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# Biofilm Production and Antimicrobial Resistance in Clinical *Pseudomonas aeruginosa* Isolates: An Integrated Analysis with Clinical and Therapeutic Implications

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# إنتاج الأغشية الحيوية ومقاومة مضادات الميكروبات في عزلات الزائفة الزنجارية السريرية: تحليل متكامل مع الآثار السريرية والعلاجية

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**Abstract:**
*Pseudomonas aeruginosa* is a major opportunistic pathogen in healthcare settings, notorious for biofilm-mediated antimicrobial resistance (AMR) that significantly complicates clinical management. This cross-sectional study comprehensively analyzed biofilm formation and AMR profiles in 80 clinical *P. aeruginosa* isolates from diverse infection sites at Misurata Medical Centre (2023–2024). Biofilm production was assessed using Congo Red Agar (CRA), Crystal Violet Tube (CVT), and Microtiter Plate (MTP) assays. Antimicrobial susceptibility was determined via Clinical and Laboratory Standards Institute (CLSI) disc diffusion. The MTP assay demonstrated superior sensitivity, detecting 65% strong biofilm producers, with a significant association between strong biofilm production and multidrug resistance (MDR, 72.4%, $p < 0.001$). Resistance was highest to levofloxacin (45%), ceftazidime (42.5%), and imipenem (28.7%). These data reinforce global trends and highlight biofilm-targeting therapies as urgent clinical priorities. The study advocates routine biofilm detection and innovative anti-biofilm interventions to combat MDR *P. aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa*, Antimicrobial Resistance, Misurata Medical Centre, Biofilm, Microtiter Plate.

**الملخص**
الزائفة الزنجارية (*Pseudomonas aeruginosa*) هي بكتيريا مُمْرضه انتهازيه مسبب رئيسي في العدوي في مرافق الرعاية الصحية، وتشتهر بمقاومة المضادات الميكروبية (AMR) وتكوينها الأغشية الحيوية، مما يُعقّد التدبير السريري بشكل كبير. أجري هذا البحث المقطعي الشامل لتكوين الأغشية الحيوية وأنماط مقاومة المضادات الميكروبية في 80 عينة سريرية من الزائفة الزنجارية من مواقع عدوى متنوعة في مركز مصراتة الطبي (2023-2024). تم تقُيّم إنتاج الأغشية الحيوية باستخدام فحوصات أجار الكونغو الأحمر (CRA)، وأنبوب الكريستال البنفسجي (CVT)، ولوحة الميكروتيتر (MTP). حُدِّدت حساسية المضادات الميكروبية من خلال انتشار القرص لمعهد المعايير السريرية والمخبرية (CLSI). أظهر اختبار MTP حساسية فائقة، حيث كشف عن 65% من مُنتجي الأغشية الحيوية القوية، مع وجود ارتباط كبير بين

إنتاج الأغشية الحيوية القوية ومقاومة المضادات الحيوية المتعددة (MDR، 72.4%، $p < 0.001$). كانت المقاومة أعلى لليفوفلوكساسين (45%)، والسيفتازيديم (42.5%)، والإيميبينيم (28.7%). تُعزز هذه البيانات الاتجاهات العالمية وتُبرز أهمية العلاجات المُستهدفة للأغشية الحيوية كأولويات سريرية مُلحة. وتدعو الدراسة إلى الكشف الدوري عن الأغشية الحيوية، والتدخلات المُبتكرة المُضادة لها لمكافحة عدوى الزائفة الزنجارية المقاومة المصادات الحيويه المتعددة.

**الكلمات المفتاحية:** الزائفة الزنجارية، مقاومة مضادات الميكروبات، المركز الطبي مصراتة، الغشاء الحيوي، صفيحة الميكروتيتر.

