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Microbial and Molecular Study of the Pseudomonas aeruginosa Virulence Factors in urinary tract infection Iraqi patients

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Abstract

This study aimed to investigate the molecular distribution of virulence factors and disinfectant resistance genes in Pseudomonas aeruginosa isolates from urinary tract infections (UTIs) in Iraq, focusing on their association with biofilm formation and antibiotic resistance. A total of 129 clinical isolates were collected from Samara Government General Hospital (94% female, 6% male). Phenotypic biofilm formation was assessed using Congo Red Agar (CRA) and microtitration plate methods, revealing 67.44% and 93.02% biofilm producers, respectively. Molecular characterization via PCR highlighted the high prevalence of significant virulence factors, including biofilm-related genes (pslA: 80.6%, pelA: 75.2%, ppyR: 69.0%) and Type-3 Secretion System (T3SS) genes (exoT: 81.4%, exoS: 86.82%, lasB: 85.3%). Disinfectant resistance genes (QacE: 66.67%, QacEΔ1: 60.47%) were also detected. Antibiotic resistance was significantly higher in biofilm-forming isolates (e.g., cefotaxime: 65.0% vs. 26.7% in non-biofilm isolates, $p = 0.001$), with strong biofilm producers exhibiting greater multidrug resistance (55.6% vs. 29.4%, $p = 0.02$). The study concludes that P. aeruginosa isolates from UTIs in Iraq carry a high burden of virulence factors and resistance genes, with biofilm formation playing a critical role in antibiotic resistance. These findings emphasize the need for targeted therapies and infection control strategies to mitigate the impact of these highly virulent and resistant strains.**Keywords:** Pseudomonas aeruginosa, virulence factors, biofilm formation, antibiotic resistance, urinary tract infection, molecular characterization

1. Introduction

Urinary tract infections (UTIs) are among the hospital-acquired infections caused by the opportunistic pathogen Pseudomonas aeruginosa, a gram-negative, motile, oxidase-positive, obligately aerobic bacillus (Kline, 2016). Its diverse virulence factors and inherent resistance to multiple drugs contribute to severe infections associated with high morbidity and mortality (Newman, 2022). Patients with indwelling medical devices or obstructed urine flow are more prone to P. aeruginosa-induced UTIs, as such conditions support bacterial colonization and biofilm formation (Mishra, 2024). Biofilm formation plays a central role in P. aeruginosa virulence. Bacteria embed themselves within a self-produced exopolysaccharide matrix that enhances persistence by protecting them from antibiotics and the host immune system. Biofilms aid in surface adherence, thereby promoting chronic device-associated infections (Sharma, 2023). Key genes regulating biofilm formation include pslA, pelA, and ppyR, which are responsible for synthesizing polysaccharides crucial to biofilm structure and development (Colvin, 2012). Understanding these molecular mechanisms is essential for designing effective therapies against persistent infections.Another critical virulence mechanism is the Type III Secretion System (T3SS), a molecular apparatus that injects effector proteins into host cells (Coburn, 2007). T3SS-associated genes—exoS, exoT, exoU, and exoY—disrupt host cellular functions and contribute to bacterial survival and dissemination. While exoS and exoT interfere with cytoskeletal functions, exoU encodes a cytotoxic phospholipase. These factors, involved in tissue damage and immune evasion, also interact with biofilm pathways, contributing to the pathogen's virulence in bloodstream and other serious infections (Horna Quintana, 2021).

2.Methodology

This study was conducted at Samara Government General Hospital, Iraq, from February 2023 to December 2024. A total of 129 Pseudomonas aeruginosa isolates were collected from UTI patients, including 6% males (n=8) and 94% females (n=121), reflecting the common gender distribution in UTIs. Standard microbiological and biochemical techniques were used for isolation and identification.Biofilm formation was assessed

phenotypically using Congo Red Agar (CRA) for qualitative screening and the microtitration plate assay for quantitative evaluation. In the latter, crystal violet-stained biofilms were measured at 570 nm using a BioTek ELx800 microplate reader after 24-hour incubation at 37°C. This allowed classification of isolates based on optical density values. Molecular characterization was carried out using PCR to detect three groups of genes: biofilm-associated genes (pslA, pelA, ppyR), Type III Secretion System (T3SS) effector genes (exoS, exoT, exoU, exoY), and disinfectant resistance genes (QacE, QacEΔ1). DNA was extracted using commercial kits

