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Prevalence of *bap* and *ompA* immune evasion genes, biofilm formation ability, antibiotic resistance pattern, and motility of *Acinetobacter baumannii*

Hafez Mozayyan Esfahani[1], Babak Beikzadeh[1*], Soodabeh Rostami[2], Mohammad Rabbani Khorasgani[1]

[1] *Department of Cell and Molecular Biology & Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran.*
[2] *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.*

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Abstract

Acinetobacter baumannii (*A. baumannii*) is recognized as a significant pathogen responsible for hospital-acquired infections. This research aims to investigate the frequency of *bap* and *ompA* immune evasion genes, thereby determining the profile of antibiotic resistance in bacteria and the biofilm-producing ability among isolates obtained from patients with respiratory infections in Isfahan, Iran. In the present study, among 100 isolates collected from respiratory tract infections, 96 isolates were confirmed as *A. baumannii* by biochemical tests and molecular analysis. The presence of *bap* and *ompA* genes in these isolates was checked by PCR, and antibiotic susceptibility was assessed using the disc diffusion method. Finally, the ability to form biofilm and motility were investigated. Results showed that 100% of the isolates carried the *ompA* gene. However, for the *bap* gene, 95.83% of isolates were positive. Investigation of antibiotic resistance showed that *A. baumannii* isolates exhibited resistance to most antibiotics. The results of the biofilm test revealed that 97.91% of the isolates could form biofilm, including 39.58% with weak biofilm, 44.79% with medium biofilm, and 13.54% with strong biofilm, leaving the remaining 2% unable to form biofilm. Moreover, our results show that 6.4% of isolates were non-motile, 45.9% had an intermediate ability for twitching motility, and 47.7% showed a high ability for twitching motility. No correlation was observed between twitching motility, biofilm production, and antibiotic resistance. The present study demonstrates that the *bap* and *ompA* genes are highly abundant in lung infections, and most of these isolates are multidrug-resistant, exhibiting a high ability to form biofilms and display motility.

1. Introduction

The Gram-negative bacterium *Acinetobacter baumannii* poses a growing challenge to public health worldwide (Abdi et al., 2020). As a key human pathogen, this bacterium is frequently associated with healthcare-related infections in clinical and community environments. *A. baumannii* can survive, spread, and quickly acquire resistance factors to a wide range of antibiotics in hospitals (Liu et al., 2024). Infections caused by this bacterium present with diverse clinical manifestations, such as ventilator-associated pneumonia, catheter-related bloodstream and urinary tract infections, septicemia, meningitis, and skin and soft tissue

*** Corresponding author.** *Babak Beikzadeh. Email: b.beikzadeh@bio.ui.ac.ir*
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infections that arise from burns and wounds. This bacterium is responsible for 17% of nosocomial infections, especially in individuals with weakened immune systems (Munier et al., 2019). Today, *A. baumannii* is the second most common bacterium in clinical laboratories after *Pseudomonas aeruginosa,* with a mortality rate of 41% (Pourhajibagher et al., 2016).

