Original Research Article Iran J Med Microbiol. 2025; 19(5): 330-340

Category: Antibiotic Resistance Peer Reviewed | Open Access

10.30699/ijmm.19.5.330

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Relationship between Biofilm Formation and Antibiotic Resistance in Clinical *Acinetobacter baumannii* Isolates from Hospitalized Patients in Iran

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ABSTRACT

Background and Aim: *Acinetobacter (A.) baumannii* is an opportunistic pathogen in humans, affecting immunocompromised patients and is becoming increasingly important as a hospital-acquired infection. These strains exhibit resistance to essential current antibiotics, posing a real health threat worldwide. The aim of this study was to investigate the relationship between biofilm formation and antibiotic resistance in clinical *Acinetobacter baumannii* isolates.

Materials and Methods: A total of 40 clinical isolates were collected from Iranian hospitalized patients. Disk diffusion method was performed to assess the antimicrobial resistance. Crystal violet staining was used to assess the biofilm-forming ability and polymerase chain reaction (PCR) was employed to determine the frequency of biofilm-related genes (*bfm*R, *bfm*S, *pga*A, *bap*, *omp*A, *csu*E, and *aba*I). The relationship between biofilm-forming ability and resistance to antibiotics was statistically analyzed using Fisher's exact test and P≤0.05 was considered significant.

Results: All *A. baumannii* isolates were multidrug-resistant (MDR). The frequency of extensively drug-resistant (XDR) was 80%, and the rest were pan-drug-resistant (PDR). Most of the MDR isolates (85%) were strong biofilm formers, and the rest of them were moderate biofilm formers. All PDRs and 81.25% of XDRs formed strong biofilms. The *bfm*R, *bfm*S, *pga*A, *bap*, and *omp*A genes were existed in all MDRs. The *csu*E and *aba*I genes were detected in 97.5% and 82.5% of MDRs, respectively. All XDRs carried *csu*E gene, and 90.62% possessed *aba*I gene. Among PDR isolates, 87.5% presented *csu*E gene, and 50% exhibited *aba*I gene.

Conclusion: The presence of biofilm-forming genes may be related to antibiotic resistance in clinical *A. baumannii* isolates, although a clone of PDR is evolving with strong biofilm-forming ability and lacking *aba*I gene.

Keywords: *Acinetobacter baumannii*, Biofilm, Extensively Drug-resistant, Multidrug-resistant, Pan-drug-resistant

Received: 2025/06/10; **Accepted**: 2025/09/20; **Published Online:** 2025/11/11;

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1. Introduction

Acinetobacter (A.) baumannii is one of the main causative agents of severe hospital-acquired infections. These strains exhibit resistance to essential current antibiotics, including beta (β)-lactams, carbapenems, quinolones, aminoglycosides, and colistin, posing a real health threat worldwide in recent years **(1-3)**.

According to Clinical and Laboratory Standard Institute 2020 (CLSI, 2020), *A. baumannii* strains are classified based on their antibiotic resistance ability into these phenotypes: multi-drug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR). Strains resistant to three or more antibiotic classes are categorized as MDR. Strains resistant to all antibiotic classes except two or fewer are classified as XDR. Strains resistant to all members of all antibiotic classes are defined as PDR **(4)**.

The emergence of antibiotic resistance in *A. baumannii* strains is often due to chromosomal mutations as well as the horizontal transfer of resistance genes between bacteria. Mobile genetic elements, including plasmids, integrons, and transposons, are responsible for the simultaneous transfer of resistance genes (carbapenemases, β-lactamases, metallo-β-lactamases, etc.) in *A. baumannii* strains, leading to resistance to multiple antibiotics **(5)**. Carbapenem resistance in MDR *A. baumannii* occurs through class D β-lactamases, which are encoded in *A. baumannii* both chromosomally and plasmid-mediated. The intrinsic carbapenemase-encoding gene in *A. baumannii* is provided by the $bla_{\text{OXA-51-like}}$ gene. The main genes encoding CHDLs (carbapenem-hydrolyzing class D β-lactamases) in *A. baumannii* strains are $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51-like}}$.