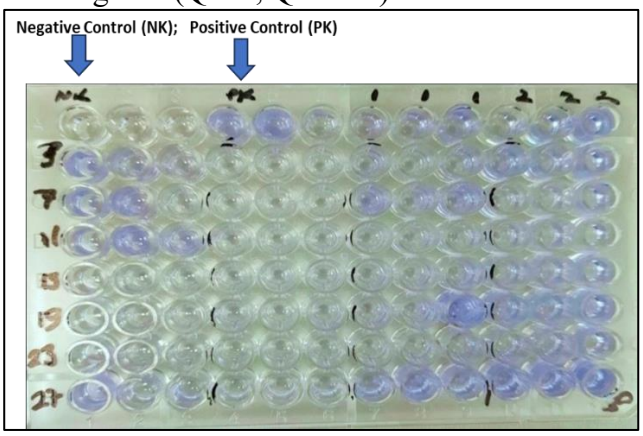

Figure-1. Evaluation of biofilm formation by microtitration plate method

.١،١Evaluation of Amplification Results of Selected Genes The PCR amplification of selected virulence and disinfectant resistance genes in P. aeruginosa isolates yielded clear and specific bands corresponding to the expected sizes for each target gene, confirming their presence in the tested strains. Genes such as exoS (450 bp), exoT (450 bp), pslA (650 bp), pelA (350 bp), and ppyR (400 bp) were successfully amplified across a substantial proportion of isolates, indicating the widespread distribution of biofilm-related and type-3 secretion system (T3SS) virulence determinants. Similarly, disinfectant resistance genes qacE (180 bp) and qacEΔ1 (240 bp) (Table 1) were detected in multiple isolates, suggesting

Table 1: Primers Used in This Study

.٢،٢Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM Corp., Armonk, NY, USA) .

.٢Results and discussion

٣،١Distribution of Virulence Genes in P. aeruginosa

Of the 129 P. aeruginosa isolates from urine samples, 8 (6%) were from males and 121 (94%) from females. PCR-based molecular screening revealed high prevalence of virulence genes grouped into toxin-related, biofilm-associated, and resistance determinants.Toxin genes exoS (86.82%), exoT (82.95%), lasB (78.29%), and toxA (73.64%) were predominant, underscoring the importance of exotoxins and the Type III secretion system in tissue damage during UTIs. Similarly, biofilm-related genes pslA (75.97%), pelA (70.54%), and ppyR (68.99%) were frequently detected, highlighting their role in exopolysaccharide matrix formation and adhesion to urinary catheters.Resistance genes QacE (66.67%) and QacEΔ1(٦٠،٤٧٪)

Table 2: Distribution of Virulence Genes in P. aeruginosa Isolates (n = 129)
*p < 0.05, **p < 0.01, ***p < 0.001

3.2 Distribution of Biofilm Formation Among P. Aeruginosa Isolates on Congo Red Agar (CRA) Medium

In this study, biofilm formation was phenotypically assessed using Congo Red Agar (CRA), a qualitative method that

Gene Name	Primer Direction	Primer Sequence (5' → 3')	Resulting Base Pair (bp)
qacE	Forward	AGCGTTGCTGATGCTGGA	180
	Reverse	CCGTTCAGCGTAGGATGTAG	
qacEΔ1	Forward	GATGAGCGTTGCTGATGCTG	240
	Reverse	TTGCCGATCAGCGTAGGATG	
exoS	Forward	ATGGCCTTCCAGTCCGCGAC	450
	Reverse	TTTGCCAGGACGCGGTTG	
exoT	Forward	CGGGAAGAGTTTGACGAGC	450
	Reverse	CGTTGAGGAGGCGGTAGAG	
pslA	Forward	CAGGAGGAGAGCGAGGATGA	650
	Reverse	TTGCGTGTAGCGTGGAAGAG	
pelA	Forward	ATCGTGGTGCACAGGAGAGT	350
	Reverse	TCGGCGTCGATGAAGAGTTA	
ppyR	Forward	GACGATCGTGGAAGCGATGA	400
	Reverse	TGCGTAGCTGTTGGTGGTAG	
lasB	Forward	CGTCTGGAAGGATGCGTGAC	500
	Reverse	TGGCACACCATCTTCTTGTC	
toxA	Forward	GTGCGTGAACTTCCTCGGAT	600
	Reverse	CTGCGTTCGTTGGTCTTGAG	

differentiates biofilm-producing strains by their characteristic colony morphology and dye binding. Out of the 129 P. aeruginosa isolates tested, biofilm formation was observed in 87 isolates (67.44%), which produced black, dry, crystalline colonies indicative of positive biofilm production on CRA medium. The remaining 42 isolates (32.56%) showed red, smooth colonies, classifying them as biofilm-negative. The distribution of biofilm-positive and biofilm-negative isolates stratified by gender was as follows (Figure 2) :

