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Synergistic Roles of Antibiotic Resistance, Biofilm Formation, and Virulence in *Pseudomonas aeruginosa* Clinical Isolates from Hospitalized Patients in Sulaymaniyah

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***Abstract* — *Pseudomonas aeruginosa* is a versatile opportunistic pathogen causing a wide range of hospital-acquired infections, particularly in immunocompromised patients. This study examined clinical specimens, antimicrobial resistance patterns, biofilm-forming capacity, and hemolysin production in isolates from hospitalized patients in Sulaymaniyah, Iraq (August 2024 - January 2025). A total of 85 isolates were recovered from 168 clinical specimens, including burn wound swabs (54.1%), endotracheal aspirates (21%), urine (14.1%), various body fluids (8.2%), and wounds (2.4%). Most isolates were obtained from male patients (56%), primarily aged 21–40 years, with a higher prevalence in general medical wards (70.6%) than intensive care units (29.4%). Identification and antimicrobial susceptibility testing were conducted using standard microbiological methods and the automated VITEK 2 system. Antimicrobial profiling showed high resistance rates to erythromycin (96.5%), ampicillin /sulbactam (95.3%), and sulfamethoxazole/ trimethoprim (77.6%), while colistin demonstrated the lowest resistance rate (29.4%). Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) profiles were detected in 72.9%, 18.8%, and 5.9% of isolates, respectively. Highlight the clinical threat posed by MDR *P. aeruginosa* and underline the need for routine monitoring of biofilm and resistance markers to guide infection control and therapy. Biofilm production was evaluated using the microtiter plate method. A high prevalence of biofilm formation was recorded (96.5%), including 42.4% moderate, 34.1% strong, and 20% weak biofilm producers. Hemolysin activity was tested on 5% sheep blood agar, with β-hemolysis detected in 50 isolates (58.8%), σ-hemolysis in 11 (12.9%), and γ-hemolysis in 24 (28.3%). Strong biofilm producers were more resistant to multiple β-lactams, fluoroquinolones, and sulfonamides. In addition, β-hemolysin production occurred more frequently among strong and moderate biofilm producers.**

***Keywords: Pseudomonas aeruginosa,* Multidrug-resistant bacteria (MDR), Biofilm formation, Hospital acquired infections, Infection control, hemolysin activity**

I. INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative opportunistic pathogen of major clinical relevance, particularly in hospital environments. Its significance stems from both intrinsic resistance mechanisms and a remarkable ability to acquire additional resistance genes. As a result, it is one of the principal causes of healthcare-associated infections (HAIs), including ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and surgical or wound infections (del Barrio-Tofiño et al., 2020). Its capacity to survive under diverse environmental conditions, combined with resistance to multiple antibiotic classes, has made it a central focus of antimicrobial resistance (AMR) research and surveillance (Reig et al., 2022, Ribeiro et al., 2019).

The global rise of AMR has further complicated the treatment of bacterial infections, leading to higher mortality rates, prolonged hospital stays, and increasing financial pressure on healthcare systems (Aslam et al., 2021). Among resistant pathogens, *P. aeruginosa* is particularly concerning because of

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its robust defense systems against a wide spectrum of antimicrobials. It predominantly affects immunocompromised patients, especially those in intensive care units (ICUs) or undergoing invasive procedures, where infections are often life-threatening (Pang et al., 2019). In addition to antimicrobial resistance, *P. aeruginosa* possesses numerous virulence factors that enhance pathogenicity. One of the most significant is Biofilm formation-structured microbial communities embedded in a self-produced extracellular polymeric substance (EPS) matrix. Biofilms are strongly associated with chronic and device-related infections, such as those seen in cystic fibrosis and with catheters and ventilators (Flemming et al., 2016). The biofilm matrix shields bacteria from desiccation and immune clearance while restricting antimicrobial penetration, making eradication particularly challenging (Høiby, 1993).

