Evolutionary Guidance for Antibiotic Discovery and Development

Saad Ahmad[1,*], Arooj Fatima[2], Sawaira Dastgir[3], Aziz-ur-Rehman[4], Ramisha Kamran[3] and Ieman Tariq[2]

[1]Lanzhou Institute of Animal Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Sciences, China
[2]Department of Microbiology Cholistan University of Veterinary and Animal Sciences Bahawalpur
[3]Department of Biochemistry Cholistan University of Veterinary and Animal Sciences Bahawalpur
[4]Department of Patho-physiology, college of veterinary and animal sciences, Jhang
*Corresponding author: saadahmad.uaf@gmail.com

Abstract

Antimicrobial resistance (AMR) is a global health threat and undermines the effectiveness of antibiotics and many modern medical practices. In light of the increasing prevalence of multidrug-resistant bacteria, especially Gram-negative species, there is an urgent need for new strategies to discover and develop antibiotics. With recent advancements in technologies such as genome mining, clustered regularly interspaced short palindromic repeats (CRISPR), and synthetic biology, the search for new antibiotics is being revitalized by exploring biosynthetic pathways that were previously overlooked. Resistance to fluoroquinolones —and now β-lactam antibiotics—emerges rapidly due to selective pressure exerted by antibiotic use. Innovative technologies like artificial intelligence have facilitated the discovery of breakthrough antibiotics like halicin, while CRISPR technology enables precise genetic modifications to combat resistance mechanisms. Effective management of AMR calls for a holistic approach, involving prudent drug use and the integration of advanced technologies. Additionally, international cooperation, the establishment of robust monitoring frameworks, and support for collaborative research initiatives are crucial to maintain a sustainable pipeline of new antibiotics. By bridging scientific advances with global health policy efforts, we can address AMR and preserve the efficacy of antibiotic therapies for future and public health.

Keywords: CRISPR technology, Genome exploration, Fluoroquinolones, Biosynthetic

Cite this Article as: Ahmad S, Fatima A, Dastgir S, Aziz-ur-Rehman, Kamran R and Tariq I, 2025. Evolutionary guidance for antibiotic discovery and development. In: Farooqi SH, Kholik K and Zaman MA (eds), One Health Horizons: Integrating Biodiversity, Biosecurity, and Sustainable Practices. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 308-316. https://doi.org/10.47278/book.HH/2025.267

A Publication of Unique Scientific Publishers

Chapter No: 25-041

Received: 13-Feb-2025
Revised: 16-March-2025
Accepted: 18-Apr-2025

Introduction

Antimicrobial resistance is a global threat to public health and the World Health Organization (WHO) therefore calls for intensified investigations into novel antibiotics. Since the 1980's, no entirely new antibiotic classes have been discovered, with recent developments focusing primarily on optimizing existing compounds. Actinobacteria and fungi, traditionally soil dwelling organisms, have historically provided the majority of antimicrobial agents. Recent breakthroughs—including genome mining, CRISPR-Cas9 technology, and revitalized culture methods—have reinvigorated antibiotic discovery, yielding notable examples like teixobactin and lugdunin. Exploring the vast repertoire of human-associated microbiota, particularly within the gut and oral cavity, using advanced techniques holds significant promise for uncovering novel antibiotic classes and addressing the urgent need for effective antimicrobial solutions (Mohr, 2016). Antimicrobial resistance is widely recognized as a crucial public health concern by international organizations and local agencies alike. The US Centers for Disease Control and Prevention (CDC) reports approximately 23,000 deaths annually in the US due to antibiotic resistance. Furthermore, studies predict millions of deaths globally in the coming decades. In response, the United Nations has established a high-level assembly to coordinate global efforts against antimicrobial resistance.

Despite alarming predictions, global deaths associated with transmittable diseases decreased from 10.7 million in 2005 to 8.6 million in 2015. Recent studies suggest that mortality rates attributable to antimicrobial resistance may be lower than previously estimated. To combat multidrug-resistant bacteria, researchers have revisited "old" antibiotics, uncovering their remarkable efficacy against such resistant isolates. The World Health Organization (WHO) emphasizes the importance of rational antibiotic use, enhanced surveillance, and intensified research into novel tools and molecules to address antimicrobial resistance. Recently marketed antibiotics, such as tedizolid and dalbavancin, belong to existing classes, highlighting the urgent need for innovative approaches to discover entirely new antibiotic classes (Johnson et al., 2014).

Developing new antibiotics has become increasingly challenging, with most recently introduced agents being modifications or combinations of existing compounds. One of the foremost obstacles lie in researching and creating entirely novel class of antibiotics. To unravel the roots of this problem, it is essential to revisit the history of antibiotic development, analyze their chemical compositions, and evaluate the methods used for their discovery. Furthermore, adopting innovative strategies for identifying new antibiotic classes (Durand et al., 2019) represents a promising avenue for future progress.

