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# IN-VITRO EFFICACY OF PIPERACILLIN/SULBACTAM AGAINST MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA*: A COMPARATIVE STUDY WITH PIPERACILLIN/TAZOBACTAM AND CEFOPERAZONE/SULBACTAM

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## Abstract

*Multidrug-resistant (MDR) Pseudomonas aeruginosa represents an important challenge for the treatment of healthcare-associated infections because of its capacity to develop resistance to multiple classes of antimicrobial agents. The organism possesses several mechanisms of resistance, including reduced outer-membrane permeability, active efflux, modification of antibiotic targets, and enzymatic inactivation of antimicrobial agents. The development and use of β-lactam/β-lactamase-inhibitor combinations have therefore become important strategies for improving the activity of β-lactam antibiotics against resistant Gram-negative organisms. This research paper examines the in-vitro efficacy of piperacillin/sulbactam against MDR P. aeruginosa and compares its activity with piperacillin/tazobactam and cefoperazone/sulbactam. The paper is based principally on the uploaded thesis by Ambreen Malik, completed in the Department of Microbiology, Quaid-i-Azam University, Islamabad, in 2012. The original laboratory study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi. A total of 170 P. aeruginosa isolates recovered from different clinical specimens were examined during three months from April to June 2011. One hundred isolates were identified as multidrug-resistant and selected for further investigation. Identification involved conventional microbiological procedures, while antimicrobial susceptibility was determined through the modified Kirby-Bauer disk diffusion method according to CLSI guidelines. The study compared three β-lactam/β-lactamase-inhibitor combinations: piperacillin/sulbactam, piperacillin/tazobactam, and cefoperazone/sulbactam. The thesis concludes that piperacillin/sulbactam demonstrated effective in vitro activity against MDR P. aeruginosa and merits consideration as an alternative therapeutic combination. The findings also underline the continuing importance of antimicrobial susceptibility testing and surveillance of resistance patterns.*

## 1. Introduction

Antimicrobial resistance has become an increasingly important problem in modern infectious disease management (Zavaleta-Monestel et al., 2025). Among Gram-negative pathogens, *Pseudomonas aeruginosa* is particularly significant because of its ability to survive in diverse environments, cause opportunistic infections, and

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develop resistance during antimicrobial exposure (Elfadadny et al., 2024). The organism is associated with infections involving wounds, the respiratory tract, urinary tract, bloodstream, and other sites, particularly among hospitalized and critically ill patients (World Health Organization, 2024).

The emergence of multidrug-resistant *P. aeruginosa* complicates treatment because resistance may occur simultaneously against several classes of antibiotics (Anjum and Mir, 2010). The original thesis emphasizes that increasing antimicrobial use can exert selective pressure on bacterial populations and contribute to the emergence of resistant strains. Consequently, antimicrobial susceptibility testing is essential for determining which agents retain activity against locally circulating isolates.

β-lactam antibiotics constitute an important group of antibacterial agents (Mora-Ochomogo and Lohans, 2021). Their principal action is directed toward bacterial cell-wall synthesis. Piperacillin, a broad-spectrum penicillin, has historically been used against a range of Gram-negative organisms, including *P. aeruginosa* (Holmes et al., 1984). However, its effectiveness may be compromised by β-lactamases, enzymes produced by bacteria that hydrolyze susceptible β-lactam antibiotics.

One strategy for overcoming β-lactamase-mediated resistance is to combine a β-lactam antibiotic with a β-lactamase inhibitor (Annunziato, 2019). Sulbactam and tazobactam are examples of such inhibitors. The combination of a β-lactam with a β-lactamase inhibitor can protect the active antibiotic from enzymatic destruction and potentially improve its antibacterial activity (Miller, 2001).

The paper examined three combinations: piperacillin/sulbactam, piperacillin/tazobactam, and cefoperazone/sulbactam. These combinations were investigated because of their relevance to infections caused by Gram-negative bacteria and their potential activity against resistant *P. aeruginosa*.

