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Research Article:

Genetic Diversity and Molecular Characterization of Clinically Isolated *Pseudomonas aeruginosa*

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Abstract:
Background: *Pseudomonas aeruginosa* is a major opportunistic pathogen and a member of the ESKAPE group, known for causing serious nosocomial infections and exhibiting high levels of antimicrobial resistance. Its resistance arises from intrinsic, acquired, and adaptive mechanisms, complicating treatment, particularly in healthcare settings.
Aim: This study aimed to identify clinical *P. aeruginosa* isolates using molecular methods, assess their genetic diversity, detect key virulence genes, and evaluate biofilm formation and antibiotic resistance profiles.
Methodology: A total of 36 clinical isolates were collected from public and private hospitals in Sulaymaniyah City between September 2024 and July 2025. Samples included bronchoalveolar lavage, endotracheal aspiration, pleural fluid, wounds, blood, catheters, and ear swabs. Initial identification was based on morphological and biochemical characteristics, followed by confirmation using Vitek® 2 and BD Phoenix M50 systems. Molecular identification targeted the *gyrB* gene. Antimicrobial susceptibility was assessed using the Kirby–Bauer disk diffusion method and automated systems. Virulence genes (*lasB, toxA, exoS,* and *algD*) were detected by polymerase chain reaction (PCR). Biofilm formation was evaluated using a 96-well plate assay, and genetic relatedness was determined by Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR).
Results: All isolates (100%) carried the *gyrB* gene. High levels of antibiotic resistance were observed: 47.22% were multidrug-resistant (MDR), 36% extensively drug-resistant (XDR), and 8% pan-drug-resistant (PDR). Three isolates were resistant to colistin and all tested antibiotics. The *lasB* and *algD* genes were detected in all isolates, while *toxA* and *exoS* were present in 32 and 19 isolates, respectively. Biofilm analysis showed that 92% of isolates were strong producers. ERIC-PCR revealed clonal relatedness among isolates from the same hospitals and specimen types.
Conclusion: Wound samples were the most common source of *P. aeruginosa*. The high prevalence of drug-resistant, strong biofilm-forming strains highlights a significant clinical concern. ERIC-PCR proved effective for assessing clonal relationships, indicating persistence of identical genotypes within individual hospitals.

Keywords: *Pseudomonas aeruginosa, Biofilm, ERIC-PCR, Virulence gene.*

1. Introduction

Pseudomonas aeruginosa infections are common because of the bacterium's adaptability, minimal growth requirements, inherent antibiotic resistance and its virulence factors. The healthcare setting presents a major challenge with these infections. Recognizing these features is critical to better patient outcomes and to the design of effective therapies. The traditional factors of pathogenicity in this organism are exotoxins, flagella, pili and LPSs that promote bacterial surface adhesion, colonization and tissue penetration. The secretion system, by allowing toxins and effector proteins to directly enter host cells, is a vital element of its pathogenic process. Other enzymes, like proteases, directly harm tissues. *P. aeruginosa* enhances its own pathogenicity

and supports chronic infections by quorum sensing and the control of multiple parameters [1, 2].

P. aeruginosa is one of the most challenging bacteria to treat in clinical settings due to its diverse mechanisms of antibiotic resistance. Intrinsic resistance is primarily mediated by the active efflux of antimicrobial compounds from the cell and the restricted permeability of the outer membrane. In contrast, acquired resistance arises through the horizontal transfer of resistance genes within and between bacterial populations via mobile genetic elements such as transposons, plasmids, and integrons. Adaptive resistance is frequently associated with biofilm formation, in which bacterial cells are embedded within a protective extracellular matrix. Within these biofilms, bacteria, particularly persister cells, represent a small dormant subpopulation of metabolically inactive cells capable of surviving high dose antibiotic exposure and therefore exhibit enhanced tolerance to antimicrobial agents, thereby reducing the effectiveness of antibiotic treatment [3]. Several studies have investigated the virulence factors associated with antibiotic-resistant *P. aeruginosa* strains. Key factors include exotoxins, biofilm formation, and bacterial defenses against environmental stresses. The *algD* gene encodes alginate, an exopolysaccharide capsule that protects bacteria from the host immune system and antimicrobial agents. The *toxA* gene encodes toxin A, which inhibits protein synthesis in eukaryotic cells. Elastase, a zinc metalloprotease encoded by the *lasB* gene, degrades collagen and elastin in lung tissue. Additionally, the *exoS* gene encodes exoenzyme S, which facilitates bacterial invasion, colonization, and dissemination [4].

Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR)-is one of the most popular molecular typing methods because it is easy to use, quick, and inexpensive. This method creates strain-specific fingerprinting patterns based on the number and location of ERIC sequences in the bacterial genome. This makes it easier to tell apart isolates that are genetically related and those that are not. ERIC-PCR has been useful in epidemiological studies, helping to choose the right antimicrobial treatment, and figuring out how *P. aeruginosa* infections spread [5].

The present study primarily aims to employ molecular approaches for the identification of clinical *P. aeruginosa* isolates and the detection of selected virulence genes associated with biofilm formation. In addition, the study evaluates the biofilm-forming capacity of the isolates through both phenotypic and genotypic analyses. It also investigates the antimicrobial susceptibility patterns of the isolates obtained from different clinical samples using the disc diffusion method and automated systems. Finally, the genetic relatedness among the isolates is assessed using the ERIC-PCR genotyping technique to determine their genetic diversity.

2. Materials and Methods

2.1. Sample Collection

A total of 36 clinical isolates of *P. aeruginosa* were collected from various public and private hospitals, including Burn and Plastic Surgery, Hiwa, Shar, and Pediatric Hospitals, as well as Smart and Bakhshin Private Hospitals in the city of Sulaymaniyah, Iraq. The isolates were obtained from diverse clinical specimens, including bronchoalveolar lavage (BAL), endotracheal aspirates (ETA), pleural fluid, wound swabs, blood, urinary catheters, and ear swabs, following the principle of one isolate per patient. All isolates included in this study were collected between September 2024 and July 2025.

2.2. Isolation and Identification

The bacterial isolates were first identified by their morphological features on MacConkey agar, cetrimide agar, blood agar, and nutrient agar, then subjected to Gram staining and biochemical tests like catalase and oxidase assays. In addition, the Vitek® 2 (BioMérieux, France) or BD Phoenix M50 automated systems (BD Diagnostic Systems, USA) were used for confirming identification according to the manufacturer's instructions [6-8].

2.3. Antimicrobial Susceptibility Assay

Antimicrobial susceptibility testing of *P. aeruginosa* isolates was performed using automated identification and susceptibility testing (ID/AST) systems, including the Vitek 2 (AST-GN 417 and AST-GN 419 cards; BioMérieux, France) and BD Phoenix M50 (BD Diagnostic Systems, USA) [8]. Minimum inhibitory concentrations were determined for ampicillin (AMP), piperacillin/tazobactam (P/T), ceftazidime (CAZ), cefepime (FEP), ceftriaxone (CRO), imipenem (IMP), meropenem (MEM), amikacin (AK), levofloxacin (LEV), ciprofloxacin (CIP), gentamicin (GEN), tigecycline (TGC), and trimethoprim–sulfamethoxazole (SXT). Susceptibility to selected antibiotics was further assessed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to the 2025 guidelines of the Clinical and Laboratory Standards Institute (CLSI), providing standardized phenotypic confirmation of resistance profiles [9]. Isolates were classified as (MDR), (XDR) and (PDR) based on established criteria: MDR was defined as non-susceptibility to at least one agent in ≥3 antimicrobial classes, XDR as non-susceptibility to at least one agent in all but ≤2 antimicrobial classes, and PDR as non-susceptibility to all agents in all antimicrobial classes [10].

2.4. DNA Extraction

Genomic DNA was extracted using the Presto Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to manufacturer's instructions. The quantity and quality of extracted DNA samples were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.5. Molecular Identification of *P. aeruginosa*

The *gyrB* gene was amplified using a thermal cycler (Techne, USA), which confirmed the molecular identification of *P. aeruginosa*. Each PCR reaction was a 50 µL volume, containing 25 µL of 2X OnePCR™ Ultra SuperMix (GeneDireX/Taiwan), 1 µL of the forward primer, 1 µL of

the reverse primer, 3 µL of template DNA (50 ng), and 20 µL of nuclease-free dH2O. The specific PCR conditions, primer sequences, and amplicon size are shown in Table 1.

2.6. Molecular Detection of Virulence (*lasB*, *toxA*, *exoS*, and *algD*) Genes in *P. aeruginosa*

Through the utilization of a polymerase chain reaction (PCR), the virulence genes *lasB*, *toxA*, *exoS*, and *algD* were detected in the 36 isolates. The primer sequences and the settings for the PCR amplification are presented in Table 1.

