB	41.1	58.9	0.074	57.5	15.1	9.6	17.8	0.091	34.2	15.1	30.1	17.8	2.8	0.791
plcH	43.8	56.2	0.254	53.4	17.8	11	17.8	0.029	32.9	15.1	34.2	15.1	2.7	0.529
plcN	43.8	56.2	0.254	54.8	17.8	9.6	17.8	0.029	31.5	22	26	17.8	2.7	0.529
exoU	49	51	0.867	63.9	4.3	12.7	19.1	0.052	29.8	12.8	29.8	23.4	4.2	0.631
exoS	39.5	60.5	0.129	51.1	20.9	7	21	0.399	34.9	9.3	27.9	23.2	4.7	0.549
toxA	44.1	55.2	0.442	57.4	14.7	10.3	17.6	0.124	30.9	16.2	33.8	16.2	2.9	0.516
nan1	54.5	45.5	0.254	45.4	27.3	18.2	9.1	0.415	27.3	18.2	36.3	9.1	9.1	0.362
pilB	53.5	46.5	0.440	67.9	17.8	10.7	3.6	0.107	28.6	21.5	39.3	7.1	3.5	0.230

Figure 1. Gel electrophoresis of PCR products of *P. aeruginosa* virulence genes. Lane 1, *algD* (313 bp); Lane 2, *lasB* (284 bp); Lane 3, *plcH* (608 bp); Lane 4, *plcN* (481 bp); Lane 5, *exoU* (428 bp); Lane 6, *exoS* (444 bp); Lane 7, *toxA* (270 bp); Lane 8, *nan1* (1316 bp); Lane 9, *pilB* (826 bp); Lane 10, negative control; and Lane M, ladder (100 bp).

acid alterations in the GyrA (Thr83Ile and Asp87Asn) and ParC (Ser87Leu and Ser87Trp) subunits of DNA gyrase and topoisomerase IV enzymes are involved in *P. aeruginosa* resistance to ciprofloxacin in Ardabil (19). Some aminoglycosides, such as amikacin and tobramycin, are commonly used for the treatment of pulmonary infections in patients with cystic fibrosis (21). Altogether, the aminoglycoside-resistant *P. aeruginosa* rate was found to be high in this study (Table 2). However, it seems that gentamicin and tobramycin are more effective than netilmicin and amikacin against *P. aeruginosa* infections in Ardabil. Aminoglycoside-modifying enzymes, rRNA methylases, and efflux pumps are involved in *P. aeruginosa* resistance to aminoglycosides (21). Overall, differences in *P. aeruginosa* drug resistance rates between this study and other studies in Iran can be attributed to self-medication, as well as inappropriate prescription and overuse of antibiotics.

Irrational use of antibiotics and the ensuing rise in the prevalence of antibiotic resistance, especially MDR, in clinical isolates of *P. aeruginosa* in Ardabil hospitals have a significant public health impact and may lead to increased hospitalization period medical costs, and mortality. On the other hand, it has been suggested that there is a relationship between drug resistance and virulence-associated genes in opportunistic bacteria such as drug-resistant *P. aeruginosa* (23). In the current study, a significant association was observed between resistance to some antibiotics and the prevalence of virulence genes in *P. aeruginosa*. These resistant and highly virulent bacteria can easily colonize in new environments or specific ecological niches with high antibiotic pressure, such as hospitals, and can cause diseases more efficiently (23). The current findings suggest that the prevalence of three virulence genes, i.e., *lasB*, *plcH*, and *plcN* (86.9%), in *P. aeruginosa* was higher than that of other virulence-associated genes in Ardabil. The most prevalent virulence genes of *P. aeruginosa* observed in other studies in Iran were as follows: (1) *lasB* gene (95.4%) (11); (2) *exoS* gene (92.9%) (12); (3) *toxA* gene (79.4%) (24); (4) *lasB* gene (92.9%) (25); (5) *toxA* gene (100%) (26); (6) *lasB* gene (100%) (27); and (7) *lasB* gene (100%) (28).

5.1. Conclusions

The current study revealed a high prevalence of resistance to all assessed antibiotics, except for doripenem, gentamicin, and tobramycin, in clinical isolates of *P. aeruginosa*. On the other hand, the number of MDR isolates was also alarmingly high. The highly virulent strains were prevalent in *P. aeruginosa* isolated from different specimens and hospital wards. This condition can be problematic for the efficient treatment of *P. aeruginosa*-associated infections in Ardabil hospitals. Hence, the continuous monitoring of *P. aeruginosa* isolates in terms of drug resistance trend, and virulence gene profile is needed.

Footnotes

Authors' Contribution: Study concept and design, F.K., M.A., and H.P.D.; Acquisition of data, S.A.B.; Analysis and interpretation of data, F.K. and H.V.; Wrote the manuscript, F.K.; Revision of the manuscript, H.V. and A.S. All authors read and approved the final manuscript.

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Ethical Approval: This research was approved by the Regional Research Ethics Committee (approval ID: IR.ARUMS.REC.1398.386).

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