The central purpose of the research was therefore to determine the prevalence of MDR *P. aeruginosa* among clinical isolates and to investigate the in-vitro susceptibility of these isolates to piperacillin/sulbactam, with comparison against piperacillin/tazobactam and cefoperazone/sulbactam.

## 2. Background and Literature Review

### 2.1 *Pseudomonas aeruginosa* and antimicrobial resistance

*P. aeruginosa* is a Gram-negative organism capable of causing serious opportunistic infections (Anjum and Mir, 2010). Its clinical importance is closely related to its adaptability and its capacity to acquire or express multiple resistance mechanisms. The research identifies several major mechanisms through which *P. aeruginosa* can become resistant to antimicrobial agents.

The first mechanism is antibiotic efflux. Bacterial efflux systems can actively transport antimicrobial compounds out of the bacterial cell, reducing the intracellular concentration of the drug and thereby limiting its effectiveness (Gaurav et al., 2023). Efflux pumps also perform physiological functions in bacterial cells, but their ability to export antimicrobial agents contributes to resistance.

A second mechanism involves modification of antibiotic targets. Antibiotics act on specific cellular targets, and changes in these targets can reduce the ability of an antimicrobial agent to bind effectively (Uddin et al., 2021). Target modification is therefore an important component of bacterial resistance.

A third mechanism involves changes in outer-membrane permeability. The outer membrane of Gram-negative bacteria forms a barrier to many antimicrobial compounds. Alterations in membrane proteins, particularly porins, can decrease antibiotic entry into the bacterial cell (Delcour, 2009). Because an antibiotic must reach its intracellular or membrane-associated target to exert its effect, reduced permeability can contribute substantially to resistance.

The fourth major mechanism described in the thesis is enzymatic modification or destruction of antibiotics. β-lactamases can hydrolyze β-lactam antibiotics and prevent them from reaching their intended antibacterial target (Schito, 2006). This

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mechanism provides an important rationale for combining β-lactam antibiotics with β-lactamase inhibitors.

### 2.2 β-lactam antibiotics

β-lactam antibiotics include penicillins, cephalosporins, carbapenems, and monobactams. Their antibacterial action is primarily associated with inhibition of bacterial cell-wall synthesis (Bush and Bradford, 2016). They interfere with enzymes involved in the construction of the bacterial cell wall, ultimately compromising bacterial survival.

Piperacillin is a β-lactam antibiotic with activity against a range of Gram-negative bacteria. However, its activity can be reduced by β-lactamases. The development of combinations such as piperacillin/tazobactam and piperacillin/sulbactam represents an attempt to preserve the activity of piperacillin in the presence of β-lactamase-producing bacteria (Gin et al., 2007).

Carbapenems, including imipenem and meropenem, have also been important agents against resistant *P. aeruginosa* (Papp-Wallace et al., 2011). However, the emergence of resistance to multiple antimicrobial classes means that dependence on any single antimicrobial group is problematic.

### 2.3 β-lactamase inhibitors and combination therapy

β-lactamase inhibitors have limited antibacterial activity when used alone but can protect β-lactam antibiotics from enzymatic degradation (Bush and Bradford, 2016). Sulbactam and tazobactam are examples of inhibitors used in combination therapy.

The research discusses three relevant combinations. Piperacillin/sulbactam combines piperacillin with sulbactam; piperacillin/tazobactam combines piperacillin with tazobactam; and cefoperazone/sulbactam combines cefoperazone with sulbactam.

Piperacillin/sulbactam could demonstrate activity comparable to, or in some circumstances greater than, other β-lactam/β-lactamase-inhibitor combinations. For example, investigated the comparative in-vitro activity of piperacillin, piperacillin/sulbactam, and piperacillin/tazobactam against nosocomial pathogens (Frank et al., 2003). The thesis notes that the two piperacillin combinations showed similar activity against *P. aeruginosa* in that investigation.

## 3. Research Objectives

The original investigation had several related objectives.

