Antimicrobial Properties and Combination for management of Pseudomonas aeruginosa with Difficult-to-Treat Resistance

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Abstract

Pseudomonas aeruginosa is a highly adaptable pathogen recognized for its intrinsic resistance and ability to develop difficult-to-treat resistance (DTR) against multiple antibiotic classes, posing significant challenges in clinical management. This review focuses on the antimicrobial properties of fosfomycin, rifampicin, and azithromycin, and their potential in combination therapies to manage DTR *P. aeruginosa* infections. These agents, although not traditionally first-line treatments for *P. aeruginosa*, have demonstrated promising synergistic effects when used in combination, particularly against biofilm-associated infections. Fosfomycin, with its ability to inhibit bacterial cell wall synthesis, has shown activity against certain resistant *P. aeruginosa* strains, especially when combined with other agents to overcome efflux-mediated resistance. Rifampicin, an RNA polymerase inhibitor, has limited standalone efficacy against Gram-negative bacteria but exhibits synergistic effects in combination therapies, enhancing bacterial killing. Azithromycin, a macrolide, targets protein synthesis and demonstrates immunomodulatory and anti-biofilm properties, making it a valuable adjunct in treating chronic *P. aeruginosa* infections. This review also explores the mechanisms underpinning the synergistic interactions of these agents and their role in enhancing antibiotic penetration, disrupting biofilms, and reducing the bacterial load. In vitro and in vivo studies indicate that combinations of these drugs can effectively target DTR *P. aeruginosa* by exploiting complementary mechanisms of action and minimizing the development of resistance. Emphasizing the need for innovative therapeutic strategies, this review highlights the clinical potential of fosfomycin, rifampicin, and azithromycin as part of combination regimens. By addressing the challenges posed by DTR *P. aeruginosa*, this article aims to provide insights into optimizing treatment approaches for one of the most challenging pathogens in modern healthcare.

Keywords: Antimicrobial Properties, Combination, Pseudomonas aeruginosa

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Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that poses a significant threat to public health, particularly in healthcare settings. It is a leading cause of healthcare-associated infections (HAIs) such as ventilator-associated pneumonia, bloodstream infections, and urinary tract infections. Critically ill and immunocompromised patients are most at risk, and infections caused by *P. aeruginosa* are associated with high morbidity, mortality, and healthcare costs. Its remarkable ability to resist a wide range of antibiotics has made it a priority pathogen in global antimicrobial resistance (AMR) efforts [1].

Intrinsic resistance mechanisms make *P. aeruginosa* inherently difficult to treat. Its outer membrane has low permeability, limiting the entry of many antibiotics. Additionally, it possesses efflux pumps that actively expel antimicrobial agents and enzymes, such as β-lactamases, that inactivate antibiotics. These intrinsic traits provide a baseline resistance to many first-line treatments, complicating therapeutic options [2].

Compounding this issue is the bacterium's ability to acquire resistance determinants through horizontal gene transfer and mutational adaptation. Mobile genetic elements such as plasmids, transposons, and integrons play a key role in the dissemination of resistance genes, including those encoding carbapenemases and extended-spectrum β-lactamases (ESBLs). This acquired resistance has led to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains, which are particularly concerning [3].

Biofilm formation further exacerbates the challenge of treating *P. aeruginosa* infections. Biofilms are structured bacterial communities encased in an extracellular polymeric matrix that provides protection against antibiotics and the host immune system. Bacteria within biofilms exhibit a dormant metabolic state, making them less susceptible to antibiotics targeting actively dividing cells. Biofilm-associated infections, such as those in cystic fibrosis patients or on medical devices, are particularly persistent and challenging to eradicate [4].

In clinical practice, infections caused by *P. aeruginosa* are often difficult to diagnose and manage. Delays in identifying resistant strains can result in inappropriate initial treatment, leading to worsened outcomes. Rapid diagnostic methods capable of detecting resistance genes and mechanisms are critical for improving the management of these infections. However, the widespread implementation of such technologies, especially in low- and middle-income countries, remains limited [5].

