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Antibiotic resistance pattern and biofilm formation among clinical *Acinetobacter baumannii* isolates: A cross-sectional study

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ABSTRACT

Objective: To investigate the pattern of antibiotic resistance and biofilm production capabilities of clinical *Acinetobacter baumannii* (*A. baumannii*) isolates in this study.

Methods: *A. baumannii* isolates were collected from Tehran Imam Khomeini Hospital in this cross-sectional study, and the minimum inhibitory concentrations for 16 antibiotics were determined using Vitek2® systems. All isolates were analyzed for biofilm production, then presence of biofilm-associated genes, and class Ⅰ and Ⅱ integron genes.

Results: 60 non-replicate *A. baumannii* isolates were included in this study. The resistance rates reached 100% for aztreonam, cefepime, ceftazidime, ciprofloxacin, piperacillin-tazobactam, piperacillin, ticarcillin, and trimethoprim-sulfamethoxazole. *A. baumannii* isolates were most sensitive to colistin and rifampicin being the most effective treatments. Multi-drug resistant and extensively drug-resistant isolates accounted for 83.3% and 16.7%, respectively. Of the isolates, 91.6% formed biofilms, categorized as 10% strong, 31.6% moderate, and 50% weak. No correlation was found between antibiotic resistance and biofilm formation. The genes *csuE*, *abaI*, and *ompA* were prevalent, but their distribution was similar across biofilm categories. A relationship between *Int1* and biofilm production was noted.

Conclusions: The high rates of antibiotic resistance and biofilm formation, alongside the presence of integrons including class Ⅰ and Ⅱ, underscore the necessity for ongoing monitoring of *A.baumannii*. Notably, class Ⅰ integron presence was significantly linked to biofilm formation. Further research is needed to explore the connection between antibiotic resistance and biofilm production in *A. baumannii*.

KEYWORDS: *Acinetobacter baumannii*; Antibiotic resistance; Biofilm; Biofilm-associated genes; Integron

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Summary

Question: What are the patterns of antibiotic resistance and biofilm production capabilities of clinical *Acinetobacter baumannii* isolates, and how do associated genetic factors influence these characteristics?

Findings: MDR and XDR isolates made up 16.7% and 83.3%, respectively. Biofilm formation was observed in 91.6% of isolates, though no correlation with antibiotic resistance was found. The *csuE*, *abaI*, and *ompA* genes were common across all biofilm categories, while class Ⅰ integrons showed a significant association with biofilm production.

Meaning: Rising antibiotic resistance in *Acinetobacter baumanii* highlights an urgent need for new treatment strategies. The strong association between class Ⅰ integrons and biofilm strength prompts further investigation into the genetic factors influencing pathogenicity in *Acinetobacter baumannii.*

1. Introduction

Antibiotic-resistant infections present major challenges in clinical management, often resulting in treatment failures and increased complications, along with significant financial burdens for both individuals and society[1]. The increase in antibiotic resistance is typically driven by genetic changes, such as acquisition of resistance genes or mutations[2]. In some instances, genetic alterations may not be directly responsible for antibiotic resistance; in these cases, factors such as biofilm formation, persistence, and the stationary growth phase are crucial, a phenomenon referred to as phenotypic resistance. Consequently, phenotypic resistance can often lead to the development of genetic resistance[3]. Biofilm formation is a well-recognized form of phenotypic resistance, and bacteria residing within biofilms can exhibit high resilience to the immune system and elevated concentrations of antibiotics and other treatments, contributing to chronic infections[4]. Recent reports suggest that biofilms may enhance horizontal gene transfer, suggesting that such transfer may occur more frequently in biofilm communities compared to free-living bacterial states[5]. Additionally, extracellular DNA (eDNA), a component of the biofilm matrix, can facilitate biofilm production and enhance antibiotic resistance within biofilms[6]. It is well established that bacterial cells within biofilms demonstrate 10 to 1 000 times greater antibiotic resistance than their planktonic form[7]. Notably, biofilm formation may be linked to multidrug-resistant (MDR) bacteria, with MDR isolates exhibiting significantly higher biofilm production than non-MDR isolates[8-10]. Therefore, investigating the relationship between phenotypic and genotypic resistance is essential.