First, it aimed to isolate *P. aeruginosa* from different clinical specimens. Second, the study sought to identify the organism through conventional microbiological and biochemical procedures. Third, it investigated antimicrobial susceptibility using disk diffusion testing. Fourth, it determined the prevalence of multidrug-resistant *P. aeruginosa* among the isolates examined. Finally, it compared the in vitro activity of piperacillin/sulbactam with piperacillin/tazobactam and cefoperazone/sulbactam against MDR isolates.

Together, these objectives connected epidemiological observation with laboratory susceptibility testing. The research was therefore not limited to determining whether resistance existed; it also attempted to identify an antimicrobial combination that retained useful activity against resistant isolates.

## 4. Materials and Methods

### 4.1 Study design and setting

The research was a laboratory-based comparative study conducted in the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. Clinical specimens were obtained from patients admitted to outpatient departments and intensive care units of Combined Military Hospital, Rawalpindi. Outdoor patients were also included.

### 4.2 Sample collection

A total of 170 *P. aeruginosa* isolates recovered from clinical specimens were included. The specimens represented different clinical sources, including

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pus, pus swabs, urine, blood, sputum, ear swabs, nasal swabs, and throat swabs.

Following screening for multidrug resistance, 100 isolates were selected for further study. The selection of these MDR isolates allowed the comparative antimicrobial investigation to focus specifically on strains presenting the resistance problem targeted by the research.

### 4.3 Isolation and identification

Clinical specimens were processed using conventional microbiological methods. Except for blood and urine specimens, samples were inoculated onto appropriate culture media, including 5% sheep blood agar, MacConkey agar, and chocolate agar.

Identification of *P. aeruginosa* involved examination of colony morphology and conventional microbiological characteristics. Gram staining, oxidase testing, motility testing, and biochemical identification procedures were among the methods used to establish the identity of the isolates. The thesis also reports the use of API 20NE for identification.

### 4.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility was investigated using the modified Kirby-Bauer disk diffusion method. Testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline M2-A9 (2006).

The three antimicrobial combinations examined were:

1. Piperacillin/sulbactam
2. Piperacillin/tazobactam
3. Cefoperazone/sulbactam

The comparison was designed to determine the relative in-vitro activity of the three combinations against the MDR isolates.

## 5. Results

The investigation identified *P. aeruginosa* in a variety of clinical specimens, demonstrating its distribution across several infection-associated sources. Of the 170 isolates examined, 100 were categorized as multidrug-resistant and were therefore included in the principal comparative analysis.

The study examined the distribution of MDR *P. aeruginosa* according to clinical specimen, gender, and age. The thesis presents the results graphically, demonstrating variation in the occurrence of MDR isolates among the different specimen categories.

The antimicrobial susceptibility results provide the central finding of the study. The thesis reports that piperacillin/sulbactam demonstrated effective activity against MDR *P. aeruginosa*. Its activity was compared directly with piperacillin/tazobactam and cefoperazone/sulbactam.

The findings are important because resistance in *P. aeruginosa* is not necessarily uniform across antimicrobial agents or combinations. A strain resistant to one β-lactam may retain susceptibility to another combination, depending upon the particular resistance mechanisms expressed. Consequently, local susceptibility testing remains important in determining the usefulness of individual antimicrobial combinations.

The study's findings also need to be interpreted in the context of its laboratory design. The results demonstrate in vitro susceptibility patterns rather than clinical treatment outcomes. In vitro activity is an important component of antimicrobial evaluation, but clinical effectiveness also depends on factors such as infection site, drug pharmacokinetics, patient characteristics, dosage, and the presence of additional resistance mechanisms.

## 6. Discussion

The results of this study demonstrate the continuing significance of MDR *P. aeruginosa* as a laboratory and clinical problem. The identification of 100 MDR isolates among the 170 *P. aeruginosa* isolates examined illustrates the substantial resistance burden within the study population.

The high level of resistance can be understood in relation to the biological characteristics of *P. aeruginosa*. Its outer membrane restricts the entry of many antimicrobial agents, while efflux mechanisms can actively remove antibiotics from

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the bacterial cell. In addition, enzymatic mechanisms can inactivate β-lactam antibiotics. These mechanisms may operate simultaneously, producing resistance to several antimicrobial classes.

