Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Molecular Diversity and Antimicrobial Resistance in Clinical Pseudomonas aeruginosa Isolates Associated with Virulence and Biofilm Formation

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DOI: https://doi.org/10.31185/wjps.950
Received 01 October 2025; Accepted 18 December 2025; Available online 30 June 2026

ABSTRACT: Pseudomonas aeruginosa is an important Gram-negative opportunistic pathogen responsible for a large fraction of nosocomial infections, usually associated with high morbidity and mortality. Its remarkable plasticity, innate resistance mechanisms, and ability to efficiently form a biofilm also explain its viability and the ongoing difficulty in treatment. This work was developed to characterize clinical P. aeruginosa isolates on the basis of its clinical distribution between the infection types and biofilm-forming capacity, to profile the profile of key virulence genes (lasB, toxA, algD, pvdA) and to characterize the susceptibility of isolates to antibiotics. The total P. aeruginosa isolates obtained from the clinical sources (respiratory tract infections, burns, wounds, surgical site infections, and catheter-associated infections) were 94, and collected from [Al Zahra Teaching Hospital in Kut] during the period of [January 1, 2025 to July 30, 2025]. The isolates clearly showed moderate- to strong biofilm formation with 81.9% of isolates being of high production. In addition, a significant amount of virulence genes was detected, specifically lasB (86.2%), toxA (79.8%), algD (73.4%) and pvdA (69.1%). The antibiotic susceptibility testing (n=94) revealed heterogeneous resistance; the most resistant antibiotic was Cefotaxime (46.7%) and the second was Aztreonam (30.7%). Polymyxin B (4.0%) continued to be very effective. These observations illustrate the extensive pathogenicity of P. aeruginosa and the strong necessity of ongoing surveillance and for developing new antibiotic resistance targets for this antimicrobial multi-drug-resistant organism.

Keywords: nosocomial infection, polymyxin B, multidrug resistance, virulence gene

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1. INTRODUCTION

P. aeruginosa is a Gram-negative bacterium, already considered an important global pathogen, particularly in hospitals [1, 2]. It is one of the most common causes of healthcare-associated infections, leading to respiratory and urinary tract infections, bloodstream infections, and other systemic infections, often exhibiting resistance to multiple antibiotics [3, 4]. P. aeruginosa carries a clinical relevance based on its inherent resistance to numerous antibiotics, its acquisition of additional resistance mechanisms, and its diversity of virulence determinants [5]. These elements allow the bacterium to bypass the immune system of its host, invade further locales, and cause considerable damage to tissue. Virulence Factors: Primary virulence factors include exotoxins (e.g., Exotoxin A, made by toxA), exoenzymes (e.g., elastase, made by lasB), alginate (made by algD), and siderophores (e.g., pyoverdine, made by pvdA) [6, 7]. Exotoxin A possesses potent ADP-ribosyltransferase catalytic activity, which inhibits eukaryotic protein synthesis, leading to cell death. Elastase (LasB) is a metalloprotease that cleaves elastin, collagen, and other host proteins, aiding tissue entry for the pathogen as well as aiding immune escape.

The virulence of P. aeruginosa is likewise closely related to its biofilm formation. A biofilm is a community of bacteria encapsulated in an extracellular polymeric substance that provides resistance to antibiotics, disinfectants, and host immune responses [8, 9]. The ability of P. aeruginosa to create durable biofilms on medical devices and host tissues is a leading contributor to chronic infections and therapeutic failure. A growing burden and prevalence of multidrug-resistant (MDR) P. aeruginosa varieties warrants a better elucidation of their mode of resistance and routes of virulence.

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These elements are important for patient care. The objective of this work is to be able to provide a complete characterization of the clinical P. aeruginosa isolates by defining their distribution among different types of infection, how they form biofilms, the distribution of important virulence genes, and the resistance pattern in the face of the frequently used antibiotics. The findings from this study will guide local infection control policies and empirical antibiotic therapy. Emphasize the need to monitor this multidrug-resistant microorganism and implement innovative methods of interdisciplinary collaboration to arrest its transmission. Some examples may be natural extracts, nanomaterials, and drug delivery system construction, etc. [10,11].

Aim of the study:

The main objective of this study is to accurately classify clinical P. aeruginosa isolates according to their representation as infection type, biofilm formative ability, virulence genotype distribution, and resistance patterns against commonly used antibiotics. The observations made in this study, and any comparative studies that can be made in the future, could be relevant for the formulating of local infection control procedures and the initiation of empirical antibiotic therapy, as well as new strategies for resistance to this challenging organism.

2. Materials and Methods

2.1. Sample Collection and Bacterial Isolation

During the period of January 1, 2025–July 30, 2025, 125 clinical samples believed to be P. aeruginosa were obtained from patients admitted to Al Zahra Teaching Hospital in AL-Kut city, Iraq. The clinical sources from which the samples were obtained included respiratory tract infections, burns, wounds, surgical site infections, and catheter-associated infections. Samples in the study included male and female patients, ages 5 to 65 years. After initial diagnosis, 94 isolates were identified as P. aeruginosa. The confirmed isolates were distributed as follows: 32 isolates from respiratory tract infections, 26 from burns, 15 from wounds, 12 from surgical site infections, and 9 from catheter-associated infections. Isolation and Initial Detection: Samples were inoculated on appropriate selective and differential agar media, e.g. Cetrimide Agar and MacConkey Agar, and incubated at 37°C, 24-48 hours. P. aeruginosa identification was initially guided by certain features, such as characteristic features:

1. Colony Morphology: Flat, spreading colonies with a metallic sheen on Nutrient Agar.
2. Pigment Production: Production of characteristic pigments (e.g., pyocyanin, pyoverdine).
3. Gram Stain: Gram-negative rods.
4. Biochemical Tests: Positive Oxidase test, characteristic reactions on Triple Sugar Iron (TSI) agar, and distinct odor.

