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Original Article

Genotypic and Phenotypic Insights into Biofilm-Associated Genes in Clinical *Acinetobacter baumannii* Isolates from Hospitalized Patients in Duhok, Iraq

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Abstract

Introduction*: Acinetobacter baumannii* (*A. baumannii*) is an opportunistic pathogen responsible for various nosocomial infections, and it is also capable of forming biofilms. It commonly develops multi-drug resistance (MDR). **Objectives:** This study aimed to investigate the prevalence of biofilm formation in *Acinetobacter baumannii* isolates that were taken from various clinical specimens and to identify genetic markers associated with biofilm development. Furthermore, the study examined the relationship between the isolate's genetic makeup and phenotypic characteristics. **Methods**: In this cross-sectional study, 514 consecutive clinical samples (urine, sputum, blood, CSF, wound swab, throat swab) were collected. Bacterial identification was achieved through colony morphology, Gram staining, and biochemical tests. The VITEK 2 system confirmed the isolates, and molecular confirmation was accomplished by amplifying the housekeeping gene 16srRNA. The isolates were stored at -80°C for further analysis. The phenotypic micro-titer plate test and the PCR-based identification of biofilm-forming genes identification were performed on isolated *A. baumannii*. **Results**: Among 514 clinical samples, *Acinetobacter baumannii* was isolated in (n = 57, 11.1%), most commonly from sputum (n = 17, 29.8%) and urine (n = 16, 28.1%). Strong biofilm formation was observed in (n = 36, 63.2%) of isolates, predominantly associated with extensively drug-resistant (XDR) profiles. Biofilm-related genes were highly prevalent: *csuE* (n = 55, 96.5%), *bap* (n = 54, 94.7%), *bfmS* (n = 50, 87.7%), and *ompA* (n = 49, 86.0%), whereas *blaPER-1* was detected in a smaller fraction (n = 11, 19.3%). Resistance was widespread across *β*-lactams, carbapenems, aminoglycosides, and fluoroquinolones, while colistin remained the most effective antibiotic (n = 45, 78.9%). No pan-drug-resistant (PDR) isolates were identified, highlighting the persistent threat of biofilm-associated multidrug-resistant *A. baumannii* in clinical settings. **Conclusion**: Pneumonia is the primary infection caused by *A. baumannii*. It demonstrates significant biofilm formation accompanied by *β*-lactam/carbapenem drug resistance while remaining sensitive to colistin. *CsuE* and *Bap* have a strong relationship with biofilm formation.

Keywords: *Acinetobacter baumannii; Antibiotic Resistance; Biofilms; Biofilm-Related Gene*

Introduction

Acinetobacter baumannii is an opportunistic Gram-negative pathogen that has emerged as one of the most significant causes of healthcare-associated infections worldwidewith mortality rate 26% (Moubareck & Halat, 2020; Lucidi *et al.,* 2024). It is frequently associated with severe infections such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and wound infections, particularly among hospitalized and immunocompromised patients. The clinical importance of *A. baumannii* has increased dramatically during the past two decades due to its remarkable ability

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to develop resistance to multiple classes of antimicrobial agents. Consequently, multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *A. baumannii* have become a major public health concern and are responsible for increased morbidity, mortality, prolonged hospital stays, and higher healthcare costs (Al-Shamiri *et al.,* 2021). One of the key virulence factors contributing to the persistence and pathogenicity of *A. baumannii* is its ability to form biofilms. Biofilms are structured communities of bacterial cells embedded within a self-produced extracellular polymeric matrix that adheres to both biotic and abiotic surfaces (Mea*et al.,* 2021; Lucidi *et al.,* 2024). The formation of biofilms allows bacteria to survive under hostile environmental conditions, evade host immune responses, and exhibit enhanced resistance to antimicrobial agents (Gautam *et al.,* 2023). In clinical settings, biofilm formation on medical devices and hospital surfaces significantly contributes to the persistence and transmission of *A. baumannii* infections (Pozo, 2021).

