Antibiotic Imipenem Resistance of Multidrug-Resistant Pathogen Pseudomonas Aeruginosa

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**Abstract:**The study investigated the inhibition of biofilm formation by multidrug-resistant *Pseudomonas aeruginosa* upon treatment with the antibiotic Imipenem. Biochemical identification confirmed the pathogen as a Gram-negative, rod-shaped *Pseudomonas species*, exhibiting motility and various metabolic characteristics typical of the genus. A 48-hour-old biofilm of *Pseudomonas aeruginosa* was established and subsequently treated with Imipenem for 12 to 48 hours. Microscopic analysis revealed a significant reduction in biofilm density post-treatment, indicating the antibiotic's efficacy in disrupting biofilm formation and persistence. Additionally, cell viability analysis demonstrated a clear dose-dependent effect, with Imipenem concentrations of 750µg/ml and 1000 µg/ml completely eradicating viable cells, highlighting the antibiotic's potent activity against the pathogen. Histamine release assays further supported these findings, showing a dose-dependent increase in histamine release, correlating with higher concentrations of cell lysate. These results underscore imipenem's potential as an effective agent against biofilm-associated infections caused by multidrug-resistant *Pseudomonas aeruginosa*, offering promising insights for therapeutic strategies targeting biofilm-related bacterial infections. The study's comprehensive approach provides an understanding of the dynamics of antibiotic-biofilm interactions and the potential of Imipenem in combating resistant bacterial strains.

**Keywords:** Imipenem; Multidrug-Resistant; *Pseudomonas aeruginosa;* Histamine; Cell viability

# Introduction

*Pseudomonas aeruginosa*, a Gram-ve bacteria of significant importance, is commonly found in healthcare environments and is a major contributor Healthcare infections [(Madhumitha & Muralidharan, 2021; Moradali et al., 2017; Reynolds & Kollef, 2021)](https://paperpile.com/c/JRliSp/IMuf+Roae+Ub2B). *Pseudomonas aeruginosa* also demonstrates substantial infections caused by pathogenic microorganisms in various hosts, including animals, plants and humans[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/JRliSp/omm46+tuJKw+8hqO4). Treating *Pseudomonas aeruginosa* infections is challenging due to its potential to thrive in diverse environmental conditions and its resistance to many antimicrobial drugs [(Laborda et al., 2021)](https://paperpile.com/c/JRliSp/bh3jG). *Pseudomonas aeruginosa* is a significant nosocomial pathogen exhibiting increasing multidrug resistance, particularly to carbapenems like imipenem[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/JRliSp/3F8we+R9t1F+xFAkf). Studies have reported varying resistance rates to imipenem, ranging from 20% to 30% (Nagaveni & Chandrakanth, 2020). A key characteristic of *Pseudomonas aeruginosa* is its capacity to form biofilms, a complex process where bacterial cells cluster within an Extracellular matrix produced by the organism itself[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/JRliSp/vDMe4+3FR0v+LV9LD). This biofilm formation, which involves a sophisticated structural arrangement, makes the bacteria highly resistant