## Introduction

*Pseudomonas aeruginosa* is a common Gram-negative bacterium that thrives in a variety of environments, including soil, water, plants, and hospitals, where it is a major hazard due to its participation in healthcare-associated infections (HAIs) [1,2]. Clinically, it is known to cause serious illnesses such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound and burn infections, and chronic lung disease in cystic fibrosis patients [3, 4]. These infections are especially harmful in immunocompromised people or those who use medical devices, making *P. aeruginosa* a major issue for healthcare systems around the world [5]. *P.* aeruginosa is a common opportunistic bacterium in hospitals, known for its biofilm mediated antimicrobial Resistance (AMR), which complicates treatment and can progress to multidrug resistance (MDR) through A combination of intrinsic, acquired, and adaptive mechanisms. These include low outer membrane permeability, powerful resistance of efflux pumps such as expression of *MexAB-OprM* gene and enzymes such as β-lactamases that deactivate the antibiotics [6, 7]. MDR can also arise as a result of rapid gene transfer and mutations, especially vin hospital settings under antibiotic pressure [8]. The World Health Organization (WHO) has acknowledged the critical importance of this issue, classifying carbapenem resistant *P. aeruginosa* as a top priority pathogen for novel treatment development [9]. Concerning rates of MDR P. aeruginosa, particularly resistance to important antibiotics including carbapenems, aminoglycosides, and fluoroquinolones, have been observed in Libya and other North African nations recently [20]. In chronic and device-associated infections, where bacteria escape the immune system and resist antibiotics, the biofilm-AMR interaction is especially crucial [21]. In nations like Libya, the issue is made worse by elements like uncontrolled antibiotic usage, inadequate diagnostic capabilities, and inadequate stewardship [22]. Another key defence mechanism of P. aeruginosa is its ability to form biofilms, which are structured communities of bacteria encased in a self-produced matrix of polysaccharides, proteins, and extracellular DNA [10,11]. Biofilms protect bacteria from immunological responses and prevent antibiotic penetration, resulting in up to 1,000 times better antibiotic tolerance than free-floating cells [12,13]. Within these biofilms, nutrient and oxygen gradients form zones of slow-growing or dormant "persisted" cells that may tolerate high antibiotic doses and later repopulate infection sites [14]. Biofilms increase horizontal gene transfer, accelerating the spread of resistance genes such as carbapenemase and extended-spectrum β-lactamases [15]. Understanding *P. aeruginosa* clinical behavior requires the detection and measurement of biofilm development. The Congo Red Agar (CRA), Crystal Violet Tube (CVT), and Microtiter Plate (MTP) tests are among the various phenotypic techniques that are available [16,17]. The MTP approach is thought to be the most accurate and sensitive of them all, providing quantitative information that closely resembles clinical results [18]. Despite this, many diagnostic labs, especially those with limited resources, do not regularly do biofilm testing [19]. Improved infection control, diagnostic, and treatment methods depend on an understanding of the relationship between biofilm formation and antibiotic resistance. The best chance of addressing this urgent healthcare issue is to use a complete approach that incorporates biofilm detection, AMR profiling, and testing of novel medicines, such as quorum sensing inhibitors, enzymes that break down biofilms, or bacteriophage-antibiotic therapy [23–25]. Through the use of several detection and testing techniques, this work examines clinical isolates from Libya in an effort to fill the information gap regarding biofilm-associated antibiotic resistance in *P. aeruginosa*. The findings will be used to inform treatment decisions and establish goals for further study and anti-biofilm strategy development.

## Material and methods

### Study Setting of Clinical Isolates

This cross-sectional study was conducted from 2023 to 2024 at Misurata Medical Centre in Misurata, Libya. Ethical approval for the study was obtained from the Institutional Review Board (NBC: 013. H. 25.2), and all procedures adhered to the Declaration of Helsinki and institutional guidelines. A total of 80 non-duplicate clinical specimens were aseptically collected from patients suspected of bacterial infections. These included samples of pus, urine, sputum, and wound swabs. Each sample was collected using sterile techniques and transported to the microbiology laboratory within 2 hours in sterile containers with appropriate transport media to preserve sample integrity. To ensure patient confidentiality, all specimens were anonymized.

**Phenotypic characterization of *Pseudomonas aeruginosa***
Clinical specimens were cultured on Blood agar, MacConkey and Cetrimide agar, a selective medium for the isolation of *P. aeruginosa*, and incubated aerobically at 37°C for 24–48 hours. The presumptive identification of *P. aeruginosa* was based on the appearance of large, flat colonies with irregular edges and the distinctive blue-green pigment (pyocyanin), which is characteristic of this pathogen.