Biofilm development in *A. baumannii* is a complex process that is regulated by several genetic determinants. Among the most important biofilm-associated genes are *csuE*, *ompA*, *bap*, *EspA*, *BfmS*, *blaPER-1* gene (Upmanyu *et al.,* 2024). The *csuE* gene is involved in the chaperone–usher pili assembly system, which facilitates the initial attachment of bacterial cells to abiotic surfaces (Olawuwo *et al.,* 2022). The *ompA* (Outer Membrane Protein A) gene plays a crucial role in bacterial adhesion, biofilm maturation, and interaction with host cells (Skerniškytė *et al.,* 2021; Delfan *et al.,* 2024). Similarly, the bap (Biofilm-Associated Protein) gene contributes to the structural stability and maturation of biofilms (Upmanyu *et al.,* 2024). Bacterial cells interact with their surroundings through the use of Extracellular Serine Protease A (*EspA*), which consist of organic polymers of microbial origin (Mohamed *et al.,* 2023). The principal components of *EspA* are hydrolytic enzymes derived from antibiotics and alginate, which are immobilized on biofilm; they operate by preventing antimicrobial compounds from reaching their targets and diminishing their antibacterial efficacy (Mohamed *et al.,* 2023). The Biofilm Sensor Kinase (*BfmS*) gene enables cells to make a sensor kinase as part of the *BfmRS* two-component regulatory system that controls biofilm formation as well as virulence and antibiotic resistance (Palethorpe *et al.,* 2022). Additionally, the *blaPER-1* gene, which encodes an extended-spectrum *β*-lactamase, has also been associated with increased biofilm formation and antimicrobial resistance in *A. baumannii*. The presence and expression of these genes may significantly influence the pathogenic potential and persistence of this organism in hospital environments (Mohamed *et al.,* 2023). Haque discovered no association between the production of *PER1* beta-lactamase and biofilm development (Haque, 2023). Thus, the presence of *blaPER1* presumably improves the adhesion capacity of cells expressing this gene; however, it does not uniformly promote biofilm formation. Biofilm-forming ability in *A. baumannii* plays an important role in the pathogenesis and subsequently antibiotic resistance (Jabbar *et al.,* 2025).

Although numerous international studies have investigated the relationship between biofilm formation and biofilm-associated genes in *A. baumannii,* limited data are available from Iraq, particularly from the Kurdistan Region. Understanding the distribution of biofilm-related genes and their association with phenotypic biofilm formation among clinical isolates is essential for improving infection control strategies and guiding effective antimicrobial therapy. The lack of regional molecular epidemiological data represents a significant knowledge gap that requires further investigation.

Therefore, the present study aimed to investigate the prevalence of biofilm formation among clinical isolates of *Acinetobacter baumannii* obtained from hospitalized patients in Duhok, Iraq. In addition, the study aimed to determine the presence of selected biofilm-associated genes (*csuE, ompA, bap, EspA, BfmS, blaPER-1* gene) using Polymerase Chain Reaction (PCR) and to evaluate the relationship between the genotypic determinants and the phenotypic capacity of isolates to form biofilms.

Materials and Methods

Bacterial Isolates

In this hospital-based cross-sectional study, a total of 514 clinical specimens were collected over a four-month period in 2024 from patients treated in four hospitals in Duhok Province, Iraq. The specimens were obtained using a convenience sampling approach, whereby eligible samples available during the study period were consecutively included. The specimens were obtained from various clinical sources, including urine (n = 174), sputum (n = 85), wound swabs (n = 106), cerebrospinal fluid (CSF) (n = 35), throat swabs (n = 37), and blood (n = 77). Primary bacterial isolation and preliminary identification were performed using standard microbiological methods, including culture on MacConkey agar, followed by colony morphology assessment and Gram staining. Further biochemical characterization was conducted using the oxidase test (Bioanalyse, Turkey) and catalase assay (Merck, Germany). Definitive identification of bacterial isolates was subsequently carried out using the automated VITEK® 2 compact version (bioMérieux, France) with GN ID cards according to the manufacturer's instructions. Molecular confirmation was performed by polymerase chain reaction (PCR) amplification of the 16S ribosomal ribonucleic acid (16S rRNA) housekeeping gene. Confirmed isolates were preserved for further analysis by storage at −80 °C in brain heart infusion (BHI) broth supplemented with 50% (v/v) glycerol. The study included hospitalized patients of all age groups and both sexes who had been admitted for more than three days, while patients who had received antibiotic therapy within one week prior to sample collection and non-hospitalized individuals were excluded. Ethical approval for the study was obtained from the Duhok Directorate General of Health, Ministry of Health (Reference No.: 31072024-6-1, issued on 31 July 2024).

Antimicrobial Susceptibility Test

The antimicrobial susceptibility profile of the bacterial isolates was determined using the VITEK® 2 Compact automated system (bioMérieux, France). Briefly, a single well-isolated bacterial colony from a fresh culture was selected and suspended in 0.45% sterile saline solution to achieve a turbidity equivalent to 0.5 McFarland standard. The standardized bacterial suspension was then used to inoculate the antimicrobial susceptibility testing card (AST-N222) according to the manufacturer's instructions. The inoculated cards were subsequently loaded into the VITEK® 2 Compact system, where automated incubation was performed at 37 °C. During incubation, the system continuously monitored bacterial growth using optical detection technology, allowing determination of the minimum inhibitory concentrations (MICs) for the tested antimicrobial agents. The obtained MIC values were automatically interpreted by the system according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and isolates were categorized as susceptible (S), intermediate (I), or resistant (R)