Biofilm-associated resistance arises from several mechanisms, including reduced antibiotic diffusion, metabolic dormancy of cells, activation of efflux pumps, and production of antibiotic-inactivating enzymes (Colvin et al., 2011, Flemming et al., 2016, Hall and Mah, 2017). The biofilm microenvironment also promotes horizontal gene transfer, accelerating the dissemination of resistance determinants (Madsen et al., 2012). Hemolysin activity represents another important component of *P. aeruginosa's* pathogenic strategy. Mediated primarily by phospholipase C and rhamnolipids, hemolysis damages erythrocyte membranes and releases hemoglobin, providing iron and other essential nutrients. This supports bacterial survival and acts synergistically with biofilm development and antimicrobial resistance. Nutrient release promotes bacterial growth within biofilms, while hemolysin-induced tissue damage facilitates colonization and persistence (Mackinder et al., 2024). Hemolytic activity should therefore be considered as part of a broader virulence framework that integrates resistance, nutrient acquisition, and biofilm-mediated tolerance. Taken together, these mechanisms—biofilm development, hemolysin activity, and multiple resistance strategies—explain the persistence and therapeutic challenges associated with *P. aeruginosa*.

Based on these considerations, it is hypothesized that strong biofilm producers would display higher levels of multidrug resistance and that hemolysin production would correlate positively with both biofilm formation and antimicrobial resistance. Therefore, this study aimed to characterize the antimicrobial resistance profiles, biofilm-forming capacities, and hemolytic activity of *P. aeruginosa* isolates collected from hospitalized patients in Sulaymaniyah Governorate, Iraq, to inform treatment strategies, strengthen infection control, and support efforts to combat multidrug-resistant and highly virulent strains in healthcare settings.

II. MATERIALS AND METHODS

Clinical Sample Collection

A total of 168 clinical specimens were collected from patients in both public and private hospitals across Sulaymaniyah governorate from August 2024 to January 2025. Samples were obtained from various sources, including wounds, burn sites, endotracheal aspirates, urine, snake bites, pleural fluid, peritoneal fluid, and bronchoalveolar lavage. Patients ranged in age from over 10 to above 70 years and were admitted to both general medical wards and ICUs.

Bacterial Isolation, Culture, and Identification

Isolates of *P. aeruginosa* were identified using standard microbiological procedures. Samples were inoculated onto nutrient agar, MacConkey agar, and cetrimide agar (Himedia, India) and incubated at 37°C for 24–48 hours to support optimal growth and pigment production. Gram staining was performed to examine cell morphology, and biochemical characteristics were assessed through oxidase and catalase tests. Phenotypic features, including biofilm formation, hemolysin production, and motility, were evaluated for each isolate. Final confirmation was achieved using the automated VITEK 2 Compact system (bioMérieux, France).

Antimicrobial Susceptibility Testing

Antimicrobial resistance profiles of *P. aeruginosa* isolates were assessed using the VITEK 2 Compact automated system (bioMérieux, France). A single pure colony from each isolate were suspended in sterile saline to prepare a bacterial suspension, adjusted to a 0.5 McFarland standard (approximately 1–2 × 10^8 CFU/mL). The standardized suspension was inoculated into VITEK 2 AST cards containing a predefined panel of antibiotics. Bacterial growth was monitored through colorimetric changes at 37°C. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines.

The antibiotic panel included: Ampicillin/ Sulbactam (AMP/SUL), Piperacillin/Tazobactam (PIP/TAZ), Cefotaxime (CTX), Ceftazidime (CAZ), Cefepime (FEP), Imipenem (IPM), Meropenem (MEM), Amikacin (AMK), Gentamicin (GEN), Ciprofloxacin (CIP), Colistin (COL), Sulfamethoxazole/Trimethoprim (SXT), Ceftriaxone (CRO), Erythromycin (ERY), and Levofloxacin (LEV). The minimum inhibitory concentration (MIC) for each antibiotic was determined, and isolates were classified as susceptible, intermediate, or resistant (CLSI, 2020). The VITEK 2 system provides rapid and reliable results, supporting timely clinical decision-making. (Khurana et al., 2020, Pincus, 2006, Ling et al., 2001).

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Phenotypic Characterization: Motility, Biofilm Formation, and Hemolysin Production

Phenotypic characterization of 85 clinical *P. aeruginosa* isolates included motility tests, biofilm formation, and hemolysin production. Motility was assessed using the semisolid agar tube method (0.4% agar), whereby isolates were inoculated via s tab technique and incubated at 37°C for 24_48 hours. Diffuse growth radiating from the stab line indicated motility (Shields and Cathcart, 2011, Aygan and Arikan, 2007, Adler and Dahl, 1967).

