MECHANISMS OF ANTIMICROBIAL RESISTANCE IN *PSEUDOMONAS AERUGINOSA*: AN OVERVIEW

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ABSTRACT

Background: Infections due to antibiotic resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) presents a major challenge to the health care systems due to limited treatment options leading to longer hospital stays, higher healthcare costs, and increased mortality rates.

Aim: The aim of this narrative review was to present a comprehensive overview of the strategies *P. aeruginosa* used to counteract the effect of antimicrobials and render them ineffective.

Materials and Methods: Databases such as Scopus, PubMed, google scholar, Hinari, Up-to-date and AJOL were search for relevant literatures using the search terms: Resistance mechanism, *Pseudomonas aeruginosa* resistance, Innate resistance, acquired resistance, Mobile genetic element and *P. aeruginosa* resistance.

Results: In this review article, we describe the types of antibiotic resistance, factors contributing to the development of antibiotic resistance, we also provide an overview of the mechanisms that contribute to antibiotic resistance in *P. aeruginosa*. We describe the molecular mechanisms involved in the development of antibiotic resistance, including efflux pumps, alterations in membrane permeability, and enzymatic inactivation of antibiotics. We also discuss the role of quorum sensing, biofilm formation, and horizontal gene transfer in the acquisition and spread of antibiotic resistance in *P. aeruginosa*. Finally, we highlight the potential strategies to combat antibiotic resistance in *P. aeruginosa*, including the development of novel antibiotics, combination therapy, and the use of alternative therapies such as bacteriophages and probiotics.

Conclusion: This review provides valuable insights into the complex mechanisms of antibiotic resistance in *P. aeruginosa* and highlights the need for a multifaceted approach to

BACKGROUND OF THE STUDY

Globally, antimicrobial-resistant infections kill almost 700,000 people annually, and it is predicted that it will increase to 10 million deaths annually by 2050 (Spaulding *et al*., 2018). The World Bank estimated that the antimicrobial resistance (AMR) would not only be a health burden but also cause a reduction in the gross domestic product in 2050 that would be comparable to the 2008–2009 global financial crisis (Padiyara *et al*., 2018). In recent years, infections caused by *P. aeruginosa* are one of the major problems in hospitals and are linked to high mortality and morbidity rates, ranging from 18% to 61% (Moghaddam *et al*., 2012; Tam *et al*., 2010: Zavascki *et al*., 2010).

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Pseudomonas aeruginosa was reportedly resistant to many antibiotics, including aminoglycosides, cephalosporins, fluoroquinolones, and carbapenems (Chaudhary *et al*., 2016; Pang *et al*., 2018). Hence, *P. aeruginosa* was placed on the top of the priority pathogen list (critical) by the World Health Organization for prioritizing the new antibiotic development against multidrug resistance (MDR) pathogens (Tacconelli *et al*., 2017).

Pseudomonas aeruginosa, a Gram-negative bacterium, is a leading cause of healthcare-associated infections and is known for its ability to rapidly develop resistance to multiple classes of antibiotics (Raman *et al*., 2018).

Monotherapy and combination therapy are used in empirical antibiotic therapy for suspected *P. aeruginosa* infections; this therapy reduces mortality in patients with severe *P. aeruginosa* infections (El Solh and Alhajhusain, 2009; Park *et al*., 2012). However, due to this bacterium's ability to resist many of the currently available antibiotics, treating *P. aeruginosa* infections has become extremely difficult (Lister *et al*., 2009). The World Health Organization (WHO) recently identified carbapenem-resistant *P. aeruginosa* as one of three bacterial species where new antibiotics are urgently needed to treat infections (Tacconelli *et al*., 2017). Furthermore, overuse of antibiotics during treatment hastens the development of multidrug-resistant *P. aeruginosa* strains, rendering empirical antibiotic therapy ineffective against this microorganism (Hirsch and Tam, 2010). Multidrug resistance *P. aeruginosa* infections in the hospital setting are associated with poor outcomes including increased resource utilization and costs, morbidity, and mortality (Raman *et al*., 2018).