In Iran, the $bla_{\text{OXA-23}}$ gene has been identified as the principal factor of carbapenem resistance and is also considered the most abundant OXA gene globally **(6-8)**. Biofilm formation is one of the main mechanisms used by *A. baumannii* to develop resistance to antibiotics. Under antibiotic stress conditions, bacteria enter a physiological dormancy, in which they can tolerate antibiotics and thus achieve successful colonization **(9)**. Through biofilm formation, *A. baumannii* gains resistance to desiccation stress and antibiotic treatment, as well as the ability to interact with host epithelial cells and matrix proteins **(10)**. The *csu*E, *bfm*R, *bfm*S, *pga*A, *bap*, *omp*A, and *aba*I genes are involved in different stages of biofilm formation in *A. baumannii* isolates; *csu*E, *bfm*R, and *bfm*S are involved in the primary attachment of *A. baumannii* to surfaces; *omp*A mediates adhesion to human epithelial cells, biofilm formation, modulation of host immune response, causes host cell apoptosis, and induction of antibiotic resistance; *pga*A facilitates cell adhesion, maintains biofilm integrity, and contributes to the stability of *A. baumannii*; the bap gene leads biofilm maturation, facilitating the adhesion of *A. baumannii* to bronchial epithelial cells **(11)**. Moreover, during biofilm growth and maturation, quorum sensing emerges as a vital mechanism for intercellular communication in *A. baumannii*, allowing coordinated responses to environmental signals. The quorum sensing system in *A. baumannii* is regulated by the AbaI/AbaR two-component system through *aba*I gene encoding **(10)**.

The existence of a synergistic relationship between biofilm formation and the emergence of drug resistance in multidrug resistant *A. baumannii* strains has been investigated by many authors. In a study by Khoshnood et al **(12)**, 50 clinical isolates of *A. baumannii* were examined and 74% of them were detected as MDR phenotype. It was also found that all of the isolates were biofilm formers. Strong biofilm producers were able to develop higher antimicrobial resistance. Furthermore, a high rate of the XDR phenotype was observed in strong biofilm-producing isolates. There was a significant association between XDR and strong biofilm production **(12)**. In another research by Fallah et al **(13)** on 100 isolates of *A. baumannii*, 88% of the isolates were detected as MDR and 75% of them were biofilm formers. A significant relationship was found between the MDR phenotype and strong biofilm ability **(13)**. Moreover, in a study by Chukamnerd et al **(14)**, higher antimicrobial resistance was shown in biofilm former strains compared to those without the ability of producing biofilm **(14)**. Another research reported by Guddeti et al **(15)** found a significant association between antibiotic resistance and biofilm formation in clinical *A. baumannii* isolates **(15)**.

In recent years, excessive and irregular antibiotics use has led to the emergence of a PDR phenotype of *A. baumannii*, which also produces strong biofilms. There are few studies on the relationship between biofilm-producing genes and the occurrence of drug resistance in these types of *A. baumannii* species. This study aims to investigate the relationship between biofilm formation ability and related genes to MDR, XDR, and PDR clinical *A. baumannii* isolates of hospitalized patients in Iran. We also want to assess whether PDR clinical isolates differ from XDR strains in biofilm-related genes?

2. Materials and Methods

2.1 Study Design and Specimen Collection

Clinical *A. baumannii* isolates (n=115) were collected from hospitalized patients (five hospitals in Shiraz and one in Tabriz, Iran) from September 2020 to October 2022. Among them, 103 isolates were phenotypically identified as *A. baumannii* using standard biochemical tests such as urea, nitrate, citrate, gelatin, and SIM (Sulfur, Indole, Motility) **(16)**. The isolates were collected from patients aged between 1 and 84 who were hospitalized in intensive care units (ICUs) of Shiraz (n=29) and non-ICU wards of Tabriz (n=11). The clinical specimens were collected from various sources, including blood, abdominal fluid, chest tube fluid, endotracheal tube fluid, urine, wound, nasal swab, and sputum.