2.7. Gel Electrophoresis and Visualization

The PCR-amplified products were stained with ethidium bromide in 1% agarose and subjected to gel electrophoresis (Cleaver Scientific, UK) in 1X TBE buffer at 80V for 60 minutes. Images were acquired using a Bio-Rad Gel Doc XR+ imaging system (Bio-Rad, USA) [11].

2.8. Molecular Typing (ERIC-PCR)

ERIC-PCR was used for molecular typing of all 36 clinical isolates. The procedure was done with a thermocycler and the primers listed in Table 1. Banding patterns were investigated in order to find the genetic relatedness among the isolates.

Table 1: The primer sequences, PCR settings, amplicon sizes, and references for the targeted genes.

Gene Primers	Primer Sequence Oligo sequences 5'→ 3 (10-50 bp)	Amplicon Size (bp)	PCR Condition for Amplification	References
gyrB-F	CCTGAC-CATCCGTCGCCACAAC	225	Initial denaturation at 94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 63°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[12]
gyrB-R	CGCAGCAGGATGCCGACGCC			
exoS-F	CTTGAAGGGACTCGACAAGG	504	Initial denaturation at94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 53°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[13]
exoS-R	TTCAGGTCCGCGTAGTGAAT			
algD-F	ATGCGAATCAGCATCTTTGGT	1311	Initial denaturation at94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 55°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[14]
algD-R	CTACCAGCAGATGCCCTCGGC			
toxA-F	GGAGCGCAACTATCCCACT	352	Initial denaturation at94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 52°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[15]
toxA-R	TGGTAGCCGACGAACACATA			
lasB-F	TCTACCCGAAGGACTGATAC	300	Initial denaturation at 94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 52°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[16]
lasB-R	AACACCCATGATCGCAAC			
ERIC-F	ATGTAAGCTCCTGGGGAT-TCAC	Variable	Initial denaturation at 94°C for 4 min, (Denaturation at 94°C for 30 Sec, annealing at 55°C and 1 min, extension at 72°C for 2 min, this was repeated for 35 cycles). With Final extension at 72°C for 5 min.	[17]
ERIC-R	AAGTAAGTGACTGGGGTGAGCG			

2.9. Biofilm Formation

Biofilm formation was assessed using the 96 well microtiter plate assay. Bacterial cultures were grown in TSB at 37°C for 24 hours. The resulting cultures were then adjusted to match McFarland standard No. 0.5 using the same medium as a diluent. A 200 µl suspension of each bacterial isolate was transferred into three separate wells of a 96-well flat-bottomed polystyrene plate and incubated at 37°C for another 24 hours.

After incubation, each well was washed three times with distilled water, then thoroughly dried. The adhered bacterial cells were stained with 200 µl of 0.1% crystal violet for 20 minutes. Washing the biofilm several times, and 200 µl of ethanol in each well dissolved the crystal violet that had stuck to it, and wells with broth that didn't have bacteria in them were used as negative controls. This assay was performed in triplicate, ELISA microplate reader

(BioTek, USA) reader was used to measure the optical density (OD) at 595 nm. The absorbance values showed how much biofilm had formed on the surface of the microtiter plate [18]. The results were divided into four groups based on the measured OD values, as follows:

ODT ≤ ODC = non-biofilm producer.

ODC < ODT ≤ 2 ODC = Weak biofilm producer.

2 ODC < ODT ≤ 4 ODC = Moderate biofilm producer.

4 ODC < ODT = Strong biofilm producer. ODT shows how much the tested isolates absorb, and ODC shows how much the control wells absorb.

2.10. Ethical Approval

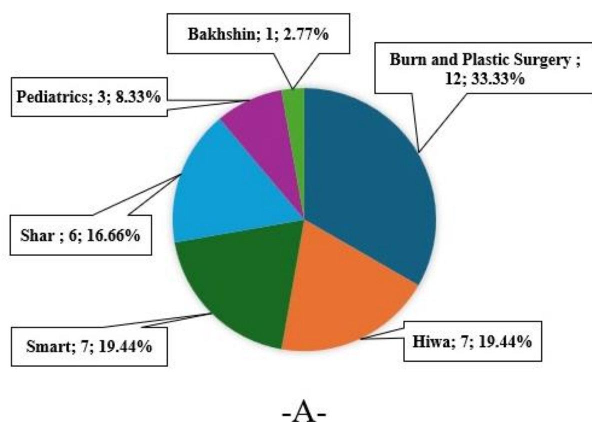
This study was ethically approved by the Research Ethics Committee of the College of Medicine University of Sulaimani, Kurdistan Region of Iraq. Approval was granted under the reference number 24102024-25-340 on October 10, 2024.

3. Results and Discussions

3.1. 3.1 Distribution of *P. aeruginosa* from Clinical Specimen

This study collected samples from different hospitals and clinics, including Burn and Plastic Surgery (12, 33.33%), Shar (6, 16.66%), Pediatric (3, 8.33%), Hiwa (7, 19.44%) public hospitals and Smart (7,19.44%) and Bakhshin (1, 2.77%) private hospitals which located in the Sulaymaniyah city. Variations in isolation rates may reflect differences in patient population, sampling frequency, or infection control strategies used in these healthcare settings. However, neither the implementation nor efficacy of infection control measures are directly evaluated in this study.

The percentages of the clinical sample sources were as follows: wounds (burn and surgical) (18, 50%), respiratory (bronchoalveolar lavage/endotracheal aspirates) (11, 30.55%), blood (4, 11.11%), ear swabs (2, 5.55%) and urinary catheter (1, 2.77%) specimens (Figure 1).



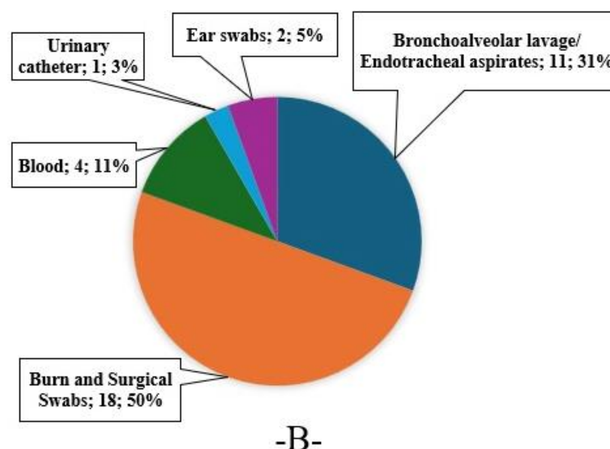


Figure 1: The distribution of clinical samples across various medical facilities. A: Percentages of the sample varied across different hospitals and clinics. B: Percentages of the clinical sample sources.

The results show that most of the isolates came from samples of wounds and respiratory tract samples. Wound sepsis is the main cause of death after burn injuries. *P. aeruginosa* is one of the highly concerns bacteria that can make it harder for physicians to treat patients and even kill them [19]. Infections frequently originate from a patient's own skin flora or from the surrounding hospital environment. Upon admission, patients are increasingly vulnerable to healthcare-associated infections (HAIs) and colonization by (MDR) microorganisms. Such risks are commonly associated with contaminated hospital surfaces, cross-transmission by healthcare personnel and other patients, or exposure to invasive medical devices [20]. Several factors drive the incidence of HAIs, including non-compliance with hygiene protocols, insufficient sterilization of reusable medical equipment, and prolonged use of indwelling catheters. These risks are further intensified by inappropriate antibiotic use, patient immunosuppression, and overcrowded healthcare facilities, highlighting the critical need for stringent infection control practices [21].

Infections caused by *P. aeruginosa* in BAL samples, indicating involvement of the lower respiratory tract, are typically associated with increased clinical severity. Lower respiratory tract infections, including pneumonia and ventilator-associated pneumonia, pose significant risks, particularly in immunocompromised patients or those with severe underlying illnesses. In the pulmonary environment, *P. aeruginosa* frequently forms biofilms, which contribute to persistent infection, increased resistance to antimicrobial therapy, and challenges in clinical management can lead to infections that last a long time and are hard to treat. Infections in the lungs can also lead to sepsis, which makes problems in other parts of the body more likely. Overall, respiratory infections with *P. aeruginosa* are associated with higher morbidity and mortality rates, making them more dangerous compared to infections in other sites [22].

3.2. Phenotypic Identification of *P. aeruginosa*

The bacterial isolates samples were morphologically analyzed and confirmed as *P. aeruginosa*. They were Gram-negative, rod-shaped, and had a characteristic grape-like odor. On Cetrimide agar and Muller Hinton agar, their colonies looked bluish-green. The oxidase and catalase tests came back positive for all of the isolates. The Vitek 2 compact systems or the BD Phoenix^{TM} automated system were used to confirm the identity (Figure 2).

