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RESEARCH ARTICLE

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Antimicrobial resistance and biofilm formation capacity among *Acinetobacter baumannii* strains isolated from patients with burns and ventilator-associated pneumonia

Saeed Khoshnood[1] | Nourkhoda Sadeghifard[1] | Nahid Mahdian[2] | Mohsen Heidary[3] | Somayeh Mahdian[1] | Maryam Mohammadi[2] | Abbas Maleki[1] | Mohammad Hossein Haddadi[1]

[1]Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran

[2]Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

[3]Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran

Correspondence
Mohammad Hossein Haddadi, Clinical Microbiology Research Centre (CMRC), Ilam University of Medical Sciences, Ilam, Iran.
Email: haddadi-m@medilam.ac.ir, haddadi841@gmail.com

Abstract

Background: *Acinetobacter baumannii* is a pathogen responsible for nosocomial infections, especially in patients with burns and ventilator-associated pneumonia (VAP). The aims of this study was to compare the biofilm formation capacity, antimicrobial resistance patterns and molecular typing based on PFGE (Pulsed-Field Gel Electrophoresis) in *A. baumannii* isolated from burn and VAP patients.

Materials and Methods: A total of 50 *A. baumannii* isolates were obtained from burn and VAP patients. In this study, we assessed antimicrobial susceptibility, biofilm formation capacity, PFGE fingerprinting, and the distribution of biofilm-related genes (*csuD*, *csuE*, *ptk*, *ataA*, and *ompA*).

Results: Overall, 74% of the strains were multidrug resistant (MDR), and 26% were extensively drug-resistant (XDR). Regarding biofilm formation capacity, 52%, 36%, and 12% of the isolates were strong, moderate, and weak biofilm producers. Strong biofilm formation capacity significantly correlated with XDR phenotype (12/13, 92.3%). All the isolates harbored at least one biofilm-related gene. The most prevalent gene was *csuD* (98%), followed by *ptk* (90%), *ataA* (88%), *ompA* (86%), and *csuE* (86%). Harboring all the biofilm-related genes was significantly associated with XDR phenotype. Finally, PFGE clustering revealed 6 clusters, among which cluster No. 2 showed a significant correlation with strong biofilm formation and XDR phenotype.

Conclusion: Our findings revealed the variable distribution of biofilm-related genes among MDR and XDR *A. baumannii* isolates from burn and VAP patients. A significant correlation was found between strong biofilm formation capacity and XDR phenotype. Finally, our results suggested that XDR phenotype was predominant among strong-biofilm producer *A. baumannii* in our region.

KEYWORDS
Acinetobacter baumannii, antimicrobial resistance, biofilm, biofilm-related genes, PFGE

1 | INTRODUCTION

Burnt skin provides a suitable environment for colonization and proliferation of bacteria. Patients with burns and ventilator are two groups at high risk for bacterial infections. In these patients, *Acinetobacter baumannii* (*A. baumannii*) can be transmitted by invasive clinical procedures, such as mechanical ventilation, and indwelling devices.[1] Multidrug-resistant (MDR) *A. baumannii* is an important ubiquitous pathogen responsible for a variety of community and hospital infections and forms biofilms in healthcare settings.[2] Eradicating *A. baumannii* faces dramatic problems due to antimicrobial therapy failure secondary to the emergence of MDR and extensively-drug resistant (XDR) isolates. In fact, antimicrobial resistance is a great threat increasing *A. baumannii*-related morbidity and mortality,[3] and biofilm formation provides the driving force for the emergence of new and more antimicrobial resistant phenotypes, which are more strongly associated with nosocomial infections.[4]

Although *A. baumannii* is naturally resistant to many available antibacterial agents, the development of antimicrobial resistance against other generation antimicrobials such as carbapenems, leading to antimicrobial therapy failure, highlights the importance of the infections caused by these bacteria as a significant health problem.[3] Biofilm formation is a main virulence factor and a hallmark characteristic of this bacterium. *Acinetobacter* spp. can form biofilm at solid–liquid and air-liquid interface. The biofilm formation rate in *A. baumannii* at the solid–liquid interface is higher than other *Acinetobacter* species.[5] Within biofilm, *A. baumannii* can acquire genes encoding antimicrobial resistance from other bacteria through mobile genetic elements including plasmids, integrons, or transposons.[3]

Biofilm formation and antimicrobial resistance have been found to be directly correlated in *A. baumannii* isolates, suggesting that biofilm formation is a necessary step in the development of MDR bacteria.[4,6]

Multidrug resistant and XDR *A. baumannii* are commonly found in healthcare-associated infections, generally in the context of nosocomial infections. Multidrug resistance profile as defined by the isolate being non-susceptible to at least one agent in ≥3 antimicrobial categories. Isolates of *A. baumannii* with resistance to at least one agent in all but two or fewer antimicrobial categories were considered XDR.[7]