Biofilm formation was evaluated using the 96 well microtiter plate assay. Isolates were cultured in tryptic soy broth (TSB) supplemented with 1% glucose and incubated at 37°C for 24 hours. Wells were then stained with 0.1% crystal violet, and adherent dye was solubilized with ethanol (Coffey and Anderson, 2014a, O'Toole, 2011). The optical density was measured at 570 nm (OD_{570}), and the biofilm index (BI) was calculated using the following formula:

BI = OD 570 (sample) - OD 570 (blank) / /OD 570 (control) (Coffey and Anderson, 2014b).

Hemolysin activity was tested on 5% sheep blood agar. After 24 hours at 37°C, hemolysis was categorized as β-hemolysis (complete, clear zone), α-hemolysis (partial, greenish zone), or γ-hemolysis (none, no zone) (Henkelman et al., 2009). Delta-type synergistic hemolysis was not observed.

Data Analysis

Data were analyzed using SPSS version 26 (IBM, Armonk, NY, USA). Associations among categorical variables, including biofilm formation, hemolysin production, and antimicrobial resistance, were examined using the Chi-square test (χ^2). For cases where expected cell counts were low, Fisher's exact test was applied. Differences in biofilm optical density (OD) across groups were assessed using one-way analysis of variance (ANOVA), followed by post hoc comparisons when appropriate. A p-value of less than 0.05 was considered statistically significant. The analysis focused on evaluating the relationships between virulence factors and antimicrobial resistance in *P. aeruginosa* isolates

III. RESULT

Patients and Bacterial Isolates

In this study, *P. aeruginosa* was recovered from 85 of 168 clinical specimens. The patient cohort ranged in age from over 10 to above 70 years and included 48 males (56%) and 37 females (44%). The largest proportion of isolates originated from the 21–40-year age group (29.4%), followed by 41–60 years (24.7%) and patients under 10 years (22.4%) (Table 1).

The majority of *P. aeruginosa* isolates were obtained from burn wound swabs (54.1%), followed by endotracheal aspirates (ETA) (21.2%) and urine samples (14.1%). Fewer isolates were recovered from body fluids (3.5%), pleural fluid (3.5%), bronchoalveolar lavage (BAL) fluid (1.2%), snake bite sites (1.2%), and purulent discharges (1.2%). Isolates were more commonly obtained from patients in general medical wards (60; 70.6%) than from ICUs (25; 29.4%) (Table 2).

Table 1: Demographic characteristics of patients with *P. aeruginosa* isolated (N = 85)

Variable	Category	Frequency N (%)	X^2 Values	P Values
Sex	Male	48 (56.5)	1.42	0.23 (N.S)
	Female	37 (43.5)		
Age group (years)	≤10	19 (22.3)	25.3	P<0.0001
	11–20	8 (9.4)		
	21–40	25 (29.4)		
	41–60	21 (24.7)		
	61–70	6 (7.1)		
	>70	6 (7.1)		
Hospital location	Ward	60 (70.6)	14.4	P<0.0001
	ICU	25 (29.4)		

Chi-square analysis revealed no significant difference by gender (χ^2 = 1.42, p = 0.23). However, age (χ^2 = 25.3, p < 0.0001) and hospital location (χ^2 = 14.4, p < 0.0001) showed significant associations, with the highest prevalence among adults (21–60 years) and ward patients

Table 2: Distribution of *P. aeruginosa* isolates by clinical specimen type (N = 85)

Specimen Type	Frequency N (%)	Ward N (%)	ICU N (%)
Burn wounds	46 (54.1)	45 (75.5)	1 (4.5)
Endotracheal aspirates	18 (21.2)	0 (0.0)	18 (72.0)
Urine	12 (14.1)	9 (15.0)	3 (12.0)
Pleural fluid	3 (3.5)	1 (1.7)	2 (8.0)
Peritoneal fluid	3 (3.5)	2 (3.3)	1 (4.0)
BAL fluid	1 (1.2)	0 (0.0)	1 (4.0)
Wound discharge	1 (1.2)	1 (1.7)	0 (0.0)
Surgical bed wound swab	1 (1.2)	1 (1.7)	0 (0.0)
Overall test result (χ^2=63.52, p<0.000001)			