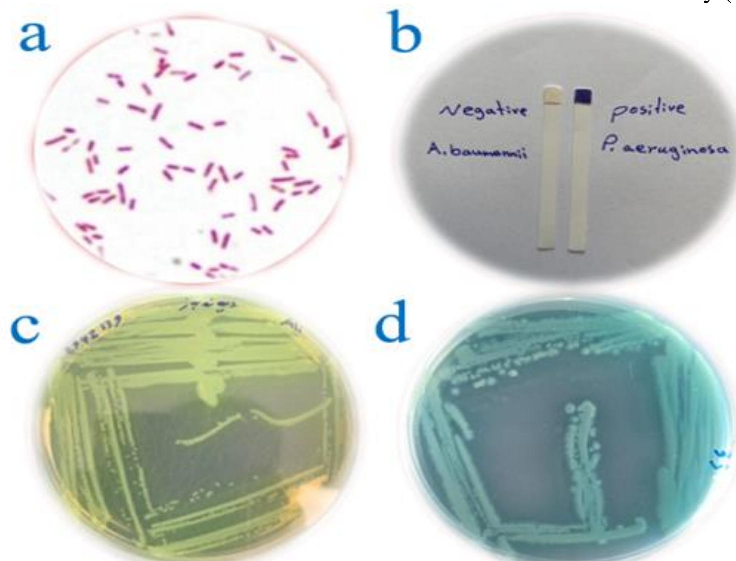


Figure 2: An example of phenotypic identification of P. aeruginosa. A. Microscopical examination (Gram stain) of the bacteria B. Positive for oxidase reaction C. Cetrimide agar without emphasizing pigment production. D. Bluish-green colony on Cetrimide agar production of pyocyanin.

3.3. Molecular Identification of *P. aeruginosa*

Polymerase chain reaction (PCR) was used to confirm the molecular identity of *P. aeruginosa*. All thirty-six samples (100%) tested positive for the *gyrB* gene (Figure 3). The *gyrB* gene (which codes for DNA gyrase subunit B) is a very specific and reliable target for PCR to confirm the identification of *P. aeruginosa*. This method is faster and more accurate than traditional methods, which is crucial for finding infections in clinical samples or environmental sources [12, 23, 24].

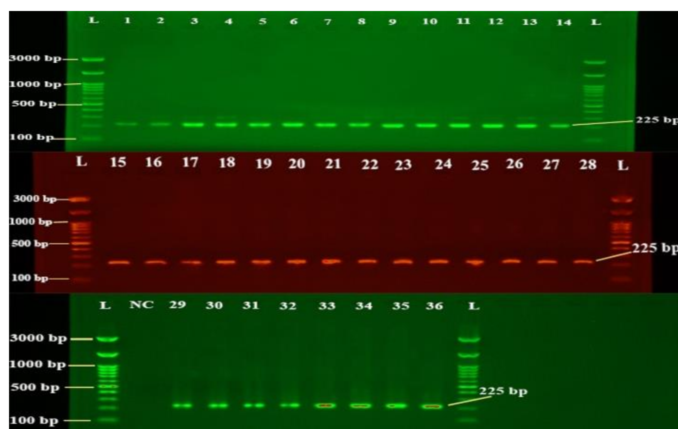


Figure 3: Agarose gel electrophoresis conducted for the *gyrB* gene (225 bp) of *P. aeruginosa*, *Lane* 1-36: Positive samples, NC: negative control, L: 100 bp DNA ladder.

3.4. Antibiotic Susceptibility Test (AST)

The results of the AST results of the bacterial samples were shown a remarkable resistance patterns to most antibiotic classes, particularly antibiotics chosen for treating *P. aeruginosa* infections. The bar chart (Figure 5) shows the percentage of *P. aeruginosa* isolates that are resistant to a group of antibiotics, including both common and more advanced ones.

Resistance to ticarcillin/clavulanic acid was observed in 93% of the bacterial isolates, indicating that this β-lactam/β-lactamase inhibitor combination is largely ineffective against the tested strains. This finding indicates that this β-lactam/β-lactamase inhibitor combination is largely ineffective against the tested isolates. High resistance rates were also detected for aztreonam (82%), tobramycin (72%), norfloxacin and levofloxacin (69% each), and ciprofloxacin (67%), reflecting a clear trend of resistance to both fluoroquinolones and aminoglycosides.

Among the carbapenems, resistance to imipenem and meropenem was observed in 56% and 39% of isolates, respectively. Cephalosporins also demonstrated moderate to high resistance levels, with resistance rates of 56% for ceftazidime and 42% for cefepime. In contrast, newer β-lactam/β-lactamase inhibitor combinations showed comparatively lower resistance rates, including ceftolozane/tazobactam (30%), ceftazidime/avibactam (43%), and piperacillin/tazobactam (42%), suggesting these agents may still retain some therapeutic value. Notably, colistin exhibited the lowest resistance rate (10%), indicating that it remains one

of the last-resort treatment options for infections caused by resistant *P. aeruginosa*.

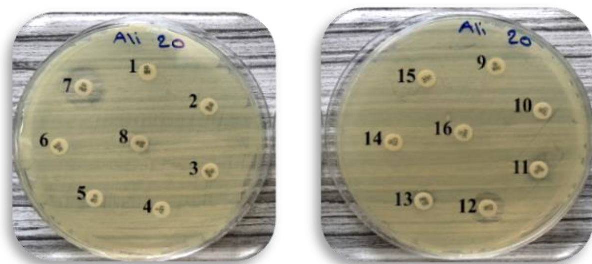


Figure 4: Kirby-Bauer Disk Diffusion Assay Results. 1:Ampicilin (AM), 2:Ticarcillin–Clavulanic acid (TIM), 3:Norfloxacin (NOR), 4:Levofloxacin (LEV), 5:Ciprofloxacin (CIP), 6:Trimethoprim–Sulfamethoxazole (SXT), 7:Imipenem (IMP), 8:Doxycycline (DO), 9: Tobramycin (TOB), 10:Ceftazidime (CAZ), 11:Cefepime (FEP), 12:Colistin (CT), 13:Piperacillin (PRL), 14:Cefotaxime (CTX),15:Ticarcillin (TIC), 16:Amoxicillin-Clavulanate (AMC).

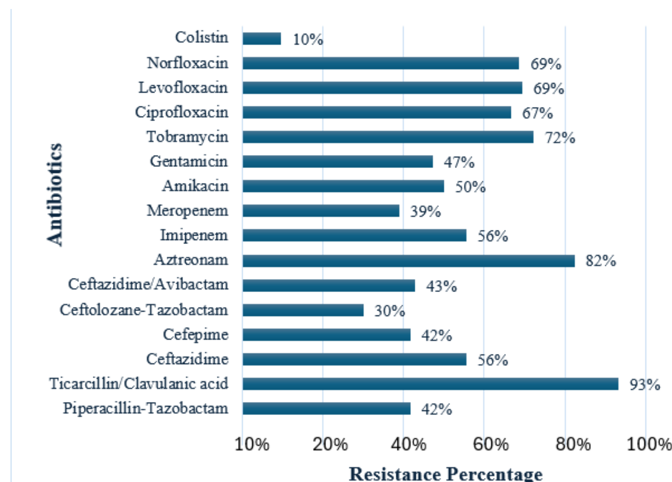


Figure 5: Percentage of *P. aeruginosa* isolates resistant to different antibiotics. Colistin exhibited the lowest resistance, while ticarcillin/clavulanic acid showed the highest resistance.

Table 2: Phenotypic resistance patterns of *pseudomonas aeruginosa* isolates classified as MDR, XDR, and PDR. R: resistance, S: sensitive.

Antimicrobial Classes	Resistance Pattern of *P. aeruginosa*											
	MDR Patterns (P)								**XDR Patterns (P)**			**PDR Patterns(P)**
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P1**	**P2**	**P3**	**P1**
Piperacillin/ Tazobactam	R	R	S	S	S	S	S	S	R	S	R	R
Cephalosporins	S	S	R	R	R	R	S	R	R	R	R	R
Polymyxins	S	S	S	S	S	S	S	S	S	S	S	R
Carbapenems	S	S	S	R	R	S	S	S	S	R	R	R
Aminoglycosides	S	S	R	S	S	S	S	S	R	R	R	R
Fluoroquinolones	S	R	S	R	S	S	R	R	R	R	R	R
Total (%)	**17/36 (47.2%)**								**13/36 (36.11%)**			**3/36 (8.3%)**

The distribution of drug-resistant *P. aeruginosa* isolates varied across the six hospitals (Figure 6).