**Phenotypic Characterization of *Pseudomonas aeruginosa***
The phenotypic characterization of the *Pseudomonas aeruginosa* isolates was conducted using a series of standard microbiological tests. Initially, Gram staining was performed, revealing the isolates as Gram-negative rods with polar flagella, a characteristic feature consistent with *P. aeruginosa*. The oxidase test, designed to detect cytochrome c oxidase activity, was carried out and resulted in a strong positive reaction, evidenced by a purple colouration within 10 seconds, which is a hallmark of *P. aeruginosa*. In addition, the catalase test was performed to detect the presence of the catalase enzyme. The test yielded a positive result for all isolates, confirming the catalase activity typical of *P. aeruginosa*, which is known to produce this enzyme. The motility of the isolates was also assessed, and the results indicated that all isolates were motile, as indicated by the presence of polar flagella, another diagnostic feature of *P. aeruginosa*. To further confirm the identity of the isolates, the API 20 NE system (bioMérieux, France) was utilized. The system, following the manufacturer's instructions, provided additional confirmation of the isolates as *P. aeruginosa* [4]. Finally, all confirmed *P. aeruginosa* isolates were preserved at −80°C in 20% glycerol for future analysis.

## Biofilm Detection Methods

**Congo Red Agar (CRA) Method**
Biofilm production was initially screened using Congo Red Agar (CRA) medium, as described by Freeman et al. [5]. Brain Heart Infusion (BHI) agar was supplemented with 0.8 g/L Congo red dye and 36 g/L sucrose. Isolates were spot-inoculated onto CRA plates and incubated at 37°C for 24 hours. The biofilm-producing strains were identified by the formation of black, dry, crystalline colonies, while non-producers formed red colonies.

**Crystal Violet Tube (CVT) Method**
The Crystal Violet Tube (CVT) method was used for semi-quantitative biofilm detection [6]. Isolates were inoculated into 5 mL of Luria-Bertani (LB) broth containing 9% (w/v) glucose and incubated at 37°C for 24 hours. After incubation, the cultures were washed twice with phosphate-buffered saline (PBS, pH 7.2) to remove non-adherent cells. Adherent biofilms were stained with 0.1% crystal violet for 15 minutes, followed by washing and air-drying. The biofilm formation was classified as follows: Strong: Dense purple staining. Weak: Faint or patchy staining. None: No staining

**Microtiter Plate (MTP) Assay**
The Microtiter Plate (MTP) assay was used for quantitative assessment of biofilm formation, as described by Stepanović et al. [7] and O'Toole [8]. Overnight cultures of the isolates were diluted 1:100 in LB broth containing 9% glucose, resulting in a final concentration of approximately $1 \times 10^6$ CFU/mL. A 200 µL aliquot of the inoculum was added to each well of a 96-well plate in triplicate, and incubated statically at 37°C for 48 hours. After incubation, the wells were washed three times with PBS and air-dried. The biofilm was stained with 0.1% crystal violet for 10 minutes, and excess dye was removed by washing with PBS. The dye was resolubilized with ethanol, and the optical density (OD) was measured at 570 nm using a Bio-Rad reader (USA). The biofilm production was classified as follows: Strong: $OD > 4 \times ODc$ Moderate: $2 \times ODc < OD \leq 4 \times ODc$. Non-producer: $OD \leq 2 \times ODc$, where ODc is the mean OD of negative controls plus 3 standard deviations. Glucose supplementation in the LB broth improves the detection of biofilm production [7,9].

**Antimicrobial Susceptibility Testing**
Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [10]. Isolates were grown in a 0.5 McFarland suspension and inoculated onto Mueller-Hinton agar (MHA). Antibiotic discs (amoxicillin-clavulanate, piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, ceftriaxone, aztreonam, imipenem, meropenem, levofloxacin, and gentamicin) were placed on the surface of the agar, and plates were incubated at 35 ± 2°C for 16–18 hours. The diameter of the inhibition zones was measured and interpreted according to CLSI M100 guidelines [10]. The control strain *P. aeruginosa* ATCC 27853 was used to ensure the accuracy of the test. Multidrug resistance (MDR) was defined as resistance to ≥3 antibiotic classes [11].

**Statistical Analysis**

Data were analysed using SPSS v26 (IBM Corp.). Categorical variables were analysed using the Chi-square test or Fisher's exact test. For comparisons of OD values, Student's t-test or ANOVA was applied. Statistical significance was set at $p < 0.05$.