Chi-square analysis revealed no significant difference by gender (χ^2 = 1.42, p = 0.23). However, age (χ^2 = 25.3, p < 0.0001) and hospital location (χ^2 = 14.4, p < 0.0001) showed significant associations, with the highest prevalence among adults (21–60 years) and ward patients

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Biochemical Identification

All *P. aeruginosa* isolates exhibited typical phenotypic characteristics: Gram-negative, catalase-positive, oxidase-positive, and motile.

Antimicrobial Resistance Profile (MDR, XDR, and PDR)

Table 3 presents the resistance rate of *P. aeruginosa* isolates (n = 85) against 15 antimicrobial agents. The highest resistance was observed for erythromycin (96.5%) and ampicillin/sulbactam (95.3%), followed by sulfamethoxazole/ trimethoprim (77.6%) and cefotaxime (63.5%). Moderate resistance was detected for ciprofloxacin (60%), ceftazidime (51.8%), amikacin (49.4%) and gentamicin (47.1%). Resistance to carbapenems was also notable, with imipenem at 41.2% and meropenem at 37.7%. Lower resistance rates were recorded for piperacillin/tazobactam (30.6%), levofloxacin (38.8%), and colistin (29.4%). These results highlight a concerning multidrug-resistant pattern and reduced efficacy of several commonly used antibiotics.

Table 3: presents the resistance rate of *P. aeruginosa* isolates (n = 85)

Antimicrobial Class	Antimicrobial Agent	Resistance (%)	Intermediate (%)	Sensitive (%)
Macrolides	Erythromy-cin	96.5	2.3	1.2
Beta -lactamase inhibitor	Ampicillin/S ulbactam	95.3	2.4	2.3
	Piperacillin/ta zobactam	30.6	14.2	55.2
Folate pathway inhibitors	Sulfamethoxa zole/tri-methoprim	77.6	11.8	10.6
3rd generation Cephalosporins	Cefotaxime	63.5	23.5	13
	Ceftriaxone	55.3	34.1	10.6
	Ceftazidime	51.8	10.6	37.6
	Cefepime	45.9	10.6	43.5
Fluroquinolones	Ciprofloxacin	**60**	7	33
	Levofloxacin	38.8	5.9	55.3
Aminoglycosides	Amikacin	49.4	14.1	36.5
	Gentamicin	47.1	9.4	43.5
Carbapenems	Imipenem,	41.2	11.8	47
	Meropenem	37.7	12.9	49.4
Polymyxins	Colistin	29.4	42.4	28.2

Chi-square test (χ^2 = 63.52, p < 0.000001) showed a highly significant distribution across specimen types, with burn wounds (54.1%) as the predominant source. Notable frequencies in endotracheal aspirates (21.2%) and urine (14.1%) indicate respiratory and urinary tract involvement.

As shown in Table 4, most isolates (72.9%) were multidrug-resistant (MDR), while 18.8% were extensively drug-resistant (XDR) and 5.9% were pan drug-resistant (PDR). These resistance patterns indicate a substantial burden, including reduced susceptibility to critical and last-resort antibiotics.

Table 4: Antimicrobial Resistance Patterns (MDR, XDR, and PDR) among Clinical *P. aeruginosa* Isolates (N = 85).

Resistance Type	Number of Antibiotic Classes	No. of Isolates (N/85)	Percentage (%)
MDR (≥3 classes)	3 to 6 (most common = 4-6)	62	72.9
XDR (non-susceptb- le to all but ≤2 classes)	7 classes	16	18.8
PDR (non-suscptibl-e to all classes)	8 classes	5	5.9

*Chi-square (χ^2): 66.10 df: 2 p-value: < 0.00001

Chi-square test (χ^2 = 66.10, df = 2, p < 0.00001) showed a highly significant difference in resistance patterns. The majority of isolates were multidrug-resistant (MDR, 72.9%), while 18.8% were extensively drug-resistant (XDR) and 5.9% were pan-drug resistant (PDR).