to standard antibiotic treatments and potentially enhances its clinical effect [(Babu et al., 2021; Sharma et al., 2023)](https://paperpile.com/c/JRliSp/FLgjQ+6sOSw). Resistance mechanisms include the production of metallo-beta-lactamases and the presence of integron-located genes. Multidrug resistance was observed in 33.7% to 60% of isolates across studies[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/JRliSp/pgMwy+QSbqg+PhinO). *Pseudomonas aeruginosa* is a forefront example of antibiotic resistance and is noted as a “priority pathogen” by the WHO [(Balakrishnan, 2022)](https://paperpile.com/c/JRliSp/6FVEy). This bacterium poses a significant therapeutic challenge due to its resistance to multiple antibiotics, including meropenem, imipenem, and third-generation cephalosporins, which are among the most effective treatments for multidrug-resistant (MDR) bacteria[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/JRliSp/ZAcXO+ALu03+ogv7l)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/JRliSp/ZAcXO+ALu03+ogv7l+oCBee). It is part of the “ESKAPE” group of pathogens *Enterobacter spp. Acinetobacter baumannii, Enterococcus faecium, Staphylococcus aureus, Klebsiella* pneumoniae*, Pseudomonas aeruginosa* which are considered of utmost concern due to their critical need for new antimicrobial agents [(Mulani et al., 2019)](https://paperpile.com/c/JRliSp/qt24z). Genomic analysis revealed a reduced flexible genome, primarily consisting of phages and defense mechanisms, which play a crucial role in evolution and pathogenicity[(Pranati et al., 2021; Sakthi et al, 2021)](https://paperpile.com/c/JRliSp/UFtRI+J69cZ). The microdiversity in strains without carbapenemases suggests multiple resistance mechanisms in the core genome [(López-Pérez et al., 2021)](https://paperpile.com/c/JRliSp/4LlmO). To combat the emergence of resistant strains, researchers recommend continuous monitoring, restricted antibiotic usage, and implementation of infection control policies [(Yadav et al., 2020)](https://paperpile.com/c/JRliSp/nmNsZ). Cefiderocol and imipenem-cilastatin are currently in the developmental stages [(Bassetti et al., 2019)](https://paperpile.com/c/JRliSp/lAFs3). Magiorakos et al. defined multidrug-resistant (MDR) *P. aeruginosa* strains as those showing non-susceptibility to at least one agent in three or more antimicrobial categories[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/JRliSp/6nfpK+7Ucmd)). Biofilm formation is a notable survival strategy utilized by *P. aeruginosa.* During this intricate process, *P. aeruginosa* communities attach to surfaces and become encased in a matrix of extracellular polymeric substances, which comprises proteins, metabolites, exopolysaccharides & extracellular DNA. The biofilms of *P. aeruginosa* are distinguished by a complex matrix containing a variety of EPS components, including elaborate proteins, lipids, polysaccharides and e DNA [(Devi et al., 2021; Thi et al., 2020)](https://paperpile.com/c/JRliSp/x6BLi+thd1I). This study aims to investigate the resistance mechanisms in multidrug-resistant *P. aeruginosa*, particularly against Imipenem and to explore the effectiveness of various strategies, including histamine release, cell viability, and antibiofilm activities.