Acinetobacter baumannii (*A. baumannii*), an aerobic, gram-negative coccobacillus, is a significant opportunistic pathogen implicated in various nosocomial infections such as pneumonia, bloodstream infections, urinary tract infections, meningitis, and wound infections. This pathogen has become a serious infectious agent and is now considered a global threat to public health[11]. Alarmingly, the emergence of MDR, extensively drug-resistant (XDR), and pan-drug-resistant A. baumannii strains has led to a rise in difficult-to-treat infections associated with high mortality rates[12]. *A. baumannii* employs several mechanisms to resist most clinically available antibiotics, including the upregulation of efflux pumps, modification of aminoglycosides, production of β-lactamases, alterations to target sites, and permeability defects[13]. Colistin- and carbapenem-resistant strains of *A. baumannii* are recognized as a critical nosocomial pathogen urgently requiring novel treatment strategies[14,15]. Alongside its genotypic resistance, this human pathogen's ability to form biofilms significantly contributes to its capacity for rapid antibiotic resistance acquisition, making it one of the most alarming pathogens of the 21st-century[16]. The ability of *A. baumannii* to form biofilms on diverse surfaces can facilitate persistent and chronic infections, drug resistance, and survival in harsh environments, ultimately leading to the emergence of fatal infections[17].

As discussed, biofilm formation can contribute to the development of antibiotic resistance and the emergence of MDR/XDR bacteria. However, a study[12] reported a correlation between strong biofilm production and antibiotic resistance, while another two studies[18,19] found no significant correlation. Interestingly, other research[20,21] indicated that non-MDR isolates were more prevalent among strong biofilm formers compared to weak ones. Given the existence of these contradictory findings and the ongoing crisis of antibiotic resistance in *A. baumannii,* further research is warranted to clarify whether biofilm formation is associated with increased antibiotic resistance or, more specifically, an increase in MDR isolates. Given these conflicting results and the urgent need for clarity in the context of *A. baumannii*'s antibiotic resistance crisis, this study investigates the relationship between biofilm formation, integron presence, and antibiotic resistance in clinical isolates.

2. Patients and methods

2.1. Bacterial isolates and antimicrobial susceptibility testing

This cross-sectional study included 60 unique clinical isolates of *A. baumannii* collected from various specimens at Tehran Imam Khomeini Hospital, including urine, blood, sputum, and catheter samples from June 2023 to December 2023. Identification of the isolates was achieved through conventional biochemical tests and confirmed by polymerase chain reaction (PCR) targeting the

blaOXA-51 gene. The isolates were inoculated in Brain Heart Infusion Broth (Condalab, Spain) with 20% glycerol and stored at 80 ℃ until analysis. Minimum inhibitory concentrations (MICs) for 16 antibiotics, including aztreonam, cefepime, ceftazidime, ciprofloxacin, levofloxacin, meropenem, imipenem, colistin, gentamicin, tobramycin, piperacillin-tazobactam, piperacillin, ticarcillin, rifampicin, trimethoprim/sulfamethoxazole, and tigecycline, were determined using the Vitek2® system (bioMérieux, software version 4.03) with results interpreted according to Clinical and Laboratory Standards Institute breakpoints[22]. Exceptionally, the results of tigecycline susceptibility were evaluated based on the criteria suggested by Jones *et al.*[23]. MDR and XDR isolates were defined as those exhibiting non-susceptibility to at least one in three or more antimicrobial classes and non-susceptibility to at least one agent in all but two or fewer antimicrobial classes, respectively[24].

2.2. Ethical statement

The study was approved by the Ethics Committee of Ilam University of Medical Sciences, Iran (ethical code: IR.MEDILAM.REC.1400.002).

2.3. Biofilm formation assays

Biofilm formation was evaluated through the crystal violet assay[25], as previously described. Briefly, an overnight culture of each *A. baumannii* isolate was inoculated into trypticase soy broth (TSB) (Merk, Germany) supplemented with 1% glucose and incubated overnight at 37 ℃. Bacterial cultures were then diluted 1:100 into fresh TSB, and 200 µL of each suspension was transferred in triplicate to 96-well polystyrene microtiter plates (Coster, USA). After overnight incubation at 37 ℃ in aerobic conditions, free-floating cells were washed with phosphate-buffered saline. Adherent cells were fixed with 99% methanol for 15 min and stained with 200 µL of 0.1% *w/v* crystal violet. Excess stain was washed off with phosphate-buffered saline, and the plates were left to dry at room temperature. Remaining crystal violet was solubilized with 200 µL of absolute ethanol for 20 min, and optical density (OD) was measured at 490 nm. *A. baumannii* ATCC 19606 served as the reference strain, while un-inoculated TSB was used as a negative control. The mean OD non-inoculated TSB defined the cut-off OD (ODc). Biofilm formation was categorized as follows: OD≤ODc = non-biofilm producer, ODc<OD≤2×ODc= weak biofilm producer, 2×ODc<OD≤4×ODc=moderate biofilm producer, and 4×ODc<OD=strong biofilm producer[26].