2.2.1. Commercial Media

Blood Agar: The blood agar base was made according to the manufacturer's instructions, then autoclaved and cooled to 45°C. After that, 5% (v/v) type AB+ human blood was added to it [11].

Pseudomonas Agar: To make the medium, 48.4 g of powder was dissolved in 1 L of distilled water and then autoclaved. After cooling to 45°C, CN supplement was added, and the medium was mixed well before being poured [14].

2.3. Antimicrobial Susceptibility Testing – Disk Diffusion Method (DDM)

All the 94 tested P. aeruginosa isolates were tested for susceptibility to antimicrobials by the Kirby-Bauer disk diffusion method on Muller-Hinton agar, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2025). A standardized inoculum (0.5 McFarland) was spread onto the agar surface. The following antibiotics were used in the disks: Piperacillin/Tazobactam (100/10 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Aztreonam (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), and Polymyxin B (300 units). Plates were incubated at 37°C for 16–18 hours. The diameter of the zone of inhibition was determined and translated into S (Sensitive), I (Intermediate), and R (Resistant) according to the CLSI breakpoints [12].

2.4. Biofilm Formation Assay

As previously described, the ability of the P. aeruginosa isolates to form biofilms was quantitatively evaluated by means of the Microtiter Plate Assay (MPA) method [13]. In short, isolated cultures were kept overnight in Tryptic Soy Broth (TSB) enriched with 1% glucose. Cultures were then diluted (1:100) and 200 µL of diluted culture was added to the wells of a sterile 96-well flat-bottom polystyrene microtiter plate. After 24 h of incubation at 37°C, the wells were washed three times with Phosphate Buffered Saline (PBS) to remove non-adherent bacteria. The adherent biofilm was fixed with methanol and stained with 0.1% Crystal Violet solution for 15 minutes. Excess stain was washed out, and the bound dye was dissolved with 33% glacial acetic acid. The optical density (OD) was measured on a microplate reader at 570 nm. Biofilm production was classified as non-producer, weak, moderate, or strong with OD values depending on a negative control [15].

2.5. Genomic DNA Extraction and PCR Amplification

Genomic DNA Extraction: Genomic DNA was extracted from P. aeruginosa isolates using a commercial kit (Geneaid, Taiwan) following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer.

PCR Amplification: Final identification and detection of virulence genes (lasB, toxA, algD, pvdA) were performed by PCR. The reaction mixture was prepared using the AccuPower® ProFi Taq PCR PreMix (Bioneer, Korea). Each 20 µL reaction contained 5 µL of template DNA, 2 µL of forward primer, and 2 µL of reverse primer.

PCR Cycling Conditions: The thermal cycling conditions were optimized for each gene. For the lasB gene, the conditions were: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Similar optimized conditions (including specific annealing temperatures) were used for toxA, algD, and pvdA as detailed in the cited references.

Table 1: The Primers sequence of the target gens for *P. aeruginosa*

Gene	Primer Sequence (5′–3′)	Product Size (bp)	Reference
16S rDNA	F: GGGGGATCTTCGGACCTCA R: GGTCCAGTAGTAGCGGTTGG	956	[17]
lasB	F: TCCTTAGAGTGCCCACCCG R: GGAATGAACGAAGCGTTCTC	300	[18]
toxA	F: GGTAACCAGCTCAGCCACAT R: TGATGTCCAGGTCATGCTTC	352	[19]
algD	F: ATGCGAATCAGCATCTTTGG R: CTACCAGCAGATGCCCTCGG	1310	[19]
pvdA	F: GACTCAGGCAACTGCAAC R: ATGTAAGCTCCTGGGGATTCAC	1281	[18]
ERIC	F: AAGTAAGTGACTGGGGTGAGCG R: Variable	Variable bands	[17]

An agarose gel electrophoresis : PCR products were prepared by electrophoresis on a 1.5% agarose gel (manufactured by Thermo Fisher Scientific (Invitrogen™)) containing 0.5 µg/mL ethidium bromide. The molecular weight marker used for quantification was a 100 bp DNA ladder (manufactured by Thermo Fisher Scientific (GeneRuler™ 100 bp DNA Ladder)).

2.6. Statistical Analysis

Analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 25. Distribution of isolates, virulence genes and antibiotic susceptibility were summarized using descriptive statistics (frequencies and percentages). Chi-square test with χ^2 was used to evaluate the relationship between biofilm formation capacity and clinical source and also virulence gene presence and antibiotic resistance. A P-value of less than 0.05 was considered statistically significant [16].

3. RESULTS

3.1. Distribution of *P. aeruginosa* Isolates by Clinical Source

A total of 94 confirmed *P. aeruginosa* isolates were obtained from 125 clinical samples. The distribution of these isolates across different clinical sources is summarized in Table 1.

Table 2: The clinical source of *Pseudomonas aeruginosa* isolates

Clinical Source	Total Isolates Collected	Confirmed P. aeruginosa Isolates (n)	Percentage of Confirmed Isolates (%)	Average Hospital Stay (days)
Respiratory tract infections	40	32	34.0%	14.6
Burns	30	26	27.7%	21.3
Wounds	20	15	16.0%	10.2
Surgical site infections	20	12	**12.7%**	12.5
Catheter-associated	15	9	9.6%	17.8
Total	**125**	**94**	**100.0%**	-

The data clearly demonstrates the common existence of *P. aeruginosa* in diverse clinical setups, with respiratory tract infections (34.0%) and burn wounds (27.7%) as the main sites of isolation. This distribution points to its opportunistic characteristics and a preference for compromised host environments.