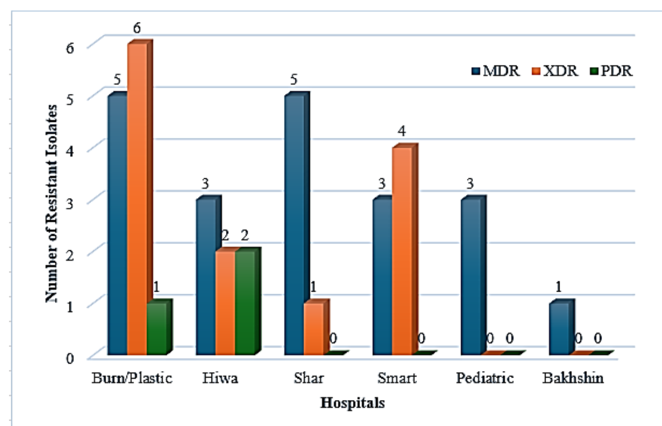


Figure 6: Distribution of (MDR), (XDR), and (PDR) isolates across different hospitals (Burn/Plastic, Hiwa, Shar, Smart, Pediatric, and Bakhshin). MDR isolates were detected in all hospitals, while XDR and PDR iso-lates were mainly observed in Burn/Plastic and Hiwa hospitals.

Burn/Plastic Hospital showed the highest burden of resistance, with 5 MDR, 6 XDR, and 1 PDR isolates (12 resistant isolates including those resistant to colistin), indicating a major clinical concern. Hiwa Hospital reported seven resistant isolates, including 3 MDR, 2 XDR, and 2 PDR strains, and together with Burn/Plastic was the only hospital where PDR isolates were detected. Shar Hospital had six resistant isolates (5 MDR and 1 XDR) with no PDR strains. Smart Private Hospital recorded seven resistant isolates consisting of 3 MDR and 4 XDR strains, while Pediatric Hospital had only 3 MDR isolates and no XDR or PDR cases. Bakhshin Private Hospital showed the lowest resistance burden, with only a single MDR isolate and no XDR or PDR detected. Overall, these findings demonstrate

notable variation in antimicrobial resistance profiles among the hospitals, with Burn/Plastic and Hiwa showing the most concerning resistance patterns.

The overall prevalence of resistance among the studied isolates was significant, with MDR, XDR, and PDR rates of 47.2%, 36.11%, and 8.3%, respectively. Local studies have documented variable findings: in Erbil, MDR and XDR rates were 76% and 20% [25], in Basrah, 72.6% and 91.3% [26] in Sulaymaniyah, 36.7% and 22% [27], and in Diyala, 30.8% and 33.3% [28]. Resistance levels also differed in nearby countries, with higher rates reported in Iran (58% MDR, 26.8% XDR) [29]and Syria (54% MDR) [30], moderate levels in Jordan (52.5% MDR, 34.1% XDR) [13], and lower prevalence in Kuwait (13.8% MDR, 6.2% XDR) [31] and Saudi Arabia (7.3% MDR, 4.1% XDR) [32].

In Europe, resistance patterns were also very different. For example, Greece had the highest rates (38.3% MDR, 30.6% XDR) [33], Spain had the second highest (27.2% MDR, 14.4% XDR) [14], and Poland had the third highest (32.7% MDR, 15.2% XDR) [34], France, on the other hand, had the lowest rates (19% MDR, 7% XDR) [35].

As a conclusion, Iraq has one of the highest rates of (MDR) and (XDR) *P. aeruginosa* in the region, higher than most of its neighbors and many parts of Europe. These results suggest that we must enhance our management of antimicrobials and improve infection control measures to prevent untreatable outbreaks and those occurring in healthcare environments.

3.5. Molecular Detection of Virulence Genes (*lasB, toxA, exoS, algD*) in *P. aeruginosa*

Molecular analysis was conducted to detect the presence of virulence genes (*lasB, toxA, exoS, and algD*) in all clinical isolates of *P. aeruginosa*. Gel electrophoresis of the PCR-amplified products revealed that 36 isolates (100%) possessed the *lasB* gene (Figure 7), 32 isolates (88.9%) contained the *toxA* gene (Figure 8), 19 isolates (52.7%) carried the *exoS* gene (Figure 9), and 36 isolates (100%) carried the *algD* gene (Figure 10).

In total, 18 isolates (50%) were positive for all of the virulence genes that were investigated. 32 isolates (88.9%) had *lasB*, *algD*, and *toxA*; 19 isolates (52.7%) had *lasB, algD*, and *exoS*; and 18 isolates (50%) had both *toxA* and *exoS*.

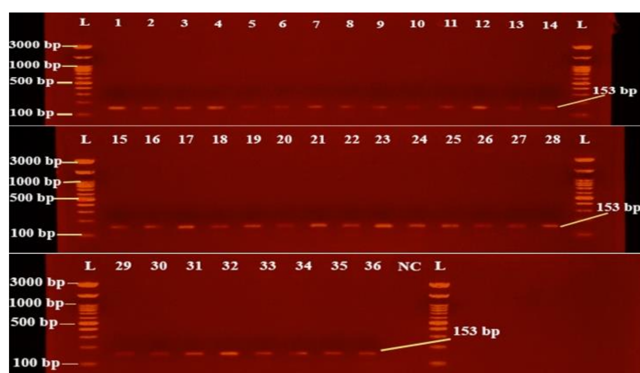


Figure 7: Agarose gel electrophoresis conducted for the *lasB* gene (153 bp) of *P. aeruginosa*, Lane 1-36: Positive samples, NC: negative control, L: 100 bp DNA ladder.

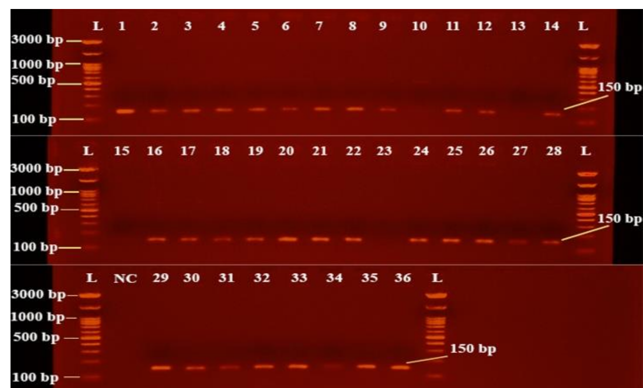


Figure 8: Agarose gel electrophoresis conducted for the *toxA* gene (150 bp) of *P. aeruginosa*, Lane 1-9, 11-12, 14, 16-22, and 24-36: Positive samples, NC: negative control, L: 100 bp DNA ladder.

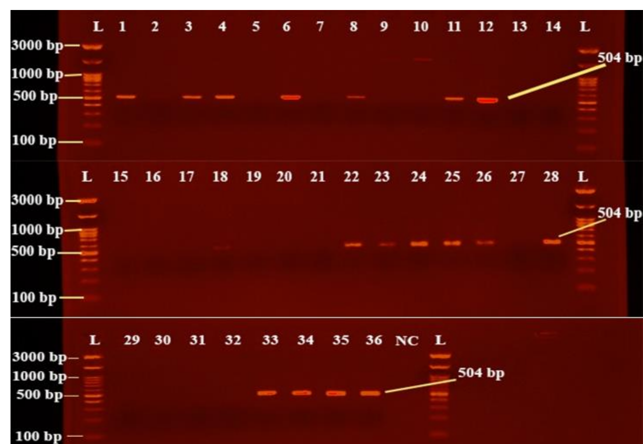


Figure 9: Agarose gel electrophoresis conducted for the *exoS* gene (504 bp) of *P. aeruginosa*, Lane 1-4, 6, 8, 11-12, 18, 22-26, 28, and 33-36: Positive samples, NC: negative control, L: 100 bp DNA ladder.

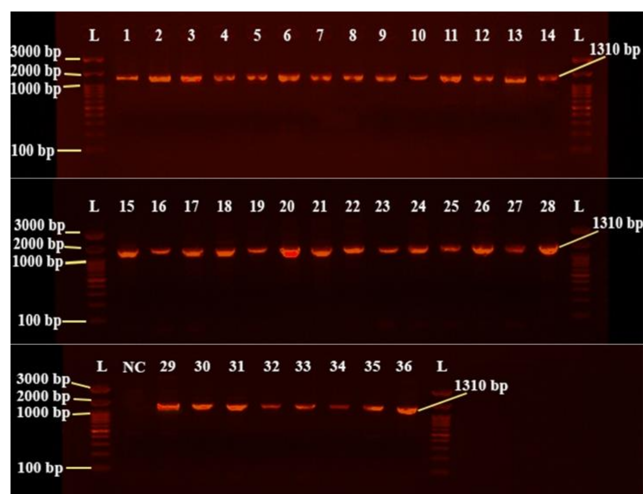


Figure 10: Agarose gel electrophoresis conducted for the *algD* gene (1310 bp) of *P. aeruginosa*, Lane 1-36: Positive samples, NC: negative control, L: 100 bp DNA ladder.

The present data indicated that the virulence genes were not evenly spread among *P. aeruginosa* isolates. PCR analysis showed that all isolates had the *lasB* and *algD* genes, which suggests that these genes are important for pathogenicity. Interestingly, approximately half of the isolates

carried all four virulence genes, suggesting a higher potential for pathogenicity.