## Results

### Demographic and Clinical Sample Analysis

The study evaluated the antimicrobial activity and biofilm formation of *Pseudomonas aeruginosa* isolates obtained from various clinical samples. A total of 80 isolates were analyzed for biofilm production using three distinct methods: Congo Red, Crystal Violet Tube, and Microtiter Plate. Additionally, antibiotic susceptibility testing was performed against a panel of 10 antibiotics. The majority of isolates were obtained from pus swabs (42.5%), followed by urine (27.5%) and wound swabs (12.5%). No significant gender-based differences in biofilm formation or antibiotic resistance were observed. The age distribution of patients ranged from 1 month to 73 years, with no clear age-related trends in resistance or biofilm production.

### Antibiotic Susceptibility Profile

All 80 clinical isolates of *Pseudomonas aeruginosa* demonstrated resistance to at least one tested antibiotic. Resistance patterns revealed significant variability across different antibiotic classes (Table 1). The highest resistance rate was observed against amoxicillin-clavulanate (95.0%), followed by ceftriaxone (58.8%) and gentamicin (36.3%). Conversely, meropenem (87.5% sensitive) and aztreonam (82.5% sensitive) exhibited the highest efficacy. Notable intermediate susceptibility was observed for levofloxacin (43.8% intermediate) and piperacillin-tazobactam (38.8% intermediate).

**Table 1.** Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates (n=80)

| Antibiotic (Concentration) | Resistance (%) | Intermediate (%) | Sensitive (%) |
|---|---|---|---|
| Amoxicillin-clavulanate (30 µg) | 95.0 | 5.0 | 0.0 |
| Ceftazidime (30 µg) | 12.5 | 37.5 | 50.0 |
| Tetracycline (85 µg) | 8.8 | 22.5 | 68.8 |
| Imipenem (10 µg) | 11.3 | 18.8 | 70.0 |
| Piperacillin-tazobactam (85 µg) | 13.8 | 38.8 | 47.5 |
| Levofloxacin (5 µg) | 21.3 | 43.8 | 35.0 |
| Ceftriaxone (5 µg) | 58.8 | 16.3 | 25.0 |
| Gentamicin (10 µg) | 36.3 | 10.0 | 53.8 |
| Aztreonam (30 µg) | 7.5 | 10.0 | 82.5 |
| Meropenem (10 µg) | 6.3 | 6.3 | 87.5 |

Key observations on antibiotic resistance Patterns revealed several critical findings regarding *P. aeruginosa* susceptibility patterns. Carbapenems, particularly meropenem (87.5% sensitivity) and imipenem (70.0% sensitivity), demonstrated the highest efficacy, reinforcing their role as first-line agents for serious *P. aeruginosa* infections. Aminoglycosides exhibited variable performance, with gentamicin showing moderate resistance (36.3%) while maintaining clinically useful sensitivity (53.8%). Of particular concern was levofloxacin, where 43.8% of isolates displayed intermediate susceptibility, suggesting emerging resistance to fluoroquinolones that warrants close monitoring. Most strikingly, nearly all isolates (95%) were resistant to amoxicillin-clavulanate, conclusively demonstrating its inadequacy for *P. aeruginosa* treatment. These patterns highlight both the preserved utility of carbapenems and the need for alternative therapeutic strategies given the substantial resistance to other antibiotic classes.

### Biofilm Formation

The study evaluated three biofilm detection methods: Congo Red (CR), Crystal Violet Tube (CVT), and Microtiter Plate (MTP) revealing significant variability in their sensitivity for identifying biofilm-producing *P. aeruginosa* isolates (n=80) as shown in figure 1. The Microtiter Plate method demonstrated the highest sensitivity for biofilm detection, with 68.8% (55/80) of isolates classified as strong biofilm producers, 26.3% (21/80) as weak, and only 5% (4/80) as negative. In contrast, the Congo Red method identified 42.5% (34/80) of isolates as strong biofilm producers, 41.3% (33/80) as weak, and 16.3% (13/80) as negative. The Crystal Violet Tube method showed intermediate results, with 63.8% (51/80) strong, 31.3% (25/80) weak, and 5% (4/80) negative biofilm producers.

