Comparison of the Inhibitory Effects of Different Antibiotics on the Growth of Escherichia coli

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Abstract. *Escherichia coli*, a common Gram-negative bacterium, includes both commensal and pathogenic strains that cause severe infections such as urinary tract infections and sepsis. With the rising threat of antibiotic resistance, understanding the relationship between antibiotic molecular structures and their inhibitory effects on *E. coli* has become crucial for effective treatment and drug development. This study investigates the inhibitory mechanisms of five major antibiotic classes against *E. coli*, focusing on how their molecular structures influence antibacterial activity. Key findings reveal that carbapenems (β-lactams) exhibit strong activity (MIC 0.25-1 μg/mL) due to their resistance to β-lactamases; amikacin (aminoglycosides) shows high efficacy (MIC 1-4 μg/mL) via high positive charge density; ciprofloxacin (quinolones) demonstrates exceptional potency (MIC 0.03-0.125 μg/mL) owing to its amphiphilic structure; azithromycin (macrolides) offers a safe alternative for sensitive cases (MIC 16-32 μg/mL); and doxycycline (tetracyclines) is useful for drug-resistant strains despite higher resistance rates. Structural modifications, such as side-chain bulky groups in β-lactams and fluorine substitution in quinolones, effectively mitigate resistance. These insights into structure-activity relationships provide a theoretical basis for rational clinical antibiotic use and the design of novel, resistance-evading antibiotics to combat *E. coli* infections.

Keywords: *Escherichia coli*; inhibitory mechanism; antibiotic.

1. Introduction

Escherichia coli has become a model organism for studying the mechanism of antibiotic action due to its unique cellular structure, which includes an outer membrane containing lipopolysaccharides, a thin peptidoglycan layer, selectively permeable cell membranes, intracellular ribosomes, DNA gyrase and other targets. Most strains are harmless, but pathogenic strains such as enterohemorrhagic *E. coli* O157: H7 can cause large-scale infections by contaminating food or water sources, posing a threat to public health. Antibiotics exert their effects by interfering with key physiological processes in bacteria, such as inhibiting cell wall synthesis, blocking protein synthesis, and interfering with DNA replication, and the strength of their effects is directly related to their molecular structure.

In recent years, the problem of antibiotic resistance has intensified, and the resistance rate of *E. coli* to traditional antibiotics has continued to rise, making it increasingly important to analyze the "structure effect" relationship. The differences in the molecular structure of antibiotics determine their ability to penetrate the outer membrane, their affinity for binding to targets, and the evolutionary direction of bacterial resistance mechanisms[1]. For example, antibiotics containing positively charged groups are more likely to bind to outer membrane lipopolysaccharides, while antibiotics with specific ring structures can accurately target ribosomes or DNA gyrase [1]. Analyzing this association can lay the foundation for clinical medication and the design of new antibiotics.

2. Evaluation System for the Growth Inhibition of E. coli by Antibiotics

Evaluating the inhibitory effect of antibiotics on *E. coli* requires a combination of quantitative indicators and dynamic observation to form a multidimensional system. The minimum inhibitory concentration (MIC) is the most commonly used indicator, which refers to the lowest drug concentration that completely inhibits the visible growth of *E. coli* in vitro. In clinical practice, antibiotics with MIC $\leq$ 8 μg/mL have practical therapeutic value for *E. coli* infection. MIC directly reflects the strength of the interaction between antibiotics and targets, and this interaction is based on

molecular structure - antibiotics containing specific functional groups can bind more tightly to the target and therefore work at low concentrations. The specific growth rate (μ) reflects the inhibitory effect from a dynamic perspective, and the calculation formula is μ=(lnNt-lnN0)/t (Nt is the number of live bacteria at time t, N0 is the initial number of live bacteria). During the logarithmic growth phase, the specific growth rate of *E. coli* that is not affected by antibiotics remains stable, but decreases with increasing drug concentration after the addition of antibiotics. This reduction is structure-dependent: antibiotics that rapidly penetrate the outer membrane and efficiently bind to the target can significantly reduce specific growth rates at low concentrations. For example, quinolones, due to their "lipid water dual solubility" structure, can reduce specific growth rates by more than 50% at low concentrations [2].