The gene combinations that were found to be most common were *lasB, algD*, and *toxA* (88.9% of the isolates), while a lower percentage (52.7%) had *lasB*, *algD*, and *exoS*. Fifty percent of the isolates also had *exoS* and *toxA*. Different clinical strains of *P. aeruginosa* exhibit different levels of virulence, which can be explained in part by the genetic variety of this bacterium. Similarities and differences were documented in the results when we compared this report to others from Iraq and the neighboring countries. We found that in Erbil *lasB* and *algD* genes were very common (100% and 91.1%, respectively) but *exoS* was less common (62.4%)[25], while in Baghdad *exoS* was more common (87%), comparing to *lasB* and *algD gens* (28% and 29%, respectively) [36].

Similarly, Iranian studies found *toxA* levels between 55% and 60% and consistently high levels of *lasB* at 95%. However, the prevalence of *exoS* varied greatly, with some studies finding as many as 90% and others finding as little as 5%[37, 38]. Much to our isolates, Turkish isolates showed a high incidence of *lasB* (98-100%) and *algD* (87-93%), whereas *toxA* (62.9-66.6%) and *exoS* (50-62.8%) differed among investigations [16, 39].

Contrarily, Egyptian isolates consistently exhibited high frequencies of all four genes: *toxA* (76-86%), *exoS* (82-84%), *algD* (80-91%), and *lasB* (89-90%), indicating that the community had numerous virulence factors [33, 40]. Polish isolates in Europe exhibited elevated levels of *toxA* (96.3%), *lasB* (90%), and *algD* (92.5%), while *exoS* levels were moderate (58.9%), aligning with our findings [34].

The high levels of *lasB* and *algD* in our samples, along with reports from nearby countries and Europe, suggest that they are likely important in the development of disease. On the other hand, the different levels of *toxA* and *exoS* in different places may have an effect on how bad the disease is and how it spreads in different areas.

3.6. Prevalence of all Virulence Genes across Different Sample Sources

When the isolates that contained all of the virulence genes simultaneously were investigated, ear samples reported the highest rate at (1/1, 100%), followed by bronchoalveolar lavage samples at (5/6, 83.3%) and endotracheal aspiration samples at (3/4,75%). Blood samples showed the lowest rate, (2/5, 40%), and wound samples, which have the highest total count 18, recorded a relatively low rate of 28%. Figure 11 shows how the presence of virulence genes varies between clinical isolation sites.

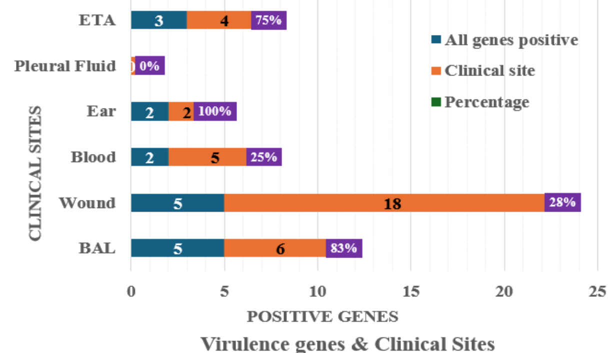


Figure 11: Distribution of all virulence genes (*lasB, toxA, exoS, and algD*) among various clinical isolation sites. Orange bar indicating showing the total number of samples collected. Blue bar indicating the number of samples positive for all tested virulence genes. Yellow bar shows the percentage of positive samples compared to the total for that source.

3.7. Relation between Virulence Genes and Resistance Profile of Studied *P. aeruginosa* Isolates

The isolates were divided into four patterns based on whether or not they have particular virulence genes: Pattern I (PI) was the most common, making up 50% (n = 18) of the isolates. All isolates in this group tested positive for all four virulence genes, which means they had a very virulent profile. Pattern II (P II) made up 39% (n = 14) of the isolates. These strains were positive for *lasB, toxA*, and *algD* but negative for *exoS*. This shows that the virulence is a little lower than that of PI. Eight percent (n = 3) of the isolates showed Pattern III (P III), which means they were positive for *lasB* and *algD* but negative for both *toxA* and *exoS*. This means they had a less virulent profile.

Finally, Pattern IV (P IV) made up just 3% (n = 1) of the isolates. It was positive for *lasB*, *exoS*, and *algD*, but negative for *toxA*. This was a unique gene combination. MDR isolates were most common in PI (67%), followed by PII (50%) and PIII (33%), while no MDR isolates were detected among PIV. The highest XDR profile was recorded in PIV (1/1, 100%), followed by PIII (67%) and PII (36%), while PI recorded only 28%. Among 3 PDR isolates, 2 of them were in PII(14%) and 1 in PI(6%).

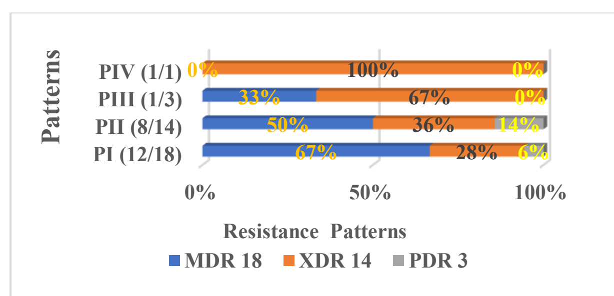


Figure 12: Illustrates the interaction among various *P. aeruginosa* virulence gene patterns (P I, P II, P III, P IV) and their corresponding antibiotic resistance profiles. P I: positive for all genes *lasB, toxA, exoS; and algD*; P II: lack *ExoS*; P III: lake *toxA, exoS*; and P IV: lake *toxA*.

In this investigation, wound isolated samples (18) recorded the highest resistance profiles, and formed strong biofilms which help bacteria stick to their environment protect them from medications and the body's defenses, and make infections endure longer. Hence, it will be hard to treat these diseases because there aren't many antibiotics left to kill them. They all had *lasB* and *algD* virulence genes, which break down tissue by breaking down elastase and protease (*lasB*) and keeping biofilms alive through alginate (*algD*). There were 14 isolates-with *toxA* and 7 with *exoS*. These made-exotoxin A and type III secretion effectors, which made them more dangerous.

This distribution pattern shows that respiratory tract isolates (BAL) are much more likely to have all of the virulence genes than systemic (blood) or superficial wound sources. The elevated frequency of virulence genes in respiratory isolates likely reflects their selective advantage in colonizing and persisting within the pulmonary environment, particularly in mechanically ventilated patients. Conversely, the lower prevalence in blood and wound isolates may suggest reliance on alternative pathogenic strategies, or that these infections arise opportunistically without requiring the full complement of virulence factors. Respiratory isolates that have multiple virulence genes are more likely to spread and cause serious illnesses in healthcare settings [35].

3.8. Detection of Biofilm Formation

Following 24 hours of incubation at 37°C in TSB medium, all 36 tested *P. aeruginosa* isolates demonstrated biofilm formation. The mean OD595 of all isolates was 0.70, which was approximately 7.6 times higher than that of the negative control (mean ODC = 0.092), indicating that the isolate was a strong biofilm producer. Among all isolates tested, 92% exhibited strong biofilm formation, 8% moderate, 0% weak, and 0% showed no adherence. (Figure 13).

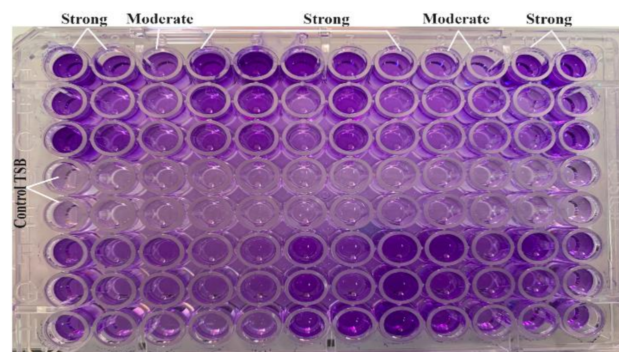


Figure 13: Detection of *P. aeruginosa* biofilm using 96-well microtiter plates assay.

Significant amounts of biofilm are produced by *P. aeruginosa* isolates from wound and BAL samples, but less is produced by ear isolates and moderate amounts are produced by blood isolates.

In the present work, we also investigate the correlation between strong biofilm formation (OD > 4X of the control), virulence genes, and different clinical isolation sites. It was found that the *exoS* gene was related to the clinical source more than other virulence genes, as 100% of the ear samples were positive for this gene, while BAL samples counted 68%, followed by wound (39%) and blood (25%) samples, On the other hand, the genes *algD, lasB*, and *toxA* are consistently present in isolates that generate strong biofilms, irrespective of the clinical sites (Figure 14).

	Strong Biofilm Formation	toxA	lasB	exoS	algD
BAL	100%	100%	100%	64%	100%
WOUND	100%	78%	100%	39%	100%
BLOOD	75%	100%	100%	25%	100%
EAR	0%	100%	100%	100%	100%
Pleural	100%	100%	100%	0%	100%
ETA	100%	100%	100%	75%	100%

Figure 14: Relationship among strong biofilm production, virulence genes and different clinical isolation sites. BAL: Bronchoalveolar lavage, ETA: Endotracheal aspiration.