Phenotypic Characterization of Biofilm Formation Using the Microtiter Plate

Biofilm formation was evaluated using the crystal violet micro-titer plate assay. Briefly, bacterial isolates were initially cultured in brain heart infusion (BHI) broth and incubated overnight at 37 °C. The resulting colonies were suspended in 0.85% NaCl and adjusted to 0.5 McFarland turbidity (1.5×10^8 CFU/mL). Subsequently, 20 µL of the standardized bacterial suspension was inoculated into wells of a 96-well micro-titer plate containing 180 µL of BHI broth, followed by incubation at 37 °C for 24 h. After incubation, the contents of the wells were discarded, and non-adherent planktonic cells were removed by three washes with sterile Phosphate-Buffered Saline (PBS). The plates were then air-dried at room temperature for 30 min, and the attached biofilms were stained with 150 µL of 1% crystal violet for 20 min. Excess stain was removed by washing the plates five times with distilled water. The bound crystal violet was subsequently solubilized using 200 µL of 95% ethanol, followed by incubation at room temperature for 10–15 min. Biofilm biomass was quantified by measuring the optical density (OD) at 570 nm using a micro-plate reader. Three negative control wells containing sterile medium were included in each plate. The optical density cut-off value (ODc) was calculated as the mean OD of the negative control plus three standard deviations (ODc = mean OD of negative + 3 × SD). Based on OD values, isolates were classified as strong biofilm producers (OD > 4ODc), moderate biofilm producers (2ODc < OD of samples ≤ 4ODc), weak biofilm producers (ODc< OD of samples ≤ 2ODc),

or non-biofilm producers (OD ≤ODc). All experiments were performed in triplicate (Joshua *et al.,* 2021).

Genotypic Detection of Biofilm

Genomic DNA was extracted using the boiling method as previously described by (Li *et al.,* 2023). Briefly, several colonies from freshly cultured isolates were suspended in 500 µL of deionized water and heated at 100 °C for 20 min, followed by rapid freezing at −50 °C for 6 min. The lysates were subsequently centrifuged at 14,000 rpm for 10 min, and the resulting supernatant containing genomic DNA was collected and stored at −50 °C until further analysis. DNA concentration and purity were assessed using a Nano-Drop Spectrophotometer (Thermo Fisher Scientific, USA). A positive control strain (Accession No. OP810499) was kindly provided by Meqdad Saleh Ahmed from the Duhok Research Center, College of Veterinary Medicine, University of Duhok (Ahmed *et al.,* 2025). Detection of *bfmS, espA, bap, csuE, ompA*, and *blaPER-1* genes in *Acinetobacter baumannii* isolates was performed using polymerase chain reaction (PCR) with an Eppendorf Thermal Cycler. Each PCR reaction was carried out in a 25 µL volume containing 12.5 µL of 2× HotStarTaq Master Mix (SinaClon, Iran), 1 µL of each primer (20 pmol), 2 µL of DNA template, and 8.5 µL of nuclease-free water. The Primer sequences and the amplification conditionsare presented in (Table 1). The amplified products were separated by 2% agarose gel electrophoresis stained with a safe DNA dye, using a 100–1000 bp DNA Ladder as a molecular size marker. Electrophoresis was conducted at 100 V for 1 h, and the PCR amplicons were visualized under ultraviolet illumination.

Table 1: *List of Primers and Amplification Conditions Used in this Study*

Target Gene	Primer Sequence	Base Pair	Annealing Temperature (35 cycles)	References
16SRNA	CAGCTCGTGTCGTGAGATGT CGTAAGGGCCATGATGACTT	150	58℃/1min	(Al-Miyah, 2023)
EspA	AGCAAGTGGTTATCCAATCG ACCAGACTCACCCATTACAT	451	60℃/1min	(Joshua *et al.*, 2021)
OmpA	GTTAAAGGCGACGTAGACG CCAGTGTTATCTGTGTGACC	578	58℃/1min	(Joshua *et al.*, 2021)
blaPER-1	GCAACTGCTGCAATACTCGG ATGTGCGACCACAGTACCAG	340	58℃/1min	(Mahmood & Alhuda, 2024)
Bap	TGCTGACAGTGACGTAGAACCACA TGCAACTAGTGGAATAGCAGCCCA	184	58℃/1min	(Joshua *et al.*, 2021)
CsuE	CATCTTCTATTTCGGTCCC CGGTCTGAGCATTGGTAA	168	60℃/1min	(Mahmood &Alhuda, 2024)
BfmS	TTGCTCGAACTTCCAATTTATTATAC TTATGCAGGTGCTTTTTTATTGGTC	196	58℃/1min	(Choi *et al.*, 2024)

Results

Demographic Characteristics

A total of 514 clinical samples were analyzed in this study, of which (n= 57, 11.1%) were confirmed as positive for *A. baumannii*. The patients' mean age was 42.54 ±23.9.7 years. The highest prevalence was found in patients aged ≥70 year at 20.3%, with ($\chi 2$ = 12.46, p= 0.188). Among the collected samples, (n=265, 51.56%) were from female patients, with (n=27, 48.3%) testing positive for *A. baumannii*. In comparison, 249 samples (48.44%) were from male patients, of which (n=30, 51.7%) were positive (Table 2).