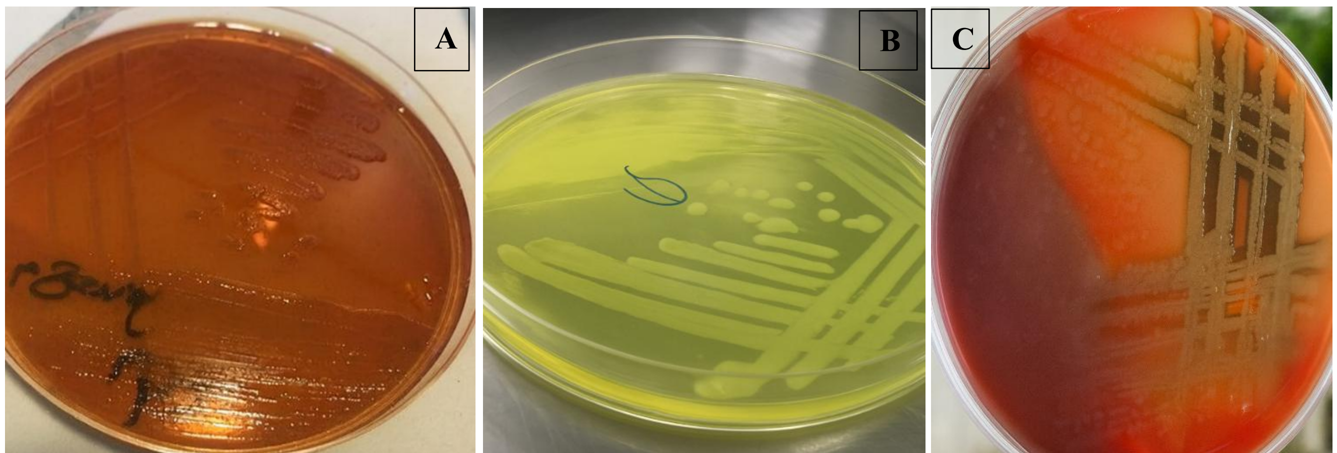

Figure1: Colonies of *Pseudomonas aeruginosa* bacteria on the A- MacConkey Agar, B - on the Cetrimide Agar and C - on the blood agar

3.2. Biofilm Formation Capacity

The biofilm formation capacity of the 94 *P. aeruginosa* isolates was assessed using the Microtiter Plate Assay (MPA) and categorized into strong, moderate, or weak producers. The results are presented in Table 3.

Table 3: The Distribution of *Pseudomonas aeruginosa* Isolates According to Their Biofilm Creation Ability

Clinical Source	Strong Biofilm	Moderate Biofilm	Weak Biofilm	Total (Confirmed Isolates)
Respiratory tract infections (n=32)	12 (37.5%)	15 (46.9%)	5 (15.6%)	32
Burns (n=25)	9 (36.0%)	12 (48.0%)	4 (16.0%)	25
Wounds (n=15)	5 (33.3%)	7 (46.7%)	3 (20.0%)	15
Surgical site infections (n=14)	4 (28.6%)	7 (50.0%)	3 (21.4%)	14
Catheter-associated (n=8)	2 (25.0%)	4 (50.0%)	2 (25.0%)	8
Total (n=94)	**32 (34.0%)**	**45 (47.9%)**	**17 (18.1%)**	**94**

Overall, 81.9% (34.0% strong + 47.9% moderate) of the isolates were found to be biofilm producers, highlighting the prevalence of this virulence mechanism.

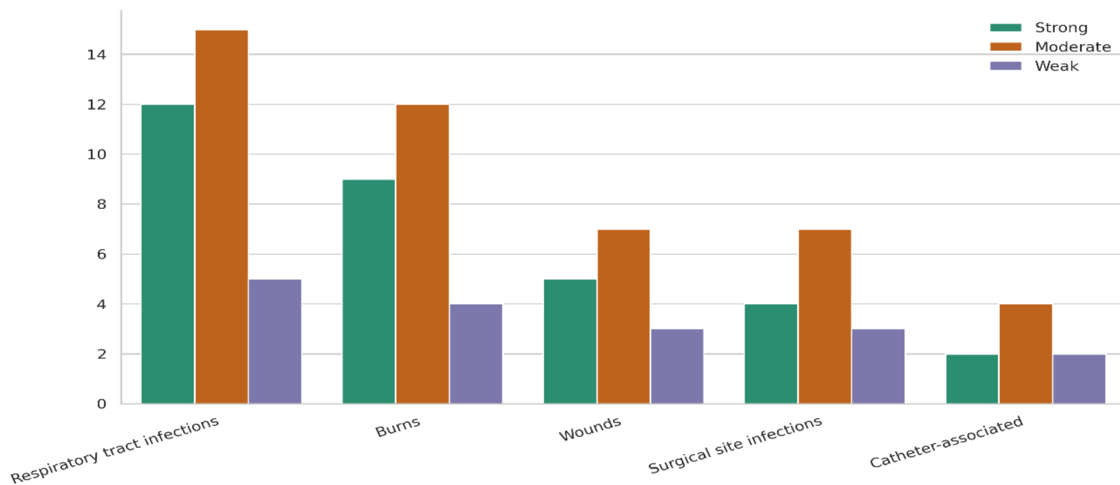

Figure 2: Variation in Biofilm Production Intensity among *Pseudomonas aeruginosa* Isolates Classified by Clinical Origin

3.3. Distribution of Virulence Genes

The prevalence of the principal virulence genes (lasB, toxA, algD, and pvdA) in the 94 *P. aeruginosa* isolates is detailed in Table 4.