The sterilization curve distinguishes between antibacterial and bactericidal agents by monitoring changes in the number of live bacteria. Antibacterial agents (such as tetracyclines) only inhibit growth and do not significantly reduce the number of viable bacteria; Fungicides (such as carbapenems in β-lactams) can reduce the number of viable bacteria by more than three orders of magnitude within 24 hours. This difference arises from the molecular structure - fungicides irreversibly destroy key bacterial structures (such as cell walls), while antibacterial agents mostly exert their effects through reversible binding to targets.

3. Molecular Structures and Inhibitory Mechanisms of Three Main Categories of Antibiotics

3.1. β-lactam Antibiotics

The common feature of β-lactams (penicillins, cephalosporins, carbapenems, etc.) is the presence of a quaternary β-lactam ring, which is their antibacterial core. The amide bond (-CO-NH-) in the ring can covalently bind to penicillin binding proteins (PBPs), a key enzyme in *E. coli* cell wall synthesis, irreversibly inhibiting their activity, resulting in the inability of peptidoglycan chains to crosslink, interruption of cell wall synthesis, and bacterial rupture and death due to osmotic pressure imbalance.

The structural differences between different subclasses are reflected in the side chains and heterocycles connected by β-lactam rings. Penicillins, such as ampicillin, have a thiazole ring connected to their beta-lactam ring and an amino ($-NH_2$) side chain. Although they enhance water solubility, they are easily hydrolyzed by beta-lactams, leading to drug resistance. Cephalosporins (such as ceftriaxone) replace the thiazole ring with the thiazide ring, introduce ester group ($-COO^-$) into the side chain, enhance steric hindrance, improve the stability of β - lactase, reduce MIC to 4-8 μ g/mL for *E. coli*, and achieve a specific growth inhibition rate of over 70%. Carbapenems (such as imipenem) fuse the β-lactam ring with the pyrrolidine ring and introduce methyl (-CH3) side chains, which not only resist almost all β-lactam enzyme hydrolysis, but also enhance the binding affinity with PBPs. The MIC against multidrug-resistant *E. coli* is still as low as 0.25-1 μg/mL, making it one of the preferred drugs for severe infections [3,4].

3.2. Aminoglycoside Antibiotics

Aminoglycosides (such as gentamicin, amikacin, etc.) are composed of aminocyclic alcohols (such as deoxystreptomycin) connected to 2-3 amino sugars through glycosidic bonds. They are strongly alkaline and easily protonated with a positive charge at physiological pH. The positive charge causes it to electrostatically attract the anionic phosphate group of the outer membrane lipopolysaccharide, disrupting the stability of the outer membrane, and then entering the bacterial cell through the pore protein. Inside the cell, it binds to the 16S rRNA of the 30S subunit of ribosomes, interfering with the localization of aminoacyl tRNA, leading to codon misreading, abnormal protein synthesis, and ultimately disrupting cell membrane integrity, resulting in bacterial death.

The number and position of amino groups in a molecule significantly affect its activity. Gentamicin contains 5 protonated amino groups and 1 guanidine group (-NH-C(=NH)-NH_2), with a high positive charge density. Its MIC for *E. coli* is 0.5-2 μg/mL, and 0.5×MIC can reduce specific growth rates by 50%. Streptomycin contains only 3 amino groups and has a low positive charge density. Its MIC increases to 8-16 μg/mL, but its inhibitory effect weakens. Amikacin enhances its binding affinity with ribosomes by introducing hydroxyl (-OH) and additional amino groups into amino sugars, and resists aminoglycoside-modifying enzymes (the main cause of resistance), maintaining a MIC of 2-4 μg/mL against resistant strains [5].