It has been proven that several factors are associated with biofilm-related genes, and in fact, biofilm formation largely governs the severity of infections and triggers antimicrobial resistance. For example, in catheter-related bacteremia and aspiration pneumonia, the prevalence of *A. baumannii* harboring biofilm-related genes, including *ompA*, *ataA*, *csuA*, *csuE*, and *ptk*, was reported to be high among antimicrobial -resistant strains.[8] The outer membrane protein A (OmpA), a 38-kDa protein of *A. baumannii*, is encoded by the *ompA* gene and acts as a major porin that allows for biofilm formation on biotic surfaces, such as epithelial cells, through facilitating porin/fibronectin interactions.[9]

A. baumannii is generally non-motile; however, it possesses several genes, known as chaperone-usher pilus (*csuA/BABCDE*) assembly operon, that are required to assemble pilus to produce strong biofilm on polystyrene and glass surfaces such as catheter and ventilators.[2] Interestingly, biofilm maturation is promoted by *csu*-operon, and the absence of the *csuE* gene results in the lack of pilus production, disrupting biofilm formation.[10] *A. baumannii* colonization is influenced by the presence of the acinetobacter trimeric autotransporter adhesion (*ata*) gene that contributes to adhesion to and invading human endothelial and epithelial cells.[11] Besides, the *ata* gene has a wide variety of molecular activities and participates in most biological processes such as adhesion, biofilm formation, immune evasion, angiogenesis, and apoptosis. On the other hand, Ptk is a putative protein tyrosine kinase encoded by the *ptk* gene, required for capsule polymerization. This is without a doubt one of the most important factors to promote biofilm formation by *A. baumannii*.[9] Based on population genetic studies and epidemiological investigations of *A. baumannii*, there are several typing methods, including multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeats (VNTRs) analysis (MLVA), and whole genome sequencing (WGS).[12,13] Among these methods, PFGE is considered the gold standard due to its sensitivity, reproducibility and discriminatory power, and to determine the prevalence of pathogens within and between hospitals and their stability in the environment are used.[14] In this study, we aimed to investigate the presence of biofilm-related genes (*ompA*, *csuA*, *csuE*, *ptk*, and *ataA*) and their association with biofilm formation and perform molecular typing based on PFGE in *A. baumannii* isolated from burn and VAP patients.

2 | MATERIALS AND METHODS

2.1 | Study population and bacterial isolates

This cross-sectional study was in accordance with the Declaration of Helsinki (between October 2020 and July 2021). All samples were collected from two hospitals in Tehran (Rasool Akram and Shahid Motahhari). Informed consent and ethical approval were obtained from the hospitals' authorities and the institutional ethics committee, respectively, prior to the study. Non-replicating *A. baumannii* bacteria were collected from burn and VAP patients. Primary identification *A. baumannii* isolates was based on the Gram staining reaction and colony morphology. Standard biochemical tests such as catalase, citrate, triple sugar iron agar, urease test, oxidase, methyl red, Voges Prausker, and indole production were used to identify the *A. baumannii* isolates.[15] All the isolates were confirmed using molecular (*gyrB*)[16] and bacteriological identification tests (API 20NE).

2.2 | Antimicrobial ausceptibility testing

Antibacterial susceptibility patterns were assessed using the disk-agar diffusion method, applying piperacillin-tazobactam

(100/10 μg), ampicillin/sulbactam (10/10 μg), imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), cefepime (30 μg), ceftriaxone (30 μg) gentamicin (10 μg), and ticarcillin-clavulanic acid (75/10 μg) (Himedia) antimicrobials. Minimum inhibitory concentrations (MIC) of polymyxin B and colistin were determined by the E-test method (AB BIODISK), and results were interpreted using Clinical and Laboratory Standards Institute 2020 (CLSI, 2020) guidelines.[17] All breakpoints were available for the antibacterial agents. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used as internal controls. MDR isolates of *A. baumannii* exhibit resistance to at least one agent from three antimicrobial classes, whereas XDR isolates of *A. baumannii* exhibit resistance to at least one agent from all, but two or fewer antimicrobial categories. PDR-*A. baumannii* isolates were non-susceptible to all antimicrobial agents.[18]

2.3 | Biofilm formation

Biofilm formation was assessed using the crystal violet quantification test. Briefly, isolates were inoculated in the LB broth culture medium (Conda) and incubated at 37°C for 24 h. The bacterial concentration was then measured by a spectrophotometer at 650 nm (OD = 0.1–0.08). The bacterial suspension (190 μl LB medium + 10 μl cultured bacteria) was poured into each well of a 96-well microplate and incubated at 37°C for 24–48 h. The biofilm formation assay was carried out three times for each sample, and the LB medium was used as a negative control in all experiments. Planktonic cells were removed, and after three times of washing with PBS, biofilm plates were fixed with 150 μl of 99% v/v methanol (Merck), and then each well was stained with crystal violet (1%, w/v) and incubated at room temperature for 20 min.[19]

Biofilm was decolorized by ethanol-acetone 33% (80, 20, v/v) for 20 min, and the supernatant was collected. Lastly, the absorbance was measured at 595 nm, and biofilm production capacity was quantified by calculating a score based on OD595 and categorized as no (OD < optical density cutoff value, ODc, −), weak (ODC < OD ≤2ODC, +), moderate (2ODc < OD ≤3ODc, + +), and strong (OD > 3ODc) biofilm formation. For the evaluation of biofilm formation, Mueller Hinton Broth (MHB) and *A. baumannii* ATCC 19606 were used as negative and positive controls, respectively. Triplicates of all experiments were conducted.