Therapeutic options for difficult-to-treat *P. aeruginosa* are increasingly limited. While polymyxins, β-lactam/β-lactamase inhibitor combinations, and aminoglycosides are often used as last-resort treatments, their efficacy is compromised by the emergence of resistance. Furthermore, the toxic side effects of certain drugs, such as colistin, pose additional challenges, particularly in vulnerable patient populations [6].

Given the formidable resistance mechanisms of *P. aeruginosa*, innovative approaches are urgently needed to combat this pathogen. Strategies such as combination therapies, anti-biofilm agents, quorum sensing inhibitors, and bacteriophage therapy are under investigation to overcome resistance and improve treatment outcomes. This review explores these approaches, with a focus on the challenges and opportunities in managing infections caused by *P. aeruginosa* with difficult-to-treat resistance [7].

A notable research gap in the study of *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR) lies in the lack of comprehensive data on the efficacy and optimization of combination therapies, particularly involving non-traditional agents like fosfomycin, rifampicin, and azithromycin. While in vitro and preclinical studies suggest promising synergistic effects, there is limited evidence from large-scale clinical trials assessing the effectiveness, safety, and pharmacokinetics of these combinations in real-world scenarios, particularly in critically ill or immunocompromised patient populations.

Additionally, while biofilm-associated resistance mechanisms are well-documented, there is insufficient understanding of how combination therapies interact with biofilms in vivo. The precise dynamics of antibiotic penetration, biofilm disruption, and eradication within host environments remain unclear, limiting the development of targeted anti-biofilm strategies.

Another gap exists in the application of advanced diagnostic tools to guide personalized treatment strategies. Rapid molecular diagnostics capable of identifying resistance profiles and predicting therapeutic efficacy for combination regimens are underutilized in clinical practice, especially in resource-limited settings where the burden of antimicrobial resistance is highest.

Addressing these gaps through clinical trials, translational research, and diagnostic innovation could provide critical insights into optimizing treatment strategies for *P. aeruginosa* with DTR and improving patient outcomes.

Antimicrobial Properties for *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacterium with inherent resistance to many antibiotics, owing to its robust intrinsic defense mechanisms. These include its low outer membrane permeability and efflux pump systems, which reduce the intracellular concentration of antibiotics. Additionally, its ability to produce β-lactamases and form biofilms further complicates treatment. The combination of these traits has necessitated the development and use of diverse antimicrobial agents targeting specific mechanisms to combat this pathogen effectively [8].

Beta-lactams, including penicillins, cephalosporins, and carbapenems, are among the most commonly used antibiotics against *P. aeruginosa*. These agents inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs). However, the efficacy of β-lactams is often limited by the production of β-lactamases, particularly extended-spectrum β-lactamases (ESBLs) and carbapenemases, which hydrolyze these antibiotics and render them ineffective [9].

Polymyxins, such as colistin, are considered last-resort antibiotics for multidrug-resistant (MDR) *P. aeruginosa*. These cationic peptides disrupt the bacterial outer membrane by interacting with lipopolysaccharides (LPS), leading to cell death. However, the emergence of colistin-resistant strains due to modifications in LPS, combined with the drug's nephrotoxicity, limits its clinical utility. Despite these challenges, polymyxins remain critical in the treatment of extensively drug-resistant (XDR) infections [10].

Aminoglycosides, such as gentamicin and tobramycin, target bacterial protein synthesis by binding to the 30S ribosomal subunit. These antibiotics are often used in combination with β-lactams or polymyxins to enhance their efficacy against *P. aeruginosa*. However, resistance mediated by aminoglycoside-modifying enzymes and efflux pump overexpression has reduced their standalone effectiveness in treating MDR strains [11].

Quinolones, including ciprofloxacin and levofloxacin, inhibit bacterial DNA replication by targeting DNA gyrase and topoisomerase IV. They are effective against a wide range of Gram-negative bacteria, including *P. aeruginosa*. However, the overuse of quinolones has led to the emergence of resistant strains, primarily through mutations in target enzymes and increased efflux pump activity, limiting their clinical applicability [12].

Fosfomycin, a phosphonic acid derivative, inhibits bacterial cell wall synthesis by targeting the MurA enzyme. Although traditionally used for urinary tract infections, fosfomycin has shown promise against *P. aeruginosa*, particularly in combination with other antibiotics. Its ability to penetrate biofilms and evade efflux mechanisms makes it a valuable agent in the fight against MDR strains [13].