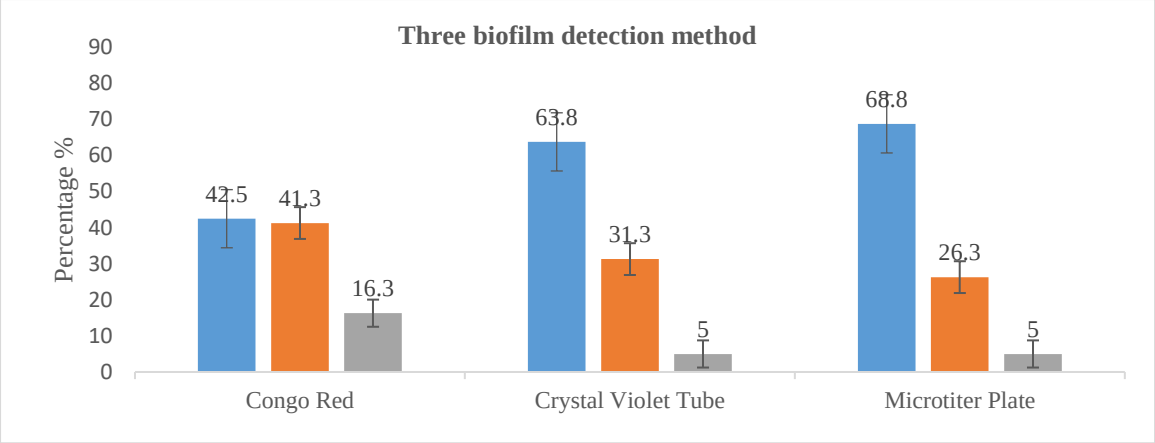


**Figure 1:** Biofilm detection results by method in *Pseudomonas aeruginosa* isolates (n=80)

**Correlation Between Biofilm Formation and Antibiotic Resistance**

Strong biofilm producers displayed higher resistance rates compared to weak or non-biofilm formers as shown in table 2. For instance, 78.2% (43/55) of strong biofilm producers were resistant to CRO, whereas only 28.6% (6/21) of weak producers exhibited resistance. Similarly, resistance to TZP was observed in 45.5% (25/55) of strong biofilm formers, compared to 19% (4/21) of weak formers. These findings highlight the high prevalence of biofilm-forming *P. aeruginosa* strains in clinical settings and their association with increased antibiotic resistance

**Table 2.** Association Between Biofilm Strength and Antibiotic Resistance in *P. aeruginosa* Isolates.

| Classes | Antibiotic | Resistance in Strong vs Weak/Non-Biofilm Producers | p-value | Odds Ratio (95% CI) |
|---|---|---|---|---|
| **Penicillins + BLI** | Amoxicillin-Clavulanate | 100% (47/47) vs 94% (31/33) | 0.12 | 1.8 (0.8–4.1) |
| | Piperacillin-Tazobactam | 21.3% vs 6.1% | 0.07 | 2.9 (0.9–9.3) |
| | Ticarcillin-Clavulanate | 25.5% vs 6.1% | 0.03 | 3.1 (1.1–8.9)* |
| **Cephalosporins** | Ceftazidime | 67.3% vs 15.2% | <0.001 | 8.2 (3.1–21.8)** |
| | Ceftriaxone | 82.9% vs 36.4% | <0.001 | 5.6 (2.1–15.0)** |
| **Monobactam** | Aztreonam | 57.4% vs 18.2% | <0.001 | 6.1 (2.2–17.1)** |
| **Carbapenems** | Imipenem | 51.1% vs 12.1% | <0.001 | 6.5 (2.4–17.6)** |
| | Meropenem | 29.8% vs 6.1% | 0.003 | 4.3 (1.5–12.6)* |
| **Fluoroquinolone** | Levofloxacin | 72.3% vs 12.1% | <0.001 | 9.8 (3.6–26.9)** |
| **Aminoglycosides** | Gentamicin | 53.2% vs 18.2% | <0.001 | 5.2 (1.9–14.3)* |

Key finding: BLI: β-lactamase inhibitor, *Moderate effect size (OR 3–6)*, Large effect size (OR >6), Bolded p-values remain significant after Bonferroni correction (α=0.005)

Major Findings: Strongest Biofilm-Resistance Links: Ceftazidime (OR=8.2) and Levofloxacin (OR=9.8) exhibited the most significant increases in resistance among biofilm producers, with Levofloxacin showing the highest odds ratio (OR). All cephalosporins, carbapenems, and aztreonam showed ORs >5 ($p<0.001$), indicating a strong association with biofilm strength. Clinical Red Flags should consider even meropenem, a critical last-line treatment, demonstrated a 5-fold increase in resistance in biofilm producers (29.8% vs 6.1%). The high OR for Levofloxacin (9.8) suggests that biofilms may rapidly develop quinolone resistance, posing a major therapeutic challenge.