2.4 | Identifying biofilm-related genes

Whole DNA was extracted from all samples by boiling.[20] Biofilm-related genes, including *ompA*, *csuA*, *csuE*, *ptk*, and *ataA* were amplified utilizing specific primers listed in Table 1. The PCR reaction was performed at the final volume of 25 μl, containing 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 10 pmol of each primer, and 50 ng of template DNA. PCR mixtures were subjected to the following thermal cycling: 5 min at 94°C, followed by 35 cycles with denaturation at 94°C for 50 s, annealing at 55°C–57°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min.

2.5 | Pulsed field gel electrophoresis genotyping

Genetic relatedness of collected isolates was carried out by Pulsed-Field Gel Electrophoresis (PFGE) as described previously.[21] Briefly, all pure-cultured isolates were embedded in agarose plugs and then treated with a cell suspension buffer (CSB) containing EDTA and proteinase K (20 mg. mL^{-1}). The plaques were thoroughly washed and

TABLE 1 Primers list was usded in this study

Genes	Primers	Sequences 5′–3′	Weight (bp)	References
ataA	ataA-F	ACGACTATCAACATTTTTAAGCTGG	101	In this study
	ataA-R	TTGGGTCGGCTGGAAAAGAA		
csuD	csuD-F	ATACCGACCTTTCACGGCTG	335	In this study
	csuD-R	GCCAGTATCGCCCTGCTTAT		
csuE	csuE-1F	CTTTAGCAAACATGACCTACC	702	[51]
	csuE-1R	TACACCCGGGTTAATCGT		
	csuE-2F	GGCGAACATGACCTATTT	580	
	csuE-2R	CTTCATGGCTCGTTGGTT		
ompA	ompA-1F	GATGGCGTAAATCGTGGTA	355	[51]
	ompA-1R	CAACTTTAGCGATTTCTGG		
	ompA-2F	GACCTTTCTTATCACAACGA	343	
	ompA-2R	CAACTTTAGCGATTTCTGG		
ptk	ptk-F	AGCCATAACCATAGCCAGCG	465	In this study
	ptk-R	ACTCGTGGTAAGAGCCCAAC		

Note: The primers for *ataA*, *csuD* and *ptk* were designed using Gene Runner (Version 3.05, Hastings Software).

then digested by the Apa I restriction enzyme (TaKaRa, Dalian) at 20°C for 5 h. All PFGE samples were loaded into the CHEF-DR III system (Bio-Rad) under the condition described by Qi et al.[22]

Finally, PFGE patterns were analyzed and processed by the GelCompare II system (Applied Maths, Sint-Martens-Latem), and a genetic similarity dendrogram was generated based on the UPGMA algorithm with a position tolerance of 1.5%. The genetic relatedness was determined according to the criteria described by Tenover et al.[23] and PFGE patterns were distinguished at a similarity cutoff of 80%.

2.6 | Statistical analysis

Statistical analysis was performed in SPSS version 20 software (SPSS, Inc.) and GraphPad Prism version 8 software (GraphPad Software Inc.). The Chi-square test and Fisher's exact test were used to determine statistically significant associations between main variables at a p value of <0.05.

3 | RESULTS

3.1 | Population and antimicrobial susceptibility

A. baumannii isolates were identified by various tests that included: Gram-negative coccobacilli, catalase positive, urease negative, H_2S negative, oxidase negative, gas negative, citrate positive, indole negative, Voges-Proskauer negative and methyl red positive. Isolates of *A. baumannii* were collected from burn patients (23/50) and patients with VAP (27/50), of whom 36 (72%) were male and 14 (28%) were female, with a mean age of 44.9 ± 12 years (range: 10–75 years). Overall, 74% (37/50) were MDR and 26% (13/50) were XDR. All the isolates were intermediate to colistin (MICs range from 0. 25 to 1 μg/ml) and polymyxin-B. (MICs range from 0. 5 to 2 μg/ml). Resistance to meropenem was the most common observation (92%, 46/50) while the least common resistance was related to ampicilin/sulbactam (42%, 21/50). The resistance rates against gentamicin, imipenem, cefepime, ceftazidime, ceftriaxone, piperacillin/tazobactam, and ticarcillin/clavulanic acid were 44% (22/50), 88% (44/50), 86% (43/50), 88% (44/50), 84% (42/50), 86% (43/50), and 66% (33/50), respectively.

There was no statistically significant difference in the antimicrobial resistance rate between the bacteria isolated from burn or VAP patients ($p = 0.1$). The prevalence of XDR in the isolates from burn and VAP patients was 39.13% (9/23) and 14.81% (4/27), respectively. No significant association was found between XDR phenotype and infection outcome ($p = 0.9$).

