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Molecular Mechanisms of Antibiotic Resistance in Bacteria and Alternative Treatment Strategies: From Evolution of Resistance to Precision Antimicrobial Therapy

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Abstract. Drug-resistant bacteria refer to microorganisms that possess resistance to one or multiple antibiotics, rendering these medications ineffective in treating infections and significantly increasing treatment difficulty. Between 1990 and 2021, over 1 million deaths annually worldwide were directly attributed to antibiotic resistance, with an average of 4.71 million deaths indirectly associated with it. Therefore, research into the molecular mechanisms of resistant bacteria and alternative treatment methods is urgently needed. Currently identified molecular mechanisms of bacterial resistance are diverse, primarily including: production of antibiotic-inactivating enzymes, modification of drug targets, reduced cell membrane permeability, and upregulation of active efflux pump systems. To address bacterial drug resistance, we have identified various alternative therapies targeting resistant bacteria, such as phage therapy, antimicrobial peptide therapy, gene editing therapy, and nanotechnology-based treatments. This paper will elaborate on the molecular mechanisms of resistant bacteria and alternative treatment strategies, while integrating artificial intelligence and machine learning data models in medical applications to provide more efficient and precise treatment approaches.

Keywords: Antibiotic resistance, Molecular mechanisms, Alternative therapy, Precision antimicrobial strategies, Multidrug-resistant bacteria

1. Introduction

In recent years, multidrug-resistant bacterial infections have grown exponentially, constituting one of the most urgent global healthcare and economic threats. According to The Lancet predictions, by 2050, infections from resistant bacteria will cause 8.22 million deaths annually, 1.7 times the number in 2021 (4.71 million). The World Health Organization (WHO) has classified multidrug-resistant bacteria as a global public health threat requiring urgent international response measures. Traditional therapies for resistant bacteria heavily rely on antibiotic treatment, which increases bacterial resistance and leads to rising treatment failure rates, especially for multidrug-resistant bacteria such as carbapenem-resistant Klebsiella pneumoniae and Acinetobacter baumannii, where drug options are extremely limited and often accompanied by serious side effects including nephrotoxicity and neurotoxicity.

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Antibiotic misuse is one of the main causes of the increase in resistant bacteria. With the widespread use of antibiotics, bacteria acquire resistance through mechanisms such as mutation and horizontal gene transfer, creating a vicious cycle of "new drug introduction-resistant bacteria emergence." This not only increases medical costs and burdens patients financially but also accelerates the evolution and spread of resistant bacteria due to antibiotic misuse. For example, opportunistic pathogens such as Pseudomonas aeruginosa have developed resistance to multiple antibiotics, becoming one of the main pathogens in hospital-acquired infections [1].

This research focuses on addressing the following key questions: What are the primary molecular mechanisms of resistant bacteria? What existing alternative antimicrobial treatment strategies are available? How can precision antimicrobial therapy be achieved to improve treatment efficacy? In-depth research into the molecular mechanisms of resistant bacteria and alternative treatment strategies has significant clinical and social implications: promoting deeper understanding of resistance mechanisms, providing a theoretical foundation for developing novel antimicrobial agents, exploring antibiotic alternative treatment options to reduce antibiotic use and delay resistance development, advancing precision antimicrobial technology to improve infection treatment outcomes and reduce adverse reactions, and providing new therapeutic approaches and antimicrobial strategies for clinical and pharmaceutical fields.

This paper is divided into the following sections: first introducing the main molecular mechanisms of resistant bacteria, then discussing the challenges and impacts caused by resistance, followed by exploring alternative treatment methods and cutting-edge technologies, and finally summarizing research findings and outlining future development directions.