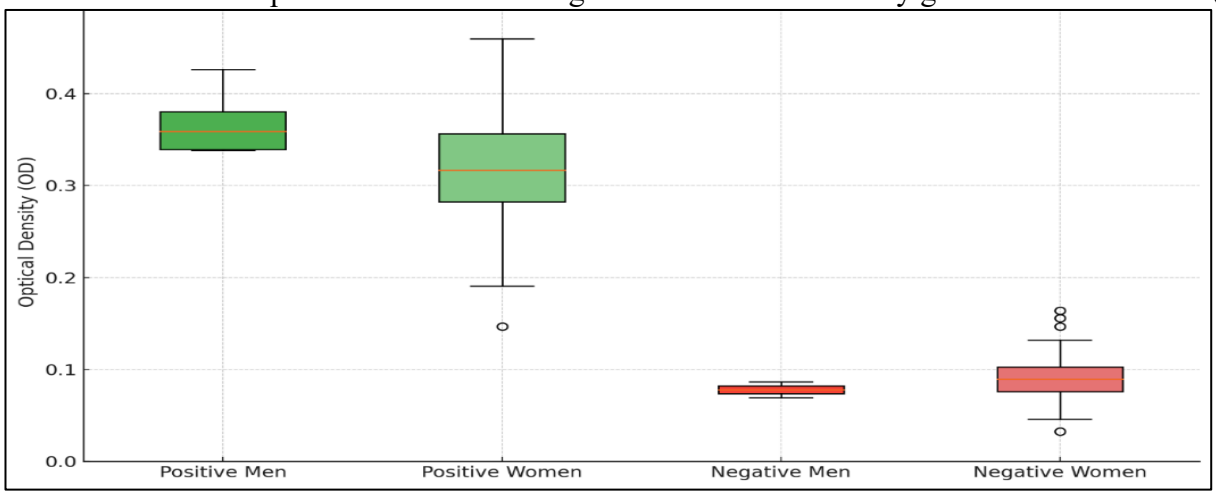

Figure 2: Biofilm Production Status Among Male and Female UTI Patients Statistical analysis using the Chi-square test showed no significant difference in biofilm formation prevalence between male and female isolates ($p > 0.05$).These phenotypic biofilm results correlated well with the high prevalence of biofilm-associated genes (pslA, pelA, and ppyR) detected by PCR, supporting the role of these genes in biofilm production and persistence of P. aeruginosa in urinary tract infections.

٣،٣Distribution of P. aeruginosa Strains by Clinic and Severity of Biofilm Formation

Clinical isolates of P. aeruginosa were collected from various clinics within Samara Government General Hospital during the study period. These isolates' level of biofilm development was assessed using the microtitration plate method, which enabled them to be categorized as strong, moderate, weak, or non-biofilm

producers. The ability of P. aeruginosa isolates to form biofilms is a generally virulent trait, regardless of the clinic source, according to statistical analysis using the Chi-square test

٣،٤Comparison of Congo Red Agar and Microtitration Plate Methods for Biofilm Detection

Biofilm formation by P. aeruginosa isolates was assessed using two standard methods: the qualitative Congo Red Agar (CRA) and the quantitative Microtitration Plate (MTP) assay. The CRA method identified 67.44% (87/129) of isolates as biofilm producers based on black colony formation, while 32.56% (42/129) appeared red and non-producers. Though cost-effective for initial screening, CRA showed limited sensitivity for weak producers. In contrast, the MTP method detected 93.02% (120/129) of isolates, offering a broader detection range. While both methods showed 97.7% concordance for strong producers, MTP identified 83.3% of CRA-negative weak producers. CRA's sensitivity and specificity were 70.8% and 85.4%, respectively, with moderate agreement ($\kappa = 0.62$, $p < 0.01$). A combined approach—CRA screening followed by MTP confirmation—may enhance accuracy while conserving resources (Harika ,2020).