3.2 | Biofilm formation capability

All the isolates evaluated were biofilm forming, and according to the quantitative assay for biofilm formation, 52% (26/50), 36% (18/50),

TABLE 2 The distribution of antimicrobial resistance patterns and biofilm-related genes among differenbiofilmproduction phenotypes

Biofilm formation capacity	Antimicrobial								
	MEM	GEN	IMI	CFP	CTZ	CTX	TZP	T/C	AMP/S
Strong (p value)	25 (0.26)	15 (0.04)	26 (0.007)	24 (0.18)	23 (0.91)	23 (0.37)	24 (0.18)	21 (0.02)	17 (0.000)
Moderate (p value)	15 (0.09)	6 (0.25)	14 (0.09)	14 (0.20)	16 (0.88)	14 (0.36)	14 (0.20)	11 (0.58)	4 (0.03)
Weak (p value)	6 (0.44)	1 (0.15)	4 (0.08)	5 (0.84)	5 (0.70)	5 (0.96)	5 (0.84)	1 (0.07)	0 (0.02)
Total	46	22	44	43	44	42	43	33	21

and 12% (6/50) of them were strong, moderate, and weak biofilm producers, respectively. The prevalence of strong biofilm producers was 61.53% (16/26) in VAP samples and 38.46% (10/26) in burn samples. In this study, a significant association was observed between being a strong biofilm producer and XDR antimicrobial resistance ($p = 0.003$). Table 2 shows the distribution of antimicrobial resistance patterns with regarding the of various biofilm-related genes and different biofilm formation categories. The pattern of biofilm related-genes among strong and moderate biofilm producers was significantly different compared with weak biofilm producers (Table 2). The distribution of antimicrobial resistance patterns among different biofilm formation categories has been demonstrated in Table 3, indicating a statistically significant association between resistance to gentamicin, imipenem, ticarcillin/clavulanic acid, and ampicillin/sulbactam and strong biofilm formation. Also, resistance to ampicillin/sulbactam was significantly associated with moderate biofilm formation (Table 3).

3.3 | Distribution of biofilm-related genes

The most prevalent gene among all *A. baumannii* isolates was *csuD* (98%, 49/50), followed by *ptk* (90%, 45/50), *ataA* (88%, 44/50), *ompA* (86%, 43/50), and *csuE* (86%, 43/50) (Figure 1). A significantly higher prevalence of *ompA* was observed in the strains isolated from burn compared with VAP patients (96.2%, 26/27, $p = 0.03$, OR = 9.175; 95% CI = 1.693–23.80). Twenty six (52%) isolates harbored all the investigated genes. All the isolates harbored more than four biofilm-related genes. There was a significant difference in the distribution of antimicrobial resistance patterns among strong biofilm producers ($p = 0.001$).

3.4 | Biofilm production capacity among antimicrobial-resistant strains

The frequency of antimicrobial-resistant strains was significantly different among isolates with various biofilm production capacities (Figure 2A). Table 3 shows the distribution of antimicrobial resistance patterns among *A. baumannii* isolates with different capacities for biofilm generation, indicating a higher antimicrobial resistance rate in strong biofilm producers.

Regarding the prevalence of antimicrobial resistance patterns in bacteria with different biofilm formation capacities, strong biofilm producers were more commonly identified with XDR phenotype (Figure 2B). A significant relationship was observed between XDR phenotype and strong biofilm formation (38.46%, 10/26, $p = 0.005$). Also, the strains harboring all the assessed biofilm-related genes showed a strong biofilm capacity and a significantly higher prevalence of XDR phenotype (47.62%, 10/21, $p = 0.001$). However, there was no significant relationship between the distribution of biofilm-related genes and biofilm formation capacity. The prevalence of XDR *A. baumannii* with different biofilm formation capacities has been depicted in Figure 2B.

Strong biofilm producers constituted 88.7% (21/26) of the strains harboring all biofilm-related genes. Our results showed that the presence of all biofilm-related genes increased the strength of biofilm formation ($p < 0.0001$).

3.5 | Pulsed-field gel electrophoresis fingerprinting

PFGE was performed for all isolates. The PFGE results showed 6 clusters and 21 different pulsotypes, indicating a remarkable genetic diversity. Among all patients, cluster 1 was the most prevalent (38%) (Figure 3), followed by clusters 2, 5, 4, 3, and 6. The distribution of cluster 3 was restricted to patients with VAP. The lowest prevalence of clusters was in clusters 3 and 4 (0.8%). In clusters 1 and 2, clonality was higher compared to other clusters. In cluster 2, the prevalence of XDR *A. baumannii* strains with a strong biofilm formation capacity was significantly higher compared to other clusters ($p = 0.013$, Figure 3). There was no significant relationship between biofilm-related genes and clusters.