The respiratory tract is particularly vulnerable to *A. baumannii* infections and is linked to high mortality rates in patients with bacteremia. Biofilm formation is considered an important virulence factor of *A. baumannii*, enabling bacterial survival in the environment and within the immune system, particularly in cases of ventilator-associated pneumonia (Gedefie et al., 2021). Additionally, *A. baumannii* quickly becomes resistant to multiple antibiotics. At present, a significant proportion of isolates exhibit resistance to common antibiotics, such as aminopenicillins, ureidopenicillins, broad-spectrum cephalosporins, most aminoglycosides, quinolones, chloramphenicol, and tetracyclines (Towner, 2009; Kyriakidis et al., 2021). In this way, the World Health Organization has categorized carbapenem-resistant *A. baumannii* as the first (critical) priority on its list of bacteria that urgently need new antibiotics. Hence, this bacterium is also known as multi-drug resistant (MDR) (Chen & Microbiology, 2020). *A. baumannii* possesses both specific and non-specific virulence factors, such as adhesion molecules, biofilm formation, iron acquisition mechanisms, various enzymes (e.g., phospholipase and elastase), outer membrane porins, and resistance to serum, all of which contribute to its ability to colonize and infect the host (Chen & Microbiology, 2020; Harding et al., 2018; Lee et al., 2017; Morris et al., 2019; Sadr et al., 2021). The biofilm-associated protein (Bap) is involved in the process of biofilm formation, attachment to tissue, disease spread, and antibiotic resistance (De Gregorio et al., 2015). Outer membrane protein A (ompA), a key component of outer membrane proteins in Gram-negative bacteria, plays a crucial role in pathogenicity by mediating biofilm formation, eukaryotic cell infection, antibiotic resistance, complement system evasion, and immune system resistance (Oh et al., 2025). The overexpression of *ompA* has been identified as a major risk factor contributing to mortality in cases of nosocomial pneumonia and bacteremia caused by *A. baumannii* (Nie et al., 2020). It may be concluded that downregulation of *ompA* gene expression reduces mortality of *A. bauamnii* infections; accordingly, it can be used as a potential candidate for treatment.

According to previous studies, the incidence of infection with MDR *A. baumannii* in medical centers has been reported to be increasing (De Blasiis et al., 2024). Still, limited studies have been conducted on the frequency of *bap* and *ompA* genes in isolates of respiratory infections. Therefore, this study aims to investigate the frequency of *bap* and *ompA* immune evasion genes, determine the antibiotic resistance profiles of bacteria, assess biofilm formation, and evaluate motility in isolates obtained from patients with respiratory tract infections.

2. Materials and methods

2.1. Collection and identification of isolates

In this cross-sectional study, 100 isolates of *A. baumannii* were collected from respiratory tract infections in hospitals in Isfahan, Iran, between 2022 and 2023, and 96 isolates were confirmed as *A. baumannii* through molecular analysis and biochemical tests. The remaining four isolates were excluded due to mixed growth or failure to amplify the *bla* OXA-51 gene during PCR testing. The isolates were initially identified by Standard laboratory methods and biochemical tests (Ahmad & Mohammad, 2020; Shoaib et al., 2020). For molecular verification of *A. baumannii* isolates, the *bla*OXA-51 gene was used as a reference gene (Turton et al., 2006). DNA extraction from the isolates was performed using the boiling method, as previously described by Falah et al. (2019); the reference strain *A. baumannii* (ATCC 19606) was used as a positive control. All identified isolates were analyzed to detect the *bap* and *omp*A genes (Table 1).

Table 1: *The Primer Sequences used in this Study*

Genes	Sequence	Annealing temperature	Product size (bp)	Reference
OXA51-F	TAA TGC TTT GAT CGG CCT GG	54 ^{oC}	353	(Turton et al., 2006)
OXA51-R	TGG ATT GCA CTT CATCTT GG			
bap-F	GAG GGA ACT TCT GCA AAA CTT TC	60 ^{oC}	108	(Al-Shamiri et al., 2021)
bap-R	CAG ACG TAT GAC TGC ATT GGT			
*omp*A-F	TGA GTC GTA TTG CAC TTG CTA C	59 ^{oC}	594	(Al-Shamiri et al., 2021)
*omp*A-R	CAG GCT TCA AGT GAC CAC C			

2.2. Antibiotics Susceptibility testing of bacterial isolates

The Kirby-Bauer disc diffusion method was used to determine antibiotic sensitivity against nine common clinical antibiotics in seven antibiotic classes, based on clinical and laboratory standards (CLSI 2024 guidelines) for identifying MDR isolates. MDR isolates are characterized by resistance to drugs from at least three different classes of antimicrobial agents (Magiorakos et al., 2012). Antibiotic disks from Padtan Teb (Iran) were used for this study. Antibiotic disks included: Ampicillin-Sulbactam (10.10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Meropenem (10 µg), Trimethoprim-Sulfomethoxazole (1.25/23.75 µg), and Piperacillin (100 µg). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as reference strains for quality control.