2.2 Molecular Identification of *A. baumannii* Isolates

A. baumannii detected isolates were cultured in nutrient broth and incubated at 37°C for 24 hours. Subsequently, DNA extraction was carried out on isolated colonies using the boiling method **(17)**. PCR amplification for *bla*$_{\text{OXA-51-Like}}$ gene was performed using an optimal annealing temperature determined by gradient PCR. Initial denaturation was performed at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 45°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min **(18)** (Table 1). Electrophoresis was performed on 1% agarose gel and the results were analyzed using an ultraviolet (UV) illuminator. *Acinetobacter baumannii ATCC19606* was used as the standard reference strain in all steps of this study. To prepare a negative control sample, sterile distilled water was added to the PCR mixture instead of the bacterial DNA sample.

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out using disk diffusion method. Bacterial inoculum suspension was prepared using Muller Hinton agar (MHA) and Mueller-Hinton broth (MHB) at concentration of 0.5 McFarland standard (1.5×10^6 CFU/mL). The procedures were performed according to the Clinical and Laboratory Standards Institute 2020 (CLSI 2020) guidelines **(19)**. The used antimicrobial disks were as follow: trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (30 µg), piperacillin-tazobactam (100/10 µg), ampicillin-sulbactam (10/10 µg), cefepime (30 µg), gentamicin (10 µg), amikacin (30 µg), ceftazidime (30 µg), meropenem (10 µg), and colistin (10 µg).

2.4 Biofilm Formation Assay

The biofilm-forming ability of *A. baumannii* isolates was evaluated phenotypically based on previous study using 1% crystal violet microtiter plate assay and the absorbance of each well was measured at 595 nm using a microplate reader. Each isolate was tested in triplicate manner **(20)**. Based on the average optical density of the samples (ODi) and the average optical density of the negative control (ODc), the isolates were categorized as follow: non-adherent when ODi < ODc, weakly adherent when ODc < ODi ≤ 2xODc, moderately adherent when 2xODc < ODi ≤ 4xODc, and strongly adherent when 4xODc < ODi **(21)**.

2.5 Gene Targeting by Multiplex PCR

The clinical *A. baumannii* isolates were molecularly identified by *bla*$_{\text{OXA-51-Like}}$ gene PCR **(18)**. In these clinical isolates, a modified multiplex PCR program was used to simultaneously amplify three genes (*bfm*S, *bfm*R, and *aba*I), two genes (*omp*A and *csu*E), and two genes (*pga*A and *bap*) **(18)**. The PCR thermal program was as follows: denaturation at 94°C for 5 min, followed by 33 cycles of denaturation at 94°C for 1 min. Annealing temperatures of 53.2°C, 53.5°C, and 53.2°C were determined from gradient PCR, with an extension step of 1 min and a final extension of 5 min. PCR assays were performed using the primers documented in Table 1. Throughout the test, the standard strain *Acinetobacter baumannii ATCC19606* was utilized. The resulting PCR products were subjected to horizontal electrophoresis on a 1% agarose gel, and the results were analyzed using a UV illuminator. A 1000 bp molecular ladder (Cinnagen, Iran) was employed as a size reference marker.

2.6 Statistical Analysis

The software IBM SPSS Statistics version 27.0 (IBM Corp., USA) was used to explore the correlation between the presence of *bla*$_{\text{OXA-51-like}}$ gene, biofilm-forming genes, and various antibiotic resistance profiles (MDR, XDR, PDR). P values less than 0.05 were considered statistically significant.

Table 1. Primers sequences of genes used in gradient PCR program for *A. baumannii* isolates.