Overall, major mechanisms of resistance in *Pseudomonas aeruginosa* can be classified in to intrinsic, acquired and adaptive (Pang *et al*., 2018). The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell and the production of antibiotic inactivating enzymes (Pang *et al*., 2018). The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational changes (Breidenstein *et al*., 2011). The adaptive resistance of *P. aeruginosa* involves formation of biofilm, where the biofilm serves as a diffusion barrier to limit antibiotic access to the bacterial cells (Drenkard, 2003). This phenomenon is due to the complex interplay of intrinsic and acquired mechanisms of resistance that are present in this organism. Understanding these mechanisms of resistance is crucial for the development of effective treatment strategies against *P. aeruginosa* infections

In recent years, incidence of MDR *P. aeruginosa* infections has increase worldwide, making it a significant public health concern (Pang *et al*., 2019).

Antimicrobial resistance

Antimicrobial resistance (AMR) has emerged as one of the most serious public health issues of the twenty-first century, threatening the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi that are no longer susceptible to common antibiotics.

Types of resistance

1.0 Natural resistance

Natural resistance can be inherent (found in all species) or acquired (the genes are naturally occurring in the bacteria, but are only expressed to resistance levels after exposure to an antibiotic). Intrinsic resistance is a trait that is shared by all bacteria, is unrelated to previous antibiotic exposure, and is unrelated to horizontal gene transfer (Martinez, 2014; Cox and Wright, 2013). Reduced outer membrane permeability (most notably lipopolysaccharide, LPS, in gram negative bacteria) and natural efflux pump activity are the most common bacterial mechanisms involved in intrinsic resistance.

Another common induced resistance mechanism is multidrug-efflux pumps. (Cox and Wright, 2013; Fajardo *et al*., 2008). For example, *P. aeruginosa* has natural resistance to sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, and among others (Reygaert, 2018).

2.0 Acquired resistance

Resistance-inducing genetic material can be acquired via all of the main routes by which bacteria acquire any genetic material: transformation, transposition, and conjugation, all referred to as horizontal gene transfer—HGT (Pang *et al*., 2018).; additionally, the bacteria can undergo mutations to its own chromosomal DNA. The purchase could be temporary or permanent. Plasmid-mediated transmission of resistance genes is the most common method of acquiring foreign genetic material; bacteriophage-borne transmission is uncommon (Pang *et al*., 2018). Certain bacteria, such as *P. aeruginosa*., are ubiquitous and can thus acquire genetic material from their environment. After acquisition, internally, insertion sequences and integrons can move genetic material, and stressors (such as starvation, UV radiation, chemicals, and other environmental factors) between different bacteria and induce genetic mutations in bacteria (substitutions, deletions etc.) (Coculescu, 2009; Davies and Davies, 2010). Antimicrobial resistance mutations are usually found only in a few types of genes: those encoding drug targets, drug transporters, drug transporter regulators, and antibiotic-modifying enzymes (Martinez, 2014). One of the most perplexing aspects of antimicrobial resistance is that using these drugs increases resistance. Even using low or very low antimicrobial concentrations (sub-inhibitory) can result in the selection of high-level resistance in subsequent bacterial generations, may select for hypermutable strains (increase the mutation rate), may increase the ability to acquire resistance to other antimicrobial agents, and may promote the movement of mobile genetic elements (Blázquez *et al*., 2012).

Mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*

Antimicrobial resistance (AMR) is the ability of an organism to resist the action of an antimicrobial agent to which it was previously susceptible (Pang *et al*., 2019). *P. aeruginosa* has an extraordinary ability to resist the effect of antibiotics via multiple mechanisms, often at the same time, resulting in resistance to nearly all available antibiotics.

The mechanism of resistance of *P. aeruginosa* to an antimicrobial agent can be classified into three Intrinsic, acquired and adaptive. as shown in Figure 1.0

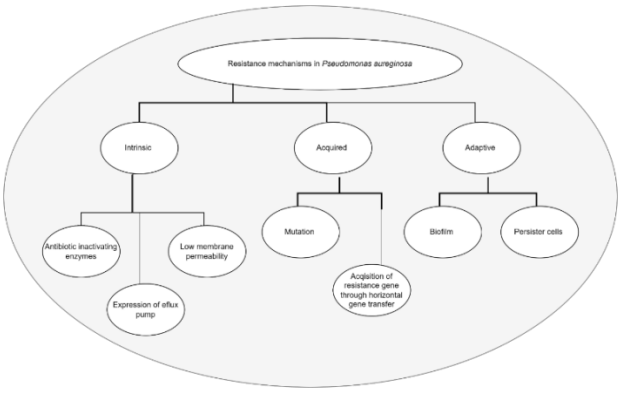

Fig. 1.0 Proposed mechanism of antibiotic resistance in *P. aeruginosa* (Pang *et al.*, 2019).