# Methods and methodology

Oral swab samples were collected from the The swabs streaked onto LB agar plates were incubated at room temperature for 24 hours to allow bacterial growth. The following morning, the bacterial strain Pseudomonas aeruginosa was identified. The bacterial pathogen Pseudomonas aeruginosa underwent biochemical analysis using samples obtained from The pathogen was streaked onto an LB agar medium, and its biochemical characteristics were evaluated according to the guidelines provided in [(Palleroni, 2015)](https://paperpile.com/c/JRliSp/Hn3Gr). Various tests were performed, including TSI agar, Methyl Red, indole production, maltose utilization, Voges-Proskauer, citrate utilization, lactose, catalase, urease, inositol, sucrose, xylose, and starch tests [(Yasuda et al., 2023)](https://paperpile.com/c/JRliSp/Sqo2J).To determine histamine concentration, overnight cultures of the Pseudomonas aeruginosa cultures were adjusted to an OD of 0.2 at 600 nm. A 10 μL aliquot of this bacterial suspension was then introduced into a 50 mL conical tube containing 10 mL of LB medium, with or without 10−3M histamine. The 10 mL mixtures were subsequently transferred to 250 mL conical flasks and incubated in an orbital shaker at room temperature. After 18 hours of incubation, 1 mL samples from each bacterial suspension were taken, placed in 1.5 mL Eppendorf tubes, centrifuged at 2,500 g for 10 minutes, and the supernatants were collected. These supernatants, now free of bacteria, were used to quantify histamine using an ELISA kit [(Dib et al., 2023)](https://paperpile.com/c/JRliSp/VLZbO).To evaluate the resistance of Pseudomonas aeruginosa to Imipenem, a cell viability assay is conducted. First, an overnight culture of Pseudomonas aeruginosa is grown in LB broth at 37°C with shaking, then adjusted to an OD of 0.1 at 600nm. Imipenem solutions of varying concentrations are prepared and added to a 96-well microtiter plate containing 100µL of the adjusted bacterial suspension in each well. After incubation at 37°C overnight, cell viability is assessed using an assay reagent such as MTT. Following incubation with the reagent, absorbance is measured using a plate reader. The results are compared to control wells without antibiotic to determine the effect of Imipenem on bacterial viability. The minimum inhibitory conc. of Imipenem, which is the minimum con. That inhibits bacterial growth, is then calculated, providing insight into the antibiotic resistance profile of Pseudomonas *aeruginosa* [(Meyer et al., 2023)](https://paperpile.com/c/JRliSp/u55xJ).The biofilm inhibition efficacy of Imipenem against the MDR pathogen *P. aeruginosa*, the methodology involved cultivating the bacteria in a microliter plate until reaching a concentration of 109cells/ml at 37°C overnight. Following biofilm formation, the cultures were treated with various concentrations of Imipenem (1000µg/ml) and incubated at 37°C for two days. The biofilm was quantified using crystal violet staining, with the optical density measured at 570 nm. Control wells contained well-grown cultures, and a sterile medium was used as a blank. The inhibition percentage was calculated using the formula:

Percentage of Inhibition = 100 - [( Sample / Control) × 100]

For the ant biofilm microscopic analysis, *Pseudomonas aeruginosa* (104cells/ml) was inoculated into a 6-well plate and was allowed to form biofilm for 24 hours. The biofilms were then treated with imipenem for a period ranging from 12 to 48 hours. Following 48 hours of treatment, the biofilms were stained with acridine orange and propidium iodide to distinguish live cells from dead ones.

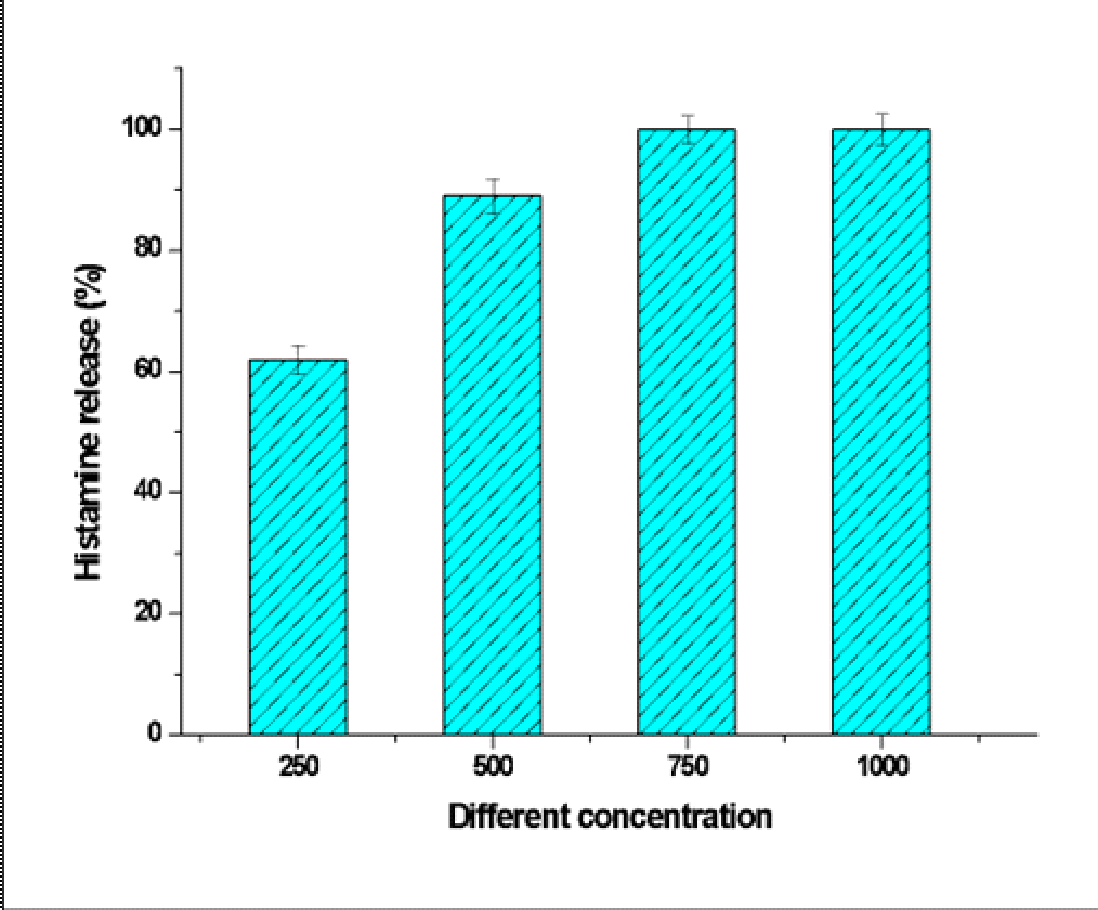
# Results

The biochemical identification of the pathogen reveals it to be a member of the genus *Pseudomonas* (Table 1)*.* The isolate was found to be Gram-negative, displaying a rod-shaped morphology under microscopic examination. The motility test confirmed the presence of flagella, indicative of motile bacteria. Indole production was negative, suggesting the bacterium does not possess the enzyme tryptophanase required for indole production. Both the Methyl Red (MR) and Voges-Proskauer (VP) tests were negative, indicating the absence of mixed acid fermentation and acetoin production, respectively. The citrate utilization test was positive, showing that the organism can use citrate as a sole carbon source, a characteristic feature of many *Pseudomonas species*. The Triple Sugar Iron (TSI) agar test results were negative for all sugar fermentations, as the isolate did not produce acid or gas from glucose, lactose, or sucrose, which is typical for non-fermentative *Pseudomonas*. A positive oxidase test confirmed the presence of cytochrome c oxidase, an essential enzyme involved in the bacterial electron transport chain. Similarly, the catalase test was positive, indicating the organism's ability to decompose hydrogen peroxide into water and oxygen. The urease test yielded a positive result, demonstrating the bacterium's ability to hydrolyze urea to ammonia and carbon dioxide. Carbohydrate fermentation tests for lactose, maltose, sucrose, and xylose were all negative, aligning with the non-fermentative nature of *Pseudomonas species*. The starch hydrolysis test was positive, showing that the bacterium produces extracellular enzymes capable of breaking down starch into simpler sugars. Finally, the inositol fermentation test was negative, further supporting the identification of the bacterium as Pseudomonas, which typically does not ferment inositol.