Table 2: *Distribution of Acinetobacter Baumannii in Different Clinical According to Age Group and Sex*

Characterizations		Positive N (%)	Negative N (%)	Chi-Square(χ^2)	*p*-value
Age group	1-30 days	1 (4%)	24 (96%)	12.46	0.188
	2-12 months	5 (14.7%)	29 (85.3%)		
	1-10 years	6 (7.9%)	70 (92.1%)		
	11-19 years	3 (7.5%)	37 (92.5%)		
	20-29 years	3 (15%)	17 (85%)		
	30-39 years	5 (12.8%)	34 (87.2%)		
	40-49 years	3 (4.7%)	61 (95.3%)		
	50-59 years	8 (11.6%)	61 (88.4%)		
	60-69 years	8 (11%)	59 (89%)		
	≥ 70 years	15 (20.3%)	59 (79.7%)		
Sex	Male	30(51.7%)	249(48.44%)	0.45	0.502
	Female	27(48.3%)	265(51.56%)		
Total		57	457	514	

Distribution of Isolates

Among the 57 confirmed isolates of *A. baumannii*, the majority were recovered from sputum samples (n = 17, 29.8%), followed by urine (n = 16, 28.1%), wound swabs (n = 12, 21.1%), blood (n = 8, 14.0%), cerebrospinal fluid (CSF) (n = 3, 5.2%), and throat swabs (n = 1, 1.8%). Regarding patient location, 29 cases (50.8%) were identified among individuals admitted to the intensive care unit (ICU), while 9 patients (15.8%) were hospitalized in other inpatient wards. In terms of clinical presentation, pneumonia was the most frequently observed condition, affecting 21 patients (36.8%), followed by burn-related infections (n = 10, 17.5%), urinary tract infections (UTIs) (n = 8, 14.0%), and meningitis or cardiovascular diseases (n = 7, 12.3%). Additionally, nephropathy was reported in 4 patients (7.0%) (Figure1). With respect to hospitalization duration, 43 isolates (75.4%) were obtained from patients with a hospital stay exceeding five days, whereas 14 isolates (24.6%) were recovered from patients hospitalized for five days or less; however, this difference was not statistically significant ($\chi^2 = 2.471$, $p = 0.480$).

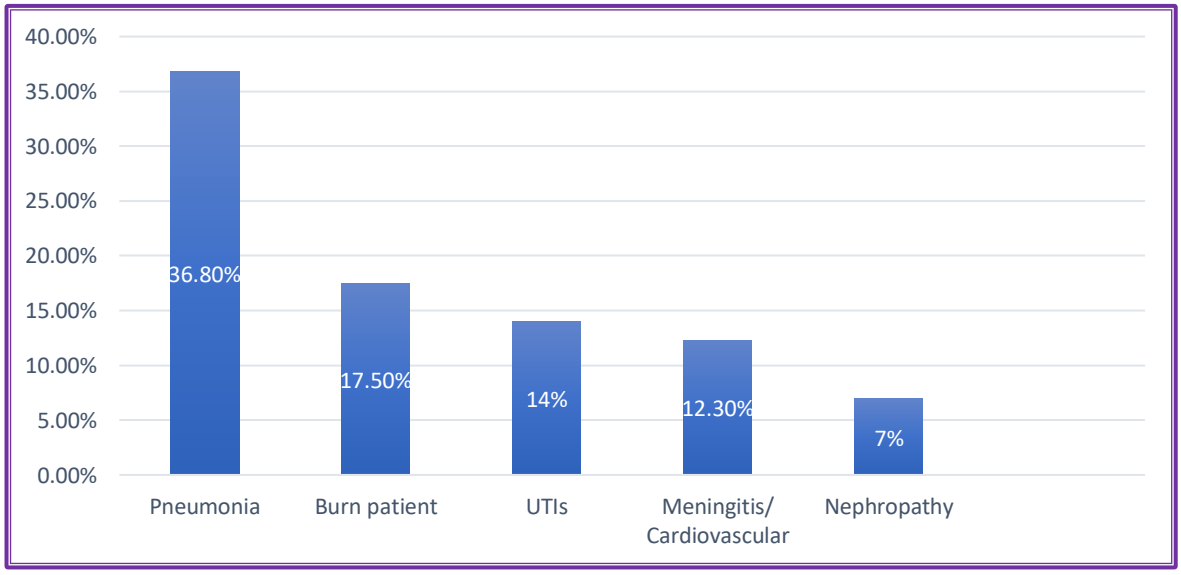

Figure 1: *Distribution of Clinical Conditions of Patients Infected with Acinetobacter Baumannii*