2.4. Detection of class Ⅰ, Ⅱ integron and biofilm associated genes

After DNA extraction from each isolate using the traditional boiling method[27], the presence of the biofilm-associated genes including *epsA*, *ompA*, *bap*, *ptk*, *abaI*, *csuE* and class Ⅰ, Ⅱ integron genes (*Int1*, *Int2*) was determined using PCR assay. The sequences and characteristics of the primers are detailed in Table 1. PCR was conducted using Taq DNA Polymerase MasterMix RED (Ampliqon, Denmark) in a thermal cycler instrument (Bio-Rad, USA). Each 25 µL reaction consisted of 3 µL of genomic DNA (100 ng/µL), 13 µL of PCR MasterMix, 1 µL (10 pmol/µL) of each forward and reverse primer, and 8 µL disulted water. PCR conditions included denaturation at 95 ℃ for 3 min, after that 37 cycles of denaturation at 95 ℃ for 30 s, annealing for 1 min, and extension at 72 ℃ for 45 s, concluding with a final extension at 72 ℃ for 8 min.

2.5. Statistical analysis

The data were analyzed by SPSS version 22 (IBM Corporation,

Table 1. Primers sequences used in this study.

Genes	Primers sequences (5-3)	Amplicon size	Reference
csuE	*csuE*-F: TTGGCTTTAGCAAACATGACCT *csuE*-R: TTGCGGGGAAAGTCCATTATTT	564 bp	[56]
bap	*bap*-F: GGTACAAACTATGTGCCGGATT *bap*-R: CTGTATTCACTCCTTGACCAGC	934 bp	
abaI	*abaI*-F: CCACACAACCCTATTTACTCGG *abaI*-R: GGCGGTTTTGAAAAATCTACGG	121 bp	
ompA	*ompA*-F: AGCATAAAGAAGCTACACCTGC *ompA*-R: AAAGTCGCCAAGAAACCTTGAT	154 bp	
epsA	*epsA*-F: AGCAAGTGGTTATCCAATCG *epsA*-R: ACCAGACTCACCCATTACAT	451 bp	[35]
ptk	*ptk*-F: GGCTGAGCATCCTGCAATGCGT *ptk*-R: ACTTCTGGAGAAGGGCCTGCAA	597 bp	
Int1	*Int1*-F: CAGTGGACATAAGCCTGTTC *Int1*-R: CCCGAGGCATAGACTGTA	160 bp	[57]
Int2	*Int2*-F: TTGCGAGTATCCATAACCTG *Int2*-R: TTACCTGCACTGGATTAAGC	288 bp	[58]

USA) and Microsoft Excel. *Chi*-square tests or Fisher's exact tests were applied for comparisons of categorical data, with a *P* value of <0.05 considered statistically significant.

3. Results

3.1. Antimicrobial susceptibility profiles

Our findings indicated that over half of the isolates exhibited complete resistance to antibiotics, with resistance rates of 100% for aztreonam, cefepime, ceftazidime, ciprofloxacin, piperacillin-tazobactam, piperacillin, ticarcillin, and trimethoprim-sulfamethoxazole. Beta-lactams were the least effective agents, while colistin and rifampicin were the most effective, showing susceptibility rates of 100% and 93.3%, respectively. Figure 1 illustrates the resistance rates for all tested antibiotics.

3.2. Biofilm formation and presence of biofilm-related genes

The average absorbance values for the *A. baumannii* ATCC 19606 reference strain and negative control were 0.455 and 0.098, respectively. The average absorbance values for the clinical isolates ranged from 0.085 to 1.650. Among the 60 *A. baumannii* isolates studied, 55 (91.6%) were identified as biofilm formers, including 6 (10.0%) classified as strong, 19 (31.6%) as moderate, and 30 (50.0%) as weak biofilm producers. Table 2 shows the genes distribution based on biofilm category. Although the all genes including *bap* (37.9%), *ompA* (50.9%), *epsA* (43.3%), *ptk* (46.9%), *abaI* (49.2%) and *csuE* (49.2%), were most common in weak biofilm, based on statistical test results, the distribution of genes was not different in biofilm category (All *P* values>0.05), suggesting that biofilm formation has not any association with different genes. Table 3 shows that, while most bacteria were resistant to antibiotics, no significant association was found between antibiotic resistance and biofilm formation (all *P* values>0.05). The distribution of biofilm-related genes *bap*, *ompA*, *epsA*, *ptk*, *abaI* and *csuE* among the 60 *A. baumannii* isolates were 48.3%, 95.0%, 50.0%, 81.6%, 98.3% and 98.3% respectively.