The Rise of Antibiotic Resistance

One of the most remarkable scientific achievements of the modern era is the development of antibiotics, which have historically saved millions of lives and revolutionized the treatment of bacterial infections. Unfortunately, antibiotic resistance has now become a critical global concern. The overuse and misuse of antibiotics have accelerated the evolution of resistant bacterial strains, rendering these essential drugs increasingly ineffective (Podolsky, 2018).

Other Gram-positive bacteria, such as *Staphylococcus aureus* and *Enterococcus* species, are primary contributors to antibiotic resistance among Gram-positive pathogens. While resistance to methicillin has been significant driver of this crisis, methicillin-resistant *Staphylococcus aureus* (MRSA) has spread globally and continues to have a substantial impact on patient outcomes (Ahmad et al., 2024). Although MRSA is still considered a manageable issue in some settings, its severity can be such that large portions of the world population are affected (Magiorakos et al., 2012; European Centre for Disease Prevention and Control, 2017).

However, the issue of resistance is even more critical in Gram negative bacteria. Strains of pathogens, including *Pseudomonas aeruginosa, Acinetobacter spp.*, and certain members of the *Enterobacteriaceae* family, have been increasingly reported as extensively drug resistant (XDR), totally drug resistant (TDR). Moreover, the growing presence of Gram-negative multidrug-resistant bacteria in community settings (Alm et al., 2014; Satlin et al., 2014) aggravates the situation further.

Importance of Evolutionary Principles in Antibiotic Development

Bacteria develop antimicrobial drug resistance when they adapt to survive in the presence of antimicrobial agents. This resistance generally arises for two main reasons: either the antibiotic fails to reach an adequate concentration at the infection site, or it does not effectively bind to its target within the bacteria, allowing them to continue multiplying. There are two primary forms of resistance: intrinsic and acquired (Varela et al., 2021). The evolution of antibiotic resistance is primarily driven by the selection of resistant organisms. Bacteria with higher mutation rates are classified as mutators. Additionally, recombination serves as a potent mechanism for the spread of antibiotic resistance genes. Enzymes associated with antibiotic resistance often exhibit considerable molecular adaptability, enabling them to evolve and counteract new drugs within a given chemical family. Genetic variability within bacterial populations is shaped by opposing forces, including processes that introduce new variations and those that eliminate existing ones. Fitness, a measure of reproductive success, is relative and is often evaluated by comparing the population growth rates of reference and target strains. Genetic drift, resulting from the limited size of a population, also influences genetic variability. Notably, research suggests that compensatory mechanisms can mitigate fitness costs, reducing the competitive advantage of drug-susceptible strains in antibiotic-free environments (González-Candelas et al., 2011).

The rise of multidrug-resistant bacteria highlights the urgent need for the development of innovative antibiotic strategies, as current infection control options are increasingly limited. To tackle this challenge, researchers are exploring alternative approaches, including biologics and non-antibiotic therapies (Brooks & Brooks, 2014). Bacteria within biofilms, complex communities of microorganisms, demonstrate enhanced resistance to antibiotics. This resistance arises from both community-level and cellular-level mechanisms. At the community level, the biofilm matrix can trap and neutralize antibiotics, reducing their effectiveness. At the cellular level, processes such as genetic mutations and horizontal gene transfer are more prevalent within biofilms, further exacerbating resistance (Mohr, 2016).

These factors, working in tandem, contribute to the resistance of biofilms to antibiotics. Additionally, biofilms facilitate the spread of antibiotic resistance by creating an environment where resistance genes can be acquired and exchanged. A deeper understanding of how bacteria interact with antibiotics within biofilms is essential for developing novel strategies to combat antibiotic resistance (Alanis, 2005).

Recent advancements in molecular biology and genetic engineering have opened new avenues for antibiotic discovery. Techniques such as metagenomics, functional screening, and combinatorial biosynthesis are being employed to identify novel antimicrobial compounds. Unfortunately, it typically takes a decade or more of intensive work by the pharmaceutical industry to bring a new antibiotic from discovery to market, with the added challenge of stringent regulatory requirements. This underscores the urgent need for increased investment in antibiotic development research (Townsend et al., 2003; Penesyan et al., 2015).

1. Evolutionary Principles

The evolution and history of antibiotics is a complex story, exemplified by the beta-lactam family, with penicillin serving as a notable example. Although a clear phenotype is evident, the molecule cannot be synthesized solely from the gene product. As a result, horizontal gene transfer has played a significant role in the evolutionary process of bacterial genes, including those involved in antibiotic biosynthesis. Consequently, genes related to regulation, resistance, and biosynthesis have become clustered within protein-coding genes (Pontes et al., 2018).