**Table 1.** Biochemical analysis of *Pseudomonas aeruginosa*

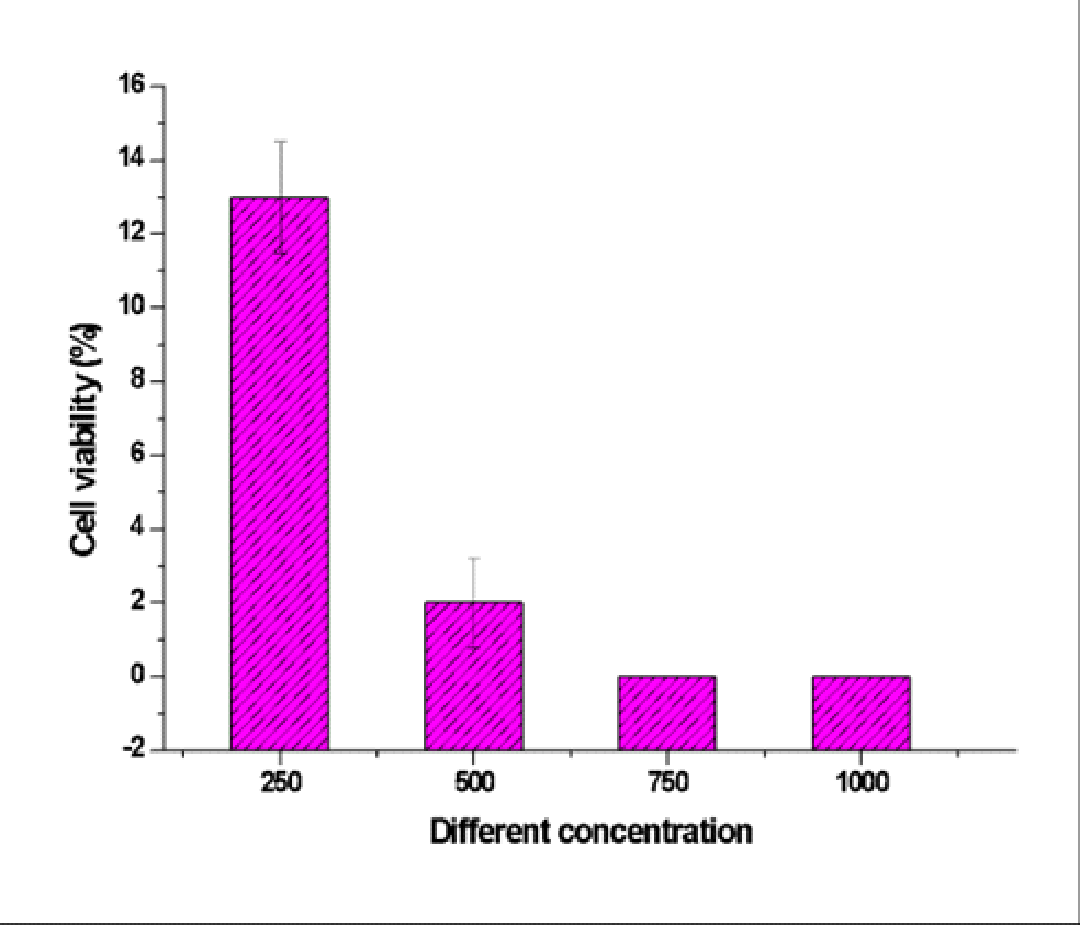
|  |  |
| --- | --- |
| **Gram stain** | **Negative** |
| Shape | Rod |
| Motility | Positive |
| Indole | Negative |
| MR | Negative |
| VP | Negative |
| Citrate | Positive |
| TSI | Negative |
| Oxidase | Positive |
| Catalase | Positive |
| Urease | Positive |
| Lactose | Negative |
| Maltose | Negative |
| Sucrose | Negative |
| Xylose | Negative |
| Starch | Positive |
| Inosital | Negative |

The study on histamine release in response to different cell lysate concentrations in multidrug-resistant *Pseudomonas aeruginosa* shows a distinct dose-dependent association (Fig.1). The histamine release was 62% with a standard deviation of 2.4 at a concentration of 250 µg/ml. Histamine release increased to 89% with a standard deviation of 2.9 when the concentration increased to 500 µg/ml histamine release maximum at 100% at 750 µg/ml, with a 2.3 standard deviation. At the highest amount evaluated 1000 µg/ml, the highest level of histamine release persisted, remaining at 100 % with a slightly increased standard deviation of 2.7. These results point to a reliable association between cell lysate concentration and histamine release in this study, suggesting that higher concentrations of cell lysate have significance resulting in higher histamine release.