3.3. Correlation between the presence of class Ⅰ, Ⅱ integron genes and MDR/XDR isolates with biofilm formation ability

As shown in Table 4, of the 60 samples, 56 (93.3%) were positive for *Int1*, and 42 (70%) were positive for *Int2*. A significant relationship was identified between integron Ⅰ and biofilm formation, with higher percentages of moderate and strong biofilm producers in *Int1*-positive isolates compared to *Int1*-negative isolates (*P*<0.001). Also, no significant relationship was found between *Int2* (*P*=0.346) or MDR/XDR status (*P*=0.473) and biofilm formation. Accordingly, the rates of XDR and MDR isolates were 83.3% and 16.7%, respectively, with no pan-drug-resistant isolates detected. Additionally, no association was detected between *Int1* or *Int2*, and antibiotic resistance (all *P* values>0.05), as shown in Table 5.

4. Discussion

A. baumannii is a highly resistant opportunistic pathogen known for its significant biofilm production capabilities, as evidenced by

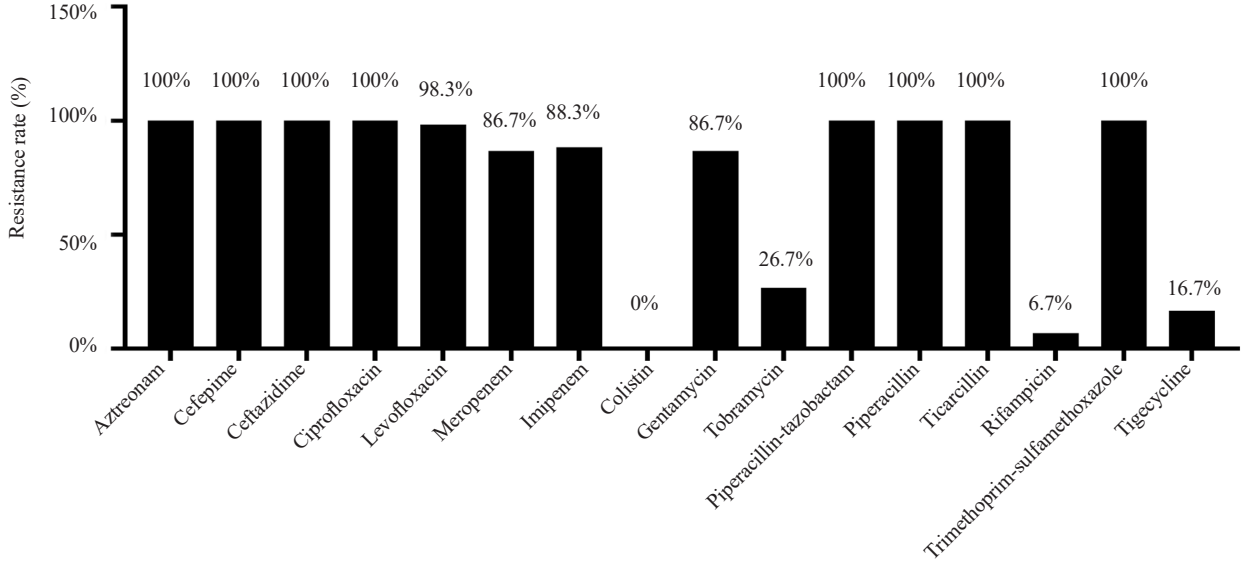

Figure 1. The antimicrobial resistance pattern and MDR/XDR prevalence of *Acinetobacter baumannii* clinical isolates.

Table 2. Relationship between the presence of biofilm-related genes and biofilm formation ability among the *A. baumannii* isolates, *n* (%).