Genes	Primer sequence (5´→ 3´)	PCR product (bp)	Annealing temperature (°c)	References
bla$_{OXA-51}$F	ATGAACATTAAAGCACT	825	45	Al-Shamiri et al **(18)**
bla$_{OXA-51}$R	CTATAAAATACCTAATTGTTC			
*csu*EF	ACCAATGCTCAGACCGGAG	751	53.5	Al-Shamiri et al **(18)**
*csu*ER	CTTGTACCGTGACCGTATCTTG			
*bfm*RF	GAAGTTGGTGTAGAAACCGATG	557	53.2	Al-Shamiri et al **(18)**
*bfm*RR	GGATTTTCAGGATCATCGCC			
*bfm*SF	CATTAGTGAAGGAGTCGCTCG	990	53.2	Al-Shamiri et al **(18)**
*bfm*SR	GGTGTACCCTGCTCTAGTTTT			
*aba*IF	CCGCTACAGGGTATTTGTTGAA	428	53.2	Al-Shamiri et al **(18)**
*aba*IR	CACGATGGGCACGAAAACC			
*omp*AF	GAGTCGTATTGCACTTGCTAC	594	53.5	Al-Shamiri et al **(18)**
*omp*AR	GCAGGCTTCAAGTGACCACC			
*bap*F	GAGGGAACTTCTGCAAAACTTTC	108	53.2	Al-Shamiri et al **(18)**
*bap*R	CAGACGTATGACTGCATTGGT			
*pga*AF	ATTCAAAAGTCAGTTGATGGGC	460	53.2	Al-Shamiri et al **(18)**
*pga*AR	TTTTTTGTCCTTGCTCCAGC			

3. Results

3.1 Biochemical Identification of *A. baumannii* Isolates

Out of 115 clinical samples collected from hospitalized patients, 103 isolates were phenotypically identified as *A. baumannii* using the standard biochemical tests.

3.2 Molecular Identification of *A. baumannii* Isolates by *bla*$_{OXA-51-like}$ Gene

PCR amplification of *bla*$_{OXA-51-like}$ gene revealed that among the total of 115 collected samples, 40 (34.78%) possessed the gene, and were identified as *A. baumannii*, which were used for further analysis (Figure 1).

3.3 Antimicrobial Susceptibility Analysis

Evaluation of the clinical *A. baumannii* isolates using disk diffusion method revealed that all isolates were of MDR phenotype, and 80% of these isolates were XDR, i.e. they were resistant to three major classes of antibiotics including: carbapenems, fluoroquinolones, and cephalosporins. From XDR isolates, 96.87% and 93.75% were resistant to aminoglycosides and t β-lactamase inhibitors or sulfonamides, respectively. Most of the XDRs (96.87%) were susceptible to polymyxins (colistin). Additionally, 20% of the MDR isolates were resistant to all antibiotic classes and categorized as the PDR phenotype. The results of the antibiotic susceptibility test for *A. baumannii* isolates are summarized in Tables 2 and 3.

3.4 Biofilm Formation Assay Results

The results of biofilm formation assay showed the optical density (OD) values of the isolates ranged from 0.775 to 3.848. The strains were scored based on the obtained OD values regarding their ability to produce biofilm. Accordingly, all our *A. baumannii* isolates were placed into moderate and strong categories. The results are presented in Table 4.

The results showed that most of the XDR isolates tended to form strong biofilms (81.25%), and the rest were moderate biofilm formers. Furthermore, it was detected that all PDRs produced strong biofilms.

3.5 Detection of Biofilm-Associated Genes by Multiplex PCR

The results of the Multiplex-PCR assay are demonstrated in Figure 2. It was revealed that all 40 isolates (100%) were positive for *bfm*S, *bfm*R, *pga*A, *bap*, and *omp*A genes. Among them, 97.5% possessed the *csu*E gene, and 82.5% had the *aba*I gene. In addition, 33 isolates (82.5%) included all genes.

Gene analysis of XDR isolates showed that all of them had the *csu*E gene, and 90.62% possessed the *aba*I gene. Among the PDR isolates, 87.5% presented the *csu*E gene, and half of them exhibited the *aba*I gene. Fisher exact test revealed a significant relationship between the deletion of the *aba*I gene and the emergence of PDR phenotype (P=0.02). As the frequency of other 5 genes

(*bfm*S, *bfm*R, *pga*A, *bap*, and *omp*A) were 100% in both XDR and PDR isolates, it was not possible to analyze the relationship between the existence of these genes and antibiotic resistance pattern in XDR or PDR isolates (Figure 3).

Table 2. The results of agar disk diffusion test for clinical *A. baumannii* isolates.