4 | DISCUSSION

MDR *A. baumannii* poses a great health challenge worldwide, and polymyxin antimicrobials such as colistin, as "salvage" therapy,

TABLE 3 The prevalence of antimicrobial resistant strains with different biofilm formation capacities

Biofilm formation capacity	Antimicrobial resistance phenotype		Biofilm-related genes					*p* Value
	MDR	XDR	csuE	csuD	ompA	ataA	Ptk	
Strong	14	12	22	26	23	23	24	0.003
Moderate	17	1	15	17	14	17	16	0.01
Weak	6	0	6	6	6	4	5	0.31
Non-biofilm	0	0	0	0	0	0	0	0
Total	37	13	43	49	43	44	45	

Abbreviations: AMP/S, ampicillin-sulbactam; CFP, Cefepime; CTX, ceftriaxone; CTZ, ceftazidime; GEN, gentamicin; IMI, imipenem; MEM, meropenem; T/C, ticarcillin-clavulanic acid; TZP, piperacillin-tazobactam.

play an important role against these infections.[24] In this study, all studied isolates were susceptible to colistin and polymyxin-B, highlighting their importance as rescuing antimicrobials against MDR *A. baumannii* that has been categorized as an urgent antimicrobial-resistant infection by the Center for Disease Control (CDC) and World Health Organization (WHO).[25] The development of MDR and especially XDR *A. baumannii* infections in burn and VAP hospitalized patients poses a great risk factor compromising their improvement and increasing morbidity and mortality in many cases.[26,27] *A. baumannii* infection is particularly common in hospitals and health environments, where its development is mediated by biofilm formation. Within a biofilm niche, bacteria are up to 1000 times more resistant to antimicrobials than the planktonic form.[25]

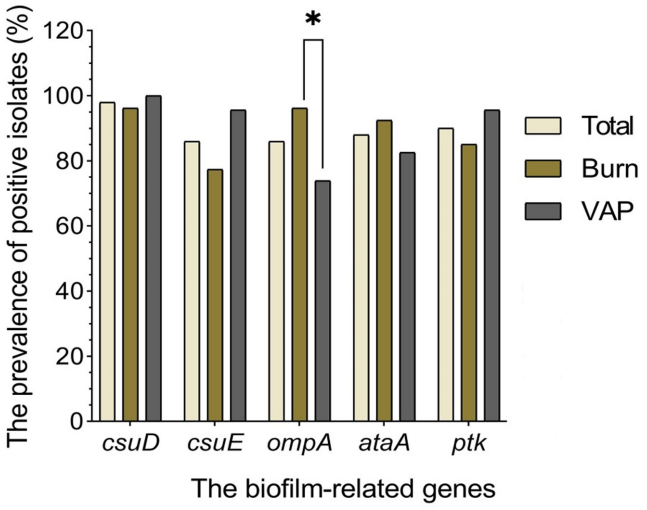


FIGURE 1 The frequency of biofilm-related genes in all examined strains isolated from different clinical sources

The increase of XDR infections greatly concerns health professionals due to the high rate of antimicrobial therapy failure in the patients admitted to the burn and ICU wards.[26,27] Regarding antimicrobial susceptibility, 26% of the assessed isolates exhibited XDR phenotype. In a recent study in Isfahan, Iran, 16.1% of 118 isolates of *A. baumannii* were XDR. In another study in Zanjan, Iran, Zighami et al. reported that 91% of *A. baumannii* isolates were XDR,[9,28] indicating different frequencies in various geographical regions of the country. In our study, since the sample size was relatively small, non-biofilm producer isolates were not found, and this limitation should be considered in future studies in the region.

Carbapenem antimicrobials such as meropenem and imipenem have activity against *A. baumannii*.[29] However, the emergence of carbapenem-resistant *A. baumannii* (CRAb) is a serious concern in Iran and other countries.[30] Recently, Beigverdi et al. have reported considerably high resistance rates against meropenem and imipenem among *A. baumannii* isolates from Iranian patients (83.6% and 81.1%, respectively).[30] In this study, we also observed a high resistance rate of *A. baumannii* against imipenem and meropenem (88% and 92%, respectively).

As antimicrobial resistance can be acquired by bacteria within a biofilm niche via different molecular mechanisms, such as