2.3. Quantitative biofilm production assay

The microtiter plate test is the gold standard and the most commonly used method to quantify biofilm formation (Stepanović et al., 2000). Biofilm formation was quantified using the microtiter plate assay, following the method previously described by Babapour et al. (2016). Briefly, each well of a 96-well round-bottom plate was filled with 200 µl of TSB containing 1% glucose and 2 µl of 0.5 McFarland bacterial suspension. The *A. baumannii strain* ATCC 19606 was utilized as a positive control. After 24 hours, the well contents were discarded, and each well was gently rinsed three times with PBS. Then, the plate was dried. 96% ethanol was added to each well to fix the formed biofilm. The wells were stained for 15 minutes with 0.1% crystal violet, and the absorbance at 570 nm was measured using a multimode reader. The test was repeated three times for each isolate, and the results were interpreted based on previously described parameters (Babapour et al., 2016). Bacterial isolates were classified by comparing their optical density (ODi) with the mean optical density of the negative control (ODc). Isolates with an ODi lower than ODc were considered non-adherent. The isolates were categorized as weakly adherent when the ODi was greater than ODc but not more than twice its value ($ODc < ODi \leq 2\times ODc$). If the ODi ranged between two and four times the ODc ($2\times ODc < ODi \leq 4\times ODc$), they were classified as moderately adherent. Isolates showing an ODi exceeding four times the ODc ($ODi > 4\times ODc$) were defined as strongly adherent (Corehtash et al., 2015).

2.4. Motility assay

Twitching motility was assessed using the stab inoculation method on 1% TSA as previously described (Al-Shamiri et al., 2021a; Selvaraj et al., 2020). Overnight bacterial cultures were inoculated by stabbing the plates from the center to the bottom using sterile toothpicks. Following inoculation, the plates were incubated at 37°C for 72 hours. The agar was discarded with care, followed by washing and staining of the plates with 0.4% crystal violet. The motility zone was measured, and the assays were carried out in duplicate. The isolates' twitching motility was classified into one of three categories: non-motile (< 5mm), intermediate (5-20 mm), and strong (>

20mm) (Al-Shamiri et al., 2021; Selvaraj et al., 2020).

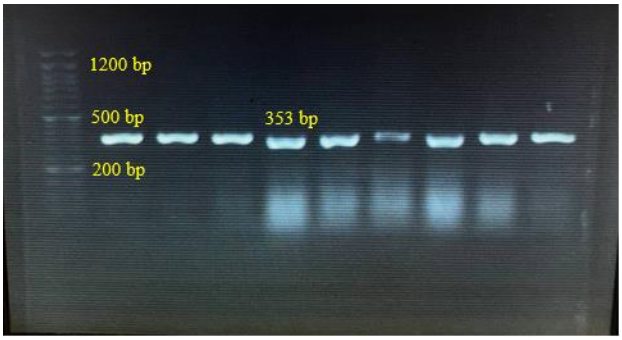
2.5. Statistical analysis

The biofilm test results were analyzed statistically using IBM SPSS Statistics software (version 27, IBM Corp, USA). GraphPad Prism software was used to measure the significance of the relationship between the presence of genes, the amount of biofilm formation, and antibiotic resistance. The p-values were calculated using Fisher's exact test, with statistical significance set at $p < 0.05$ and a confidence level of 95%.

3. Results and Discussion

In this cross-sectional study, 100 isolates were collected from respiratory tract infections, and 96 isolates were confirmed as *A. baumannii* based on biochemical tests and molecular analysis of the *bla*OXA-51 gene (Fig 1).