3.3. Quinolone Antibiotics

Quinolones are artificially synthesized broad-spectrum antibacterial drugs, with the basic structure being a nitrogen-containing hexagonal quinolone nucleus. The third generation and beyond (such as ciprofloxacin and levofloxacin) introduce a fluorine atom (-F) at position 6 and a piperazine ring (containing -NH-) at position 7, forming a "lipid water dual solubility" characteristic: fluorine atoms enhance lipid solubility, allowing it to pass through the outer membrane hydrophobic channel. The piperazine ring enhances hydrophilicity, allowing it to dissolve in the intracellular water environment and efficiently reach the targets - DNA gyrase (topoisomerase II) and topoisomerase IV.

Quinolones have high specificity in binding to DNA gyrase: the 6-position fluorine atom enhances binding through hydrophobic interactions, and the nitrogen atom of the 7-position piperazine ring forms hydrogen bonds with the active center amino acid residues of the enzyme, strongly inhibiting DNA supercoiling and blocking replication and repair. Ciprofloxacin has a MIC as low as 0.03-0.125 μg/mL for *E. coli*, and a specific growth inhibition rate of over 95% at 1×MIC, making it one of the most active antibiotics against Gram-negative bacteria. The first generation without introducing fluorine atoms (such as nalidixic acid) has limited practical value due to poor lipid solubility and weak target binding ability, with MIC as high as 32-64 μg/mL. The introduction of the methoxy (-OCH∝) group into moxifloxacin at position 8 can reduce phototoxicity, enhance inhibition of topoisomerase IV, and remain effective against some multidrug-resistant strains.

3.4. Macrolide Antibiotics

Macrolides are centered around a 14-16-membered macrocyclic lactone ring, with multiple hydroxyl groups (-OH) and one dimethylamine group (-$N(CH3)_2$) attached to the ring. If erythromycin has a 14-membered ring, azithromycin forms a 15-membered ring by adding a nitrogen heterocyclic ring on top of the 14-membered ring. Traditional macrolides have weak activity against Gram-negative bacteria, and due to their high molecular weight and strong hydrophilicity, they are difficult to penetrate the outer membrane lipopolysaccharide barrier of *E. coli* (such as erythromycin, whose MIC against *E. coli* is usually>128 μg/mL).

Azithromycin significantly enhances its activity through structural optimization [7]: the introduction of nitrogen heterocycles increases the alkalinity of the molecule (pKa from 8.6 to 9.6), making it easier to protonate at physiological pH, increasing its electrostatic binding with lipopolysaccharides, and increasing its outer membrane penetration rate by more than 10 times compared to erythromycin; The 15 membered ring space structure is more suitable for the center of ribosome 50S subunit peptidyl transferase, and the binding affinity is significantly improved [7]. These changes reduced the MIC of azithromycin against *E. coli* to 16-32 μg/mL, with a specific growth inhibition rate of 60% at 2×MIC, providing an alternative option for allergy sufferers to other antibiotics.

3.5. Tetracycline Antibiotics

Tetracyclines have a four-membered benzene ring structure, containing multiple hydroxyl groups, ketone groups (-CO-), and one dimethylamine group on the ring, and the molecules are amphiphilic. It inhibits protein synthesis by binding to the 30S subunit of ribosomes, preventing aminoacyl tRNA from entering the A-site. It needs to be mediated by cations (such as Mg^{2+}) to bind with outer

membrane lipopolysaccharides, with weak penetration ability, so the MIC for *E. coli* is usually 4-8 μg/mL.