Antibiotic disk	Resistance rate
Meropenem	100%
Ciprofloxacin	100%
Cefepime	100%
Ceftazidime	100%
Piperacillin-Tazobactam	100%
Amikacin	97.5%
Gentamicin	97.5%
Trimethoprim-Sulfamethoxazole	95%
Ampicillin-Sulbactam	95%
Colistin	22.5%

Table 3. Antibiotic resistance patterns of clinical *A. baumannii* isolates.

Antibiotic resistance phenotype	Antibiotic classes
MDR (n=40)	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides (n=28)
	Betaβ-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides/ Polymyxins (n=8)
	Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides/ Polymyxins (n=1)
	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones (n=1)
	Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides (n=1)
	β-lactamase Inhibitor/ Cephalosporin/ Carbapenem/ Fluoroquinolone (n=1)
XDR (n=32)	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides (n=28)
	Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides/ Polymyxins (n=1)
	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones (n=1)
	Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides (n=1)
	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Fluoroquinolones (n=1)
PDR (n=8)	β-lactamase Inhibitors/ Cephalosporins/ Carbapenems/ Aminoglycosides/ Fluoroquinolones/ Sulfonamides/ Polymyxins (n=8)

β-lactamase Inhibitors (piperacillin-tazobactam, ampicillin-sulbactam), Cephalosporins (cefepime, ceftazidime), Carbapenems (meropenem), Aminoglycosides (amikacin, gentamicin), Fluoroquinolones (ciprofloxacin), Sulfonamide (trimethoprim-sulfamethoxazole), Polymyxins (colistin)

Table 4. Antibiotic resistance patterns of clinical *A. baumannii* isolates.

Biofilm score	The average optical density of the isolates (ODi)	N (%)
Non-biofilm former	ODi ≤0.320	0 (0)
Weak biofilm former	0.320 < ODi ≤ 0.640	0 (0)
Moderate biofilm former	0.640 < ODi < 1.28	6 (15)
Strong biofilm former	ODi > 1.28	34 (85)

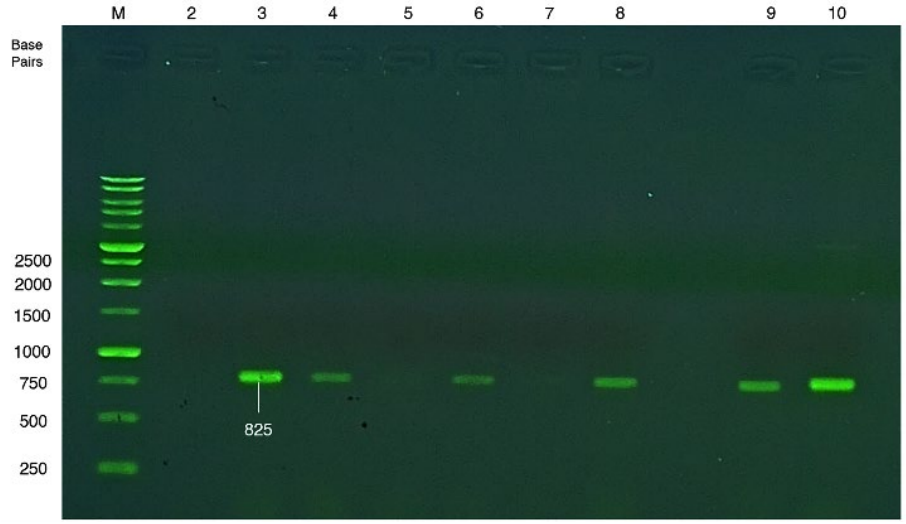

Figure 1. PCR results for the *bla*$_{OXA-51-like}$ gene. First lane: 1000 bp marker, second lane: negative control, third lane: positive control (825 bp), other wells (4-10): the tested isolates **(Prepared by Authors, 2025)**.