Figure 1: *PCR Products of the bla OXA-51 Gene in A. aumannii*

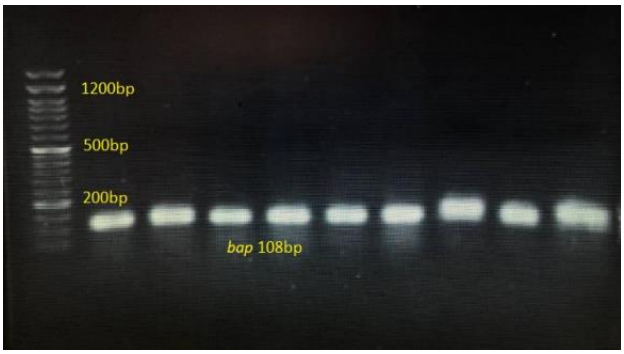


Note. The first well contains the 50bp ladder, the second well contains the positive control strain *A. baumannii* ATCC 19606, and the third to tenth wells contain the isolates.

3.1. Prevalence of *bap* and *ompA* genes in *A. baumannii* isolates

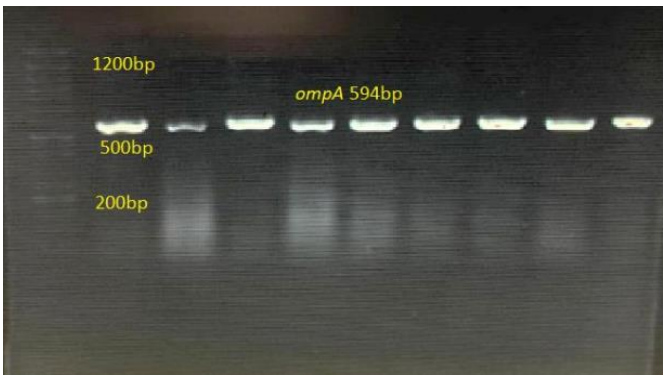
Analysis of the *bap* and *ompA* genes revealed that 95.83% of the isolates (92 isolates) carried the *bap* gene, while the *ompA* gene was present in all isolates (100%) (Fig 2, Fig 3). In a study conducted in Brisbane, the frequency of the *bap* gene was reported to be 91.7%, indicating its high frequency in certain regions (Goh et al., 2013). Another study in Tehran, Iran, found that the *ompA* gene was 100% prevalent (Badmasti et al., 2015). Fallah reported that the frequency of the *bap* gene was 92% (Fallah et al., 2017). Additionally, a study conducted in Isfahan reported a frequency of the bap gene of 70.3% (Monfared et al., 2019). In the same year, a report from Tehran revealed that the frequency of the *bap* gene was 98%, and the *ompA* gene was determined to be 96% (Mozafari et al., 2021). In a 2022 study, the frequency of both genes was reported to be 100% (Ghadiri, Doosti, & Shakhsi-Niaei, 2023). A comparative analysis of studies from Iran and other countries indicates a potentially higher frequency of the *bap* gene in Iranian clinical isolates. However, in some studies, such as a study conducted in China, the frequency of the *bap* gene was reported to be 95.5%, which is very close to our result (Liu et al., 2018).

Figure 2: *PCR Products of the bap Gene in A. baumannii*



Note. The first well is the 50bp ladder, the second well is the positive control strain, and the third to tenth wells are related to the isolates.

Figure 3: *PCR Products of the ompA Gene in A. baumannii*



Note. The first well contains the 50bp ladder, the second well contains the positive control strain, and the third through tenth wells are related to the isolates.

3.2. Antibiotic susceptibility of *A. baumannii* isolates

Based on the results of the disc diffusion test, it was found that a total of 86.4% of the isolates showed resistance to trimethoprim-sulfamethoxazole (83 isolates), 95.8% of the isolates were resistant to the ampicillin-sulbactam, cefepime, ciprofloxacin, gentamicin, amikacin, and meropenem (92 isolates), 97.9 % of the isolates were resistant to ceftazidime (94 isolates), and 96.8% of isolates (93 isolates) were resistant to Piperacillin (Table 2). Among these isolates, 1.05% (1 isolate) were non-MDR and 98.95% (95 isolates) were MDR. 80.2% (77 isolates) were resistant to all antibiotics tested, and none were sensitive to all antibiotics tested (Table 3).