Biofilm Expression Categories and Related Genes

The study demonstrates the relationship between the strength of biofilm formation and the presence of biofilm-associated genes (Table 3). Overall, the majority of isolates were classified as strong biofilm producers (n=36, 63.2%), followed by moderate producers (n=10, 17.5%), weak producers (n=9, 15.8%), while only a small proportion showed no biofilm formation (n=3, 3.5%). Among the investigated genes, *CsuE* showed the highest prevalence (n=55, 96.5%), followed by *Bap* (n=54, 94.7%), *BfmS* (n=50, 87.7%), and *OmpA* (n=49, 86%). The *EspA* gene was detected in (n=45, 78.9%) of isolates, whereas *blaPER1* demonstrated the lowest frequency (n=11, 19.3%). Most gene-positive isolates were associated with strong biofilm formation, including *BfmS* (n=32, 64%), *OmpA* (n=31, 63.3%), *CsuE* (n=35, 63.6%), *Bap* (n=34, 63%), *EspA* (n=30, 66.7%), and *blaPER1* (n=9, 81.8%). However, statistical analysis revealed no significant association between biofilm formation strength and the presence of these genes ($p > 0.05$).

Table 3:*Relationship between The Strength of Biofilm Formation and the Presence of Associated Genes*

Positive genes	Non N(%)	Weak N (%)	Moderate N(%)	Strong N(%)	Total N(%)	χ^2	*p*-value
BfmS	2(4%)	8(16%)	8(16%)	32(64%)	50(87.7%)	0.890	0.828
OmpA	2(4.1%)	8(16.3%)	8(16.3%)	31(63.3%)	49(86%)	0.686	0.877
CsuE	2(3.6%)	8(14.5%)	10(18.2%)	35(63.6%)	55(96.5%)	2.030	0.566
Bap	2(3.7%)	8(14.8%)	10(18.5%)	34(63%)	54(94.7%)	1.290	0.731
EspA	2(4.4%)	5(11.1%)	8(17.8%)	30(66.7%)	45(78.9%)	3.920	0.270
blaPER1	0(0.0%)	1(9.1%)	1(9.1%)	9(81.8%)	11(19.3%)	2.172	0.537
Total	3(3.5%)	9(15.8%)	10(17.5%)	36(63.2%)	57(100%)	0.890	0.828

Antimicrobial Susceptibility Profile

Table 4 presents the antimicrobial susceptibility profile of *A. baumannii* isolates. A high level of resistance was observed to most tested antibiotics. Resistance to β-lactam antibiotics was particularly pronounced, including piperacillin (n=55, 96.5%), clavulanic acid (n=54, 94.7%), ticarcillin (n=52, 91.2%), ceftazidime (n=49, 86%), and cefepime (n=49, 86%). Carbapenem resistance was also notable, with imipenem resistance detected in (n=47, 82.5%) isolates and meropenem resistance in (n=48, 84.2%). Similarly, high resistance rates were observed for aminoglycosides, including gentamycin (n=51, 89.5%) and tobramycin (n=51, 89.5%). Resistance to the fluoroquinolone ciprofloxacin was identified in (n=50, 87.7%) isolates. In contrast, minocycline demonstrated comparatively lower resistance (n=24, 42.1%), with a considerable proportion of isolates showing intermediate susceptibility (n=14, 24.6%) or sensitivity (n=19, 33.3%). Notably, colistin exhibited the highest activity against the isolates, with the majority remaining sensitive (n=45, 78.9%), while resistance was observed in only (n=2, 3.5%) isolates. Overall, the findings indicate a high prevalence of multidrug resistance among *A. baumannii* isolates, with colistin remaining the most effective antimicrobial agent.

Table 4: *Antimicrobial Susceptibility Profile among A. Baumanii Isolates*

Antibiotics	Resistance N (%)	Intermediate N (%)	Sensitive N (%)	MIC (µg/mL)
Ticarcillin	52(91.2%)	1(1.8%%)	4(7%)	≥128
Clavulanic acid	54(94.7%)	1(1.8%)	2(3.5%)	≥ 128
Piperacillin	55(96.5%)	1(1.8%)	1(1.8%)	≥128
Ceftazidime	49(86%)	1(1.8%)	7(12.3%)	≥ 64
Cefepime	49(86%)	1(1.8%)	7(12.3%)	≥ 64
Imipenem	47(82.5%)	4(7%)	6(10.5%)	≥ 16
Meropenem	48(84.2%)	4(7%)	5(8.8%)	≥ 16
Gentamycin	51(89.5%)	1(1.8%)	5(8.8%)	≥ 16
Tobramycin	51(89.5%)	1(1.8%)	5(8.8%)	≥ 16
Ciprofloxacin	50(87.7%)	3(5.3%)	4(7%)	≥ 4
Minocycline	24(42.1%)	14(24.6%)	19(33.3%)	≤ 1
Colistin	2(3.5%)	10(17.5%)	45(78.9%)	≤ 0.5