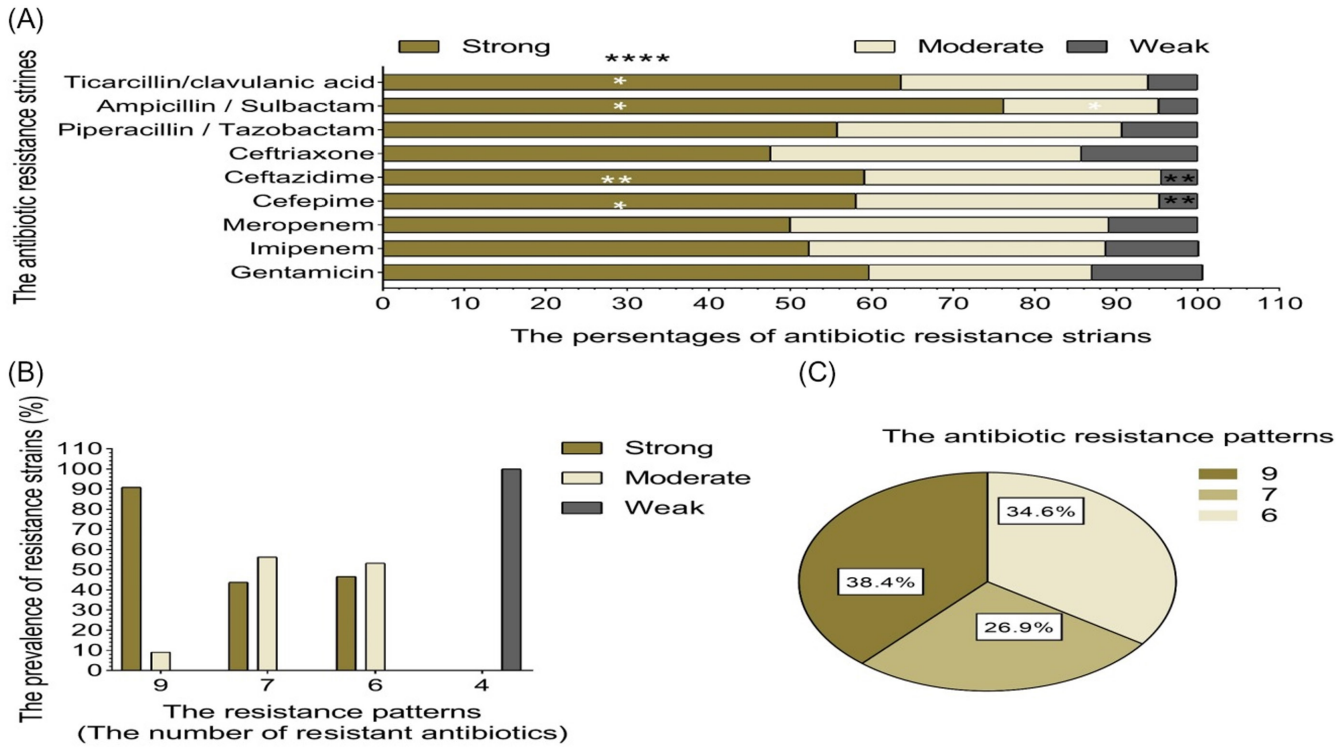
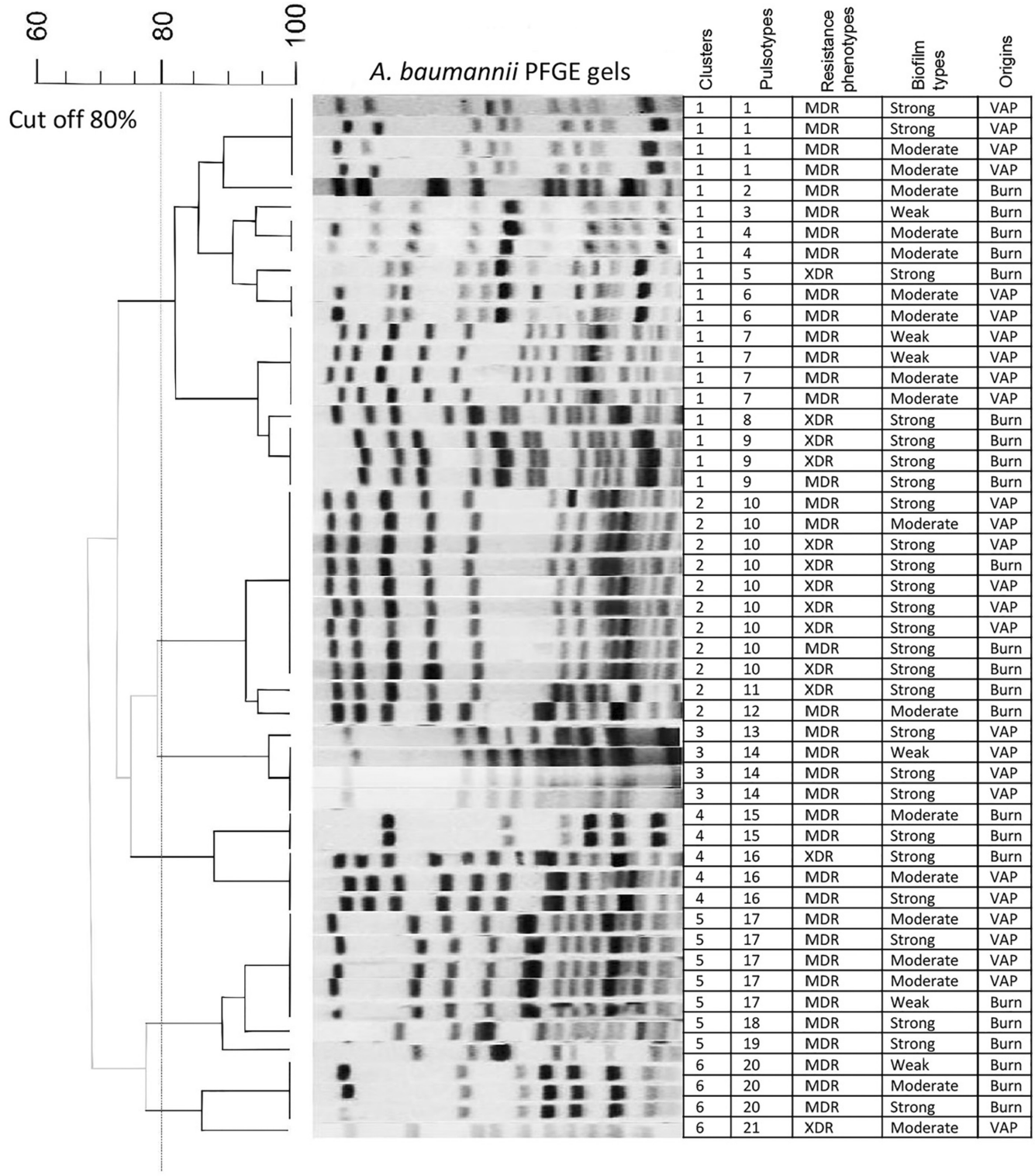