Table 2: *Antibiotic Resistance Profile of A. baumannii Isolates*

Antibiotics	Sensitive	Intermediate	Resistant
SXT	2.1% (2 isolates)	11.4% (11 isolates)	86.4% (83 isolates)
CAZ	2.1% (2 isolates)	0%	97.9% (94 isolates)
GM	2.1% (2 isolates)	2.1% (2 isolates)	95.8% (92 isolates)
AN	4.2% (4 isolates)	0%	95.8% (92 isolates)
SAM	0%	4.2% (4 isolates)	95.8% (92 isolates)
FEP	2.1% (2 isolates)	2.1% (2 isolates)	95.8% (92 isolates)
CP	2.1% (2 isolates)	2.1% (2 isolates)	95.8% (92 isolates)
MEN	4.2% (4 isolates)	0%	95.8% (92 isolates)
PIP	3.2% (3 isolates)	0%	96.8% (93 isolates)

Table 3: *Analyses of Results of Antibiotic Resistance*

Resistance	Number	Percentage
Resistant to the antibiotics	77	80.2%
Sensitive to the antibiotics	0	0%
MDR	95	98.95%
Non-MDR	1	1.05%

The analysis of antibiotic resistance demonstrated that *A. baumannii* isolates exhibit resistance to a broad spectrum of antibiotics. The highest level of resistance was to ceftazidime (97.9%), and the lowest level of resistance was to trimethoprim-sulfamethoxazole (86.4%). 98.95% of the studied isolates were MDR. Also, the isolates that formed a strong biofilm were all resistant to all tested antibiotics. However, we also had MDR strains that formed a moderate and weak biofilm, which is consistent with previous works. According to a 2018 study on hospital isolates in Isfahan, resistance to meropenem (99.3%), cefepime (97.4%), ceftazidime (96.7%), ciprofloxacin (99.3%), amikacin (82.4%), and gentamicin (94.1%) was reported, which is consistent with our results (Rezaei et al., 2018). Also, in a study conducted in Isfahan from 2016 to 2018 on pneumonia and empyema infections of *A. baumannii*, resistance to ampicillin-sulbactam was 74.07%, ceftazidime 100%, cefepime 90%, meropenem 100%, and amikacin 76.92%, gentamicin 80%, ciprofloxacin 95% and cotrimoxazole 92.31% were reported, which unlike ampicillin-sulbactam and amikacin and gentamicin, are entirely in line with our results (Mostafavi et al., 2021). In another study, conducted in 2017 in Isfahan, Polymyxins, Ampicillin/sulbactam, and Minocycline exhibited the greatest activity against *A. baumannii* isolates, and all of the isolates (100%) were MDR (Shokri et al., 2017).

3.3. Biofilm formation capacity

The quantitative biofilm test using the standard microtiter plate method showed that 2 of the isolates could not form a biofilm, 38 isolates formed a weak biofilm, 43 isolates formed a moderate biofilm, and 13 isolates formed a strong biofilm (Table 4). The association between the *bap*

and *ompA* genes and biofilm formation, as well as antibiotic resistance, was also evaluated. Our results show no significant relationship between the *bap* and *ompA* genes and biofilm formation in clinical isolates. Among isolates with moderate and strong biofilm capacity were those with MDR (Tables 5-7).

Table 4: *Results of the Quantitative Biofilm Analysis of A. baumannii Isolates*

Adherence	Number	Percentage
None	2	0.02%
Weak	38	39.58%
Moderate	43	44.79%
Strong	13	13.54%

This study reveals that 97.8% of MDR strains form biofilms, with a simultaneous correlation between the potential for biofilm formation and the level of antibiotic resistance (Table 5). Isolates that exhibited strong biofilm formation were also resistant to all tested antibiotics. However, no significant association was found between the presence of *bap* and *ompA* genes and either biofilm formation or antibiotic resistance ($p > 0.05$) (Tables 6,7). As this was a cross-sectional study, the temporal relationship between antibiotic resistance, gene presence, and biofilm formation could not be established. Longitudinal studies are needed to confirm these findings.