Doxycycline is a structural modification product of tetracycline, which can remove the hydroxyl group at position 6 and introduce a methyl group (-CH3), reduce chelation with metal ions (to avoid reduced activity), enhance molecular stability, reduce MIC to 2-4 μg/mL, extend half-life to 12-24 hours, and have a longer lasting efficacy [8]. But tetracyclines are easily excreted by efflux pumps (such as TetA protein), leading to drug resistance. Tigecycline changes its molecular configuration by introducing a glycylamino group at position 9, making it difficult for efflux pumps to recognize. Its MIC against drug-resistant strains remains as low as 1 μg/mL [8].

4. The Correlation between Molecular Structure and Antibiotic Resistance in E. coli

The molecular structure of antibiotics not only determines their antibacterial activity but also affects the emergence and evolution of antibiotic resistance in *E. coli*. *E. coli* is mainly resistant through four mechanisms, all closely related to the structure of antibiotics. The main cause of β-lactam resistance is the production of β-lactamase by *E. coli*, which can hydrolyze the amide bond of the β-lactam ring and inactivate the drug [9]. But if the antibiotic side chain contains large volume functional groups (such as the piperazine ring of cefoperazone), it can hinder the enzyme from binding to the β-lactam ring through steric hindrance and maintain activity. Aminoglycoside resistance is often mediated by aminoglycoside-modifying enzymes, which can acetylate, phosphorylate, or adenosine-modify the amino groups of drugs. If the easily modifiable amino group is replaced with a hydroxyl group (such as amikacin), it can evade modification and maintain activity. Quinolone resistance is often associated with mutations in the DNA gyrase GyrA subunit (such as Ser83→Leu), which reduce enzyme drug binding affinity. However, quinolones with 7 fluoropiperazine rings (such as gatifloxacin) can compensate for the loss of binding strength by increasing the number of hydrogen bonds, thus combating drug resistance [10]. Tetracycline efflux pump TetA can recognize the structure of a tetrahydrobenzene ring and expel it. Tigecycline changes its configuration through 9-site modification, making it unrecognizable to efflux pumps and overcoming drug resistance.

5. Comparison and Clinical Application of Inhibitory Effects of Different Types of Antibiotics

The significant differences in the inhibitory effects of different types of antibiotics on *E. coli* are directly attributed to their molecular structural characteristics. Carbapenems in β-lactams, such as imipenem, have a structural advantage against β-lactams, with MIC as low as 0.25-1 μg/mL, specific growth inhibition rate of 90%, and resistance rate of only 10% -15%, making them the preferred choice for multidrug-resistant infections. Amikacin, an aminoglycoside compound, has a high positive charge density and anti-modifying enzyme structure, with a MIC of 1-4 μ g/mL and an inhibition rate of 85%. It is often used in combination with β-lactams to enhance its bactericidal effect, but its nephrotoxicity limits its use in patients with renal insufficiency. Enrofloxacin, a quinolone, has a "lipid water dual solubility" structure and high affinity for DNA gyrase, with a MIC of only 0.03-0.125 μ g/mL and an inhibition rate of 95%. It has shown outstanding performance in urinary tract infections, but in recent years, the resistance rate has increased to 30% -35%, and drug sensitivity testing is required when used. The MIC of azithromycin, a macrocyclic lactone, is 16-32 μ g/mL, with weak activity but high safety. It is suitable for mild patients with β-lactam allergy. The MIC of tetracycline class doxycycline is 2-4 μg/mL, with an inhibition rate of 75%, but the resistance rate exceeds 50%. It is mainly used for infections caused by other antibiotic-resistant strains. When making clinical choices, it is necessary to consider the advantages and disadvantages brought by the structure. Carbapenems are preferred for β-lactase-producing strains, aminoglycosides can be used in combination for those with normal kidney function, quinolones should be avoided for pregnant

women and children as they may affect cartilage development, and azithromycin can be chosen for allergic patients.