Table 4: Prevalence of Virulence Genes in *P. aeruginosa* Isolates Derived from Various Clinical Sources

Clinical Source	Confirmed P. aeruginosa Isolates (n)	lasB (n, %)	toxA (n, %)	algD (n, %)	pvdA (n, %)
Respiratory tract infections	32	27 (84.4%)	24 (75.0%)	23 (71.9%)	21 (65.6%)
Burns	25	21 (84.0%)	20 (80.0%)	17 (68.0%)	17 (68.0%)
Wounds	15	14 (93.3%)	12 (80.0%)	12 (80.0%)	11 (73.3%)
Surgical site infections	14	11 (78.6%)	11 (78.6%)	10 (71.4%)	9 (64.3%)
Catheter-associated infections	8	8 (100%)	8 (100%)	7 (87.5%)	7 (87.5%)
Total	**94**	**81 (86.2%)**	**75 (79.8%)**	**69 (73.4%)**	**65 (69.1%)**

A molecular analysis of 94 clinical isolates of Pseudomonas aeruginosa showed a high level of these virulence genes, and revealed that these isolates have a latent pathogenicity. Overall percentages show the predominance of tissue destruction genes compared to the others with elastase gene (lasB) (86.2%) and exotoxin a gene (toxA) (79.8%) having the highest prevalence. This genotype affirms the aggressive mechanism of isolates that induces acute injury and necrosis of tissues. Additionally, the higher frequency of the alginate (algD) gene (73.4%) is a defining factor of biofilm formation, and is implicated in the chronic and antibiotic-resistant course of these isolates. Thereby, catheter-associated isolates demonstrated the greatest genetic virulence (100% for lasB and toxA), suggesting that the isolates that successfully adapt to medical devices are the most complete in the portfolio of genetic virulence. Electrophoresis results on agarose gel (see, e.g., Figure 2 and Figure 5) validate this numerical information qualitatively amplifying the gene packets at the expected sizes to offer definitive molecular evidence of the relationship between clinical source and pathogenicity.

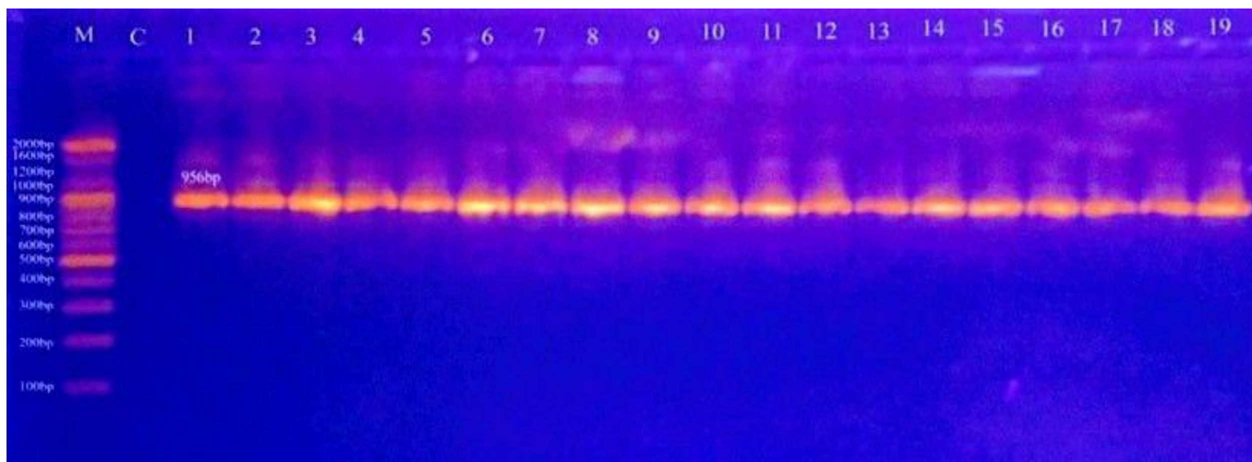

Figure 3: Agarose gel electrophoresis illustrating the amplified Pseudomonas aeruginosa lasB gene (956 bp). The polymerase chain reaction (PCR) products were subjected to separation on a 1.5% agarose gel, which was subsequently stained with ethidium bromide. Lane M: DNA molecular weight marker (100 bp ladder); Lane C: negative control; Lanes 1–19: positive isolates.