The results of the biofilm formation test showed that 39.58% of the isolates had weak biofilm capacity, 44.79% were moderate biofilm, and 13.54% could form a strong biofilm. Although two isolates did not form a biofilm, they did possess *bap* and *ompA*, indicating that various other factors are involved in biofilm formation, in addition to these two genes. Therefore, this study revealed that most isolates had weak potential to form a biofilm. However, since only two isolates could not form a biofilm, biofilm can be considered one of the main pathogenic factors of *A. baumannii*. The findings of this research align with those of many other studies. In a 2022 study, the frequency of *bap* and *ompA* genes was reported to be 100%, and all isolates were found to form biofilms (Ghadiri et al., 2023). This study shows that since 97.8% of MDR strains form biofilms, there is a simultaneous relationship between the potential for biofilm formation and the level of antibiotic resistance, a finding also mentioned in the study by (Babapour et al., 2016).

Table 5: *Correlation Between Biofilm Formation Intensity and Antibiotic Resistance*

Adherence	Number	Percentage
None	2	0.02%
Weak	38	39.58%
Moderate	43	44.79%
Strong	13	13.54%

Table 6: *The Relationship Between the Presence of bap and ompA Genes with the Strength of Biofilm Formation and Antibiotic Resistance*

Biofilm Strength	*bap*		*ompA*		MDR		
	positive	negative	positive	negative	positive	negative	Total
weak	36 (94.7%)	2 (5.3%)	38 (100%)	0 (0%)	38 (100%)	0 (0%)	38
moderate	42 (97.6%)	1 (2.4%)	43 (100%)	0 (0%)	42 (97.6%)	1 (2.4%)	43
strong	12 (92.3%)	1 (7.7%)	13 (100%)	0 (0%)	13 (100%)	0 (0%)	13
Non-biofilm formation	2 (100%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)	2
	92 (95.83%)	4 (4.17%)	96 (100%)	0 (0%)	95 (98.9%)	1 (1.1%)	96 (100%)
	0.53		0.99		0.99		

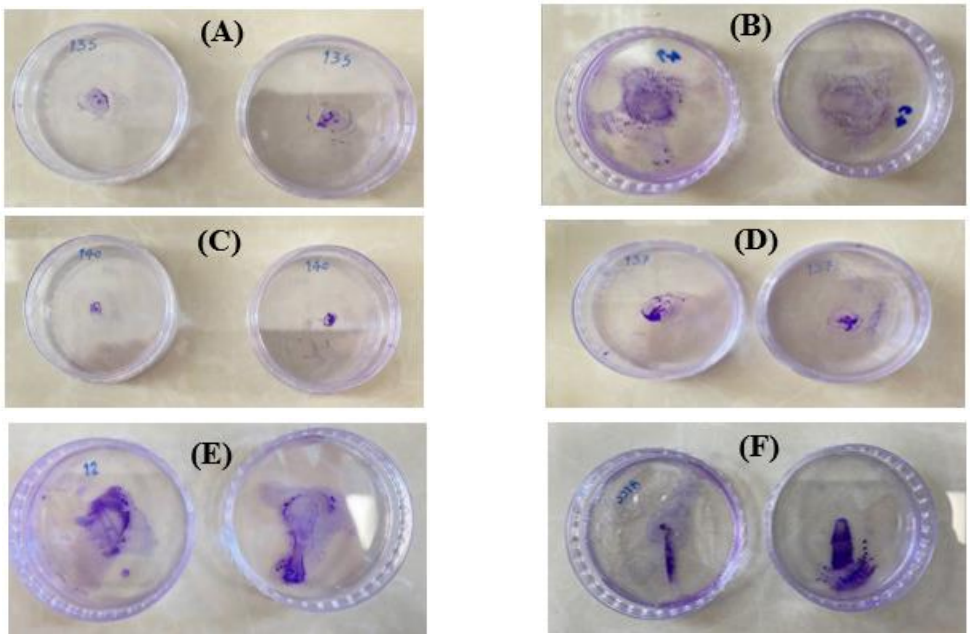
Table 7: *Comparing the Relationship Between bap and ompA Genes and Biofilm Formation*