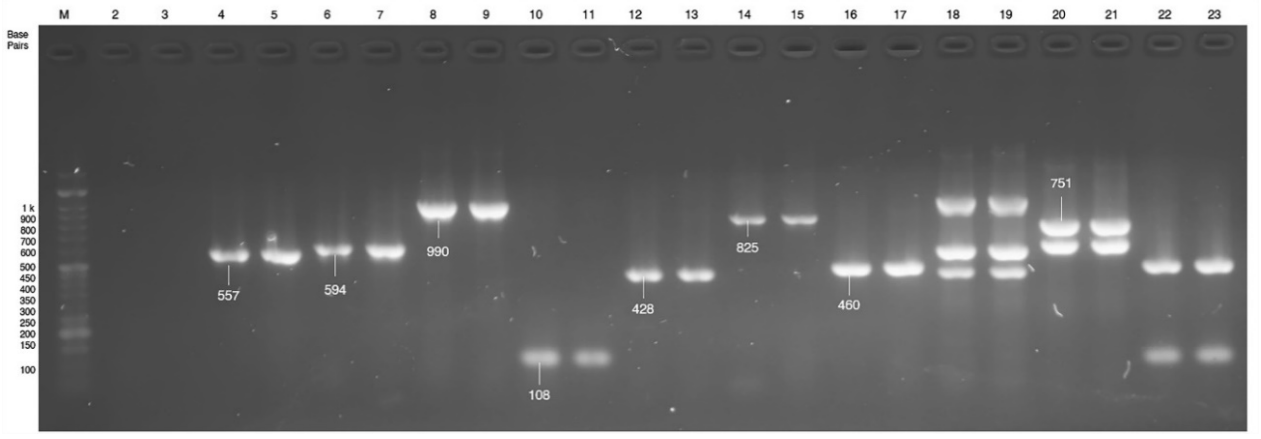

Figure 2. PCR results of seven biofilm-related genes and *bla*$_{OXA-51-Like}$ gene of *A. baumannii* on 1% agarose gel **(Prepared by Authors, 2025)**.

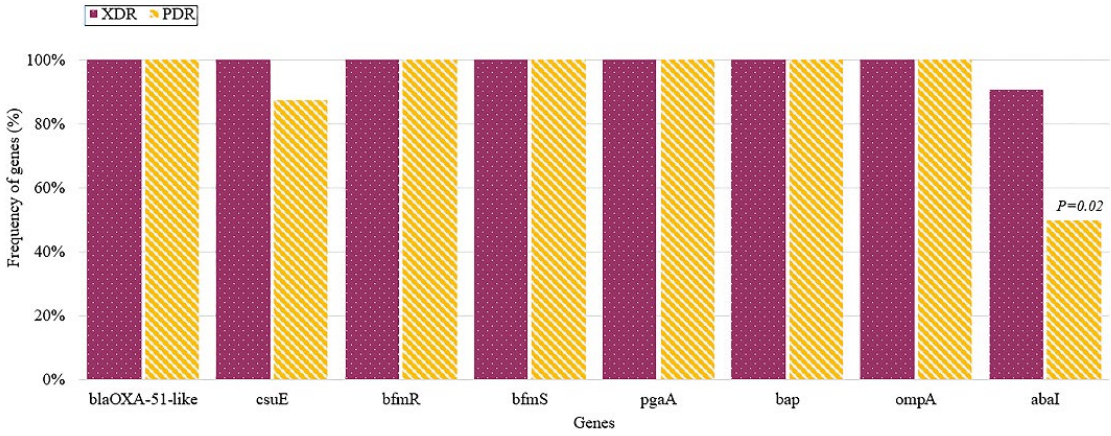

Figure 3. The frequency of biofilm-related genes in multidrug-resistant phenotypes (XDRs, PDRs) of clinical *A. baumannii* isolates. lane 1: 50 bp molecular marker (Cinnagen, Iran), 2: negative control, 4 & 5: *bfm*R (557), 6 & 7: *omp*A (594), 8 & 9: *bfm*S (990), 10 & 11: *bap* (108), 12 & 13: *aba*I (428), 14 & 15: $bla_{\text{OXA-51-Like}}$ (825), 16 & 17: *pga*A (460), 18 & 19: *aba*I/*bfm*R/*bfm*S, 20 & 21: *csu*E (751)/*omp*A, 22 & 23: *pga*A/*bap* **(Prepared by Authors, 2025)**.