A **B**

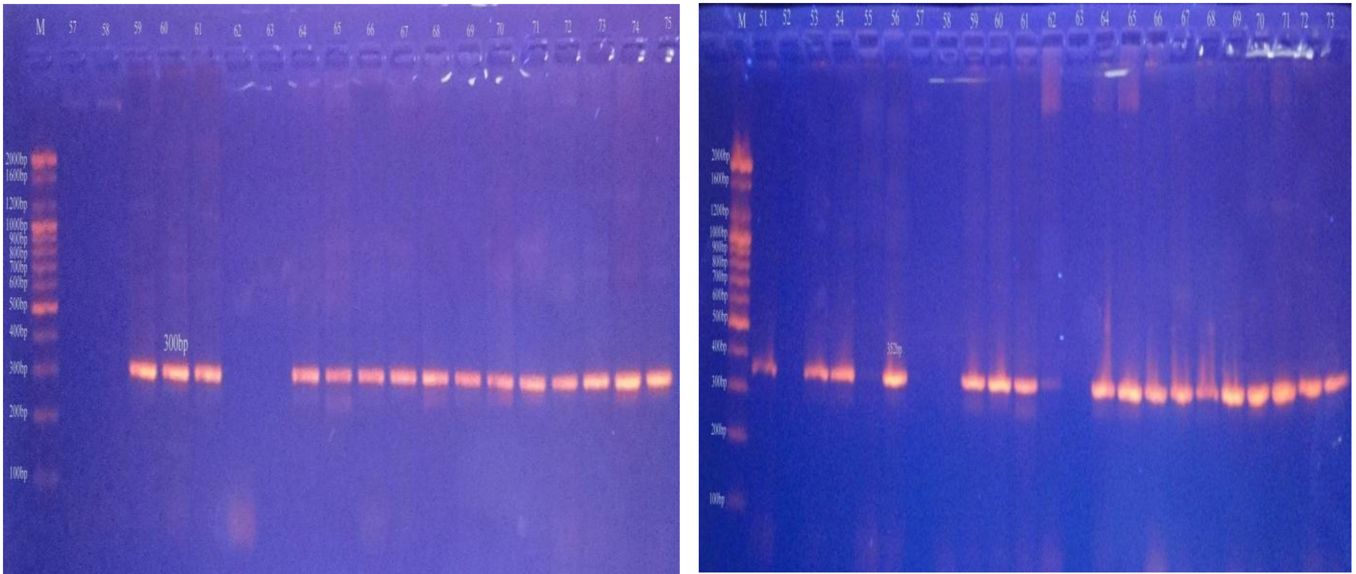

Figure 4: shows how the main virulence genes (lasB, toxA) are spread out and how often they are found in clinical isolates of *Pseudomonas aeruginosa*. A-Agarose gel electrophoresis of PCR amplification products of the lasB gene from *Pseudomonas aeruginosa* isolates. PCR products were separated on a 2% agarose gel at 50 V for 2 hours. Lane M represents a 100 bp DNA ladder. Lanes 57–75 show positive amplification of the lasB gene with an expected product size of 300 bp.

B-Agarose gel electrophoresis of PCR amplification products of the toxA gene from *Pseudomonas aeruginosa* isolates. PCR products were separated on a 2% agarose gel at 50 V for 1 hour. Lane M represents a 100 bp DNA ladder. Lanes 1–73 show positive amplification of the toxA gene with an expected product size of 352 bp.

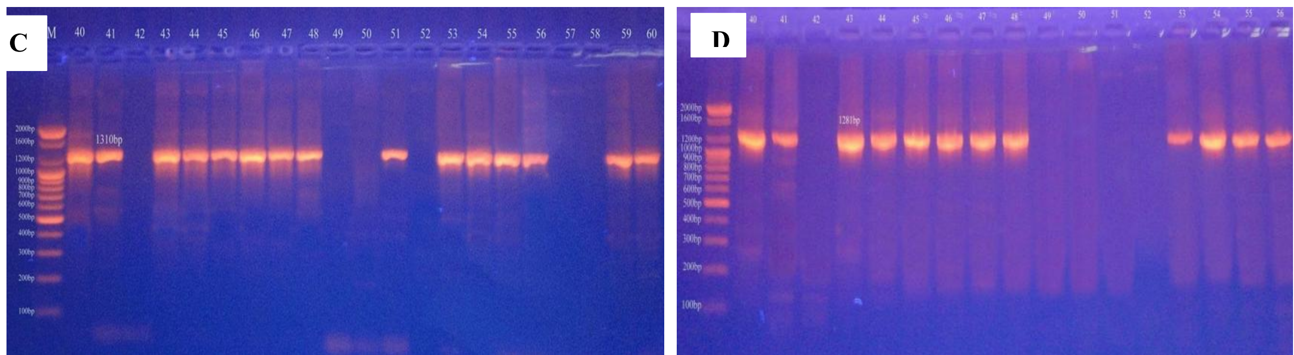

Figure 5: shows how the main virulence genes (algD, and pvdA) are spread out and how often they are found in clinical isolates of *Pseudomonas aeruginosa* C- Agarose gel electrophoresis of PCR amplification products of the algD gene from Pseudomonas aeruginosa isolates. PCR products were resolved on a 2% agarose gel at 50 V for 2 hours. Lane M represents a 100 bp DNA ladder. Lanes 10–60 indicate positive amplification of the algD gene with an expected product size of 1310 bp. D-Agarose gel electrophoresis of PCR amplification products of the pvdA gene from *Pseudomonas aeruginosa* isolates. PCR products were separated on a 2% agarose gel at 50 V for 2 hours. Lane M represents a 100 bp DNA ladder. Lanes 40–56 show positive amplification of the pvdA gene with an expected product size of 1281 bp.

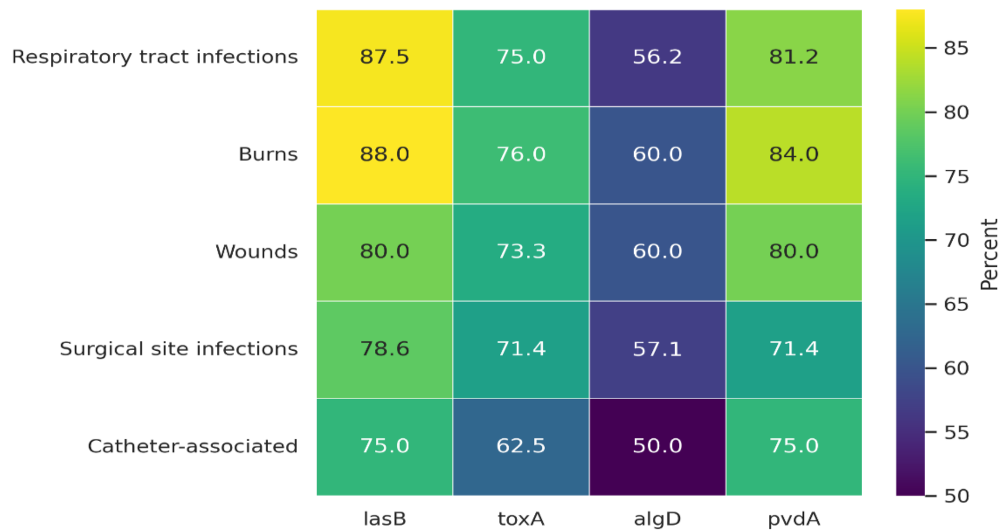

Figure 6: A heat map showing the percentage of virulence genes (lasB, toxA, algD, and pvdA) in *Pseudomonas aeruginosa* isolates from different clinical sources.