Gene	Non-biofilm	Weak	Moderate	Strong	*P*
bap					
Negative	2 (6.5%)	19 (61.3%)	9 (29.0%)	1 (3.2%)	0.172
Positive	3 (10.3%)	11 (37.9%)	10 (34.5%)	5 (17.2%)	
ompA					
Negative	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)	0.413
Positive	4 (7.0%)	29 (50.9%)	18 (31.6%)	6 (10.5%)	
epsA					
Negative	4 (13.3%)	17 (56.7%)	6 (20.0%)	3 (10.0%)	0.178
Positive	1 (3.3%)	13 (43.3%)	13 (43.3%)	3 (10.0%)	
ptk					
Negative	1 (9.1%)	7 (63.6%)	3 (27.3%)	0 (0.0%)	0.585
Positive	4 (8.2%)	23 (46.9%)	16 (32.7%)	6 (10.2%)	
abaI					
Negative	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0.797
Positive	5 (8.5%)	29 (49.2%)	19 (32.2%)	6 (10.2%)	
csuE					
Negative	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0.797
Positive	5 (8.5%)	29 (49.2%)	19 (32.2%)	6 (10.2%)	

Table 3. The relationship between biofilm formation ability of *A. baumannii* isolates with antibiotic resistance profile, *n* (%).

Antibiotic resistance	Non-biofilm	Weak	Moderate	Strong, *n* (%)	*P*
Aztreonam					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0.500
Resistant	5 (100.0%)	30 (100.0%)	18 (94.7%)	6 (100.0%)	
Cefepime					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Ceftazidime					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Ciprofloxacin					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Levofloxacin					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0.500
Resistant	5 (100.0%)	30 (100.0%)	18 (94.7%)	6 (100.0%)	
Meropenem					
Sensitive	0 (0.0%)	3 (10.0%)	3 (15.8%)	1 (16.7%)	
Intermediate	0 (0.0%)	1 (3.3%)	0 (0.0%)	0 (0.0%)	0.931
Resistant	5 (100.0%)	26 (86.7%)	16 (84.2%)	5 (83.3%)	
Imipenem					
Sensitive	0 (0.0%)	3 (10.0%)	3 (15.8%)	1 (16.7%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.795
Resistant	5 (100.0%)	27 (90.0%)	16 (84.2%)	5 (83.3%)	
Colistin					
Sensitive	5 (100%)	30 (100%)	19 (100%)	6 (100%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Gentamycin					
Sensitive	0 (0.0%)	3 (10.0%)	3 (15.8%)	1 (16.7%)	
Intermediate	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0.642
Resistant	5 (100.0%)	27 (90.0%)	15 (78.9%)	5 (83.3%)	

Table 3. Continued.

Antibiotic resistance	Non-biofilm, *n* (%)	Weak, *n* (%)	Moderate, *n* (%)	Strong, *n* (%)	*P*
Tobramycin					
Sensitive	0 (0.0%)	3 (10.0%)	3 (15.8%)	1 (16.7%)	
Intermediate	4 (80.0%)	14 (46.7%)	5(26.3%)	3 (50.0%)	0.195
Resistant	1 (20.0%)	9 (30.0%)	6 (31.6%)	0 (0.0%)	
Piperacillin-tazobactam					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Piperacillin					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Ticarcillin					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Rifampicin					
Sensitive	4 (80.0%)	21 (70.0%)	13 (68.4%)	4 (66.7%)	
Intermediate	1 (20.0%)	8 (26.7%)	4 (21.1%)	1 (16.7%)	0.839
Resistant	0 (0.0%)	1 (3.3%)	2 (10.5%)	1 (16.7%)	
Trimethoprim-sulfamethoxazole					
Sensitive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Resistant	5 (100.0%)	30 (100.0%)	19 (100.0%)	6 (100.0%)	
Tigecycline					
Sensitive	5 (100.0%)	26 (86.7%)	16 (84.2%)	3 (50.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.144
Resistant	0 (0.0%)	4 (13.3%)	3 (15.8%)	3 (50.0%)	

NA: Not applicable.

Table 4. The relationship between biofilm-forming capacities with presence of *Int1*, *Int2* genes and MDR/XDR isolates.