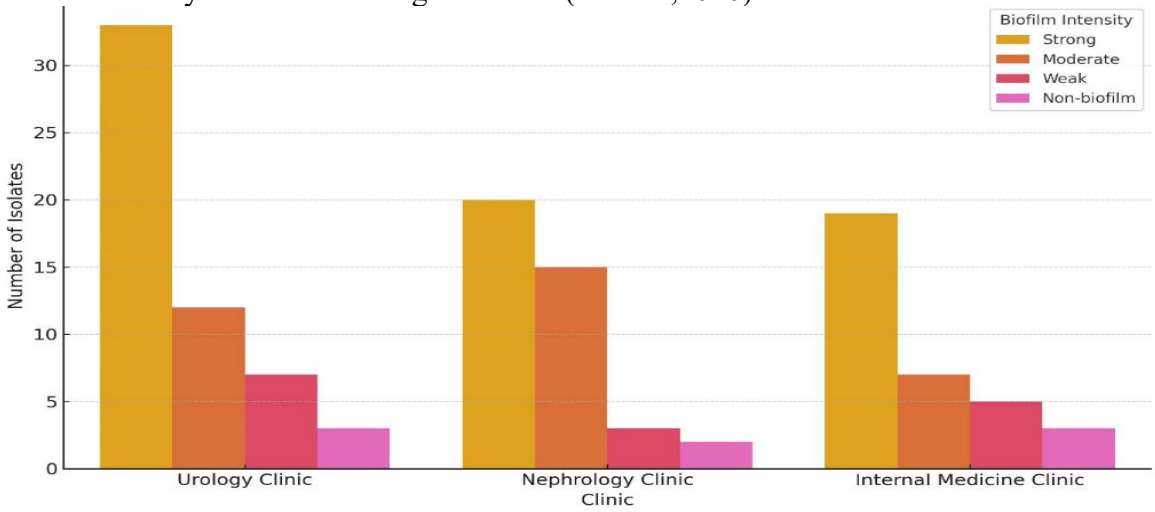

Figure 3: Biofilm Formation Intensity of P. aeruginosa Isolates Across Hospital Clinics

٣،٥Relationship Between Antibiotic Resistance and Biofilm Formation

The study evaluated the antibiotic resistance profiles of P. aeruginosa isolates against nine commonly used antibiotics and analyzed their association with biofilm formation. The antibiotics tested included Cefotaxime, Ceftazidime, Cefepime, Piperacillin/Tazobactam, Ciprofloxacin, Amikacin, Meropenem, Imipenem, and Levofloxacin. Resistance rates were compared between biofilm-producing and non-biofilm-producing isolates using the microtitration plate method (Harika ,2020).

Table 3: Antibiotic Resistance in Biofilm-Forming vs. Non-Biofilm-Forming Isolates

Antibiotic	Resistant Isolates n (%)	Biofilm-Positive Resistant n (%)	Biofilm-Negative Resistant n (%)	p-value
Cefotaxime	78 (60.5%)	70 (65.0%)	8 (26.7%)	0.001*
Ceftazidime	64 (49.6%)	58 (53.7%)	6 (20.0%)	0.002*
Cefepime	59 (45.7%)	54 (50.0%)	5 (16.7%)	0.003*
Piperacillin/Tazobactam	52 (40.3%)	48 (44.4%)	4 (13.3%)	0.005*
Ciprofloxacin	45 (34.9%)	42 (38.9%)	3 (10.0%)	0.007*
Amikacin	36 (27.9%)	33 (30.6%)	3 (10.0%)	0.043*
Meropenem	28 (21.7%)	26 (24.1%)	2 (6.7%)	0.042*
Imipenem	26 (20.2%)	24 (22.2%)	2 (6.7%)	0.048*
Levofloxacin	22 (17.1%)	20 (18.5%)	2 (6.7%)	0.123

*Significant at $p < 0.05$

The data demonstrate a clear association between biofilm formation and increased antibiotic resistance in P. aeruginosa strains isolated from urinary tract infections. Biofilm-positive isolates exhibited significantly higher resistance rates against beta-lactams (Cefotaxime, Ceftazidime, Cefepime, Piperacillin/Tazobactam), aminoglycosides (Amikacin), carbapenems (Meropenem, Imipenem), and fluoroquinolones (Ciprofloxacin), except Levofloxacin. This enhanced resistance in biofilm producers is likely due to the protective nature of the biofilm matrix, which impedes antibiotic penetration and facilitates bacterial persistence. Biofilms also promote horizontal gene transfer and upregulate resistance genes, further complicating treatment. These findings

underscore the clinical challenge posed by biofilm-forming P. aeruginosa strains, which not only resist antibiotics but also sustain chronic infections in the urinary tract. Effective management of such infections requires strategies that disrupt biofilms alongside appropriate antibiotic therapy.