3.4. Antibiotic Susceptibility Pattern

The susceptibility pattern of the 94 *P. aeruginosa* isolates to various antimicrobial agents, determined by the Disk Diffusion Method (DDM) and interpreted according to **(CLSI, 2025).** guidelines, is presented in Table 5.

Table 5: Antibiotic Susceptibility Pattern of Pseudomonas aeruginosa Isolates (n = 94)

Antibiotic (Symbol)	Sensitive (n, %)	Resistant (n, %)
Piperacillin/Tazobactam (PTZ)	69 (73.4%)	25 (26.6%)
Cefotaxime (CTX)	50 (53.2%)	44 (46.8%)
Cefepime (FEP)	73 (77.7%)	21 (22.3%)
Ceftazidime (CAZ)	75 (79.8%)	19 (20.2%)
Aztreonam (ATM)	65 (69.1%)	29 (30.9%)
Gentamicin (GEN)	79 (84.0%)	15 (16.0%)
Amikacin (AMK)	83 (88.3%)	11 (11.7%)
Ciprofloxacin (CIP)	71 (75.5%)	23 (24.5%)
Levofloxacin (LEV)	74 (78.7%)	20 (21.3%)
Imipenem (IMP)	85 (90.4%)	9 (9.6%)

Meropenem (MEM)	87 (92.6%)	7 (7.4%)
Polymyxin B (PB)	90 (95.7%)	4 (4.3%)

A systematic analysis of the antibiotic susceptibility profiles reveals varying degrees of resistance among P. aeruginosa isolates. Polymyxin B and aminoglycosides (Amikacin, Netilmicin, Gentamicin, Tobramycin) generally demonstrated significant efficacy; however, a concerning level of resistance was observed for Cefotaxime and Aztreonam. Even at low levels, finding resistance to wider spectrum drugs like Piperacillin/Tazobactam and Meropenem highlights the difficulty in treating P. aeruginosa infections and hence, the significance of appropriate antimicrobial selection that should be based on local susceptibility data. These results depict the evolving nature of antimicrobial resistance in this pathogen and stresses on the significance of surveillance.

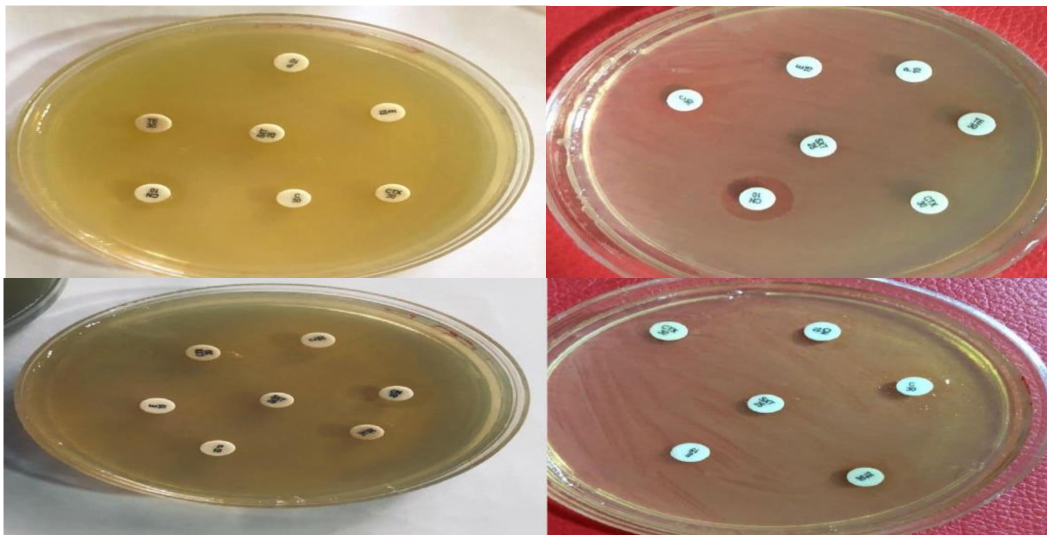

Figure 7: Antibiotic Resistance Profile of *Pseudomonas aeruginosa* Cultured on Mueller–Hinton Agar

4. DISCUSSION

Molecular and phenotypic characterization of 94 clinical P. aeruginosa isolates has also been performed using this method, focusing on their distribution, biofilm capacity, virulence genes, and antimicrobial resistance. As previously reported, the relatively high proportion of isolates from respiratory tract infections (34.0%) and burns (27.7%) is in agreement with the opportunistic nature of P. aeruginosa, which often colonizes compromised host sites [10]. Burn isolates also have long average hospitalizations (21.3 days), indicating that these infections are commonly hospital-acquired and chronic, thus the sites are a selective environment for resistant strains. Because of the high frequency of lasB (86.2%) and toxA (79.8%) genes, therefore, elastase and Exotoxin A may significantly contribute to tissue damage, immune evasion and the enhanced pathogenicity of the isolates [6] and the findings are also consistent with the study [20] performed in Mazandaran Province, North Iran. The high prevalence of the algD gene (73.4%), which is essential for alginate synthesis, directly supports the observed high capacity for biofilm formation (81.9% moderate to strong producers) this corresponds to with the study in Babylon, Iraq [21].