2. Molecular mechanisms of antibiotic resistance

2.1. Biological basis of antibiotic resistance

Antibiotic resistance is the result of bacterial adaptation to environmental pressure, primarily acquired through spontaneous gene mutations and horizontal gene transfer. Spontaneous gene mutations can lead to bacteria acquiring resistance, with these mutations potentially affecting antibiotic targets, membrane permeability, or drug metabolism. For example, quinolone resistance is often associated with mutations in DNA gyrase and topoisomerase IV genes, which alter drug binding sites and weaken antibiotic efficacy [2].

Horizontal gene transfer is an important pathway for bacteria to acquire resistance, mainly through three mechanisms: transformation (direct uptake of DNA fragments from the environment), conjugation (transfer of plasmids between bacteria through conjugation tubes), and transduction (phage-mediated DNA transfer). These mechanisms enable resistance genes to spread rapidly between different bacterial species. For instance, methicillin-resistant Staphylococcus aureus (MRSA) acquires the mecA gene, encoding the altered penicillin-binding protein PBP2a, leading to broad resistance to β-lactam antibiotics [3].

Environmental factors and antibiotic misuse have a significant driving effect on the evolution of resistant bacteria. Under antibiotic selection pressure, bacteria carrying resistance genes have survival advantages and gradually become the dominant bacterial population. Research indicates that hospital environments, animal husbandry, and wastewater treatment plants are hotspots for the transmission of resistance genes [4].

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2.2. Major resistance mechanisms

Bacteria can degrade or modify antibiotics by producing specific enzymes, rendering them inactive. These primarily include β-lactamases, such as carbapenemases that hydrolyze the β-lactam ring of carbapenem antibiotics, and aminoglycoside-modifying enzymes, which modify aminoglycoside antibiotics through acetylation, adenylation, or phosphorylation, preventing their binding to ribosomes. Bacteria inactivate antibiotics before they reach their targets by producing these enzymes, thus developing resistance. For example, resistant Pseudomonas aeruginosa and Enterobacteriaceae often produce various β-lactamases, including extended-spectrum β-lactamases (ESBLs) and carbapenemases (KPC, NDM, VIM, etc.) [5].

Bacteria can modify antibiotic targets to reduce drug affinity. Common target modifications include ribosomal protection proteins, such as bacterial Tet(M) and Tet(O) proteins that protect ribosomes from tetracycline antibiotics; DNA gyrase mutations, leading to reduced binding of quinolone antibiotics; and alterations in penicillin-binding proteins (PBPs), such as PBP2a produced by MRSA, which has low affinity for β-lactam antibiotics. These modifications prevent antibiotics from effectively binding to their targets even after entering bacterial cells, thus losing their bactericidal or bacteriostatic effects. For instance, vancomycin-resistant enterococci modify the terminal D-Ala-D-Ala of peptidoglycan precursors to D-Ala-D-Lac, reducing vancomycin binding affinity [6].

Efflux pumps are protein complexes on bacterial cell membranes that actively pump antibiotics out of cells, reducing intracellular antibiotic concentrations. Major efflux pump families include the Resistance-Nodulation-Division (RND) superfamily, common in Gram-negative bacteria such as the MexAB-OprM system in Pseudomonas aeruginosa; the Major Facilitator Superfamily (MFS), widely present in various bacteria; the Multidrug And Toxic compound Extrusion (MATE) family; the Small Multidrug Resistance (SMR) family; and ATP-Binding Cassette (ABC) transporters. Enhanced expression of efflux pumps is an important mechanism for multidrug resistance, particularly in non-fermenting Gram-negative bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii [7].

Bacteria can reduce cell membrane permeability by decreasing the expression of outer membrane proteins (porins) or modifying lipopolysaccharide structures, preventing antibiotics from entering cells. This mechanism is particularly important in Gram-negative bacteria, such as Klebsiella pneumoniae downregulating OmpK35 and OmpK36 outer membrane protein expression to reduce the permeation of carbapenem antibiotics [7].