٣,٦Evaluation of Molecular Results of Biofilm-Related Genes and Type III Secretion System Genes

In this study, molecular detection of key biofilm-associated (pslA, pelA, ppyR) and Type III Secretion System (T3SS) genes (exoS, exoT, lasB, toxA) was performed on 129 P. aeruginosa isolates from urinary tract infections in Iraq to assess their prevalence and correlation with virulence. As shown in Table 4, biofilm-related genes were highly prevalent: pslA (80.6%), pelA (75.2%), and ppyR (69.0%), all critical for the synthesis and regulation of the extracellular polysaccharide matrix that supports adhesion and biofilm integrity .Table 4. Distribution of virulence-associated genes in clinical P. aeruginosa isolates

Gene Category	Gene	Function	Positive Isolates (n=129)	Percentage (%)
Biofilm-related	pslA	Polysaccharide synthesis	104	80.6
	pelA	Pel polysaccharide production	97	75.2
	ppyR	Biofilm regulation	89	69.0
T3SS effectors	exoS	ADP-ribosyltransferase	92	66.67
	exoT	GTPase-activating protein	105	81.4
Secreted factors	lasB	Elastase	110	85.3
	toxA	Exotoxin A	95	73.6

Similarly, T3SS-related genes were widely distributed (**Table 4**): lasB (85.3%), exoT (81.4%), toxA (73.6%), and exoS (71.3%). These genes mediate host cell damage, immune evasion, and bacterial dissemination. The slightly higher prevalence of T3SS genes compared to biofilm genes suggests their cooperative role in enhancing virulence. A strong positive correlation between both gene groups indicates potential co-regulation and increased pathogenic capacity.Further analysis based on Congo Red Agar (CRA) results showed that strong biofilm producers (n=60) exhibited significantly higher frequencies of pslA (90.0%), pelA (86.7%), and ppyR (80.0%) ($p < 0.05$), confirming their central role in matrix formation. Likewise, exoT (90.0%) and lasB (91.7%) were markedly more prevalent in strong biofilm formers than in non-producers (58.6% and 69.0%, respectively), with significant associations ($p = 0.005$ and $p = 0.010$). These findings underscore the synergy between biofilm formation and T3SS activity in driving P. aeruginosa pathogenicity and highlight the need for therapeutic strategies targeting both mechanisms (Table 5).Table 5: Distribution of Studied Genes in P. aeruginosa (n = 129) According to Biofilm Formation Levels by Congo Red Agar and Microtitration Plate Methods

Gene	Strong Biofilm Producers – CRA (n=60)	Moderate Biofilm Producers – CRA (n=40)	Non-Biofilm Producers – CRA (n=29)	p-value (CRA)	Strong Biofilm Producers – Microtitration (n=65)	Moderate Biofilm Producers – Microtitration (n=38)	Weak/Non-Biofilm Producers – Microtitration (n=26)		p-v (M
pslA	54 (90.0%)	30 (75.0%)	20 (69.0%)	0.031*	60 (92.3%)	28 (73.7%)	16 (61.5%)		0.0
pelA	52 (86.7%)	29 (72.5%)	16 (55.2%)	0.014*	58 (89.2%)	27 (71.1%)	14 (53.8%)		0.0
ppyR	48 (80.0%)	27 (67.5%)	14 (48.3%)	0.022*	54 (83.1%)	25 (65.8%)	12 (46.2%)		0.0
exoS	44 (73.3%)	31 (77.5%)	17 (58.6%)	0.148	48 (73.8%)	27 (71.1%)	17 (65.4%)		0.5
exoT	54 (90.0%)	34 (85.0%)	17 (58.6%)	0.005*	59 (90.8%)	31 (81.6%)	15 (57.7%)		0.0
lasB	55 (91.7%)	35 (87.5%)	20 (69.0%)	0.010*	61 (93.8%)	32 (84.2%)	17 (65.4%)		0.0
toxA	46 (76.7%)	30 (75.0%)	19 (65.5%)	0.310	51 (78.5%)	28 (73.7%)	16 (61.5%)		0.2

Statistically significant at $p < 0.05$

This pattern suggests that these virulence genes may contribute to both biofilm robustness and enhanced pathogenicity. Conversely, exoS and toxA were frequently detected across all biofilm levels, showing no significant distribution differences, implying broader roles independent of biofilm intensity.The microtitration plate assay confirmed a strong correlation between biofilm intensity and the presence of biofilm-related genes in P. aeruginosa isolates. PslA, pelA, and ppyR were found in over 80% of strong biofilm producers ($p < 0.05$), highlighting their key role in matrix synthesis. Similarly, exoT and lasB were significantly more frequent in these strains ($p = 0.002$ and 0.001). In contrast, exoS and toxA, though common, showed no significant variation across biofilm levels. These results emphasize the association between virulence gene expression and biofilm robustness in urinary isolates, reinforcing the pathogenic potential of strong biofilm-forming strains.