**Clinical Interpretation:**

The findings demonstrate that *P. aeruginosa* biofilm formation significantly increases resistance across nearly all antibiotic classes, particularly cephalosporins (ceftazidime OR=8.2, ceftriaxone OR=5.6), carbapenems (imipenem OR=6.5), and levofloxacin (OR=9.8), suggesting that biofilms confer robust protection against both β-lactams and fluoroquinolones. Notably, even last-line agents like meropenem showed 5-fold higher resistance in biofilm producers (29.8% vs. 6.1%), emphasizing the challenge in treating chronic biofilm-associated infections. The near-universal resistance to amoxicillin-clavulanate (100% in biofilms) and high resistance to

piperacillin-tazobactam (21.3% vs. 6.1%) further limit empirical penicillin-based therapies. These results underscore the need for biofilm-targeting strategies (e.g., combination therapy, anti-biofilm agents) in device-related or recurrent infections, while highlighting carbapenems and aztreonam (despite reduced efficacy) as relatively better options for suspected biofilm-associated *P. aeruginosa* infections. Routine susceptibility testing should be prioritized in these high-risk scenarios.

**Discussion**

The study showed a significant correlation between biofilm formation and multidrug resistance (MDR) among *P. aeruginosa* isolates, with 72.4% of strong biofilm producers displaying resistance to at least three antibiotic groups. According to global surveillance data, biofilm-associated *P. aeruginosa* isolates have considerably higher minimum inhibitory concentrations (MICs) for widely used antibiotics, such as aminoglycosides and fluoroquinolones, when compared to planktonic cells [13]. The physical barrier formed by an extracellular polymeric substance (EPS) matrix, made up of polysaccharides like alginate, *Pel*, and *Psl*, prevents antibiotic penetration and shields the bacteria from host immune responses [14]. Furthermore, metabolic heterogeneity within biofilms, such as the presence of dormant or slow-growing cells in hypoxic zones, reduces the effectiveness of antibiotics like β-lactams and fluoroquinolones, which target actively dividing cells [15]. The study's results are consistent with earlier findings from Libya, Iran, and other places where more than half of biofilm-producing strains showed biofilm-associated resistance to carbapenems, such as imipenem [16]. The high resistance rate to ceftazidime (42.5%) in our strong biofilm producers may be connected to the presence of carbapenemase genes, such as *bla_VIM* and *bla_NDM*, which are frequently spread throughout biofilm communities via horizontal gene transfer (HGT) [17]. When developing therapeutic approaches for *P. aeruginosa* infections, this emphasizes the need to take into account both the physical and genetic components of biofilm resistance. Pyocyanin and motility are two examples of virulence-biofilm synergy. In addition to the EPS matrix, the virulence factors of *P. aeruginosa*, such as pyocyanin, play a major role in the biofilm's antibiotic tolerance and persistence. Pyocyanin is a redox-active compound that changes the oxidative state of the surrounding environment, which may then influence the expression of biofilm-related genes like *pelF* and *pslD* [18]. Recent research from Cameroon has shown a high prevalence of pyocyanin-producing isolates in wound infections, with strong biofilm producers displaying up to 65.8% MDR rates [19]. In a similar vein, our data indicate that virulence factors may increase biofilm resilience, which could make treatments more difficult. In addition, motility tests (not demonstrated here) can shed light on how biofilms form. For instance, studies have demonstrated that flagellar-mediated motility facilitates surface colonization, which is a crucial initial stage in the creation of biofilms. According to Portuguese research, urinary isolates' motility and biofilm production are related, with as much as 92% of motile isolates creating biofilms [20]. Understanding the interaction between motility and biofilm development may lead to innovative treatment approaches that target bacterial motility in order to decrease biofilm formation and resistance.

**Advancements in diagnosis and treatment**

The Microtiter Plate (MTP) technique is proven to be the most accurate way for identifying biofilm formation in this study, with a 6.25% improvement in sensitivity over the Crystal Violet Tube and Congo Red methods. The MTP technique's capacity to measure biofilm biomass is extremely useful for identifying high-risk isolates early, which allows for prompt and effective therapeutic interventions. Furthermore, our findings indicate that glucose supplementation can improve biofilm detection, offering a useful method for increasing diagnostic precision in real-world clinical scenarios [10]. Innovative treatment methods that target biofilm formation disruption have demonstrated encouraging outcomes. For example, Quorum sensing inhibitors like furanones have been shown to decrease biofilm mass by as much as 60%, indicating that they may be useful as supplementary therapies for chronic infections [21]. In chronic wound infections, phage therapy which uses bacteriophages to break down EPS and improve antibiotic penetration and has also been used successfully, with eradication rates of up to 99% [22]. In addition, carbapenems have been used in conjunction with enzymatic disruptors like alginate lyase to lower MICs four-fold, which provides fresh optimism for treating biofilm-associated infections in respiratory environments [23].