Bacteria can form biofilms, which are microbial communities encased in an extracellular polymeric substance (EPS) matrix secreted by bacteria. Biofilms protect bacteria from antibiotics and host immune systems through mechanisms including physical barriers, altered metabolic states, and quorum sensing regulation. Biofilm-related infections are often difficult to eradicate, such as biofilms formed by Pseudomonas aeruginosa, which are associated with chronic pulmonary infections and catheter-related infections [8].

3. Challenges and problems posed by resistant bacteria

3.1. Clinical challenges

Resistant bacterial infections significantly increase patient mortality risk. According to World Health Organization data, over 1 million deaths globally each year are directly attributed to antibiotic-resistant infections, with even higher numbers indirectly associated. Research shows that MRSA

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infections have a 64% higher mortality rate than infections caused by methicillin-sensitive Staphylococcus aureus [3].

With the spread of resistance, clinically available antibiotics are becoming increasingly limited, especially for extensively drug-resistant (XDR) and pandrug-resistant (PDR) strains. For example, for Enterobacteriaceae carrying carbapenemases such as NDM-1, polymyxins may be the only effective antibiotic option, and these drugs often come with serious adverse reactions like nephrotoxicity [2].

Resistant bacteria are the main pathogens in hospital-acquired infections. These infections not only prolong hospitalization and increase medical costs but may also lead to serious complications or even death. Common multidrug-resistant hospital infection pathogens include MRSA, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae, which often develop resistance to multiple antibiotics simultaneously [1,7].

3.2. Social and economic impacts

Resistance genes can spread in hospitals, communities, and the environment, posing a serious public health threat. For example, the emergence and spread of community-acquired MRSA has led to global infection outbreaks. Additionally, resistance genes can spread globally through food chains, water sources, and international travel [3,4].

The treatment cost of resistant bacterial infections is far higher than that of sensitive bacterial infections, including more expensive drugs, prolonged hospitalization, and additional isolation measure costs. Meanwhile, antibiotic development faces challenges of high costs, long cycles, and low return on investment, leading to decreased research enthusiasm from pharmaceutical companies. Since 2000, only a few new types of antibiotics have been approved, far from meeting clinical needs [4].

3.3. Limitations of existing treatment methods

Traditional antibiotics have limited therapeutic effects on multidrug-resistant and extensively drug-resistant strains. For example, non-fermenting Gram-negative bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii can simultaneously develop resistance to multiple antibiotics including carbapenems, aminoglycosides, and quinolones, resulting in extremely limited treatment options [1].

The development of new antibiotics faces numerous challenges, including long development cycles (typically 10-15 years), low success rates (only about 1% of candidate molecules reach the market), low return on investment (new antibiotic use is restricted to delay resistance development), and technical bottlenecks (traditional screening methods have difficulty discovering antibiotics with entirely new mechanisms of action). These factors collectively lead to insufficient antibiotic innovation, failing to meet the urgent need to combat resistant bacteria [4].

4. Alternative treatment strategies

4.1. Phage therapy

Bacteriophages are viruses that specifically infect bacteria, completing their life cycle by lysing host bacteria. Phage therapy as an alternative to antibiotics offers advantages including high specificity, self-replication, biofilm penetration ability, and high safety. Phage therapy has been applied to

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various resistant bacterial infections, including urinary tract infections caused by Pseudomonas aeruginosa and Escherichia coli [8,9].

Through genetic engineering technology, scientists can modify bacteriophages to enhance their therapeutic effects, including expanding host range, enhancing lytic ability, improving stability, and delivering CRISPR-Cas systems. For example, research shows that genetically modified bacteriophages have stronger penetration and bactericidal effects against Pseudomonas aeruginosa biofilms [8].

4.2. Antimicrobial Peptides (AMPs)

Antimicrobial peptides are short peptides produced by organisms with broad-spectrum antimicrobial activity, including those of animal, plant, and microbial origin. Additionally, scientists have designed various synthetic peptides based on natural antimicrobial peptide structures, such as fusion peptides and cyclic peptides, further improving their stability and activity [4].