Category	*Int1-*	*Int1+*	*P*	*Int2-*	*Int2+*	*P*	MDR	XDR	*P*
Non-biofilm	3 (75.0%)	2 (3.6%)		0 (0.0%)	5 (11.9%)		0 (0.0%)	5 (10.0%)	
Weak	1 (25.0%)	29 (51.8%)		11 (61.1%)	19 (45.2%)		4 (40.0%)	26 (52.0%)	
Moderate	0 (0.0%)	19 (33.9%)	<0.001	6 (33.3%)	13 (31.0%)	0.346	5 (50.0%)	14 (28.0%)	0.473
Strong	0 (0.0%)	6 (10.7%)		1 (5.6%)	5 (11.9%)		1 (10.0%)	5 (10.0%)	
Total	4 (6.7%)	56 (93.3%)		18 (30.0%)	42 (70.0%)		10 (16.7%)	50 (83.3%)	

Calculated by fisher exact test.

Table 5. The relationship between antimicrobial resistance profile and *Int1*, *Int2* genes among *A. baumannii* isolates.

Category	*Int1-*	*Int1+*	*P*	*Int2-*	*Int2+*	*P*
Aztreonam						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	1 (1.8%)	0.999	0 (0.0%)	1 (2.4%)	0.999
Resistant	4 (100.0%)	55 (98.2%)		18 (100.0%)	41 (97.6%)	
Cefepime						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Ceftazidime						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Ciprofloxacin						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	

Table 5. Continued.

Category	*Int1-*	*Int1+*	*P*	*Int2-*	*Int2+*	*P*
Levofloxacin						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	1 (1.8%)	0.709	1 (5.6%)	0 (0.0%)	0.300
Resistant	4 (100.0%)	55 (98.2%)		17 (94.4%)	42 (100.0%)	
Meropenem						
Sensitive	1 (25.0%)	6 (10.7%)		2 (11.1%)	5 (11.9%)	
Intermediate	0 (0.0%)	1 (1.8%)	0.445	1 (5.6%)	0 (0.0%)	0.429
Resistant	3 (75.0%)	49 (87.5%)		15 (83.3%)	37 (88.1%)	
Imipenem						
Sensitive	1 (25.0%)	6 (10.7%)		2 (11.1%)	5 (11.9%)	
Intermediate	0 (0.0%)	0 (0.0%)	0.399	0 (0.0%)	0 (0.0%)	0.998
Resistant	3 (75.0%)	50 (89.3%)		16 (88.9%)	37 (88.1%)	
Colistin						
Sensitive	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Gentamycin						
Sensitive	0 (0.0%)	7 (12.5%)		2 (11.1%)	5 (11.9%)	
Intermediate	0 (0.0%)	1 (1.8%)	0.989	1 (5.6%)	0 (0.0%)	0.429
Resistant	4 (100.0%)	48 (85.7%)		15 (83.3%)	37 (88.1%)	
Tobramycin						
Sensitive	1 (25.0%)	17 (30.4%)		9 (50.0%)	9 (21.4%)	
Intermediate	2 (50.0%)	24 (42.9%)	0.989	6 (33.3%)	20 (47.6%)	0.103
Resistant	1 (25.0%)	15 (26.8%)		3 (16.7%)	13 (31.0%)	
Piperacillin-tazobactam						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Piperacillin						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Ticarcillin						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Rifampicin						
Sensitive	3 (75.0%)	39 (69.6%)		14 (77.8%)	28 (66.7%)	
Intermediate	1 (25.0%)	13 (23.2%)	0.999	2 (11.1%)	12 (28.6%)	0.241
Resistant	0 (0.0%)	4 (7.1%)		2 (11.1%)	2 (4.8%)	
Trimethoprim-sulfamethoxazole						
Sensitive	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
Resistant	4 (100.0%)	56 (100.0%)		18 (100.0%)	42 (100.0%)	
Tigecycline						
Sensitive	3 (75.0%)	47 (83.9%)		16 (88.9%)	34 (81.0%)	
Intermediate	0 (0.0%)	0 (0.0%)	0.528	0 (0.0%)	0 (0.0%)	0.708
Resistant	1 (25.0%)	9 (16.1%)		2 (11.1%)	8 (19.0%)	

Calculated by fisher exact test.

numerous studies[28,29]. Nearly half of all *A. baumannii* strains in many parts of the world are now resistant to multiple drugs[30]. In certain Asian countries, such as India, Turkey, and Iran, treating infections caused by MDR *A. baumannii* can be challenging[31]. In response to this growing threat, the World Health Organization has classified *A. baumannii* as a Group-1 priority pathogen, necessitating the urgent development of new antimicrobials[32]. This study evaluated the antimicrobial susceptibility profiles, biofilm formation potential, and presence of integron and biofilm-related genes in 60 clinical isolates of *A. baumannii.* Our findings indicated that a majority of these isolates were resistant to multiple antibiotics, with 100% resistance observed against eight out of