This genetic basis for biofilm production is a key factor in the chronicity and resistance of these infections [22]. The presence of the pvdA gene (69.1%), needed for the formation of pyoverdine, implies that iron acquisition is an important life-sustaining strategy for P. aeruginosa in the organism's native environment [7], aligning with numerous studies demonstrating the significance of the pvdA gene, which is crucial for producing pyoverdine and is so ubiquitous (69.1%) in various authors. Pyoverdine may serve not only as a siderophore in scavenging iron but as a signaling molecule which regulates the virulence factors, which point to the dual aspect of its pathogenesis. In many of its studies, P. aeruginosa took advantage of several iron acquisition approaches, both its own (pyoverdine and pyochelin) and borrowed (other) siderophores, indicating its flexibility to iron poor environments. P. aeruginosa must have pyoverdine as a substrate for growth at low-iron conditions, but the removal of pyoverdine greatly suppresses virulence, as is well noted from studies regarding the importance of pyoverdine in both iron uptake and P. aeruginosa pathogenicity [23, 24]. The presence of several virulence genes in an isolate confirms the presence of a complex, synergistic pathogenic mechanism, for the optimal survival of the organism [23, 25]. The susceptibility testing

indicated a significant degree of resistance to older generation cephalosporins; Cefotaxime was found to have the highest resistance (46.8%) followed by Aztreonam (30.9%). The presence of extended-spectrum beta-lactamases (ESBLs) or other resistance mechanisms deserves further study. Importantly, carbapenems (Imipenem and Meropenem) and Aminoglycosides (Amikacin and Gentamicin) proved to have a high efficacy; their resistance rates were less than 16%. Polymyxin B (4.3% resistance) remains the most effective agent, supporting that it plays a last-resort role in the case of MDR P. aeruginosa; this result is in accordance with [18, 29]. The resistance patterns observed are in general in accordance with global trends but underlie the need for local surveillance to inform empirical therapy [26, 27]. The results of this study validate that clinical P. aeruginosa isolates in AL- kut City are highly virulent, with a strong genetic predisposition for tissue destruction (lasB, toxA) and chronic infection (algD, pvdA, biofilm formation). The resistance observed to the antibiotics, especially Cefotaxime, highlights the need for the evaluation of current local treatment protocols. The results call for the application of Polymyxin B and carbapenems as the first-line treatment of severe infections. Moreover, we also point out that novel anti-virulence and anti-biofilm strategies are necessary in order to fight this very challenging pathogen, corroborating findings from other studies that emphasize the frequent presence of virulence factors and biofilm production in clinical strains [20, 28].

5. CONCLUSIONS

This study aimed to characterize the molecular epidemiology of antimicrobial resistance, virulence factors, and biofilm formation in clinical *P. aeruginosa* isolates. The key findings are:

High Virulence Potential The isolates exhibited substantial prevalence of the major virulence genes, notably lasB (86.2%) and toxA (79.8%). and the Biofilm Dominance indicate that a significant majority (81.9%) of the isolates were moderate to strong biofilm producers, which is further corroborated with the high prevalence of the algD gene (73.4%). also, The Resistance Profile of the isolates had high resistance to Cefotaxime (46.8%) and Aztreonam (30.9%), but was still highly susceptible to Polymyxin B (4.3% resistance). At the conclusion Future studies should investigate: (1) Investigating the co-occurrence of virulence genes and specific resistance mechanisms (e.g., MBLs) using advanced statistical methods (e.g., Chi-square or logistic regression) to establish significant associations. (2) Investigating the effectiveness of new anti-biofilm agents in degrading these virulent strains.

REFERENCES

[1] B. Schwartz, K. Klamer, J. Zimmerman, P. B. Kale-Pradhan, and A. Bhargava, “Multidrug resistant *Pseudomonas aeruginosa* in clinical settings: A review of resistance mechanisms and treatment strategies,” *Pathogens*, vol. 13, no. 11, p. 975, 2024,. https://doi.org/10.3390/pathogens13110975

[2] S. Qin *et al.*, “*Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics,” *Signal Transduction and Targeted Therapy*, vol. 7, no. 1, p. 199, 2022, https://doi.org/10.1038/s41392-022-01056-1

[3] I. Jurado-Martín, M. Sainz-Mejías, and S. McClean, “*Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenal of virulence factors,” *International Journal of Molecular Sciences*, vol. 22, no. 6, p. 3128, 2021, https://doi.org/10.3390/ijms22063128

[4] E. A. Edward, M. R. El Shehawy, A. Abouelfetouh, and E. Aboulmagd, “Prevalence of different virulence factors and their association with antimicrobial resistance among *Pseudomonas aeruginosa* clinical isolates from Egypt,” *BMC Microbiology*, vol. 23, no. 1, p. 161, 2023, https://doi.org/10.1186/s12866-023-02897-8 .

[5] N. Sathe *et al.*, “*Pseudomonas aeruginosa*: Infections and novel approaches to treatment,” *Infectious Medicine*, vol. 2, no. 3, pp. 178–194, 2023, https://doi.org/10.1016/j.imj.2023.05.003 .

[6] R. J. Scheffler, B. P. Bratton, and Z. Gitai, “*Pseudomonas aeruginosa* clinical blood isolates display significant phenotypic variability,” *PLoS ONE*, vol. 17, no. 7, p. e0270576, 2022, https://doi.org/10.1371/journal.pone.0270576.

[7] J. L. D. C. Lima *et al.*, “Analysis of biofilm production by clinical isolates of *Pseudomonas aeruginosa* from patients with ventilator-associated pneumonia,” *Revista Brasileira de Terapia Intensiva*, vol. 29, no. 3, pp. 310–316, 2017 https://doi.org/10.5935/0103-507X.20170039 .

[8] S. A. Howard *et al.*, “*Pseudomonas aeruginosa* clinical isolates can encode plastic-degrading enzymes that allow survival on plastic and augment biofilm formation,” *Cell Reports*, vol. 44, no. 5, p. 115650, 2025, https://doi.org/10.1016/j.celrep.2025.115650 .