1.0 Intrinsic resistance mechanism

The intrinsic antibiotic resistance mechanism refers to its innate ability to reduce the efficacy of antibiotics through inherent structural or functional characteristics (Botelho *et al*., 2019).

Pseudomonas aeruginosa has been shown to possess high intrinsic resistance to most

antibiotics through low outer membrane permeability, efflux pump (systems that pump antibiotics out of the cell) and production of antibiotic-inactivating enzymes such as β-lactamase that renders antibiotic ineffective (Paprocka *et al*., 2022).

Low Outer membrane permeability of *P. aeruginosa*

The outer membrane of *P. aeruginosa* is highly restricted, 12–100 times less permeable than that of *Escherichia coli* (Paprocka *et al*., 2022). Since most antimicrobials used in treating *P. aeruginosa* infections must penetrate the cell membrane to reach its intracellular target, sublethal concentrations of such antimicrobials result in the development of resistance by the *P. aerugi*nosa. *Pseudomonas aeruginosa* possesses several specific porins, including the OprD involved in antibiotic uptake. It contains the binding sites for carbapenems, and the absence of OprD in *P. aeruginosa* increases the resistance to this antibiotic category (Chevalier *et al*., 2017). In Addition, overexpression OprH (is the smallest *P. aeruginosa* porin) is associated with increased resistance to polymyxin B and gentamicin through stabilization of the outer membrane by inducing LPS modification (Pang *et al*., 2019).

Bacterial efflux pumps are proteins anchored in the membrane of a bacteria that play an essential role in expelling toxic compounds, including antibiotics, out of the cell. *P. aeruginosa* expresses several efflux pumps that expel antimicrobials and other substances out of the bacterial cell (Pachori *et al*., 2019). They consist of cytoplasmic membrane transporters, periplasmic linker proteins and outer membrane porin channel proteins (Daury *et al*., 2016). The cytoplasmic and periplasmic components of *P. aeruginosa* RND pumps are named multidrug efflux (Mex) along with a letter. The outer membrane porin is named Opr along with a letter. *P. aeruginosa* ex- presses twelve RND family efflux pumps, four of which (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) contribute to antibiotic resistance to beta, lactams, fluoroquinolones and aminoglycosides (Langendonk *et al*., 2021).

Antibiotic-inactivating enzymes

Pseudomonas aeruginosa can produce antibiotic-inactivating enzymes that break down or modify antibiotics as one of the significant effective mechanisms of intrinsic resistance in *P. aeruginosa*. Many antibiotics have chemical bonds such as amides and esters that are susceptible to hydrolysis (Langendonk *et al*., 2021) by enzymes commonly produced by *P. aeruginosa*, such as β-lactamases and aminoglycoside-modifying enzymes (Dehbashi *et al*., 2020).

Pseudomonas aeruginosa possesses an inducible AmpC gene, encoding the hydrolytic enzyme β lactamase. This enzyme can break the amide bond of the β-lactam ring, leading to the inactivation of β-lactam antibiotics (El Zowalaty *et al*., 2015). *Pseudomonas aeruginosa* isolates have been found to produce extended-spectrum-β-lactamases (ESBLs) which confer a high degree of resistance to most β- lactam antibiotics, including penicillins cephalosporins and aztreonam (Berrazeg *et al*., 2015; Pachori *et al*., 2019).

Pseudomonas aeruginosa express aminoglycoside modifying enzymes that are associated with AMR to aminoglycoside. APIs have been found to transfer a phosphoryl group to the 3′-hydroxyl of aminoglycosides such as kanamycin, neomycin and streptomycin, thereby inactivating these antibiotics (Mancuso *et al*., 2021). The AACs of *P. aeruginosa* has been found to transfer an acetyl group to the amino group at position 3′ and 6′of aminoglycosides, which is responsible for the inactivation of gentamicin, tobramycin, netilmicin, kanamycin and amikacin (Poole, 2011). Resistance to gentamicin, amikacin and tobramycin is conferred by the ANTs of *P. aeruginosa*, which transfer an adenylyl group to either the amino or hydroxyl group of these aminoglycosides (Subedi *et al*., 2018; Mancuso *et al*., 2021).