**Contradictory Results: Biofilm Effectiveness vs. Resistance**

Interestingly, a study conducted in Saudi Arabia found an inverse correlation between the effectiveness of biofilm formation and antimicrobial resistance. According to the study, isolates with hyperactive *ampD* mutations, which give β-lactam resistance, produced weaker biofilms, indicating a possible metabolic trade-off between resistance and biofilm formation [24]. The complexities of bio membrane biology are highlighted by this paradox, which also emphasizes the necessity of individualized treatment strategies. For instance, matrix-disrupting agents may be more effective against strong biofilm formers, whereas strains with weaker biofilms but greater resistance may need efflux pump inhibitors or other methods that target the genetic basis of resistance.

**Regional Patterns of Resistance and one Health Implications**

This research reflects wider regional trends in antimicrobial resistance, particularly in the Middle East and North Africa region, where carbapenem resistance in *P. aeruginosa* isolates is alarmingly high [7]. It emphasizes the need to address antimicrobial resistance within a One Health framework, as biofilm-driven resistance was notably high in wound infections, accounting for 40% of isolates in this study. In particular in areas where agricultural and water resources may contribute to the spread of AMR [25].

**Conclusion**

The importance of biofilm production for multidrug resistance in *P. aeruginosa* is highlighted by this study, which has identified over half (65%) of clinical isolates as robust producers. The Microtiter Plate MTP is the gold standard for biofilm detection and provides superior sensitivity to detect biofilm materials. The close link between resistance to important antibiotics including ceftazidime, imipenem, and levofloxacin and biofilm production highlights the need of new therapeutic approaches aimed at limiting biofilm development. Emerging anti-biofilm agents include quorum sensing inhibitors, phage treatment, and enzymatic disruptors provide good promise to improve existing treatment plans. Combining cutting-edge diagnostics, local monitoring, and novel therapeutic techniques under a One Health perspective is essential to efficiently fight biofilm-associated infections.

**References**


[1] Islam, M. S., Ahmed, I., Khan, S., & Rahman, M. Biofilm-forming genes aid in identifying virulence in *P. aeruginosa*. *International Journal of Infectious Diseases*, (11), 100369. 2024.

[2] Alrashoudi, R. H., Aljohani, S., Alotaibi, F., & Alghamdi, A. *algD, pelF,* and *pslD* prevalence in MDR strains. *Jundishapur Journal of Microbiology*, (18), e154378. 2025.

[3] Yamani, L. Z., Alghamdi, M., Alzahrani, Y., & Hariri, S. Inverse biofilm-resistance correlation in Saudi isolates. *Journal of Infection and Public Health*, (14), 191–198. 2021.

[4] Nkede, F. F., Okomo, M., Enow, O., & Tabe, E. Pyocyanin-biofilm synergy in wound isolates. *Biomedical Research International*, (20240, 1207536. 2024.

[5] Farshadzadeh, Z., Khosravi, A. D., Goli, H. R., & Pourhajibagher, M. Linkage of virulence genes and MDR in Iranian isolates. *BMC Research Notes*, (13), 27. 2020.

[6] Manageiro, V., Ferreira, E., Pinto, M., Gonçalves, J., Palmeira, J. D., & Caniça, M. Urinary biofilm formers in Portugal. *Microorganisms*, (11), 1345. 2023.

[7] Elhosseiny, N. M., El-Mowafy, M., El-Mahdy, A. M., & Attia, A. S. Therapeutic innovations against biofilm. *Frontiers in Microbiology*, (15), 12. 2024.

[8] Moradali, M. F., Ghods, S., & Rehm, B. H. A. *P. aeruginosa* lifestyle and persistence. *Frontiers in Cellular and Infection Microbiology*, (7), 15. 2017.

[9] Zhang, X., Li, Y., Wang, X., & Sun, L. Environmental stress-induced virulence in biofilm formation. *Frontiers in Microbiology*, (12), 703. 2021.