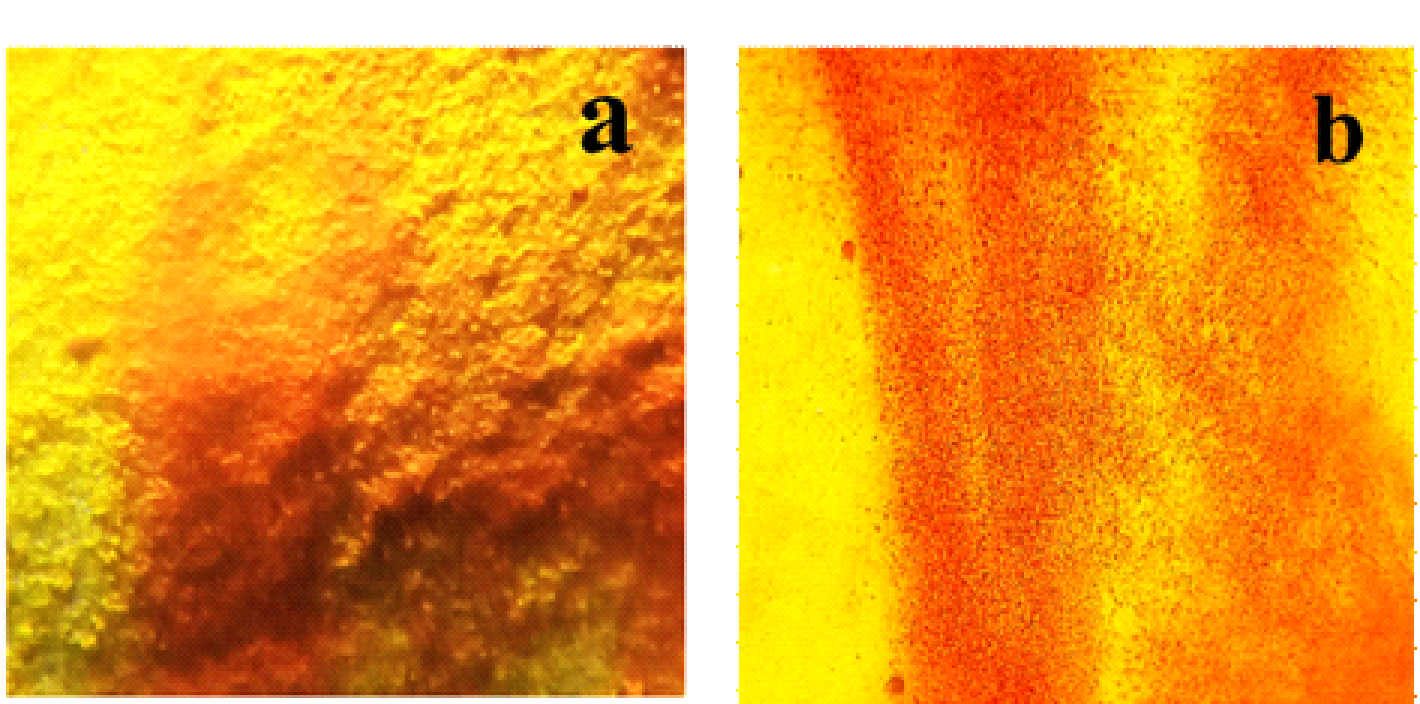
**Figure 1.** Histamine Release from Pseudomonas Treated with Antibiotic Imipenem.

In the analysis of cell viability for multidrug-resistant *P. aeruginosa* exposed to varying concentrations of the antibiotic Imipenem, the results reveal a clear dose-dependent effect (Fig. 2). At a concentration of 250µg/ml Imipenem, the cell viability was 13%, indicating a moderate impact on the pathogen. This viability significantly decreased to 2% at 500 µg/ml, suggesting increased antibiotic effectiveness at this higher concentration. At concentrations of 750µg/ml and 1000 µg/ml, cell viability decreased to 0%, indicating a total loss of viability and highlighting the strain's strong resistance to Imipenem at these levels. These findings underscore the multidrug-resistant nature of the *Pseudomonas aeruginosa* strain, demonstrating that Imipenem is ineffective even at higher concentrations.



**Figure 2.** Cell Viability of Pseudomonas aeruginosa Treated with Imipenem.

The study aimed to assess the inhibition of biofilm formation by *Pseudomonas aeruginosa* upon treatment with the antibiotic Imipenem (Fig.3). The analysis focused on a 48-hour-old biofilm of *Pseudomonas aeruginosa*. For the antibiofilm microscopic analysis, Pseudomonas aeruginosa (10⁴cells/ml) was inoculated into a 6-well plate were allowed to form a biofilm for 24 hours(Nikalje et al., 2024). The biofilm was then treated with Imipenem for 12 to 48 hours. Observations indicate that Imipenem effectively inhibits the formation and persistence of *Pseudomonas aeruginosa* biofilm(Chehelgerdi et al., 2023). Consequently, treatment with imipenem leads to a significant reduction in biofilm density, demonstrating its potential as an effective agent against biofilm-associated infections caused by *Pseudomonas aeruginosa.*