These results highlight the predominance of MDR strains, with smaller but clinically critical proportions of XDR and PDR isolates that pose serious therapeutic challenges.

Biofilm Formation and Hemolytic Activity

Biofilm formation was widespread, which is detected in 96.5% of isolates. Strong or moderate biofilm formation accounted for 76.5% (strong = 34.1%, moderate = 42.4%), while 20% were weak producers and 3.5% were non-producers (Figure 1, Table5).

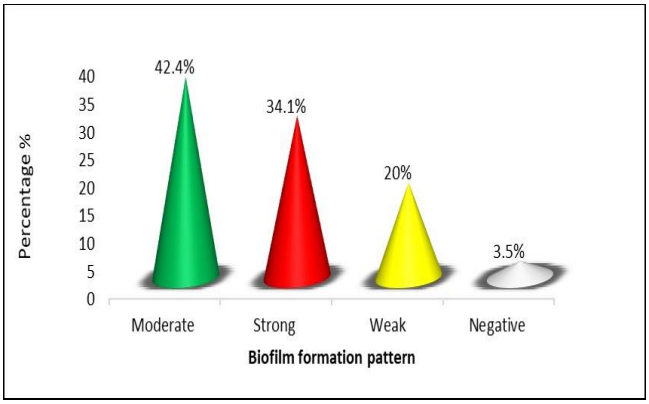

Figure 1: A total of 85 clinical isolates were assessed for their biofilm-forming abilities.

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Table 5: Mean OD values of biofilm categories in *P. aeruginosa* isolates with ANOVA results ($p < 0.001$).

Biofilm Pattern	Range of OD	Mean OD (±SD)	N (%)
Strong	0.802–<3.50	2.11 ± 0.62	29 (34.1)
Moderate	0.401–0.802	0.58 ± 0.12	36 (42.4)
Weak	0.200–0.418	0.29 ± 0.06	17 (20.0)
Negative	0.179–0.204	0.19 ± 0.01	3 (3.5)

ANOVA (F-test) result: There was a statistically significant difference in mean OD values across the four biofilm categories ($p < 0.001$), confirming that the classification system reliably distinguishes between strong, moderate, weak, and negative biofilm producers. Post-hoc comparisons (Tukey's test) showed significant differences between each group ($p < 0.05$).

Hemolytic activity differed among the isolates, with β-hemolysis present in 50 isolates (58.8%), σ-hemolysis in 11 (12.9%), and γ-hemolysis in 24 (28.3%) (Figure 2).

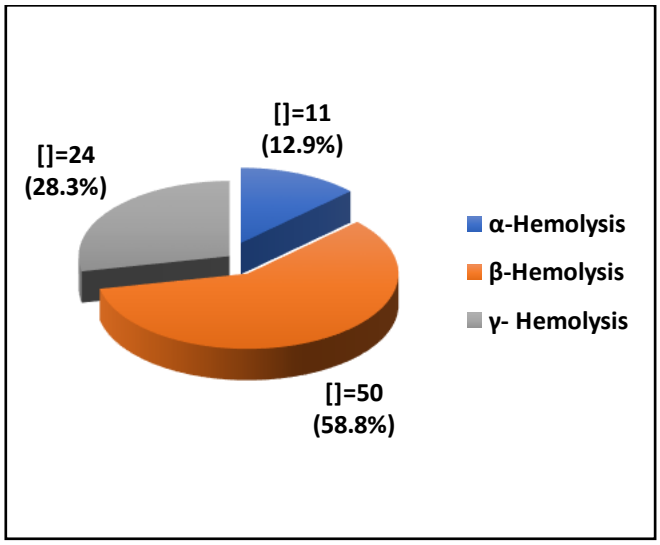

Figure 2:. Distribution of Hemolysin Types in *P. aeruginosa* Isolates. The hemolytic activity of 85 *P. aeruginosa* isolates is shown. *β*-Hemolysis was the most common, occurring in 50 isolates (58.8%), reflecting substantial cytolytic activity. γ-Hemolysis was observed in 24 isolates (28.3%), indicating no visible red blood cell lysis. α-Hemolysis was the least frequent, detected in 11 isolates (12.9%), characterized by partial or greenish hemolysis.