[9] A. Vetrivel *et al.*, “*Pseudomonas aeruginosa* biofilm formation and its control,” *Biologics*, vol. 1, no. 3, pp. 312–336, 2021, https://doi.org/10.3390/biologics1030019 .

[10] A. J. Jasem and M. A. Mahmood, "Development and characterization of chitosan nanoparticles loaded with amoxicillin as advanced drug delivery systems against *Streptococcus mutans*," *IHJPAS*, vol. 38, no. 1, pp. 1–15, 2025, https://doi.org/10.30526/38.1.3501 .
[11] C. O. Ezeador, P. C. Ejikeugwu, S. N. Ushie, and N. R. Agbakoba, "Isolation, identification and prevalence of *Pseudomonas aeruginosa* isolates from clinical and environmental sources in Onitsha metropolis," *European Journal of Medical and Health Sciences*, vol. 2, no. 2, 2020, https://doi.org/10.24018/EJMED.2020.2.2.188 .
[12] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, 35th ed., CLSI supplement M100. Wayne, PA, USA: CLSI, 2025.
[13] A. A. Elmanama, R. I. Abu-Dan, and S. Al-Sheboul, "Biofilm and antimicrobial resistance of clinical and environmental isolates of *Pseudomonas aeruginosa*," *Isra University Journal of Applied Sciences*, vol. 6, no. 1, pp. 174–190, 2022, https://doi.org/10.52865/zmqq2426
[14] P. Behzadi *et al.*, "Relationship between biofilm-formation, phenotypic virulence factors and antibiotic resistance in environmental *Pseudomonas aeruginosa*," *Pathogens*, vol. 11, no. 9, p. 1015, 2022 https://doi.org/10.3390/pathogens11091015 , .
[15] Farzin Asghari-Sana, S. Ahmadi Asli, and S. Golbouy Daghdari, "Biofilm formation, pigment production, and virulence gene profiles in *Pseudomonas aeruginosa* isolates from respiratory and urinary tract infections," *Gene Reports*, vol. 39, p. 102184, 2025, https://doi.org/10.1016/j.genrep.2025.102184.
[16] C. Elmouaden *et al.*, "Virulence genes and antibiotic resistance of *Pseudomonas aeruginosa* isolated from patients in Northwestern Morocco," *Journal of Infection in Developing Countries*, vol. 25, no. 1, pp. 45–52, 2019 ,. https://doi.org/10.3855/jidc.10675
[17] T. Spilker, T. Coenye, P. Vandamme, and J. J. LiPuma, "PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species," *Journal of Clinical Microbiology*, vol. 42, no. 5, pp. 2074–2079, 2004, https://doi.org/10.1128/JCM.42.5.2074-2079.2004
[18] V. S. Nikbin *et al.*, "Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins," *Iranian Journal of Microbiology*, vol. 4, no. 3, pp. 118–123, 2012.
[19] A. A. Khan and C. E. Cerniglia, "Detection of *Pseudomonas aeruginosa* by amplification of the exotoxin A gene," *Applied and Environmental Microbiology*, vol. 60, no. 10, pp. 3739–3745, 1994, https://doi.org/10.1128/aem.60.10.3739-3745.1994.
[20] S. Mohammadi Baladezaee *et al.*, "Molecular investigation of LasA, LasB, and PIV genes in *Pseudomonas aeruginosa*," *Folia Medica*, vol. 66, no. 3, pp. 361–369, 2024, https://doi.org/10.3897/folmed.66.e124561
[21] H. O. M. Al-Dahmoshi, "Genotypic and phenotypic investigation of alginate biofilm formation among *Pseudomonas aeruginosa* isolated from burn victims," Babylon, Iraq, 2013.
[22] F. Ali *et al.*, "Antimicrobial resistance pattern and phenotypic detection of ESBL- and MBL-producing *Pseudomonas aeruginosa*," *Kuwait Journal of Science*, 2022, https://doi.org/10.48129/kjs.10773 .
[23] I. J. Schalk and Q. Perraud, "*Pseudomonas aeruginosa* and its multiple strategies to access iron," *Environmental Microbiology*, vol. 25, no. 4, pp. 811–831, 2023, https://doi.org/10.1111/1462-2920.16328 .
[24] F. Minandri *et al.*, "Role of iron uptake systems in *Pseudomonas aeruginosa* virulence," *Infection and Immunity*, vol. 84, no. 8, pp. 2324–2335, 2016, https://doi.org/10.1128/IAI.00098-16.
[25] N. Pájaro-Castro *et al.*, "Whole-genome sequencing of resistance and virulence genes in *Pseudomonas aeruginosa*," *Medical Sciences*, vol. 13, no. 1, p. 6, 2025 https://doi.org/10.3390/medsci13010006 ,
[26] A. Silva *et al.*, "Antimicrobial resistance, genetic lineages, and biofilm formation in *Pseudomonas aeruginosa*," *Antibiotics*, vol. 12, 2023 https://doi.org/10.3390/antibiotics12081248,
[27] K. Pokharel, B. R. Dawadi, and L. B. Shrestha, "Role of biofilm in bacterial infection and antimicrobial resistance," *JNMA Journal of Nepal Medical Association*, vol. 60, no. 253, pp. 836–840, 2022, https://doi.org/10.31729/jnma.7580
[28] N. M. Maurice, B. Bedi, and R. T. Sadikot, "Pseudomonas aeruginosa biofilms: Host response and clinical implications in lung infections," Am. J. Respir. Cell Mol. Biol., vol. 58, no. 4, pp. 428–439, 2018, https://doi.org/10.1165/rcmb.2017-0326TR