2.0 Acquired antibiotic resistance

Pseudomonas aeruginosa can gain the capability to resist the action of antibiotics via spontaneous mutation or acquisition of antibiotic-resistance genes via horizontal gene transfer of mobile genetic elements that carry the antibiotic-resistant gene (Mancuso *et al*., 2021). In Addition to the high intrinsic antibiotic resistance of *P. aeruginosa*, the acquired resistance also significantly contributes to the development of multidrug-resistant strains, which increases the difficulty in eradicating this microorganism and leads to more cases of persistent infections (Paz *et al*., 2019).

Resistance by mutations or alteration of target

Interference with antibacterial targets is a common strategy that *P. aeruginosa* utilize to avoid the antimicrobial action of antibiotics. It can be achieved through the protection of the targets and modifications of the target sites (Munita and Arias, 2016). Thus, mutational alterations of the target sites in *P. aeruginosa* also contribute to its antibiotic resistance (Horcajada *et al*., 2019). Spontaneous mutations can affect the expression or function of a specific porin-small water-filled channels within membranes that mediate the diffusion of hydrophilic antibiotics, thereby reducing bacterial membrane permeability and increasing antibiotic resistance (Fernandez and Hancock, 2012; Pang *et al*., 2019). For instance, a change in the amino acid sequence of OprD in *P. aeruginosa* confers a high level of resistance to carbapenems, especially to imipenem (Fang *et al*., 2014; Paprocka *et al*., 2022).

Overexpression of MexXY–OprM induced by mexZ gene mutation led to increased resistance to aminoglycoside, β-lactam and fluoroquinolone antibiotics in clinical isolates of *P. aeruginosa* (Paprocka *et al*., 2022). The *P. aeruginosa* strains with mutations in the nfxB gene encode a transcriptional regulator, have overexpressed MexCD–OprJ, and are less susceptible to fluoroquinolones and penem antibiotics, a β-lactam subfamily (Paprocka *et al*., 2022).

Another example is mutations in genes encoding DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE), which cause a decrease in the binding affinity of the encoded proteins to quinolones, leading to resistance to quinolones in *P. aeruginosa* (Flores-Velázquez and Pérez, 2021). *Pseudomonas aeruginosa* strains with ribosomal mutations have shown a high level of resistance to aminoglycosides since this antibiotic group inhibits protein translation by targeting the 30S ribosomal subunit (Kakoullis *et al*., 2021). Modifying penicillin-binding proteins in *P. aeruginosa* has been shown to increase resistance to the β-lactam class of antibiotics (Moya *et al*., 2012). *Pseudomonas aeruginosa* clinical isolates have an overproduction of β-lactamases caused by mutations in a β-lactamase inducible gene ampC, which significantly increased the resistance to cephalosporins (Berrazeg *et al*., 2015; Kakoullis *et al.,* 2021).

Acquisition of resistance genes

Antibiotic resistance genes can be carried on mobile genetic elements (plasmids, transposons, integrons and phages), and bacteria can acquire these genes via horizontal gene transfer from the same or different bacterial species (Khosravi *et al*., 2017). The main mechanisms of horizontal gene transfer involve transformation, transduction and conjugation (Fig.1.0). Acquisition of resistant genes to aminoglycoside and β-lactam antibiotics has been reported in *P. aeruginosa* (Cavalcanti *et al*., 2015). The genes for Metallo-beta lactamases in *P. aeruginosa* have been reported to be carried by genetic elements, including integrons and plasmids (Martínez *et al*., 2020; Cavalcanti *et al*., 2015).