Antibiotic Class Relates to the Biofilm Expression Categories

The analysis of antibiotic resistance patterns across multiple antibiotic classes in relation to biofilm formation demonstrated a predominance of resistant isolates among strong biofilm producers. Resistance to β-lactam antibiotics was observed in (n=47, 82.5%) isolates, including (n=2, 4.3%) non-biofilm formers, (n=5, 10.6%) weak producers, (n=7, 14.9%) moderate producers, and (n=33, 70.2%) strong biofilm producers.

Resistance to aminoglycosides was detected in (n=51, 89.5%) isolates, distributed as (n=1, 2.0%) non-biofilm former, (n=7, 13.7%) weak producers, (n=8, 15.7%) moderate producers, and (n=35, 68.6%) strong biofilm producers. Similarly, fluoroquinolone resistance was identified in (n=51, 89.5%) isolates, including (n=2, 3.9%) non-biofilm formers, (n=7, 13.7%) weak producers, (n=7, 13.7%) moderate producers, and (n=35, 68.6%) strong biofilm producers. A statistically significant association was identified between the level of biofilm formation and resistance to β-lactams, aminoglycosides, and fluoroquinolones ($p< 0.05$).

Regarding other antibiotic classes, tetracycline resistance was observed in (n=25, 43.9%) isolates, comprising (n=1, 4.0%) non-biofilm former, (n=4, 16.0%) weak producers, (n=2, 8.0%) moderate producers, and (n=18, 72.0%) strong biofilm producers. Polymyxin resistance was rare and detected in only (n=2, 3.5%) isolates. Additionally, resistance to antifolate agents was recorded in (n=48, 84.2%) isolates, including (n=2, 4.2%) non-biofilm producers, (n=8, 16.7%) weak producers, (n=8, 16.7%) moderate producers, and (n=30, 62.5%) strong biofilm producers (Table 5).

Table 5: *Correlation between Antibiotics Class and Biofilm Formation Categories*

Antibiotics class (Resistance)	**Non N(%)**	**Weak N (%)**	**Moderate N(%)**	**Strong N(%)**	**Total N (%)**	χ^2	***p*-value**
β-lactams	2(4.3%)	5(10.6%)	7(14.9%)	33(70.2%)	47(82.5%)	8.111	0.044
Aminoglycosides	1(2.0%)	7(13.7%)	8(15.7%)	35(68.6%)	51(89.5%)	7.864	0.049
Fluoroquinolones	2(3.9%)	7(13.7%)	7(13.7%)	35(68.6%)	51(89.5%)	7.864	0.049
Tetracyclines	1(4%)	4(16%)	2(8%)	18(72%)	25(43.9%)	2.895	0.408
Polymyxins	0(0.0%)	1(50%)	0(0.0%)	1(50%)	2(3.5%)	2.030	0.566
Antifolate	2(4.2%)	8(16.7%)	8(16.7%)	30(62.5%)	48(84.2%)	0.667	0.879

Gene-Resistance Correlation

Antibiotic resistance patterns were analyzed in relation to biofilm-associated genes. The bfmS gene was detected in (n = 50, 87.7%) isolates, including (n = 2, 4.0%) susceptible, (n = 14, 28.0%) multidrug-resistant (MDR), and (n = 34, 68.0%) extensively drug-resistant (XDR) strains, with no pan-drug-resistant (PDR) isolates identified. Similarly, ompA was present in (n = 49, 86.0%) isolates, comprising (n = 2, 4.1%) susceptible, (n = 12, 24.5%) MDR, and (n = 35, 71.4%) XDR strains. The csuE gene was identified in (n = 55, 96.5%) isolates, including (n = 2, 3.6%) susceptible, (n = 14, 25.5%) MDR, and (n = 39, 70.9%) XDR isolates. Likewise, bap was detected in (n = 54, 94.7%), comprising (n = 2, 3.7%) susceptible, (n = 13, 24.1%) MDR, and (n = 39, 72.2%) XDR isolates. The *espA* gene was identified in (n = 45, 78.9%), including (n = 1, 2.2%) susceptible, (n = 12, 26.7%) MDR, and (n = 32, 71.1%) XDR isolates. In contrast, blaPER-1 was detected in (n = 11, 19.3%), all of which were resistant, comprising (n = 2, 18.2%) MDR and (n = 9, 81.8%) XDR isolates, with no susceptible or PDR strains observed (Table 6). Regarding biofilm formation, strong biofilm production was observed in (n = 36, 63.2%) isolates and was predominantly associated with XDR phenotypes (n = 30, 83.3%). No PDR strains were detected across any category. Although stronger biofilm formation tended to correspond with higher resistance levels, the association between biofilm intensity and antimicrobial resistance patterns was not statistically significant ($\chi^2 = 8.954$, $p = 0.176$).