The relationship between biofilm formation and its strength was analyzed by categorizing isolates into strong (n = 29), moderate (n = 36), weak (n = 17), and negative (n = 3) groups. Antibiotic resistance patterns were examined within each biofilm category. Notably, higher resistance rates were observed among strong and moderate biofilm producers, particularly for ampicillin/sulbactam, sulfamethoxazole/trimethoprim, and ciprofloxacin, whereas colistin demonstrated lower resistance across all groups (Table 6). Hemolysin production was also assessed in relation to biofilm formation. β-Hemolysis was the most common (n = 50), followed by γ-hemolysis (n = 24) and α-hemolysis (n = 11). Strong and moderate biofilm producers were more frequently associated with β-hemolytic activity, suggesting a potential link between biofilm-forming capacity and virulence expression (Table 6).

Table 6: Biofilm formation in relation to antimicrobial resistance and hemolytic activity (n = 85) antimicrobial resistance was categorized by biofilm.

Antimicrobial Classes	Biofilm formation patterns according to resistance of isolates N				χ^2 Value	P-value
	Strong N= 29	Moderate =36	Weak N=17	Negative N=3		
Ampicillin/ Sulbactam	27	11	15	3	8.74	0.003*
Ciprofloxacin	20	21	9	1	6.82	0.009*
Ceftriaxone	20	18	8	1	5.97	0.015*
Sulfamethoxazole/ trimethoprim	19	31	14	2	7.54	0.006*
Erythromycin	12	35	16	0	4.92	0.027*
Cefotaxime	17	20	14	3	5.45	0.020*
Ceftazidime	17	17	9	1	3.22	0.072
Cefepime	15	13	10	1	2.95	0.086
Amikacin	15	15	10	2	2.40	0.121
Gentamicin	15	15	8	2	2.75	0.098
Imipenem	13	14	7	1	3.81	0.051
Levofloxacin	12	13	7	1	2.11	0.146
Colistin	12	5	7	1	0.72	0.397
Meropenem	11	13	7	1	1.85	0.173
Biofilm formation patterns according to hemolytic activity. No						
β-Hemolysis =50	13	25	10	2	7.85	0.020*
γ-Hemolysis =24	11	6	6	1	6.43	0.040 *
α-Hemolysis =11	5	5	1	0	4.92	0.027*

* = Significance. Strong and moderate biofilm-producing *P. aeruginosa* isolates showed significantly higher resistance to β-lactams, fluoroquinolones (ciprofloxacin $\chi^2 = 6.82$, levofloxacin $\chi^2 = 6.15$), and co-trimoxazole ($\chi^2 = 7.54$, $p < 0.05$), while colistin remained effective. Strong biofilm formation was also significantly associated with β-hemolysis ($\chi^2 = 7.85$, $p < 0.05$), highlighting the link between virulence and biofilm-mediated pathogenicity.

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IV. DISCUSSION

This study investigated the distribution, antimicrobial resistance, and virulence traits of *P. aeruginosa* isolates from hospitalized patients in Sulaymaniyah Governorate. Most isolates were recovered from male patients (56.5%) and were most prevalent in the 21–60-year age group (29.4%). Burn wounds accounted for more than half of all isolates (54.1%), followed by endotracheal aspirates (21.2%) and urine samples (14.1%). Although no significant difference was observed between male and female patients ($p = 0.23$), significant associations were found for age groups and hospital wards (both $p < 0.0001$). These results are consistent with previous Iraqi studies from Sulaymaniyah and Najaf reporting a strong link between *P. aeruginosa* infections, burn injuries, and ICU stays (AL-Ameen and Ghareeb, 2022, Jubair and Alkhudhairy, 2024). All isolates exhibited the expected biochemical characteristics of *P. aeruginosa* and were accurately identified using the automated VITEK 2 Compact system, confirming its reliability for routine hospital diagnostics.