FIGURE 2 (A) The percentages of antimicrobial resistant strains – resistant strains with different biofilm formation capacities. *$p \leq 0.01$; **$p \leq 0.001$; ****$p \leq 0.0001$. (B) The biofilm formation capacity of isolates with different antimicrobial resistant strains resistance patterns. (C) The distribution of strong biofilm producing isolates among different antimicrobial resistant strains resistance patterns.



Clusters	Pulsotypes	Resistance phenotypes	Biofilm types	Origins
1	1	MDR	Strong	VAP
1	1	MDR	Strong	VAP
1	1	MDR	Moderate	VAP
1	1	MDR	Moderate	VAP
1	2	MDR	Moderate	Burn
1	3	MDR	Weak	Burn
1	4	MDR	Moderate	Burn
1	4	MDR	Moderate	Burn
1	5	XDR	Strong	Burn
1	6	MDR	Moderate	VAP
1	6	MDR	Moderate	VAP
1	7	MDR	Weak	VAP
1	7	MDR	Weak	VAP
1	7	MDR	Moderate	VAP
1	7	MDR	Moderate	VAP
1	8	XDR	Strong	Burn
1	9	XDR	Strong	Burn
1	9	XDR	Strong	Burn
1	9	MDR	Strong	Burn
2	10	MDR	Strong	VAP
2	10	MDR	Moderate	VAP
2	10	XDR	Strong	VAP
2	10	XDR	Strong	Burn
2	10	XDR	Strong	VAP
2	10	XDR	Strong	VAP
2	10	XDR	Strong	VAP
2	10	MDR	Strong	Burn
2	10	XDR	Strong	Burn
2	11	XDR	Strong	Burn
2	12	MDR	Moderate	Burn
3	13	MDR	Strong	VAP
3	14	MDR	Weak	VAP
3	14	MDR	Strong	VAP
3	14	MDR	Strong	VAP
4	15	MDR	Moderate	Burn
4	15	MDR	Strong	Burn
4	16	XDR	Strong	Burn
4	16	MDR	Moderate	VAP
4	16	MDR	Strong	VAP
5	17	MDR	Moderate	VAP
5	17	MDR	Strong	VAP
5	17	MDR	Moderate	VAP
5	17	MDR	Moderate	VAP
5	17	MDR	Weak	Burn
5	18	MDR	Strong	Burn
5	19	MDR	Strong	Burn
6	20	MDR	Weak	Burn
6	20	MDR	Moderate	Burn
6	20	MDR	Strong	Burn
6	21	XDR	Moderate	VAP

FIGURE 3 Pulsed field gel electrophoresis was analyzed via Bionumerics using the UPGMA algorithm at the position tolerance of 1.5% and the cut off of 80%.

horizontal gene transfer, plasmid transformation, and DNA uptake, the development of MDR and XDR strains has been noted to associated with biofilm formation on biotic and abiotic surfaces.[22] Accordingly, one of the key findings of this research was that XDR strains tended to form stronger biofilm than MDR strains. The results of this study are quite different from those of Qi et al. who reported that non-biofilm producer *A. baumannii* often XDR.[22] On the other hand, Zighami et al. and Khoshnood et al. reported that all MDR and XDR *A. baumannii* isolates had a strong biofilm formation capacity, highlighting that these strains were often associated

with ICU-related infections.[9,31] Shenkutie et al. showed that biofilm formation would induce irreversible resistance in XDR *A. baumannii* strains.[32]

In this study, we showed that the presence of a full set of biofilm-related genes (*ompA*, *ptk*, *ataA*, *csuE*, and *csuA*) predicted strong biofilm formation and variable antimicrobial resistance. An analysis of the biofilm-related genes of *A. baumannii* was published by Liu et al. It was found that *abaI* and *csuE* were present in 59.8% of the samples and *ompA* in 100% of the samples.[33] *A baumannii* isolates from meat of different origins are examined for the presence of biofilm-related genes by Elbehiry et al. In their study, the prevalence of *casE*, *ompA*, *bap*, and *csgA* was 72%, 60%, 52.7%, and 25%, respectively.[34]

Literature information and our results suggest that ompA-mediated adhesion contributes significantly to biofilm formation in *A. baumanni*-associated nosocomial infections, especially in burn and VAP patients.[35,36] In this study, ompA was significantly and more frequently detected in burn than in VAP samples. The ompA protein mediates the interaction between bacteria and epithelial cells.[37] Previous studies have reported a positive relationship between the presence of biofilm-related genes such as *ompA* and antimicrobial resistance.[9,38]