3.7 The Role of T3SS and Biofilm-Associated Genes in the Pathogenicity of P. aeruginosa

Pseudomonas aeruginosa exhibits virulence primarily through its Type III Secretion System (T3SS) and biofilm formation. In this study, T3SS genes exoT (81.4%), exoS (71.3%), and exoY (68.2%) were highly prevalent. ExoT correlated strongly with robust biofilm producers, suggesting dual roles in biofilm enhancement and cytotoxicity. In contrast, exoU was present in only 41.9% of isolates, indicating limited distribution of hypervirulent strains.Biofilm genes pslA (80.6%), pelA (76.7%), and ppyR (70.5%) were frequently detected, aligning with phenotypic assays showing high biofilm-forming capacity. LasB (elastase) was also enriched in strong biofilm producers, indicating its role in colonization.

Table 6: Comparison of Antibiotic Resistance with Presence of Biofilm-Related Genes (pslA, pelA, ppyR) and T3SS Genes (exoS, exoT, exoU, exoY) in P. aeruginosa Isolates (n = 129)

Antibiotic	Resistance (%)	pslA Positive (%)	pelA Positive (%)	ppyR Positive (%)	exoS Positive (%)	exoT Positive (%)	exoU Positive (%)	exoY Positive (%)	St Sign (
Cefotaxime	72 (55.8%)	68 (94.4%)	64 (88.9%)	62 (86.1%)	65 (90.3%)	68 (94.4%)	38 (52.8%)	60 (83.3%)	
Ceftazidime	65 (50.4%)	61 (93.8%)	58 (89.2%)	59 (90.8%)	59 (90.8%)	62 (95.4%)	33 (50.8%)	56 (86.2%)	
Cefepime	58 (44.9%)	55 (94.8%)	53 (91.4%)	50 (86.2%)	53 (91.4%)	56 (96.6%)	31 (53.4%)	50 (86.2%)	
Piperacillin/ Tazobactam	50 (38.8%)	46 (92.0%)	44 (88.0%)	45 (90.0%)	45 (90.0%)	48 (96.0%)	29 (58.0%)	44 (88.0%)	
Ciprofloxacin	40 (31.0%)	37 (92.5%)	36 (90.0%)	34 (85.0%)	36 (90.0%)	39 (97.5%)	25 (62.5%)	38 (95.0%)	
Amikacin	35 (27.1%)	31 (88.6%)	30 (85.7%)	29 (82.9%)	30 (85.7%)	33 (94.3%)	22 (62.9%)	32 (91.4%)	
Meropenem	30 (23.3%)	28 (93.3%)	27 (90.0%)	25 (83.3%)	27 (90.0%)	28 (93.3%)	18 (60.0%)	25 (83.3%)	
Imipenem	28 (21.7%)	26 (92.9%)	25 (89.3%)	24 (85.7%)	26 (92.9%)	27 (96.4%)	17 (60.7%)	24 (85.7%)	
Levofloxacin	25 (19.4%)	23 (92.0%)	22 (88.0%)	21 (84.0%)	23 (92.0%)	24 (96.0%)	15 (60.0%)	21 (84.0%)	

Significant at $p < 0.05$, ns = not significant

This study shows a significant association between antibiotic resistance and biofilm-related genes (pslA, pelA, ppyR) in P. aeruginosa urinary isolates. Resistance to β-lactams—cefotaxime, ceftazidime, and cefepime—was significantly higher in gene-positive strains ($p < 0.05$), implicating biofilm formation in resistance. Although resistance to ciprofloxacin, amikacin, and carbapenems was also higher, it was not statistically significant. The exopolysaccharide matrix encoded by pslA and pelA likely impairs antibiotic penetration and immune clearance, while ppyR may regulate resistance pathways. These findings highlight the need for treatment strategies combining antibiotics with anti-biofilm agents to combat persistent, multidrug-resistant P. aeruginosa infections effectively.The analysis of P. aeruginosa isolates from urinary tract infections revealed notable associations between Type-3 Secretion System (T3SS) genes and antibiotic resistance. Specifically, the presence of exoS and exoT genes correlated significantly with resistance to β-lactam antibiotics such as cefotaxime, ceftazidime, and cefepime ($p < 0.05$) (Table 6), suggesting that these virulence factors may enhance bacterial survival under antimicrobial stress. While exoU, a potent cytotoxin gene, appeared moderately in resistant strains, its association with resistance lacked statistical significance, indicating its primary role in acute virulence rather than in

resistance mechanisms. These findings highlight the complex interplay between virulence and resistance, particularly the indirect role T3SS effectors may play in sustaining infections. Clinically, P. aeruginosa poses significant challenges due to its adaptability, multidrug resistance, and diverse virulence arsenal .