4. Discussion

Our results showed that all *A. baumannii* strains examined in this study were multidrug-resistant (MDR). About 80% of MDRs were extensively drug-resistant (XDR), and the remainder (20%) were of PDR phenotype. It means that 20% of the total MDRs of clinical *A. baumannii* isolates were resistant to all available antibiotics. Moreover, all strains were biofilm producers. Approximately, 85% were strong biofilm formers and about 15% showed moderate biofilm-forming ability. Therefore, all the clinical *A. baumannii* isolates were both multidrug-resistant and biofilm formers. Similar results have been reported by the other authors. In a study by Shakib et al **(22)**, it was shown that biofilm production was significantly associated with the prevalence of MDR *A. baumannii* strains. In another research by Yousefi Nojookambari et al **(23)**, it was reported that out of 70 *A. baumannii* isolates, 80% showed MDR phenotype and 92.9% of them were biofilm producers. Similar results have been reported in a study by Alshamiri et al **(18)**, who found a significant correlation between antibiotic resistance and biofilm-forming ability of the clinical *A. baumannii* isolates. Biofilm formation by susceptible strains was significantly lower than resistant strains and the majority of biofilm-forming strains had an antibiotic resistance profile **(18)**.

In the second part of this experiment, we investigated the frequency of biofilm-related genes; *bfm*R, *bfm*S, *pga*A, *bap*, *omp*A, *csu*E, and *aba*I in multidrug-resistant *A. baumannii* clinical isolates. It was revealed that all 40 isolates were positive for *bfm*S, *bfm*R, *pga*A, *bap*, and *omp*A genes, 97.5% possessed the *csu*E gene, and 82.5% had the *aba*I gene. It was also shown that 33 isolates (82.5%) included all genes. The comparison of XDR and PDR phenotypes regarding the presence of biofilm-related genes (*bfm*R, *bfm*S, *pga*A, *bap*, *omp*A, *csu*E, and *aba*I) showed that both XDR and PDR phenotypes possessed the *bfm*R, *bfm*S, *pga*A, *bap*, and *omp*A genes. In addition, all XDRs and 87.5% of PDRs carried the *csu*E gene. Similar results were obtained by Abbaszadeh et al **(8)** who reported that 100% of the XDR *A. baumannii* strains were biofilm formers and all presented the *bfm*R, *bfm*S, *pga*A, *omp*A, and *csu*E genes. In another study by Saadulla and Muhammed **(24)**, a significant association was found between antibiotic resistance, biofilm formation, and biofilm-related genes in *A. baumannii* clinical isolates. It was also shown that strains that possessed the *omp*A, *bap*, and *csu*E genes produced stronger biofilms compared to the strains lacked these genes **(24)**. In another study by Alshmiri et al **(18)**, 25 biofilm-related genes were examined in 70 clinical isolates of *A. baumannii*. It was found that majority of the strains with these genes were biofilm-producers **(18)**. Since all strains were sensitive to colistin and none of them showed the PDR phenotype, no significant association was found between any of the biofilm-related genes examined and the emergence of PDR phenotype. The association between biofilm-related genes and antibiotic resistance in *A. baumannii* strains has been shown in another study conducted by Monfared et al **(25)**. It was found that 83.9% of the *A. baumannii* strains were MDR, 16.1% were XDR, and 7.6% were resistant to colistin. Most of the isolates (72.9%) were able to form biofilms. There was a significant relationship between biofilm-related genes and antibiotic resistance **(25)**. A high frequency of the presence of several biofilm-related genes was also reported in multidrug-resistant *A. baumannii* isolates in a study by Mazraeh et al **(26)**.

Another interesting output of our study, although statistically not significant, was that 12.5% of our PDR isolates lacked *csu*E gene while they were still strong biofilm-formers. The *csu*E gene plays a role in the early stages of biofilm formation for the initial attachment of bacteria to the surfaces. It seems that in a clone of PDR strains of *A. baumannii*, the bacteria preferred to lose this gene to preserve their antibiotic resistance. This phenomenon has been reported by some other researchers. In a study by Acharya et al **(27)**, it was observed that the colistin-resistant strains with biofilm-forming ability showed a significant decrease in *csu*E gene expression. In some resistant strains of *A. baumannii*, csu pili were replaced by type IV pili during biofilm maturation. Type IV pili were encoded by *pil*A gene, therefore, its presence in biofilm-producing strains can explain the absence of the *csu*E gene **(28)**.