P. aeruginosa has a very flexible genome that lets it change its virulence factors based on the ecological pressures of its environment. Mitov et al. (2010) found that respiratory isolates had a lot more elastase and phospholipase C–encoding loci than blood isolates, which is a genomic richness that is not often seen in blood isolates[41]. In a similar manner, Qin et al. (2022) demonstrated that BAL specimens from patients with ventilator-associated-pneumonia exhibited a significantly heightened prevalence of *exoS*, *exoU*, and *algD*, surpassing the genetic diversity observed in wound isolates [42]. Approximately 80% of respiratory isolates carried multiple virulence gene cassettes, while only about one-third of blood isolates displayed a similar genomic profile. This difference emphasizes adaptive variations that are likely shaped by the differing pressures in their microenvironments [43].

These molecular findings align with earlier research, highlighting a strong link between the types of virulence factors and the ability of *P. aeruginosa* to form biofilms. The fact that PI and PII profiles are the-most common among strong biofilm producers suggests that these genotypes are especially good at growing in biofilms. This is because virulence expression and biofilm development are linked through shared regulatory pathways [37].

This change probably helps-bacteria stay alive longer in chronic-infections, which makes them harder to treat. Because of this, isolates with PI and PII virulence profile may be linked to longer infections and more resistance to antibiotics. This shows that we-need to use targeted treatment methods and improve infection control measures.

3.9. Molecular Typing (ERIC-PCR)

In this study, the ERIC primer was used to detect variations in the distribution and copy number of this repetitive element within the genomes of clinical *P. aeruginosa* isolates. ERIC-PCR amplification of 36 *P. aeruginosa* isolates collected from different sources in different hospitals produced banding patterns ranging from 3 to 11 bands per isolate, with fragment sizes between 100 and 1500 bp (Table 3). The most frequent bands were 28 bands of about 800 bp and the least frequent bands were 7 bands of about 1000 bp. The dendrogram (Figure 15) based on the ERIC-PCR patterns grouped isolates into 29 genotypes 36 *P. aeruginosa* with 80% similarity as the cut-off with a discriminatory power (D) of 0.8175.

ERIC-PCR analysis classified the isolates into three major clusters (C1, C2, and C3), indicating notable genetic diversity. Shar Hospital included six isolates from respiratory sources, which consisted of five bronchoalveolar lavages and one endotracheal aspirate. Four of these isolates had two sets of identical genotypes in C3 (one set of them shared the same resistance and virulence profiles), and the other set had identical genotypes in C2 but not necessarily shared the same resistance profile. The Burn/Plastic hospital had 12 isolates, all from wound samples. Ten of these isolates had different genotypes and were grouped in C1. Only two of these genotypes were identical and were

grouped in C3 and almost different virulence and resistance profiles. The Hiwa Hospital had seven isolates, all from C2. They came from four wound samples, one BAL sample, and two blood samples. None of these isolates were genetically identical and the virulence and resistance profiles are often varied. The Pediatric hospital provided three isolates from respiratory sources (endotracheal aspirate). Two of these isolates exhibited similar genotypes categorized as C3 with identical resistance-profile but different virulence-pattern, while the remaining isolate was documented in C1.

Smart Private Hospital included seven-isolates, all grouped within C2. Three of these isolates showed identical genotypes (2 of them with same resistance and virulence pattern) from different sources (blood, wound, and ear). Additionally, two other identical genotypes were identified from separate sources (blood and wound) and showed different resistance pattern. The last two isolates were not related. Bakhshin Private Hospital had a single isolate located in C2, which was obtained from a catheter sample.

Table 3: Molecular weight and percentage of bacterial sample had the band.

Band	Molecular weight	No. of isolates (36)	Percentage %
ERIC 1	**100bp**	10	27.77%
ERIC 2	**200bp**	18	50%
ERIC 3	**300bp**	18	50%
ERIC 4	**400bp**	27	75%
ERIC 5	**500bp**	27	75%
ERIC 6	**600bp**	26	72.20%
ERIC 7	**700bp**	20	55.55%
ERIC 8	**800bp**	28	75%
ERIC 9	**900bp**	23	63.88%
ERIC 10	**1000bp**	4	11.11%
ERIC 11	**1500bp**	7	19.44%

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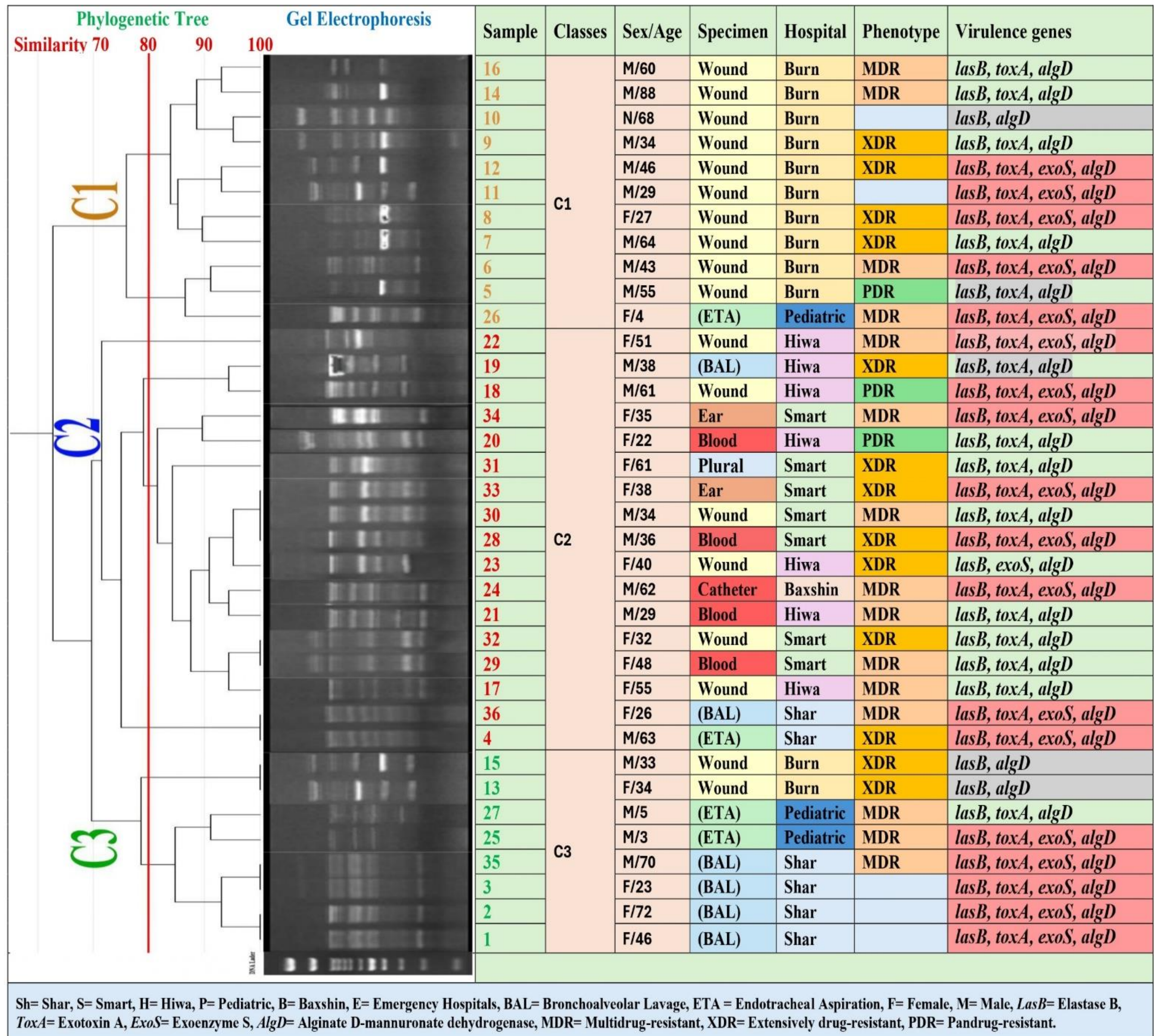