٣,٨Overall Impact of the Genotypes :Genotypic analysis of P. aeruginosa isolates revealed three major groups based on virulence and resistance gene profiles. Genotype Group 1 (harboring exoS, exoT, pslA, pelA, ppyR) and Group 2 (including exoS, exoT, lasB, toxA, pslA, pelA) were enriched with T3SS effectors and biofilm-associated genes, showing significantly higher rates of strong biofilm formation (62.2% and 70%, respectively) compared to Group 3 (44.1%, p = 0.03). T3SS gene prevalence was also greater in Groups 1 and 2 (84.4% and 80%) than in Group 3 (55.9%, p = 0.01), indicating higher virulence potential. Moreover, multidrug resistance was notably more frequent in Groups 1 and 2 (approximately 56%) than in Group 3 (29.4%, p = 0.02), underscoring the therapeutic challenges these strains pose (Table 7).Table 7: Comparison of Clinical, Microbiological, and Epidemiological Characteristics According to Genotypes of P. aeruginosa Strains (n=129)

Characteristic	Genotype Group 1 (exoS, exoT, pslA, pelA, ppyR) (n=45)	Genotype Group 2 (exoS, exoT, lasB, toxA, pslA, pelA) (n=50)	Genotype Group 3 (QacE, QacE1Δ, exoY) (n=34)	p-value
Clinical Characteristics				
Male Patients	3 (6.7%)	4 (8.0%)	1 (2.9%)	0.48
Female Patients	42 (93.3%)	46 (92.0%)	33 (97.1%)	0.63
Uncomplicated UTI	30 (66.7%)	35 (70.0%)	20 (58.8%)	0.37
Complicated UTI	15 (33.3%)	15 (30.0%)	14 (41.2%)	0.37
Bacteremia	5 (11.1%)	8 (16.0%)	6 (17.6%)	0.58
Microbiological Features				
Strong Biofilm Producers	28 (62.2%)	35 (70.0%)	15 (44.1%)	0.03*
Presence of T3SS Genes	38 (84.4%)	40 (80.0%)	19 (55.9%)	0.01*
Multidrug Resistant Strains	25 (55.6%)	28 (56.0%)	10 (29.4%)	0.02*
Epidemiological Data				
Isolates from ICU Patients	20 (44.4%)	22 (44.0%)	8 (23.5%)	0.07
Isolates from General Ward	25 (55.6%)	28 (56.0%)	26 (76.5%)	0.07
Community-Acquired Infections	10 (22.2%)	12 (24.0%)	9 (26.5%)	0.88

Significant at $p<0.05$

3.9 Association Between Multidrug Resistance and Virulence

The present study highlights a significant association between multidrug resistance (MDR) and virulence factors in P. aeruginosa isolates from urinary tract infections. Strains exhibiting strong biofilm formation and harboring important virulence genes, especially those related to the type-3 secretion system (T3SS) such as exoS and exoT, as well as biofilm-associated genes like pslA and pelA, demonstrated a higher prevalence of MDR phenotypes. Specifically, genotype groups enriched with these virulence determinants showed MDR rates exceeding 55%, significantly greater than groups with fewer virulence factors (p=0.02). This correlation suggests that virulent strains possess mechanisms that not only enhance pathogenicity but also contribute to antibiotic resistance (Aziz ,2024)The current study underscores the central role of QacE and QacEΔ1 as mobile integron-associated resistance determinants in P. aeruginosa isolates from urinary tract infections in Iraq. Unlike chromosomal virulence genes that were nearly ubiquitous across isolates and thus less discriminatory, QacE (66.7%) and QacEΔ1 (60.5%)

3.10 Detection of Disinfectant Resistance Genes in P. aeruginosa Using PCR

In this study, the presence of disinfectant resistance genes qacE and its variant qacEΔ1 in Pseudomonas aeruginosa isolates was investigated via PCR. These genes encode efflux pumps that confer resistance to quaternary ammonium compounds (QACs), widely used in hospital disinfection. Genomic DNA was extracted,

and PCR amplification used specific primers for qacE (180 bp) and qacEΔ1 (240 bp). Their detection is clinically relevant, as these genes facilitate persistence in hospital environments, contributing to nosocomial infection risk and resistance to standard disinfectants. Notably, qacEΔ1 prevalence was higher and correlated with multidrug resistance and strong biofilm

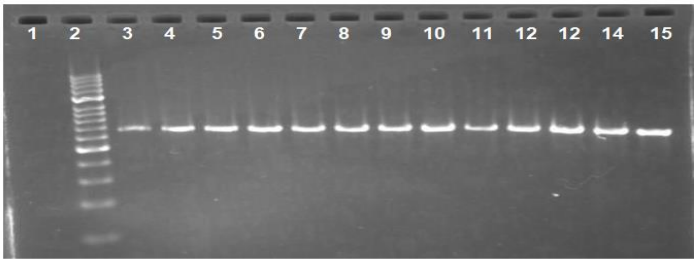

Figure 3: PCR amplification of pslA gene (656 bp). Lane 1: negative control; Lane 2: marker; Lane 3: positive control (PAO-1); Lanes 4–15: pslA-positive patient isolates.