Related Biofilm genes	*Biofilm formation (%)*	*Non-biofilm formation (%)*	*p-Value*
bap +	*90 (97.8)*	*2 (2.2)*	*0.2462*
bap -	*4 (10)*	*0 (0)*	
ompA +	*94 (98)*	*2 (2)*	*0.99*
ompA -	*0 (0)*	*0 (0)*	

3.4. Motility

Considering biofilm formation differs between drug-resistant and drug-sensitive strains, the twitching motility ability of isolates was analyzed. Isolates were divided into three categories based on their twitching motility: non-motile (< 5mm), intermediate (5-20 mm), and strong (> 20mm). Our results show that 6.4% of isolates were non-motile, 45.9% had an intermediate ability of twitching motility, and 47.7% showed a high ability of twitching motility (Fig 4). Based on the twitching motility test results, 96.95% and 3.05% isolates of MDR and non-MDR showed high twitching motility, respectively. Additionally, among the strong, moderate, and weak biofilm formers, 21.2%, 54.55%, and 21.2% exhibited a high ability to display twitch motility, respectively. Furthermore, 3.05% of isolates that could not form biofilm demonstrated a strong ability to twitch motility. No correlation was observed between twitching motility, antibiotic resistance, and biofilm formation.

Figure 4: *Twitching motility of isolates after 24 h in 1% TSA*



Note. A. The twitching motility zone of the isolate was classified as intermediate motility (5-20 mm). B. The twitching motility zone of the isolate was classified as strong motility (> 20mm). C. The twitching motility zone of the isolate was classified as intermediate motility (5-20 mm). D. The twitching motility zone of the isolate that was classified as intermediate motility (5-20 mm). E. The twitching motility zone of the isolate was classified as strong motility (> 20mm). F. The twitching motility zone of ATCC 19606 was classified as intermediate motility (5-20 mm).

4. Conclusion

Our research determined the prevalence of immune evasion genes (*bap* and *ompA*), antimicrobial resistance, biofilm formation, and motility in respiratory-derived *A. baumannii* isolates. Considering the high frequency of *bap* and *ompA* genes, which play a role in biofilm formation and immune evasion mechanisms, as well as MDR profile isolates, it can be concluded

that the resistant *A. baumannii* population is dominant in the hospital. Moreover, compared to previous reports, the frequency of these two genes is increased. No correlation was found between twitching motility and antibiotic resistance or biofilm formation, although most isolates exhibited high twitching motility. Although several studies have evaluated individual aspects of *A. baumannii* pathogenicity, few have conducted an integrated analysis of immune evasion genes, biofilm formation, antibiotic resistance, and motility in respiratory isolates. This study addresses that gap by providing a multifaceted assessment of clinical isolates from a high-risk population in central Iran. Future studies should include larger, multicenter cohorts and employ longitudinal designs to determine the causal relationships between virulence factors and clinical outcomes. Moreover, investigating the molecular mechanisms regulating biofilm development and motility in MDR strains may uncover novel therapeutic targets. These findings underscore the urgent need for stringent infection control practices in hospitals, particularly in managing multidrug-resistant *A. baumannii*, which possesses strong biofilm-forming capabilities. The high prevalence of *bap* and *ompA* genes suggests that these markers may serve as potential diagnostic or prognostic indicators in clinical microbiology.

Ethics statement

This study was approved by the Iranian National Committee for Ethics in Biomedical Research (ethics code: IR.UI.REC.1402.091), University of Isfahan, Iran.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

Data is available by request to the author.

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Open access

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Authors' Contributions

All authors contributed to the study's conception and design. HME, BB, SR, and MRK prepared materials and collected and analyzed data. HME wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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