Sample	Classes	Sex/Age	Specimen	Hospital	Phenotype	Virulence genes
16	C1	M/60	Wound	Burn	MDR	*lasB, toxA, algD*
14		M/88	Wound	Burn	MDR	*lasB, toxA, algD*
10		N/68	Wound	Burn		*lasB, algD*
9		M/34	Wound	Burn	XDR	*lasB, toxA, algD*
12		M/46	Wound	Burn	XDR	*lasB, toxA, exoS, algD*
11		M/29	Wound	Burn		*lasB, toxA, exoS, algD*
8		F/27	Wound	Burn	XDR	*lasB, toxA, exoS, algD*
7		M/64	Wound	Burn	XDR	*lasB, toxA, algD*
6		M/43	Wound	Burn	MDR	*lasB, toxA, exoS, algD*
5		M/55	Wound	Burn	PDR	*lasB, toxA, algD*
26		F/4	(ETA)	Pediatric	MDR	*lasB, toxA, exoS, algD*
22	C2	F/51	Wound	Hiwa	MDR	*lasB, toxA, exoS, algD*
19		M/38	(BAL)	Hiwa	XDR	*lasB, toxA, algD*
18		M/61	Wound	Hiwa	PDR	*lasB, toxA, exoS, algD*
34		F/35	Ear	Smart	MDR	*lasB, toxA, exoS, algD*
20		F/22	Blood	Hiwa	PDR	*lasB, toxA, algD*
31		F/61	Plural	Smart	XDR	*lasB, toxA, algD*
33		F/38	Ear	Smart	XDR	*lasB, toxA, exoS, algD*
30		M/34	Wound	Smart	MDR	*lasB, toxA, algD*
28		M/36	Blood	Smart	XDR	*lasB, toxA, exoS, algD*
23		F/40	Wound	Hiwa	XDR	*lasB, exoS, algD*
24		M/62	Catheter	Baxshin	MDR	*lasB, toxA, exoS, algD*
21		M/29	Blood	Hiwa	MDR	*lasB, toxA, algD*
32		F/32	Wound	Smart	XDR	*lasB, toxA, algD*
29		F/48	Blood	Smart	MDR	*lasB, toxA, algD*
17		F/55	Wound	Hiwa	MDR	*lasB, toxA, algD*
36		F/26	(BAL)	Shar	MDR	*lasB, toxA, exoS, algD*
4		M/63	(ETA)	Shar	XDR	*lasB, toxA, exoS, algD*
15	C3	M/33	Wound	Burn	XDR	*lasB, algD*
13		F/34	Wound	Burn	XDR	*lasB, algD*
27		M/5	(ETA)	Pediatric	MDR	*lasB, toxA, algD*
25		M/3	(ETA)	Pediatric	MDR	*lasB, toxA, exoS, algD*
35		M/70	(BAL)	Shar	MDR	*lasB, toxA, exoS, algD*
3		F/23	(BAL)	Shar		*lasB, toxA, exoS, algD*
2		F/72	(BAL)	Shar		*lasB, toxA, exoS, algD*
1		F/46	(BAL)	Shar		*lasB, toxA, exoS, algD*

Sh= Shar, S= Smart, H= Hiwa, P= Pediatric, B= Baxshin, E= Emergency Hospitals, BAL= Bronchoalveolar Lavage, ETA = Endotracheal Aspiration, F= Female, M= Male, *LasB*= Elastase B, *ToxA*= Exotoxin A, *ExoS*= Exoenzyme S, *AlgD*= Alginate D-mannuronate dehydrogenase, MDR= Multidrug-resistant, XDR= Extensively drug-resistant, PDR= Pandrug-resistant.

Figure 15: Dendrogram Phylogenetic tree and agarose gel electrophoresis (1.5%TBE) for ERIC PCR products of *P. aeruginosa*. Identical bands (S1, S2), (S28, S30 and S33), (S3, S35), (S13, S15), (S4, S36) and (S29, S32), diverse bands (S5-S12, S14, S31, S34) and (S16-S27). Lane L: 100 bp DNA ladder.

The clustering pattern seen in the ERIC-PCR dendrogram demonstrates predominantly local dissemination of *P. aeruginosa* strains, since identical genotypes were confined to specific hospitals, no evidence of inter-hospital clonal spread was observed among the isolates analyzed.

As a result, the infections in each hospital seem to have originated from distinct bacterial strains that were likely introduced independently into those specific environments, rather than from a connected outbreak. These distinct strains may have arisen from a community, the hospital environment (e.g., water systems, surfaces), or the patients' own diverse colonizing microbiota.

The analysis of the remaining isolates (23/36, 64%) showed that the ERIC-PCR banding profiles exhibited significant genetic diversity. This suggests that multiple genotypes exist within the hospitals. The analysis also showed that isolates from different hospitals did not have the same genotypic profiles. This shows that strains were spreading independently in each hospital. These results show that the spread of the disease is complicated, which could make it harder to control. To better understand how different subtypes spread and stay in healthcare settings, a thorough study of the transmission pathways that are active in these

settings is needed. The genetic differences seen suggest that cases found in different hospitals are not related in terms of disease spread, which means that there is no one dominant clone. This pattern matches what other regional studies have found. For instance, research done in Iraq found a lot of genetic variation among hospital isolates, with many major, intermediate, and unique genetic clusters [44], This diversity may show that *P. aeruginosa* can set up local reservoirs and then spread between healthcare facilities.

Similar results have been found in Iran, where a lot of genetic diversity was found. There were also a lot of (MDR) and (XDR) isolates with different ERIC-PCR profiles [45]. This indicates that resistant clones are proliferating in the area. In conclusion, this study demonstrates that multidrug-resistant *P. aeruginosa* constitutes a substantial concern in healthcare environments in Sulaymaniyah City, particularly concerning respiratory tract and wound infections. It is very hard to control these infections because they have a lot of MDR, XDR, and PDR phenotypes, form strong-biofilms, and have a lot of different clonal lineages.

The fact that key virulence genes *lasB* and *algD* are found in many places, while *toxA* and *exoS* are found in fewer places, shows that the pathogen can adapt and that there is a lot of genetic diversity in different areas.

The results show that ERIC-PCR is a low-cost option than other methods. It gives researchers a reliable and quick way to "screen" *P. aeruginosa* strains because it is very cost-effective, easy to use, reliable, quick, and able to type *P. aeruginosa* strains.

Finally, these results show how important it is to improve antimicrobial stewardship, infection control measures, and molecular surveillance all the time in order to stop the spread of high-risk *P. aeruginosa* clones and contribute to patients' recovery.

4. Conclusion

This study provides molecular and phenotypic characterization of clinical *P. aeruginosa* isolates from hospitals in Sulaymaniyah City. A high prevalence of multidrug-resistant and extensively drug-resistant strains was observed, and most isolates demonstrated strong biofilm-forming ability. Molecular analysis revealed widespread distribution of the virulence genes *lasB* and *algD*, while *toxA* and *exoS* were present in a substantial proportion of isolates, indicating considerable pathogenic potential. ERIC-PCR typing showed notable genetic diversity, with identical genotypes mainly confined to isolates from the same hospitals, suggesting local clonal dissemination. These findings highlight the need for strengthened antimicrobial stewardship, strict infection-control practices, and continued molecular surveillance to limit the spread of high-risk *P. aeruginosa* strains.

5. References

[1] Silverio MP, Schultz J, Parise MT, Parise D, Viana MVC, Nogueira W, et al. Genomic and phenotypic insight into antimicrobial resistance of Pseudomonas fluorescens from King George Island, Antarctica. Front Microbiol. 2025;16:1535420. doi:10.3389/fmicb.2025.1535420

[2] Lewandowska W, Mahillon J, Drewnowska JM, Święcicka I. Insight into the phylogeny and antibiotic resistance of Pseudomonas spp. originating from soil of the Białowieża National Park in Northeastern Poland. Front Microbiol. 2025;16:1454510. doi:10.3389/fmicb.2025.1454510

[3] Kharat AS, Makwana N, Nasser M, Gayen S, Yadav B, Kumar D, et al. Dramatic increase in antimicrobial resistance in ESKAPE clinical isolates over the 2010–2020 decade in India. J Infect Public Health. 2024;63(5):107125. doi:10.1016/j.jiph.2024.107125

[4] Adenipekun EO, Akinleye EF, Tewogbade OA, Iwalokun BA. Detection of virulence genes and multidrug resistance in Pseudomonas aeruginosa clinical isolates from a public hospital in Lagos, Nigeria. New Microbes New Infect. 2023;22:e01950. doi:10.1016/j.nmni.2023.e01950

[5] Hematzadeh A, Haghkhah M. Biotyping of isolates of Pseudomonas aeruginosa isolated from human infections by RAPD and ERIC-PCR. Heliyon. 2021;7(9):e07967. doi:10.1016/j.heliyon.2021.e07967

[6] Cunrath O, Graulier G, Carballido-López A, Pérard J, Forster A, Geoffroy VA, et al. The pathogen Pseudomonas aeruginosa optimizes the production of the siderophore pyochelin upon environmental challenges. Metallomics. 2020;12(12):2108–2120. doi:10.1039/d0mt00029a

[7] Gülhan B, Çıkman A, Aydın M, Hasbek M, Özekinci T, Akyüz S, et al. Rapid detection of pathogens and resistance genes grown in blood cultures with two multiplex tandem real-time PCR kits. Infect Dis Clin Microbiol. 2025;7(1):37–46. doi:10.36519/idcm.2025.465