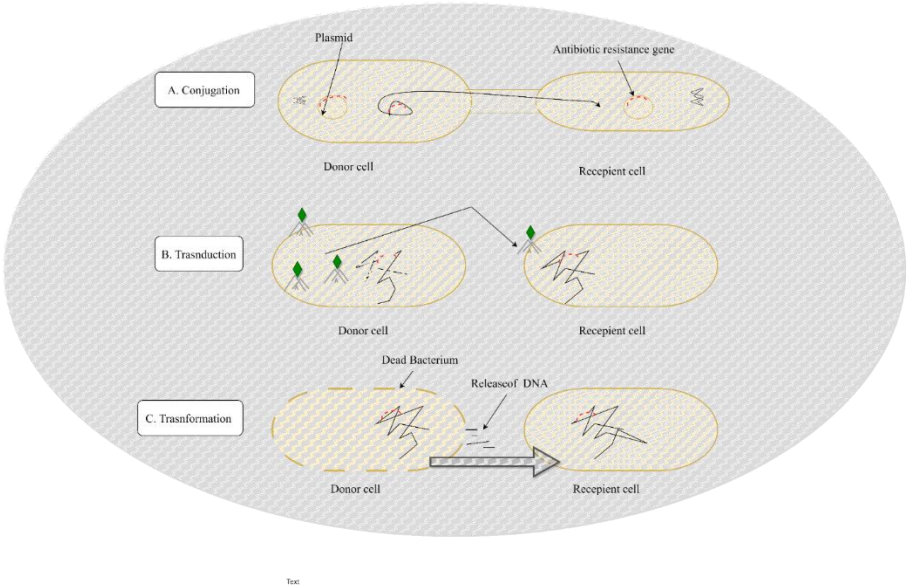

Fig. 2.0 Schematic illustration of mechanisms of horizontal gene transfer. (A) Conjugation is a process that transfers DNA through directly from donor cell to the recipient cell through physical contact facilitated by sex pili (B) Transduction is the transfer of DNA from one bacterium to another by means of a phage (C) In transformation, bacteria take up free fragments of DNA released into the environment and incorporate them into their genome (Pang *et al.,* 2019).

3.0 Adaptive antibiotic resistance

Adaptive resistance refers to *P. aeruginosa* surviving the effect of antibiotics owing to transient alterations in gene expression in response to an environmental stimulus (Spalding *et al*., 2018). In *P. aeruginosa*, the central mechanisms of adaptive mediate antibiotic resistance are the formation of biofilm and the generation of persister cells which leads to persistent infection and poor prognosis particularly in patients with cystic fibrosis (Spalding, *et al*., 2018).

Biofilm-mediated resistance

The mechanisms of biofilm-mediated resistance involve reduced penetration of antibiotics, altered microenvironment inducing slow growth of *P. aeruginosa* within a biofilm, induction of an adaptive stress response and persister cell differentiation (Pang *et al*., 2019). The microbial cells growing in biofilms are less sensitive to antimicrobial agents and host immune responses than those grown in free aqueous suspension (Maurice *et al*., 2018). Even bacteria that are deficient in intrinsic resistance or lack protective mutations can become less susceptible to antibiotics when they grow in a biofilm (Ciofu and Tolker-Nielsen, 2019).

Persister cells associated with resistance

Persister cells in antibiotic resistance are another major obstacle to treating *P. aeruginosa* infections. Persister cells refer to phenotypic variants that are not genetically resistant to antibiotics but are tolerant to high concentrations of antibiotics (Horcajada *et al*., 2019). Persister cells comprise about 1% of biofilm cells and are slow-growing, metabolically inactive and highly tolerant to antibiotics (Maurice *et al*., 2018). The majority of *P. aeruginosa* cells can be killed by antibiotics; however, persisterss can remain viable and repopulate biofilms due to a dormant state that shuts down the synthesis of the antibiotic targets (Van den Bergh *et al*., 2017). Persister cells do not proliferate in the presence of antibiotics. However, they resume growth once the antibiotics are removed (Maurice *et al*., 2018).

Potential strategies to combat antibiotic resistance in *Pseudomonas aeruginosa*

Use of new antibiotics

The discovery and development of new antibiotics is crucial in the fight against antibiotic-resistant bacteria. In recent years, several new antibiotics have been approved for use against *P. aeruginosa*. Ceftolozane/tazobactam is a novel cephalosporin/beta-lactamase inhibitor combination that has been shown to be effective against MDR *P. aeruginosa* (Papp-Wallace *et al.,* 2019). Another novel antibiotic, Cefiderocol, is a siderophore cephalosporin that has demonstrated activity against carbapenem-resistant *P. aeruginosa* (Hackel *et al.,* 2019). Additionally, plazomicin is a new aminoglycoside antibiotic that has been approved for the treatment of complicated urinary tract infections (cUTI), including infections caused by MDR *P. aeruginosa* (Kaye *et al*., 2019). While the discovery of new antibiotics is essential, their overuse and misuse can lead to the development of resistance. Therefore, it is crucial to use new antibiotics prudently, particularly in hospital settings. One approach is to reserve the use of new antibiotics for the treatment of serious infections caused by antibiotic-resistant bacteria. Another approach is to use antibiotics in combination with other therapies to improve their effectiveness and reduce the likelihood of resistance