Antimicrobial resistance was extensive and highly significant across antibiotic classes ($p < 0.00001$). The highest resistance was observed against erythromycin (96.5%) and ampicillin/sulbactam (95.3%), followed by sulfamethoxazole/trimethoprim (77.6%). Cephalosporin resistance varied, with cefotaxime (63.5%) showing the highest and cefepime (45.9%) the lowest resistance. Fluoroquinolone resistance was high for ciprofloxacin (60%) but lower for levofloxacin (38.8%). Carbapenem resistance was substantial—imipenem (41.2%) and meropenem (37.7%)—while aminoglycosides showed moderate resistance (amikacin 49.4%, gentamicin 47.1%). Colistin remained the most effective agent (resistance 29.4%), highlighting its continued role as a last-resort therapy. However, the emergence of colistin-resistant isolates emphasizes the importance of judicious antibiotic use and antimicrobial stewardship programs. These findings are consistent with previous reports from Baghdad and other regions in Iraq (Al-Byti et al., 2020, AL-Rubaye et al., 2020); (Ali et al., 2017, Seenaa et al., 2024); (Ali et al., 2017, Seenaa et al., 2024). MDR strains were the most prevalent (72.9%), while smaller but clinically important proportions of XDR (18.8%) and PDR (5.9%) isolates illustrate significant therapeutic challenges. These results reflect the global rise in antimicrobial resistance and its impact on clinical care. The proportions observed in this study are higher than those reported in earlier investigations Compared with earlier Iraqi reports (Saderi and Owlia, 2015), the proportions here suggest an alarming increase in multidrug resistance, especially in critical care settings.

Analysis of virulence factors revealed that nearly all isolates (96.5%) produced biofilms, most of them strong or moderate. Biofilm formation correlated significantly with antibiotic resistance ($p < 0.05$), particularly for ampicillin/sulbactam, ciprofloxacin, ceftriaxone, and co-trimoxazole. Similar associations between biofilm and drug resistance have been reported in Ethiopia, Egypt, and Iran (Olana et al., 2024, Akrami et al., 2024). Hemolytic activity was also prevalent, with β-hemolysis predominating (58.8%), followed by γ- and α-hemolysis. Strong and moderate biofilm producers were more frequently associated with β-hemolytic activity, suggesting that biofilm formation and virulence expression may act synergistically to enhance pathogenic potential. Such combined resistance and virulence likely contribute to treatment failures in burn and ICU patients, where isolates are both highly resistant and virulent. Statistical analysis confirmed a significant relationship between biofilm strength and hemolysin production ($p < 0.05$), supporting observations from Najaf (Jubair and Alkhudhairy, 2024). The combination of strong biofilm formation, hemolytic activity, and high resistance profiles represents a major therapeutic challenge. Experimental approaches, such as surfactant-based therapies and nanoparticle-assisted photodynamic therapy, have shown potential for disrupting biofilms and restoring antibiotic efficacy (Salem et al., 2025, Shiralizadeh et al., 2024). These findings underscore *P. aeruginosa* as a critical public health threat in Sulaymaniyah, where the dual burden of resistance and virulence contributes to increased morbidity and mortality, particularly among burn and ICU patients.

The study highlights the urgent need for effective antimicrobial stewardship, enhanced infection control, and continuous surveillance to monitor emerging resistance patterns. It also underscores the importance of exploring novel treatment strategies, including anti-biofilm agents and alternative therapies, to reduce the clinical and economic impact of *P. aeruginosa* infections in Iraq and similar healthcare settings.

CONCLUSION

Multidrug-resistant *P. aeruginosa* with robust biofilm formation and virulence factor expression poses a serious threat to hospitalized patients in Iraq. Biofilm creation, hemolysin synthesis, and antibiotic resistance act synergistically to increase persistence and complicate therapy. Regular surveillance, effective infection control, and antimicrobial stewardship are critical for reducing morbidity and mortality. Future research should combine molecular analysis with novel therapeutic strategies to successfully address resistant and virulent bacteria. The findings support integrating biofilm disruption, virulence inhibition, and antibiotic stewardship to reduce the burden of *P. aeruginosa* infections in hospitals. Future research should investigate novel anti-biofilm agents, phage therapy, and targeted virulence inhibitors as complements to conventional antibiotic treatment. This study relied on phenotypic assays; molecular characterization of

resistance genes and biofilm-associated virulence factors could provide deeper mechanistic insights. Additionally, data were limited to Sulaymaniyah; broader, multi-center surveillance is recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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