One of the important mechanisms for antibiotic resistance in *A. baumannii* strains is biofilm formation. This mechanism limits the antibiotic penetration, makes the bacteria enter a latent phase that protects them against antibiotics, and facilitates their successful colonization **(9)**. Various genetic factors are involved in *A. baumannii* biofilm formation, like CsuA/BABCDE assembly system, which is used for the initial attachment of bacteria to the surfaces and is controlled by the BfmRS two-component system; *omp*A, which is involved in the invasion of epithelial cells; poly-beta-1,6-N-acetylglucosamine (PNAG), a major component of the biofilm matrix that is synthesized by the *pga*ABCD locus; the Wza-Wzb-Wzc system, which is involved in the synthesis and secretion of biofilm matrix exopolysaccharides; and finally, the Bap protein, which aids in biofilm maturation **(11)**.

Quorum sensing is a communication network of autoinducer molecules in Gram-negative bacteria. One benefit of quorum sensing in *A. baumannii* is its contribution to biofilm formation. This is achieved through an autoinducer-receptor mechanism, in which a signaling protein called AbaI guides signals from acyl homoserine lactone (a signaling molecule) to a receptor protein (AbaR). *lux*I (AbaI synthetizer) and *lux*R (AbaR activator) are two main quorum sensing genes in *A. baumannii* and are essential for Acyl homoserine lactones (AHL) production **(8, 26)**.

Another output of our study was that we found a significant difference between XDR and PDR phenotypes concerning the presence of the *aba*I gene ($P \leq 0.05$). In PDR isolates, the frequency of this gene was about 50% that was remarkably less than that in XDR isolates (90.62%). In other words, at least in half of the PDR isolates of our *A. baumannii* strains, the *aba*I gene was deleted. The *aba*I gene is involved in the final stages of bacterial biofilm formation **(8, 26)** and encodes for some important catalysts involved in quorum sensing and recruiting other bacteria to form biofilms **(29)**. It appears that integrity of the outer membrane, especially LPS A, is critical for the formation of a strong biofilm. We speculate that the absence of the *aba*I gene in half of our PDR *A. baumannii* strains facilitates colistin resistance. Other genes may be involved in quorum sensing in addition ot the *aba*I gene in these PDR isolates, but this point needs more detailed investigations.

Limitations

We had intended to extend our project to encompass additional antibiotic sensitivity tests (including minimum inhibitory concentration (MIC)), but the high costs precluded us from pursuing this avenue further. The MIC testing and genomic validations (e.g., sequencing of PCR products) were not performed in our study.

5. Conclusion

Based on the results, all our multidrug-resistant clinical *A. baumannii* isolates were biofilm formers and the majority possessed biofilm-related genes (*bfm*R, *bfm*S, *pga*A, bap, *omp*A, *csu*E, and *aba*I). However, a clone of pan-drug resistant phenotype is also evolving with strong biofilm-forming ability but lacking *aba*I gene.

6. Declarations

6.1 Acknowledgment

We would like to gratitude the Microbiology Laboratory at the Faculty of Veterinary Medicine, Shiraz University, and the Medical Microbiology Laboratory at the School of Paramedical Sciences, Shiraz University of Medical Sciences for their kind unsparing cooperation.

6.2 Ethical Considerations

This study has received ethical approval under the code IR.SUMS.REC.1401.430. The procedures utilized in this study were non-invasive, and all information was treated anonymously.

6.3 Authors' Contributions

R.M. and A.D. and M.M. conceptualized and supervised the study. Z.Z. collected the clinical samples and conducted laboratory experiments. M.M. and Z.Z. analyzed the data and prepared the manuscript. M.M. and R.M. and Z.Z. edited the manuscript. The final version of the manuscript has been approved by all authors.

6.4 Conflict of Interests

The authors declare no conflict of interest.

6.5 Financial Support and Sponsorship

This study was funded by the University of Maragheh.

6.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript, except for grammar checking.

6.7 Data Availability Statement

All data generated or analyzed during this study are included in this article.

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