Alternative therapy

One potential strategy to combat antibiotic resistance in *P. aeruginosa* is to develop alternative therapies. One such therapy is the use of bacteriophages, which are viruses that infect and kill bacteria. Bacteriophages have been used for decades in Eastern Europe to treat bacterial infections, and there is growing interest in their use in the Western world. Recent studies have shown that bacteriophages can effectively kill *P. aeruginosa* in vitro and in animal models, and clinical trials are currently underway to test their efficacy in humans (Parracho *et al*., 2012). Another potential alternative therapy is the use of antimicrobial peptides, which are small molecules that can kill bacteria. Antimicrobial peptides are naturally occurring and have been shown to be effective against *P. aeruginosa* in vitro and in animal models (Brogden, 2005). However, their use in humans is limited by issues of toxicity and stability.

Combination therapy

Another potential strategy to combat antibiotic resistance in *P. aeruginosa* is the use of combination therapies. Combination therapy involves the use of two or more antibiotics to treat infections caused by antibiotic-resistant bacteria. The rationale behind combination therapy is that it can reduce the likelihood of resistance by targeting multiple pathways or mechanisms. Additionally, combination therapy can improve the effectiveness of antibiotics, particularly against biofilm-associated infections and another idea behind combination therapy is that the bacteria will be less likely to develop resistance to multiple antibiotics at once. Several studies have shown that combination therapy can be effective against *P. aeruginosa* (Cai *et al*., 2016). For example, a recent study by Hu *et al*. (2021) demonstrated that the combination of colistin and rifampicin was more effective than colistin alone against carbapenem-resistant *P. aeruginosa*. Similarly, the combination of ceftolozane/tazobactam and aztreonam was shown to be effective against MDR *P. aeruginosa* (Ramos-Martinez *et al*., 2021). Other studies have also demonstrated the effectiveness of combination therapy against P. aeruginosa infections, particularly those associated with biofilms (Mowbray *et al*., 2018; Vila-Dominguez *et al*., 2020). However, the optimal combination of antibiotics is not always clear, and there is a risk of increased toxicity when using multiple antibiotics.

Improved infection control measures

Improved infection control measures can also help to combat antibiotic resistance in *P. aeruginosa*.

One such measure is the use of antimicrobial stewardship programs, which are designed to promote the appropriate use of antibiotics and reduce the development of resistance. Antimicrobial stewardship programs can include the use of diagnostic tests to identify the specific bacteria causing an infection, as well as guidelines for prescribing antibiotics based on the bacteria's susceptibility patterns (Pulcini *et al*., 2018). Another infection control measure is the use of isolation precautions for patients with antibiotic-resistant infections. Isolation precautions involve placing the patient in a private room and requiring healthcare workers to wear protective clothing when caring for the patient. This can help to prevent the spread of the bacteria to other patients

CONCLUSION

In conclusion, the emergence of antibiotic-resistant strains of *P. aeruginosa* has become a significant global health threat. This review article provides an overview of the complex mechanisms involved in antibiotic resistance in *P. aeruginosa*, including the molecular mechanisms, quorum sensing, biofilm formation, and horizontal gene transfer. The knowledge of these mechanisms is crucial to understanding the development and spread of antibiotic resistance in *P. aeruginosa*, which is essential for developing effective strategies to combat this global health challenge. We have also highlighted some potential approaches to tackle antibiotic resistance in *P. aeruginosa*, such as the development of novel antibiotics, combination therapy, improvement of infection control measures, and the use of alternative therapies. The multifaceted approach is essential to overcome the challenges posed by antibiotic resistance, and further research is necessary to develop effective strategies against this threat. Therefore, this review article provides valuable insights into the current knowledge of the mechanisms of antibiotic resistance in *P. aeruginosa* and emphasizes the need for continued research to combat this global health challenge.

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