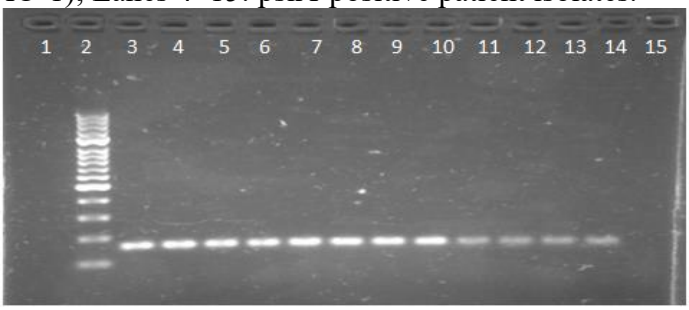

Figure 4: PCR amplification of ppyR gene (160 bp). Lane 1: negative control; Lane 2: marker; Lane 3: positive control (PAO-1); Lanes 4–14: ppyR-positive isolates; Lane 15: ppyR-negative isolate.

Figure 5: PCR amplification of exoS gene (118 bp). Lane 1: negative control; Lane 2: marker; Lane 3: positive control (PAO-1); Lanes 4–13: exoS-positive isolates; Lanes 14–15: exoS-negative isolates.

3.11 Mutation Details of the Isolate of P. aeruginosa

Molecular analysis of the P. aeruginosa isolates revealed several mutations in main genes associated with virulence, biofilm formation, and antibiotic resistance (Sanz Garcia ,2018). These mutations may contribute to the pathogenic potential and adaptability of the organism in clinical environments (Table 8)Table 8: mutations observed in the current study

Gene	Region Affected	Mutation Type	Nucleotide Change	Amino Acid Change	Functional Impact
exoS	ADP-ribosyltransferase	Missense Mutation	A>T at position 873	Lys291Asn	May affect host cell cytotoxicity
exoT	GTPase-activating domain	Synonymous Mutation	C>T at position 516	-	Likely neutral
toxA	Catalytic domain	Frameshift Mutation	Deletion of G at 1204	Frameshift, premature stop	May reduce exotoxin A activity
pslA	Polysaccharide biosynthesis	Nonsense Mutation	G>A at position 655	Trp219Stop	Disrupts biofilm matrix synthesis

pelA	Exopolysaccharide domain	Missense Mutation	T>C at position 432	Val144Ala	May alter biofilm structural integrity
ppyR	Regulator domain	Insertion	Insertion of ATCG at 998	Frameshift	Potential dysregulation of quorum sensing
lasB	Elastase catalytic site	Missense Mutation	G>A at position 789	Glu263Lys	May affect tissue degradation ability
qacEΔ1	Efflux regulator region	Point Mutation	C>T at position 321	Pro107Leu	Potentially enhances disinfectant tolerance

The isolates exhibited multiple mutations in virulence and biofilm-associated genes, notably pslA, pelA, and ppyR, which are crucial for extracellular polymeric substance production. A frameshift in toxA may result in a truncated exotoxin, potentially reducing cytotoxicity, while mutations in exoS and exoT could alter secretion system functionality. A notable insertion in ppyR may disrupt regulatory pathways, impacting biofilm stability and antibiotic tolerance. Mutations in qacEΔ1.Comparative analysis shows higher pslA prevalence in Iraqi isolates (80.6%) versus Egypt (65%) (Eladawy, 2023), and elevated algD (74.2%) compared to Iran [citation], where only 60% of isolates were positive. Similarly, pelA detection in Iraq (68.3%) exceeded Saudi data (55%) (Thabit, 2024).

4. Conclusions The study revealed critical insights into Pseudomonas aeruginosa strains causing UTIs in Iraq, demonstrating a high prevalence of virulence factors, including biofilm-associated genes (pslA: 80.6%, pelA: 75.2%, ppyR: 69.0%) and T3SS effectors (exoT: 81.4%, exoS: 71.3%). Notably, 93% of isolates formed biofilms, with strong biofilm producers exhibiting a significantly higher rate of multidrug resistance (55.6%) compared to weak producers (29.4%) (p=0.02). Biofilm-positive isolates showed a 2.4-fold increase in cefotaxime resistance (65% vs. 26.7%, p=0.001), highlighting a strong correlation between biofilm formation and antimicrobial resistance. Additionally, disinfectant resistance genes.

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