***Table 6**: Correlation between Biofilm Related Genes, Biofilm Expression Categories and Antibiotics Categories*

Parameters		MDR N(%)	XDR N(%)	PDR N(%)	Sensitive	Total N(%)	χ^2	*p*-value
Biofilm related genes	*BfmS*	14(28%)	34(68%)	0(0.0%)	2(4%)	50(87.7%)	3.114	0.211
	OmpA	12(24.5%)	35(71.4%)	0(0.0%)	2(4.1%)	49(86%)	0.339	0.844
	cusE	14(25.5%)	39(70.9%)	0(0.0%)	2(3.6%)	55(96.5%)	0.8.09	0.667
	Bap	13(24.1%)	39(72.2%)	0(0.0%)	2(3.7%)	54(94.7%)	0.223	0.895
	espA	12(26.7%)	32(71.1%)	0(0.0%)	1(2.2%)	45(78.9%)	1.414	0.493
	blaPER1	2(18.2%)	9(81.8%)	0(0.0%)	0(0.0%)	11(19.3%)	0.889	0.641
Biofilm expression categories	None	1(50%)	1(50%)	0(0.0%)	0(0.0%)	2(3.5%)	8.954	0.176
	Weak	4(44.4%)	4(44.4%)	0(0.0%)	1(11.2%)	9(15.8%)		
	Moderate	3(30%)	6(60%)	0(0.0%)	1(10%)	10(17.5%)		
	Strong	6(16.7%)	30(83.3%)	0(0.0%)	0(0.0%)	36(63.2%)		

Discussion

The opportunistic pathogen *Acinetobacter baumannii* has emerged as a major cause of hospital-acquired infections, particularly among critically ill and immunocompromised patients (Nasr, 2020). In the present study*, A. baumannii* was detected in (n = 57, 11.1%) of 514 clinical specimens, a prevalence comparable to reports from Iraq, including (n = 52, 9.1%) in Kerbala (Mohammed *et al.,* 2022) and (n = 63, 14.0%) in Erbil (Abduljabar & Mawlood, 2023). These findings confirm the continued clinical importance of *A. baumannii* in Iraqi healthcare settings. The distribution of infections was nearly equal between males (n = 30, 51.7%) and females (n = 27, 48.3%), indicating no clear sex-related predisposition. However, elderly ICU patients represented the most vulnerable group (n = 13, 22.8%), supporting previous evidence that advanced age, prolonged hospitalization, invasive procedures, and extensive antibiotic exposure significantly increase the risk of infection (Appaneal*et al.,* 2021; Jiang *et al.,* 2022).

Regarding specimen sources, respiratory samples were the most common, with sputum accounting for (n = 17, 29.8%), followed by urine (n = 16, 28.1%), wound swabs (n = 12, 21.1%), and blood (n = 8, 14.0%). These findings are consistent with studies conducted in Erbil where sputum samples represented the predominant source of *A. baumannii* isolates (Smai & Ganjo, 2020). However, other Iraqi studies reported wound infections as the primary source (Mohammed et al., 2022), suggesting that variations in patient populations, hospital wards, and sampling strategies may influence the distribution of clinical isolates.

Clinically, pneumonia was the most frequently observed condition (n = 21, 36.8%), followed by burn infections (n = 10, 17.5%) and urinary tract infections (n = 8, 14.0%). Similar patterns have been reported in regional studies where respiratory infections represent the dominant manifestation of *A. baumannii* infections in hospitalized patients (Ababneh *et al.,* 2025). In contrast, studies from other regions such as Mexico have reported meningitis and ventilator-associated pneumonia as the most frequent clinical outcomes (Vázquez-López *et al.,* 2020). These variations highlight the influence of local epidemiological factors and hospital practices on infection patterns.

Biofilm formation was highly prevalent among the isolates in the present study. Strong biofilm production was detected in (n = 36, 63.2%) of isolates, while moderate and weak biofilm formation were observed in (n = 10, 17.5%) and (n = 9, 15.8%), respectively, and only (n = 2, 3.5%) isolates were non-biofilm producers. Similar findings were reported in Baghdad where strong biofilm production was identified in (n = 32, 52.5%) of isolates (Obaid, 2025). Conversely, studies from Nepal reported a lower proportion of strong biofilm producers (Bhandari *et al.,* 2025), indicating possible geographical differences in virulence characteristics. The ability of *A. baumannii* to form biofilms significantly contributes to its environmental persistence and resistance to antimicrobial agents (De Oliveira *et al.,* 2020).