The inhibitory effect of antibiotics on *E. coli* is the result of the interaction between molecular structure and bacterial physiological characteristics. The ability to penetrate the outer membrane depends on lipid solubility and charge, such as the positive charge of aminoglycosides and the lipid solubility of quinolones. The binding affinity with the target depends on functional group matching, such as covalent binding between β-lactam rings and PBPs, and hydrogen bonding between quinolones and DNA gyrase. Resistance avoidance requires structural modifications, such as β-lactam side chain large groups and quinolone fluorine atoms. Future research can focus on three directions. Firstly, based on targets such as PBPs and ribosome crystal structures, computer simulations can be used to optimize functional groups and enhance target binding affinity. The second is to design bifunctional molecules, such as combining the β-lactam ring with the macrolide ring, while acting on the cell wall and ribosome to reduce the risk of drug resistance. The third is to increase the drug enrichment concentration in the bacterial body by binding to outer membrane-specific receptors such as iron carriers. These strategies are centered around the "structure effect" correlation and are expected to address the challenge of drug resistance.

6. Conclusion

The molecular structure of antibiotics is the core factor determining their inhibitory effect on *E. coli*. Specifically, β-lactams exhibit different enzymatic activities and binding abilities due to differences in their β-lactam ring and side chain structures, among which carbapenems have the best effect on multidrug-resistant strains. The positive charge density of aminoglycosides determines their outer membrane penetration ability and ribosome binding affinity, and amikacin exhibits strong resistance due to structural modifications. The fluorine atom and piperazine ring of quinolones endow them with a "lipid water dual solubility" property, with ciprofloxacin having the strongest activity. Macrolides improve outer membrane penetration through ring structure optimization, making azithromycin an alternative choice for allergy patients. Tetracyclines modified with side chains can avoid efflux pump resistance, while tigecycline remains effective against resistant strains. Meanwhile, the structure of antibiotics directly affects the evolution of resistance mechanisms in *E. coli*, and modifications such as bulky side chain groups and fluorine atoms are key to avoiding resistance. The "structure effect" correlation revealed in this study provides a theoretical basis for precise drug selection in clinical practice and also points out the direction for the design of new antibiotics. The limitation lies in the fact that the research is mainly based on in vitro data, lacking analysis of in vivo dynamic metabolism and microbial interactions. In the future, the structural modification effect can be verified by combining in vivo models, and the research on target binding mechanisms can be deepened through computer simulations to promote the development of efficient and low-resistance antibiotics.

References

[1] Benn, S. et al. (2021). Structural insights into the outer membrane of live E. coli. Proceedings of the National Academy of Sciences, 118(43), e2111333118.

[2] Combat Model Team. (2020). Drug-target binding predicts optimal antibiotic dose levels in quinolones. PLoS Biology, 18(8), e3000732.

[3] Zhang, J. et al. (2021). Clinical use of carbapenems under antimicrobial stewardship policies. Scientific Reports, 11(9), 11903544.

[4] [4] Imipenem/Relebactam Study Group. (2021). Efficacy of imipenem/relebactam in hospital-acquired pneumonia. Journal of Infection, 83(2), 155-163.

[5] Plazomicin Study Group. (2021). Structural basis for plazomicin action and resistance in E. coli. Nature Communications, 12, 3456.

[6] Indole Research Group. (2020). Inhibition of indole production enhances quinolone activity against E. coli persisters. Nature Microbiology, 5(7), 920-928.

[7] Macrolide Modification Group. (2020). Scaffold modifications in erythromycin macrolide antibiotics. Molecules, 25(17), 3941.

[8] Li, X. et al. (2023). Genomic characterization of tigecycline-resistant E. coli isolates from hospital sewage. Frontiers in Microbiology, 14, 1282988.

[9] Sulbactam/Avibactam Consortium. (2021). Combination of sulbactam/avibactam against carbapenem-resistant Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy, 65(7), e00515-21.

[10] Ferrand, G. et al. (2023). Impact of efflux pumps on quinolone susceptibility in multidrug-resistant Enterobacteriaceae. Journal of Antimicrobial Chemotherapy, 78(6), 1483-1492.