16 commonly used antibiotics, including aztreonam, cefepime, ceftazidime, ciprofloxacin, piperacillin-tazobactam, piperacillin, ticarcillin and trimethoprim-sulfamethoxazole that showed excessive drug resistance among them. Furthermore 16.7% of isolates were MDR and 83.3% were XDR which was in line with Monfared *et al.*, study[33] from Iran that identified 16.1% XDR and 83.9% MDR strains. But our results differed from those of Mirzaei *et al.*[34] study from Iran that reported 74.75% and 73.13% of the *A. baumannii* isolates were screened as the MDR and XDR. Likewise, in other study in Iran, Zeighami *et al.*[35] reported that 100% and 98% of isolates were considered as MDR and XDR, respectively. Nowadays, the spread of MDR and XDR *A. baumannii* poses a significant global public health challenge and effective antibiotic treatment is essential for reducing and mortality rates. In most studies, the combination of colistin and rifampicin has shown 100% synergy against MDR. *A. baumannii*[36]. In this study, all isolates including MDR and XDR isolates were susceptible to colistin, and colistin along with rifampicin was the most effective antibiotics with susceptibility rates of 100% and 93.3%, respectively. This is consistent with previous studies in Iran[37], China, and Brazil[38,39] declaring the efficacy of colistin and rifampicin against *A. baumannii* strains. However, our finding were differed from those of Sherif *et al*.[16] and Farajnia *et al.*[40] studies, which reported colistin resistance rates of 12.2% and 24%, correspondingly, in *A. baumannii*.

These variation in drug resistance pattern of our research compared with another studies may be due to differences in antibiotic therapy regimens, geographic location, hospital settings, clinical source and the specific isolates being studied. The only antibiotics that are currently effective against *A. baumannii* are colistin and tigecycline, which are used as a last resort for treating MDR strains[36]. Fortunately, in this study, all the isolates were colistin-susceptible; but, 16.7% were tigecycline-resistant which can be very important considering the significance of this antibiotic in the treatment of MDR strains.

Biofilm formation is believed to be a main pathogenic characteristic in the development and dissemination of bacterial infections. In clinical settings, the elevated capacity for biofilm formation in *A. baumannii* strains is a concerning factor since it can result in persistent infections and render these infections more challenging[41]. In terms of biofilm formation ability, we found that 91.6% of the *A. baumannii* isolates both in the susceptible and resistant groups were biofilm formers and the majority of them were weak, followed by moderate and strong biofilm producers. Our findings are comparable with previous studies reporting high levels of biofilm formation in *A. baumannii* isolates. Monfared *et al.*[33], Smitran *et al*.[42], Al-Shamiri *et al*.[31] showed 86.5%, 91.9%, and 97.1% biofilm producing, respectively. Biofilm formation from of indwelling devices such as urethral catheters, ostomy devices, intracardiac and intravascular catheters, and tubes can be a source of sepsis in patients[42].

The nature of relation between biofilm formation and resistance profiles in *A. baumannii* is a controversial topic[16]. While there are conflicting reports on the association between the biofilm formation and resistance, our results demonstrated that there was no significant statistical relation between biofilm formation capacity and resistance to any of the evaluated antibiotics that was similar to previous investigations that declared a negative relationship between biofilm production capacity and either antimicrobial resistance or multidrug resistance in *A. baumannii.* In agreement with our findings, Donadu *et al*.[43] and Baniya *et al*.[44] found no statistically significant difference between the non-MDR and MDR isolates regarding biofilm-producing capacity. Therefore, it suggests that other important mechanisms such as horizontal gene transfer, mutations, efflux pumps, *etc*. may be contribute to creating antibiotic resistance of these isolates. Furthermore, our study examined various virulence factors associated with biofilm formation in *A. baumannii*, and we demonstrated that the *csuE* (98.3%), *abaI* (98.3%), and *ompA* (95%) genes were the most prevalent genes followed by *ptk* (81.6%), *epsA* (50%), and *bap* (48.3%) genes.