The study's focus on β-lactam/β-lactamase-inhibitor combinations is therefore significant. Piperacillin by itself can be vulnerable to β-lactamase-mediated hydrolysis. The addition of sulbactam is intended to inhibit relevant β-lactamases and preserve the activity of piperacillin. Piperacillin/tazobactam has similarly been widely investigated because tazobactam can inhibit β-lactamases and thereby enhance the activity of piperacillin. Cefoperazone/sulbactam represents another combination in which sulbactam is used to protect a β-lactam antibiotic.

The principal contribution of the present investigation is its comparison of these combinations specifically against MDR *P. aeruginosa*. According to the conclusion, piperacillin/sulbactam proved effective against the MDR isolates examined and showed useful activity when compared with piperacillin/tazobactam and cefoperazone/sulbactam.

This result is consistent with the general rationale for β-lactam/β-lactamase-inhibitor combinations. However, it should not be interpreted as demonstrating universal superiority of piperacillin/sulbactam. The study was an in-vitro investigation conducted in a particular Pakistani clinical setting and during a defined period. Resistance patterns can change over time and can vary between hospitals, geographical areas, patient populations, and specimen types.

The findings also reinforce the importance of antimicrobial surveillance. Susceptibility data generated from local laboratories can help clinicians understand which drugs retain activity against circulating organisms. Such information becomes particularly important where MDR organisms are common.

The thesis further emphasizes that increasing antimicrobial resistance creates a need for careful selection of effective antimicrobial agents. Repeated or inappropriate use of antibiotics can contribute to selection for resistant organisms. Therefore, laboratory susceptibility testing should be combined with appropriate antimicrobial stewardship and infection-control practices.

## 7. Limitations

Several limitations should be considered when interpreting the findings.

First, the study was conducted at a single principal laboratory and involved specimens obtained from a defined clinical setting in Rawalpindi. Consequently, its findings cannot automatically be generalized to all hospitals or regions.

Second, the study was based on in-vitro susceptibility testing. The observation that an isolate is susceptible to an antimicrobial combination in laboratory testing does not by itself establish clinical superiority or guarantee successful treatment in patients.

Third, the study focused on susceptibility patterns rather than detailed molecular characterization of resistance mechanisms. Although the thesis discusses mechanisms such as efflux, permeability changes, target modification, and enzymatic resistance, the investigation did not establish the molecular contribution of each mechanism in the individual isolates.

Finally, antimicrobial resistance is dynamic. Results obtained from isolates collected in 2011 provide an important historical picture but should not be assumed to represent current resistance patterns. Contemporary surveillance would be necessary to determine whether the same susceptibility profile persists.

## 8. Conclusion

Multidrug-resistant *Pseudomonas aeruginosa* presents a significant challenge because of its ability to resist multiple antimicrobial agents through several complementary mechanisms (Elfadadny et al., 2024). The study examined this problem through a comparative in vitro assessment of three β-lactam/β-lactamase-inhibitor combinations.

The investigation analyzed 170 *P. aeruginosa* isolates obtained from different clinical specimens and identified 100 as multidrug-resistant. These isolates were subsequently examined using

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modified Kirby-Bauer disk diffusion testing against piperacillin/sulbactam, piperacillin/tazobactam, and cefoperazone/sulbactam.

The principal finding reported by the thesis is that piperacillin/sulbactam demonstrated effective in-vitro activity against MDR *P. aeruginosa* and represented a potentially useful alternative among the antimicrobial combinations investigated. The findings also support the broader importance of β-lactam/β-lactamase-inhibitor combinations in confronting β-lactam resistance.

At the same time, the findings should be understood within the limits of the study. They represent laboratory susceptibility patterns from isolates collected in a particular clinical environment during 2011 and should not be treated as contemporary or universally applicable treatment evidence.

The study ultimately demonstrates the value of systematic antimicrobial susceptibility testing in the management of MDR *P. aeruginosa*. Continued surveillance, appropriate antimicrobial selection, rational antibiotic use, and effective infection-control measures remain essential components of addressing antimicrobial resistance.

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