**Figure 3** Antibiofilm activity of *Pseudomonas aeruginosa* treated with antibiotic at different concentration, a) Control, b) Imipenem

# Discussion

[(Su et al., 2022)](https://paperpile.com/c/JRliSp/McmrW) reported that increased fatty acid biosynthesis contributes to ciprofloxacin resistance in P. aeruginosa. This finding highlights fatty acid biosynthesis as a potential target pathway for addressing antibiotic-resistant bacteria. Similarly, [(Tang et al., 2022)](https://paperpile.com/c/JRliSp/zL5K4) demonstrated that glucose-potentiated amikacin effectively kills Pseudomonas aeruginosa strains resistant to cefoperazone/sulbactam, thereby enhancing the efficacy of antibiotic-mediated killing.[(Herrera-Espejo et al., 2020)](https://paperpile.com/c/JRliSp/RmUQb) found that pentamidine when combined with antibiotics, demonstrated in vitro synergy against MDR Pseudomonas aeruginosa clinical strains. This suggests that pentamidine could be a substantial adjunct to antibiotics in treating infections caused by MDR Pseudomonas aeruginosa. [(Botelho et al., 2019)](https://paperpile.com/c/JRliSp/aiWvQ) explained that Pseudomonas aeruginosa develops antibiotic resistance through the acquisition of resistance genes, chromosomal mutations, and horizontal gene transfer. This leads to a diverse, non-clonal population structure and the emergence of high-risk clones globally.[(Ohadian Moghadam et al., 2020)](https://paperpile.com/c/JRliSp/gIKW7) reported that carbapenem-resistant Pseudomonas aeruginosa strains, exhibiting significant genomic diversity, have spread in an Iranian hospital, presenting a major challenge for antibiotic control measures. [(Lam et al., 2020)](https://paperpile.com/c/JRliSp/MgiIT) reported that 600-Da branched polyethylenimine (600-Da BPEI) significantly enhances the effectiveness of beta-lactam antibiotics against P. aeruginosa and its biofilms, providing a promising approach for addressing multidrug-resistant infections. [(Ugwuanyi et al., 2021)](https://paperpile.com/c/JRliSp/QwOGn) identified 39 Pseudomonas isolates: 35 were P. aeruginosa and 4 were other Pseudomonas species. All isolates were resistant to amoxicillin-clavulanate, ceftazidime, cefuroxime with varying resistance to other antibiotics. They all formed biofilms, with 28% being strong biofilm formers, and harbored the pslA and pslD biofilm genes. Efflux pump activity was observed in 59% of isolates, with mexA detected in all and mexB and oprM in most strong, moderate, and weak biofilm formers. [(Kamali et al., 2020)](https://paperpile.com/c/JRliSp/Ll7GL) it is recommended to use combination therapy, such as an anti-pseudomonal beta-lactam like piperacillin/tazobactam or ceftazidime paired with an aminoglycoside, or a carbapenem such as imipenem or meropenem combined with fluoroquinolones and an aminoglycoside, to treat Pseudomonas infections. Effective antimicrobial use and strict infection control are crucial to prevent resistance. Selecting combination strategies with appropriate anti-pseudomonal and anti-biofilm agents can help eradicate biofilm-associated infections.

# Conclusion

Pseudomonas aeruginosa poses significant clinical challenges due to its formidable Antibiotic resistance and biofilm formation capabilities. This study investigates the bacterium's biochemical profile and resistance mechanisms, underscoring the necessity for alternative treatment approaches. The multidrug-resistant pathogen exhibited notable anti-biofilm activity, surpassing the efficacy of Imipenem in counteracting biofilm formation. These findings suggest that antibiotics might offer a promising alternative or supplementary treatment for biofilm related infections caused by multidrug-resistant Pseudomonas aeruginosa. Future research is needed to elucidate the mechanisms of action and explore potential clinical applications of antibiotics to establish effective treatment strategies against this resilient pathogen.

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