Molecular analysis revealed a high prevalence of biofilm-associated genes, particularly *csuE* (n = 55, 96.5%), *bap* (n = 54, 94.7%), *bfmS* (n = 50, 87.7%), *ompA* (n = 49, 86.0%), and *espA* (n = 45, 78.9%).

These genes are widely recognized for their roles in bacterial adhesion, biofilm maturation, and persistence. Similar findings have been reported in international studies where *csuE* and *ompA* are among the most prevalent virulence determinants in biofilm-forming *A. baumannii* isolates (Silva *et al.,* 2021; Khoshnood *et al.,* 2023). The bap gene, which encodes a surface-associated protein involved in cell-to-cell adhesion, was also highly prevalent, supporting its critical role in biofilm structural stability (Mohamed *et al.,* 2023). In contrast, the *blaPER-1* gene was detected in a smaller proportion of isolates (n = 11, 19.3%), suggesting that while β-lactamase genes contribute to antimicrobial resistance, their association with biofilm formation may vary across populations (Bardbari *et al.,* 2017).

The antimicrobial susceptibility profile revealed extensive resistance to multiple antibiotic classes, including β-lactams (n = 47, 82.5%), carbapenems (n = 48, 84.2%), aminoglycosides (n = 51, 89.5%), and fluoroquinolones (n = 51, 89.5%). These findings align with global reports indicating the rapid emergence of multidrug-resistant *A. baumannii* in hospital environments (Panahi *et al.,* 2024). Most isolates were classified as extensively drug-resistant (XDR) (n = 41, 72.0%), while multidrug-resistant (MDR) isolates accounted for (n = 14, 25.0%), with no pan-drug-resistant isolates detected. Comparable resistance patterns have been reported in recent studies from the Middle East and Asia (Javadi *et al.,* 2025a; Javadi *et al.,* 2025b).

Despite the high resistance rates observed, colistin remained the most effective antimicrobial agent, with susceptibility detected in (n = 45, 78.9%) of isolates. Similar findings have been reported in previous studies conducted in Duhok, where resistance to colistin remained relatively low compared with other antibiotics (Alnakshabandie *et al.,* 2024). Nevertheless, global reports indicate a gradual increase in colistin-resistant strains, emphasizing the importance of continuous surveillance and antimicrobial stewardship (Novović & Jovčić, 2023).

Limitations

The present study identified a trend linking strong biofilm formation with higher antimicrobial resistance levels. Among strong biofilm producers, (n = 30, 83.3%) exhibited XDR phenotypes, suggesting that biofilm formation may enhance antimicrobial tolerance by limiting antibiotic penetration and facilitating bacterial persistence (Rajangam & Narasimhan, 2024). Although statistical analysis did not demonstrate a significant association between biofilm intensity and resistance patterns, the observed trend is consistent with previous studies indicating that biofilm-forming strains are more likely to exhibit MDR or XDR phenotypes (Eze *et al.,* 2021).

Future Scope

Future research should extend to multicenter studies across different regions of Iraq to monitor the evolving epidemiology of *A. baumannii*, focusing on variations in biofilm-associated genes and antimicrobial resistance patterns over time. Incorporating advanced molecular diagnostics and whole-genome sequencing into clinical microbiology laboratories is recommended to identify resistance determinants, virulence factors, and genetic linkages that contribute to biofilm development and antibiotic resistance.

Further studies should explore the expression dynamics of biofilm-related and resistance genes using transcriptomic approaches such as quantitative RT-PCR or RNA sequencing to clarify the relationship between gene presence and phenotypic expression. Investigations into innovative biofilm-disrupting strategies including quorum-sensing inhibitors, enzymatic agents, and nanoparticle-based formulations are needed to improve the effectiveness of existing antibiotic regimens and mitigate persistent infections.

Future work should examine *A. baumannii* isolates from both hospital environments and clinical sources to assess the role of environmental factors in biofilm formation, gene regulation, and resistance propagation within healthcare settings.

Conclusion

Overall, the findings of this study highlight the clinical threat posed by biofilm-forming, extensively drug-resistant *A. baumannii* in hospital environments. The high prevalence of virulence-associated genes combined with substantial antimicrobial resistance underscores the urgent need for enhanced infection-control strategies, antimicrobial stewardship programs, and the development of novel therapeutic approaches targeting biofilm-associated mechanisms. Continuous epidemiological monitoring is essential to limit the spread of this pathogen and reduce its impact on patient outcomes in healthcare settings.

Conflict of Interest

The authors have reported no conflicts of interest

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