Being the most abundant porin in *A. baumannii*, the role of OmpA in drug resistance was more prominent in disruption mutants of the gene, which indicated reduced resistance to imipenem, meropenem, nalidixic acid, and chloramphenicol. Also diffusion, studies shows that OmpA possibly couples with efflux pumps and forces out antibacterial compounds from the periplasm.[39] Overproduction of this gene is a risk factor for the mortality rate of nosocomial bacteremia and pneumonia caused by *A. baumannii*. Besides, the expression level of OmpA measured by qRT-PCR can be used as a rapid diagnostic index for antibiotic-resistant *A. baumannii*.[40] In this study, a strong capacity for biofilm formation significantly correlated with the presence of all the examined biofilm-related genes, which was in agreement with the results of Amin et al.[36] However, we observed a significant association between being a strong biofilm producer and showing antimicrobial resistance, which was in contrast with the report of Amin et al. who asserted that non-MDR strains were more capable of generating strong biofilm.[36] This was inconsistent with our observation indicating a higher biofilm formation capacity in XDR than in MDR *A. baumannii* strains. There are several reports suggesting that XDR bacterial strains form stronger biofilm than antimicrobial -sensitive strains,[9,41,42] which was in parallel to our results showing the higher biofilm formation capability of XDR compared to MDR strains. In contrast with our results; however, a number of studies have reported that sensitive strains form more strong biofilm than MDR bacteria.[22,42,43]

Besides, in our study, harboring all the assessed biofilm-related genes significantly correlated with XDR phenotype, suggesting a role for biofilm formation in the acquisition of antimicrobial resistance, especially in healthcare facilities and among the bacteria forming biofilm on biotic and abiotic surfaces.[44] Recently, Shenkutie et al. have reported that biofilm formation by *A. baumannii* during hospital colonization induces transient antimicrobial tolerance in sensitive strains but a more stable resistance in XDR strains.[32] Genetic relatedness is confirmed by several methods and has been addressed by surveillance, subtyping, and epidemiological studies on *A. baumannii* outbreaks.[45]

In this context, Salguero et al. have recently shown in an epidemiological study that matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) and repetitive-element PCR (Rep-PCR) are not suitable methods to replace PFGE in the epidemiological evaluation of *A. baumannii*.[46]

In the present study, genome fingerprinting was confirmed by *ApaI*-digested PFGE, which is the gold standard for determining genetic relatedness among *A. baumannii* clinical isolates.[47] The analysis revealed that the distribution of XDR phenotype was significantly high in cluster No. 2; however, no significant correlation was found between clusters and biofilm formation.

Our results were similar to those of Bardbari et al. Three Iranian hospitals were sampled for typing of MDR *A. baumannii* strains by PFGE to identify the strains they contained. Eight clusters were identified, with two main clusters accounting for 30% and 23% of the sample. In their study, they showed that there was no correlation between biofilm formation and PFGE patterns.[48]

The PFGE method was used in a study conducted by Ceparano et al. in which 102 *A. baumannii* strains isolated from 59 patients were genotyped by this method. Two main patterns were observed as a result of PFGE typing. The results indicated that approximately 40% of the genotyped strains were associated with healthcare-associated infections, the majority of which were VAP in both infection patterns.[49]

In another study conducted in an academic hospital in Turkey, PFGE was used to determine the type of 69 strains of *A. baumannii*. It has been suggested that different clones may be present in the same hospital departments, while the same clones may be present in different departments.[50]

This study investigated the antimicrobial resistance patterns and biofilm formation capacities of MDR *A. baumannii* strains collected from two Tehran's hospitals, Iran, by the molecular detection of biofilm-related genes. To the best of our knowledge, there are no previous reports on biofilm formation capacity and the distribution of biofilm-related genes in *A. baumannii*, followed by PFGE fingerprinting. Our findings suggested that a strong biofilm formation capacity mediated by biofilm-related genes might contribute to the acquisition of antimicrobial resistance, especially XDR phenotype, in *A. baumannii* found in the burn and ICU wards.

5 | CONCLUSION

In the present study, the distribution of biofilm-related genes and antimicrobial resistance patterns were determined in *A. baumannii* isolated from burn and VAP patients. We also checked for a possible correlation between biofilm formation and antimicrobial resistance patterns. The results demonstrated that XDR phenotype

significantly correlated with a strong biofilm formation capacity, and biofilm-related genes showed a significantly high prevalence in XDR *A. baumannii* clinical isolates. Our results indicated that the prevalence of antimicrobial resistance correlated with strong biofilm formation, suggesting the transmission of resistance mechanisms among bacterial strains within the biofilm niche. It is suggested to use biofilm disrupting agents to prevent biofilm formation, especially on hospital surfaces, to reduce the extent of the infections caused by MDR and XDR *A. baumannii* strains, particularly by designing prospective studies.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation were performed by Dr Norkhoda sadeghifard, data collection and analysis were performed by Maryam Mohammadi, Nahid Mahdian, Saeed Khoshnood, Mohammad Hossein Haddadi and Abbas Maleki. The first draft of the manuscript was written by Saeed Khoshnood, Mohammad Hossein Haddadi, Abbas Maleki and Mohsen Heidary and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this study.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

ORCID

Saeed Khoshnood https://orcid.org/0000-0002-5143-3178
Mohsen Heidary https://orcid.org/0000-0002-9839-5017

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