Rifampicin, an RNA polymerase inhibitor, has limited activity against Gram-negative bacteria, including *P. aeruginosa*, due to the impermeability of the outer membrane. However, when used in combination with other agents, rifampicin can exhibit synergistic effects, enhancing bacterial killing. This property makes it a potential adjunctive therapy for difficult-to-treat infections [14].

Azithromycin, a macrolide antibiotic, inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. While not traditionally effective against *P. aeruginosa*, azithromycin has demonstrated immunomodulatory and anti-biofilm properties. These characteristics make it a valuable component of combination therapies for chronic infections, such as those seen in cystic fibrosis patients [15].

Newer β-lactam/β-lactamase inhibitor combinations, such as ceftolozane-tazobactam and ceftazidime-avibactam, have shown excellent activity against MDR *P. aeruginosa*. These agents combine the cell wall-inhibiting properties of β-lactams with the ability to inhibit β-lactamases, restoring efficacy against resistant strains. They represent a significant advancement in the treatment of drug-resistant infections [16].

Antimicrobial peptides (AMPs) are emerging as a promising class of therapeutics against *P. aeruginosa*. These peptides disrupt bacterial membranes or interfere with essential cellular processes, and their broad-spectrum activity and low propensity for resistance development make them an attractive alternative to traditional antibiotics. Preclinical studies have shown their efficacy against biofilm-associated infections, highlighting their potential in combating MDR strains [17].

Phage therapy, which employs bacteriophages to target and lyse *P. aeruginosa*, is another innovative approach. Phages can penetrate biofilms and are highly specific to their bacterial targets, minimizing off-target effects. This specificity and their ability to co-evolve with bacterial populations make phages a promising option for managing antibiotic-resistant infections [18].

The development of anti-virulence therapies targeting *P. aeruginosa*'s resistance mechanisms, such as efflux pumps and quorum sensing systems, represents another frontier in antimicrobial research. These therapies aim to disarm the pathogen rather than kill it, reducing selective pressure for resistance. Combining these approaches with existing antibiotics holds significant promise for improving the treatment of *P. aeruginosa* infections [19].

Mechanism of Resistance and Antimicrobial Combination

Pseudomonas aeruginosa is renowned for its multifaceted resistance mechanisms, complicating treatment, particularly for multidrug-resistant (MDR) strains. Fosfomycin, rifampicin, and azithromycin are emerging agents in combination therapy to tackle difficult-to-treat *P. aeruginosa*. These combinations exploit the unique properties of each antibiotic to enhance efficacy and circumvent resistance mechanisms [20].

Fosfomycin is a broad-spectrum antibiotic that inhibits bacterial cell wall synthesis by targeting MurA, an enzyme involved in peptidoglycan biosynthesis. However, *P. aeruginosa* has intrinsic mechanisms that reduce fosfomycin's effectiveness, including the overexpression of efflux pumps (e.g., MexAB-OprM) and mutations in the uptake systems such as GlpT and UhpT transporters. These resistance mechanisms necessitate combining fosfomycin with other agents to restore its efficacy [21].

Rifampicin, an RNA polymerase inhibitor, is primarily active against Gram-positive bacteria, but its activity against *P. aeruginosa* is limited by the outer membrane's impermeability. Mutations in the rpoB gene, encoding the β-subunit of RNA polymerase, also confer resistance. Despite these challenges, rifampicin has shown significant synergistic effects when combined with other agents, making it a potential candidate in combination therapies [22].

Azithromycin, a macrolide antibiotic, inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. While its direct activity against *P. aeruginosa* is limited due to efflux pumps and ribosomal mutations, azithromycin exhibits immunomodulatory and anti-biofilm properties. These unique attributes enhance its utility as an adjunct in combination therapies targeting chronic infections [23].

The combination of fosfomycin and rifampicin leverages their complementary mechanisms of action. Fosfomycin disrupts the cell wall, increasing bacterial permeability, which facilitates rifampicin's access to its intracellular target. This synergistic interaction has been demonstrated in both in vitro and in vivo studies, significantly enhancing bacterial killing and reducing the likelihood of resistance development [24].