[8] Robert M, Ruffier d'Epenoux L, Paquin A, Boutoille D, Guillouzouic A, Corvec S, et al. Ciprofloxacin-susceptible but levofloxacin-resistant Pseudomonas aeruginosa clinical strains with Vitek® 2: mechanisms and clinical consequences. Eur J Clin Microbiol Infect Dis. 2025;44(3):549–558. doi:10.1007/s10096-024-05006-3

[9] Tang KWK, Millar BC, Moore JE. Antimicrobial resistance (AMR). Br J Biomed Sci. 2023;80:11387. doi:10.3389/bjbs.2023.11387

[10] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: international expert proposal. Clin Microbiol Infect. 2012;18(3):268–281. doi:10.1111/j.1469-0691.2011.03570.x

[11] Peña C, Suarez C, Gozalo M, Murillas J, Almirante B, Pomar V, et al. Impact of carbapenem resistance on mortality in Pseudomonas aeruginosa bloodstream infections. Antimicrob Agents Chemother. 2012;56(3):1265–1272. doi:10.1128/AAC.05991-11

[12] Jamaluddin IP, Musa SH, Ethica SN, Ansori AN, Yosephi V, Atmaja PY, et al. Detection of Pseudomonas aeruginosa using PCR targeting 16S rRNA and gyrB genes. Narra J. 2024;4(2):e774. doi:10.52225/narra.v4i2.774

[13] Al Dawodeyah HY, Obeidat N, Abu-Qatouseh LF, Shehabi AA. Antimicrobial resistance and virulence genes of Pseudomonas aeruginosa. Germs. 2018;8(1):31–40. doi:10.18683/germs.2018.1130

[14] Cotar AI, Chifiriuc MC, Dinu S, Bucur M, Iordache C, Banu O, et al. Molecular virulence markers in Staphylococcus aureus and Pseudomonas aeruginosa. Int J Mol Sci. 2010;11(12):5273–5291. doi:10.3390/ijms11125273

[15] Sabharwal N, Dhall S, Chhibber S, Harjai K. Molecular detection of virulence genes in Pseudomonas aeruginosa. Int J Appl Basic Med Res. 2014;4(2):103–107. doi:10.4103/2229-516X.136783

[16] Ertugrul BM, Oryasin E, Lipsky BA, Willke A, Bozdogan B. Virulence genes in diabetic foot infections. Infect Dis (Lond). 2018;50(4):273–279. doi:10.1080/23744235.2017.1393839
[17] Najem SA-A, Shubbar EE. Molecular analysis of Pseudomonas aeruginosa by ERIC-PCR. Int J Health Sci. 2022;6(S1):10144–10151. doi:10.53730/ijhs.v6nS1.7203
[18] Cai Z, Zhang W, Liao G, Huang C, Wang J, Zhang J. Biofilm inhibition mechanisms. J Water Process Eng. 2025;69:106613. doi:10.1016/j.jwpe.2024.106613
[19] Fuentes-González MF, Fernández-Rodríguez D, Colín-Castro CA, et al. Gram-negative bloodstream infections in burn patients. Int J Mol Sci. 2024;25(19):10458. doi:10.3390/ijms251910458
[20] Akinsulie OC, Aliyu VA, Idris I, et al. The implications of handwashing and skin hygiene on infectious disease dynamics: the African scenario. Trop Med Infect Dis. 2024;9(4):82. doi:10.3390/tropicalmed9040082
[21] Qader AR, Muhamad JA. Nosocomial infection in Sulaimani burn hospital, Iraq. Ann Burns Fire Disasters. 2010;23(4):177–181.
[22] Chen Y, Xu M, Pan J, et al. Moxifloxacin-loaded nanoparticles for overcoming multidrug resistance. ACS Appl Mater Interfaces. 2025;17(4):5695–5709. doi:10.1021/acsami.4c14991
[23] Azemin A, Alias N, Kari A. Identification of Pseudomonas aeruginosa from sheep milk. Malays J Fundam Appl Sci. 2022;18(1):30–42. doi:10.11113/mjfas.v18n1.2302
[24] Hassan KI, Abdullah SR. Detection of Pseudomonas aeruginosa using PCR. Baghdad Sci J. 2018;15(4):401–405. doi:10.21123/bsj.2018.15.4.0401
[25] Aziz SH, Abdulrahman TT, Hamad A. Molecular characterization of virulence factors in Pseudomonas aeruginosa. J Adv Pharm Educ Res. 2024;14(S1):1–10. doi:10.51847/6zXyY9bJ8L
[26] Alkhulaifi ZM, Mohammed KA. Prevalence and molecular analysis of antibiotic resistance of Pseudomonas aeruginosa in Iraq. Iran J Microbiol. 2023;15(1):45–52. doi:10.18502/ijm.v15i1.11917
[27] Omer KA, Arif SK, Othman HE. Molecular detection of MBL genes in Pseudomonas aeruginosa. Bahrain Med Bull. 2024;46(2):2075-2079. doi:10.26715/bmb.46.2.2
[28] Alsaadi LA, Al-Dulaimi AAF, Rasheed HR. Carbapenem resistance genes in Iraq. Indian J Public Health Res Dev. 2020;11(2):2550-2554.doi:10.37506/ijphrd.v11i2.195173
[29] Vaez H, Salehi-Abargouei A, Ghalehnoo ZR, Khademi F. Multidrug-resistant Pseudomonas aeruginosa in Iran. J Glob Infect Dis. 2018;10(4):212–217. doi:10.4103/jgid.jgid_113_17
[30] Mahfoud M, Al Najjar M, Hamzeh AR. Multidrug resistance in Pseudomonas aeruginosa in Syria. J Infect Dev Ctries. 2015;9(2):210–213. doi:10.3855/jidc.5643
[31] Abulhasan YB, Abdullah AA, Shetty SA, et al. Healthcare-associated infections in neurocritical care. Neurocrit Care. 2020;32(3):836–846. doi:10.1007/s12028-019-00856-8
[32] Al-Tawfiq JA, Rabaan AA, Saunar JV, Bazzi AM. Antimicrobial resistance in Saudi Arabia. J Infect Public Health. 2020;13(5):737–745. doi:10.1016/j.jiph.2020.01.004
[33] Ghanem SM, Abd El-Baky RM, Abourehab MA, et al. Virulence and resistance genes. Infect Drug Resist. 2023;16:2371–2385. doi:10.2147/IDR.S403441
[34] Bogiel T, Depka D, Rzepka M, et al. Biofilm and toxin genes. Antibiotics (Basel). 2021;10(3):241. doi:10.3390/antibiotics10030241
[35] Hibbert TM, Whiteley M, Renshaw SA, et al. Emerging strategies to target virulence in Pseudomonas aeruginosa respiratory infections. Crit Rev Microbiol. 2024;50(6):1037–1052. doi:10.1080/1040841X.2023.2285995
[36] Ali AM, Al-Kenanei KA, Hussein SA, Bdaiwi QO. Molecular study of virulence genes of Pseudomonas aeruginosa. Med J Babylon. 2020;31(1):26–41. doi:10.4103/MJOB.MJOB_35_19
[37] Ghasemian S, Karami-Zarandi M, Heidari H, et al. Molecular characterization of Pseudomonas aeruginosa. J Clin Lab Anal. 2023;37(4):e24850. doi:10.1002/jcla.24850
[38] Shariat A, Alizadeh S, Hoseinabadi MJ, Movagharnejad M. Molecular detection of virulence genes. Avicenna J Clin Microbiol Infect. 2018;5(1):28–34. doi:10.5812/ajcmi.64332
[39] Berber I, Avsar C, Yegin Z, et al. Molecular epidemiology of Pseudomonas aeruginosa. Braz J Infect Dis. 2016;20(2):224–225. doi:10.1016/j.bjid.2015.12.002
[40] Ali SA, Ghamry AA, Soliman AM, et al. Virulence and resistance in Egypt. J Infect Dev Ctries. 2025;19(5):712–722. doi:10.3855/jidc.20957
[41] Mitov I, Strateva T, Markova B. Virulence genes in Bulgaria. Braz J Microbiol. 2010;41(3):588–595. doi:10.1590/S1517-83822010000300008
[42] Qin S, Xiao W, Zhou C, et al. Pseudomonas aeruginosa: pathogenesis and resistance. Signal Transduct Target Ther. 2022;7(1):199. doi:10.1038/s41392-022-01056-1
[43] Fazeli H, Akbari R, Moghim S, et al. Pseudomonas aeruginosa infections in hospital settings. J Res Med Sci. 2012;17(4):332–338.
[44] Naji H, Saleh MJ. Genetic diversity and resistance in Iraq. J Life Sci Appl Res. 2024;5(1):14–27. doi:10.59807/jlsar.v5i1.93
[45] Elahi G, Goli HR, Shafiei M, Nikbin VS, Gholami M. Antimicrobial resistance and genetic diversity of Pseudomonas aeruginosa. BMC Microbiol. 2024;24(1):546. doi:10.1186/s12866-024-03707-5