Consistent with our findings, Zeighami *et al*.[35] detected that the *csuE* and *ompA* genes had frequencies of 90% and *ompA* 76.7%, respectively. Ghasemi *et al.*[45] in Iran showed *abaI*, *csuE*, and *ompA* genes were present in all (100%) *A. baumannii* isolates. *AbaI* (acyl homoserine lactone synthase) plays an essential role in bacterial motility and biofilm formation[46]. Likewise the *csuE* plays an important role in biofilm formation, twitching motility and irreversible attachment in *A. baumannii*. *OmpA* is the most common slow porin in *Acinetobacter* genus and plays a crucial rules in cells and closely associated with the bacterial pathogenesis and survival under harsh conditions[32]. Its absence in *A. baumannii* and *Escherichia col*i led to significant reduction of virulence. Also, it seems that some of its external loops, connecting *N*-terminal β-strands, are involved in interactions with host cells or other surfaces[47].

Despite the fact that these genes are present in most isolates, we observed no significant associations between their presence and the ability of the isolates to form biofilm, which is consistent with some previous literature[31,45]. This may indicate these genes participate in other virulence and physiological processes including maintaining cell membrane integrity, mediating drug resistance, modulating the immune response of the host, invading epithelial cells, and triggering host cell apoptosis[32]. However, measuring the expression level of these genes, particulary *ompA*, could shed more light on their role in biofilm formation.

Integrons are considered as a unique device and mechanism for the distribution of antimicrobial resistance genes, as they have the ability to cluster and express these genes, thereby enhancing

the ability of *A. baumannii* to be a successful disease-causing bacteria[48]. The study also investigated the correlation between presences of class Ⅰ, Ⅱ integron genes and antibiotic resistance, and biofilm formation ability. We revealed 93.3% of isolates was positive for class Ⅰ integron and 70% was positive for class Ⅱ integron. Similar to our results, Goudarzi *et al.*[49] reported 74.1% class Ⅰ and 12.5% class Ⅱ integrons. Another study by Adeniji *et al.*[48] revealed that 79% and 12.3% of *A. baumannii* isolates were positive class Ⅰ and Ⅱ integrons, respectively. On the other hands, Nourbakhsh *et al.*[50], showed the frequency of class Ⅰ and Ⅱ integrons was 100% and 44%, respectively. In the study of Ramírez *et al.*[51], the prevalence of the class Ⅱ integron was 68% that agrees with our results. Also, our data demonstrated that class Ⅰ and Ⅱ integrons were widely distributed among clinical isolates of *A. baumannii*. The reason for variable rate of integrons may be attributed to geographical differences, diver's health policies, especially regarding the antibiotics prescription. Interestingly, we showed that the presence of class Ⅰ integron genes was significantly associated with moderate and strong biofilm production that was in accordance with Alabdali *et al.*'s[52] study. This phenomenon could be because, integrons can exchange and uptake gene cassettes more efficiently within the biofilm[53]. Our finding may explain the emergence of the high prevalence of class Ⅰ integrons for the first time in our region, which indicates a concern in the future and increased biofilm production and antibiotic resistance associated with the presence of class Ⅰ integron. In contrast to previous studies that have shown a relation between class Ⅰ or Ⅱ integron and acquisition of MDR/XDR phenotype[52,54,55]. Our results demonstrated that there was no association between antibiotic resistance and the presence of class Ⅰ or Ⅱ integron genes. These results suggest that the mechanisms underlying biofilm formation and antibiotic resistance in *A. baumannii* are not necessarily interlinked.

In conclusion, high prevalence of antibiotic resistance, biofilm formation capability and class Ⅰ and Ⅱ integron, and virulence factor genes in our study highlights the importance of continuous monitoring biofilm production and antibiotic resistance in *A. baumannii*, especially in hospital settings where these bacteria are a common cause of infections. We demonstrated that class Ⅰ integron significantly associated biofilm formation capacity and further investigations are required to explore the role of its exact role in biofilm formation. Colistin and rifampicin was the most effective antibiotic against *A. baumannii.* Negative relationship was found between biofilm formation ability and antimicrobial resistance.

This study also has some limitations. While the study provides valuable insights into the patterns of antibiotic and resistance biofilm formation among *A. baumannii* isolates, more isolates and a larger cohort could enhance the reliability of the results and enable a more nuanced analysis of potential associations.

Conflict of interest statement

The authors report no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

Study concept and design: VHK and SK; Acquisition of data: VHK, SK, NO and MH; Analysis and interpretation of data: ZF and ADK; Drafting of the manuscript: SK, FS, VHK; Critical revision of the manuscript for important intellectual content: MHH and NS; Statistical analysis: FS; Administrative, technical and material support: VHK; Study supervision: FS, VHK and SK.

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