When combined with azithromycin, fosfomycin's cell wall disruption augments the macrolide's penetration into bacterial cells and biofilms. Azithromycin's anti-biofilm effects further complement fosfomycin's activity, making this combination particularly effective in biofilm-associated infections, such as those seen in cystic fibrosis patients or medical device-related infections [25].

Rifampicin and azithromycin also exhibit synergistic effects in combination with fosfomycin. Rifampicin's intracellular activity against RNA polymerase complements azithromycin's inhibition of protein synthesis, creating a multi-targeted attack on the pathogen. These combinations are especially promising in addressing resistance mechanisms such as efflux pump overexpression and biofilm-mediated resistance [26].

Biofilm-associated resistance is a major challenge in treating *P. aeruginosa* infections. The extracellular polymeric matrix (EPM) of biofilms acts as a barrier to antibiotic penetration. Fosfomycin's ability to disrupt biofilm integrity, combined with azithromycin's quorum sensing inhibition and rifampicin's intracellular activity, makes these combinations highly effective against biofilm-embedded bacteria [27].

The pharmacokinetic and pharmacodynamic properties of these combinations also support their clinical potential. Fosfomycin's broad tissue distribution and high urinary concentrations make it ideal for treating urinary tract infections caused by *P. aeruginosa*. When combined with rifampicin or azithromycin, it addresses systemic and chronic infections more effectively [28].

Clinical studies have shown that fosfomycin-rifampicin combinations significantly reduce bacterial loads in MDR *P. aeruginosa* infections. Similarly, the addition of azithromycin enhances the efficacy of fosfomycin in respiratory and biofilm-associated infections, reducing inflammatory markers and improving patient outcomes [29].

Despite their potential, the emergence of resistance to these combinations remains a concern. Mutations in target sites, efflux pump overexpression, and biofilm persistence can compromise the

efficacy of fosfomycin, rifampicin, and azithromycin. Continuous monitoring and optimization of these combinations are essential to maintain their clinical utility [30].

The synergistic effects of fosfomycin, rifampicin, and azithromycin extend beyond antimicrobial activity. Azithromycin's immunomodulatory properties, including the inhibition of pro-inflammatory cytokines and neutrophil recruitment, complement the bactericidal effects of fosfomycin and rifampicin, reducing tissue damage in chronic infections [31].

Combination therapies targeting resistance mechanisms also reduce the selective pressure for resistance development. By employing multiple agents with complementary targets, these combinations minimize the likelihood of bacterial survival and subsequent resistance mutations, addressing one of the key challenges in antimicrobial therapy [32].

The role of fosfomycin, rifampicin, and azithromycin in combination therapy highlights the importance of personalized medicine in treating *P. aeruginosa*. Tailoring these combinations based on the resistance profile of the pathogen and the infection site can optimize treatment outcomes and reduce unnecessary antibiotic exposure [33].

Further research is needed to validate the long-term efficacy and safety of these combinations. Large-scale clinical trials assessing their use in different patient populations, including critically ill and immunocompromised individuals, are essential to establish their role in routine clinical practice [34].

The integration of rapid diagnostic tools to identify resistance mechanisms and guide combination therapy is another critical area for development. Molecular diagnostics capable of detecting efflux pump expression, biofilm formation, and specific resistance genes can enhance the precision of fosfomycin, rifampicin, and azithromycin use [35].

Exploring the potential of adjunctive therapies, such as anti-biofilm agents and quorum sensing inhibitors, alongside these combinations could further enhance their efficacy. These strategies would provide a comprehensive approach to overcoming *P. aeruginosa*'s resistance mechanisms [36].

Phage therapy and nanotechnology-based drug delivery systems also offer innovative ways to improve the effectiveness of fosfomycin, rifampicin, and azithromycin combinations. Encapsulation in nanoparticles can enhance drug stability, reduce toxicity, and improve penetration into biofilms, providing a promising avenue for future research [37].

The combination of fosfomycin with rifampicin or azithromycin represents a paradigm shift in the management of MDR and DTR *P. aeruginosa*. By addressing both planktonic and biofilm-associated bacteria, these combinations offer a multifaceted approach to tackling one of the most challenging pathogens in modern medicine [38].

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