The mechanisms of action of antimicrobial peptides are diverse, mainly including membrane disruption, inhibition of intracellular targets, and immunomodulatory effects. Due to these unique mechanisms, bacteria find it difficult to develop resistance to antimicrobial peptides, making them promising alternatives to antibiotics [4].

4.3. Gene editing technology

The CRISPR-Cas system, as a revolutionary gene editing tool, can be used to target bacterial resistance genes, including specifically knocking out resistance genes, diversifying delivery systems, and developing antimicrobial CRISPR therapies. Research shows that the CRISPR-Cas9 system can effectively cut resistance genes in Escherichia coli and Staphylococcus aureus, restoring their sensitivity to antibiotics [1].

CRISPR technology can not only target resistance genes but also enhance antibiotic efficacy by targeting virulence genes, disrupting biofilm formation, and combining with antibiotics. This intervention strategy provides new therapeutic approaches for resistant bacterial infections, especially for persistent infections that are insensitive to traditional antibiotics [1,4].

4.4. Nano-antimicrobial technology

Nanometal materials, such as nanosilver, nanocopper, and nano-zinc oxide, possess unique antimicrobial properties, including direct contact killing, free radical production, metal ion release, and biofilm inhibition. Research shows that nanosilver has good antimicrobial activity against various resistant strains, including MRSA and ESBL-producing Enterobacteriaceae [7].

Nano-drug delivery systems can improve the therapeutic effects of antibiotics, including targeted delivery, controlled release, enhanced permeability, and synergistic effects. For example, research shows that liposome-encapsulated vancomycin has significantly higher antimicrobial activity against MRSA than free vancomycin and can effectively penetrate biofilms [3,7].

4.5. Artificial intelligence-driven precision antimicrobial therapy

Artificial intelligence and machine learning technologies can be used in multiple aspects of resistant bacteria research, including resistance gene prediction, rapid resistance detection, resistance evolution modeling, and epidemiological surveillance. For example, machine learning algorithms have been used to predict nitrofurantoin resistance, significantly improving prediction accuracy [10].

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Artificial intelligence has enormous potential in the development of novel antimicrobial drugs, including antimicrobial peptide design, drug target identification, drug repurposing, and combination therapy optimization. AI-assisted drug design has successfully predicted multiple candidate molecules with good antimicrobial activity and low toxicity, providing new approaches to solving the resistance crisis [4].

5. Conclusion

This paper systematically summarizes the molecular mechanisms of resistant bacteria and alternative treatment strategies. The molecular mechanisms of resistant bacteria are diverse, including antibiotic-inactivating enzyme production, target modification, efflux pump enhancement, reduced cell membrane permeability, and biofilm formation. The complex combination of these mechanisms poses severe challenges to traditional antibiotic therapy, especially for multidrug-resistant and extensively drug-resistant bacteria.

To address the resistance crisis, various alternative treatment strategies have emerged, such as phage therapy, antimicrobial peptides, gene editing technology, and nano-antimicrobial materials. These novel methods exert antimicrobial effects through mechanisms different from traditional antibiotics, potentially breaking through resistance bottlenecks. Meanwhile, artificial intelligence and machine learning technologies provide new approaches for precision antimicrobial therapy, optimizing resistance detection and new drug development processes.

Future research on resistant bacteria should focus on the following directions: in-depth analysis of resistance mechanisms, optimization of alternative treatment strategies, exploration of combination therapy regimens, improvement of clinical application feasibility, and development of precision medicine. Combating resistant bacteria requires global collaborative efforts, including strengthening global antimicrobial drug regulation, establishing resistance monitoring networks, promoting knowledge sharing and technology transfer, increasing research and development investment, and promoting interdisciplinary collaboration. Only through multi-faceted, multi-level comprehensive responses can we effectively alleviate the resistance crisis and safeguard global public health security.

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