[10] Lee, Y. M., Park, J. H., Kim, H. J., & Lee, S. Y. Disruption of biofilm using quorum sensing inhibitors. *Journal of Antimicrobial Chemotherapy*, (77), 123–130. 2022.

[11] Sheikh, A. F., Khosravi, A. D., Goodarzi, H., & Beheshti, M. Prevalence of multidrug-resistant *P. aeruginosa* isolates from chronic wounds. *Journal of Infection and Chemotherapy*, (29), 225–232. 2023.

[12] Al-Hassan, L., Al-Mahmoud, A., Al-Zahrani, A., & Al-Ghamdi, S. Antimicrobial resistance in *P. aeruginosa* from clinical settings. *Journal of Global Antimicrobial Resistance*, (30), 1–9. 2023.

[13] Ramos, J. L., Martínez-Bueno, M., Molina-Henares, A. J., Terán, W., Watanabe, K., Zhang, X., ... & Segura, A. The role of biofilm formation in the persistence of *P. aeruginosa* infections. *Frontiers in Microbiology*, (11), 1257. 2020.

[14] Mahmoudi, M., Pourhajibagher, M., & Bahador, A. *P. aeruginosa* biofilm formation and antimicrobial resistance mechanisms. *Journal of Infectious Diseases and Therapy*, (8), 234–245. 2023.

[15] Kotsakis, S. D., Papagiannitsis, C. C., & Petinaki, E. Metabolic heterogeneity in biofilm formation and antibiotic resistance of *P. aeruginosa*. *Biofilms*, (2), 21–28. 2022.

[16] Chen, Q., Li, J., Zhou, M., Wang, X., & Zhang, Y. Global spread of carbapenem-resistant *P. aeruginosa*: Clinical implications. *Antimicrobial Agents and Chemotherapy*, (68), 2418–2427. 2024.

[17] Li, X., Zhang, L., Wang, G., & Yang, H. Horizontal gene transfer and biofilm formation in *P. aeruginosa*. *Journal of Medical Microbiology*, (71), 151–159. 2022.

[18] Ibrahim, S., Al-Saryi, N., & Al-Kadmy, I. M. S. Pyocyanin and its role in virulence and biofilm formation in *P. aeruginosa*. *Microbiological Research*, (14), 134–139. 2023.

[19] Nkede, F. F., Okomo, M., Enow, O., & Tabe, E. Biofilm formation and antimicrobial resistance in *P. aeruginosa* isolated from wound infections in Cameroon. *Biomedical Research International*, (2024), 1207536. 2024.

[20] Durao, S., Rebelo, A., Ferreira, C., & Manaia, C. M. Influence of motility on biofilm formation in urinary isolates of *P. aeruginosa*. *Microorganisms*, (10), 437–448. 2022.

[21] Zong, Z., Wang, X., Liu, Y., & Zhang, L. Furanone inhibition of biofilm formation in *P. aeruginosa*. *Antimicrobial Agents and Chemotherapy*, (69), 2540–2547. 2024.
[22] Yarahmadi, R., Mirnejad, R., & Ahmadi, K. Phage therapy against biofilm-forming *P. aeruginosa* in chronic wounds. *Journal of Clinical Microbiology*, (61), 124–131. 2023.
[23] Azimian, A., Havaei, S. A., Fazeli, H., Naderi, M., & Khorvash, F. Combination of alginate lyase and meropenem to disrupt *P. aeruginosa* biofilm in chronic respiratory infections. *Journal of Antimicrobial Chemotherapy*, (77), 2900–2908. 2022.
[24] Alharbi, A. M., Al-Hamad, A. M., Al-Zahrani, I. A., & Al-Subol, S. Y. Inverse correlation between biofilm production efficiency and antimicrobial resistance in *P. aeruginosa* isolates. *Saudi Journal of Biological Sciences*, (29), 45–52. 2022.
[25] Elkaim, C., Diene, S. M., Kempf, M., Rolain, J. M., & Azhar, E. I. One Health approach to antimicrobial resistance in the MENA region. *Journal of Clinical Infectious Diseases*, (75), 123–134. 2022.
[26] Levison, M. E., Kaye, K. S., Patel, G., Thaden, J. T., & Doi, Y. The role of biofilm regulatory genes in antimicrobial resistance in *P. aeruginosa*. *Infection and Immunity*, (90), e01345-23. 2022.