Natural Selection and Antibiotic Resistance

Evolution occurs through natural selection, genetic drift, and historical constraints. Surprisingly, evolution is highly predictable across different environments and lineages, as long as selection pressures are strong, as demonstrated in several well-documented cases of evolution. When chance and historical factors are accounted for, natural selection favors the fittest genotype in a given environment, making the evolutionary process more predictable (Bailey et al., 2015, Lassig et al., 2017). However, natural selection is only as predictable as our ability to understand genetic drift and past events—both of which are, to some extent, governed by chance.

Genetic Variation and Mutation

In general, two methods of population adaptation to new environments are recognized: the addition and exploitation of existing genetic variation, or the introduction of new mutations. These pathways can lead to distinct evolutionary outcomes and genetic influences. Adaptation is typically achieved more rapidly through the utilization of existing genetic variation, where the fixation of multiple small-effect alleles is common, and recessive traits are often spread. However, adaptation driven by new mutations may result in different genomic patterns of selection. Studying these mechanisms allows researchers to determine which process is being employed, providing insights into how populations adapt to

environmental changes and how the source of genetic variation influences this process (Palumbi, 2001; Barrett & Schluter, 2008).

Horizontal Gene Transfer and Antibiotic Resistance Spread

One of the serious threats to addressing the growing problem of antibiotic resistance in pathogenic bacteria is the widespread presence of antibiotic resistance genes (ARGs). This has led to the realization that ARGs are not confined to clinical pathogens but are also present in commensal bacteria, environmental sources, and mobile genetic elements, forming a large reservoir of resistance genes, known as the resistome. These genes are spread through horizontal gene transfer (HGT) via various processes, including conjugation, transformation, and transduction (Perron et al., 2015). Conjugation is the most significant factor in the dissemination of ARGs among bacterial populations (Figure 1). To develop effective strategies for combating the spread of antibiotic resistance, it is essential to gain a deeper understanding of the resistome within these bacteria and how it is transferred to pathogenic strains.

Identifying Antibiotic Targets

A large number of infectious and harmful microorganisms pose a significant risk to human health. These infections are caused by both Gram-positive and Gram-negative bacteria. Antibacterial drugs are commonly used to treat bacterial infections. Antibiotics exert their effects through at least three key mechanisms:

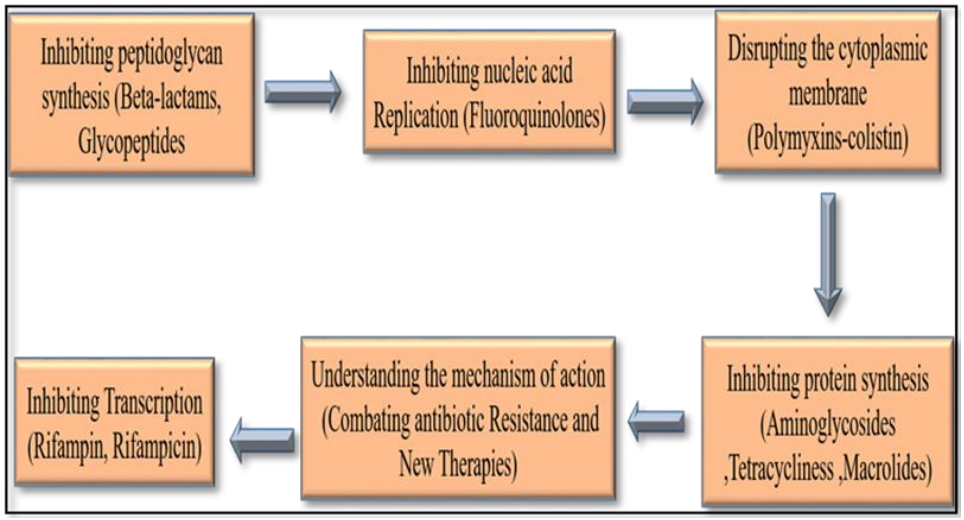

Fig. 1: Mode of Antibiotic Resistance

Essential Genes and Pathways in Pathogens

Through comprehensive genome analysis, it is possible to identify genes that are essential for the survival of a cell or organism. When these genes are found across an entire genus, they become potential targets for broad-spectrum drugs, as disrupting such genes can lead to cellular dysfunction or death. Many of these essential genes have been identified in various organisms, particularly in pathogens. To develop new treatments, it is crucial to understand the roles of these genes. Essential genes are commonly identified using various gene-inactivation methods, including targeted gene knockout, genetic footprinting, and transposon-based mutagenesis. Additionally, computational techniques such as subtractive genomics, essentiality-based mapping, and phylogenetic profiling are employed to predict essential genes. Data from experimental studies are also analyzed using bioinformatics tools. This chapter outlines the various approaches for identifying essential genes, understanding their functions, and discusses the advantages and challenges associated with these methods (Chen et al., 2017).

Phylogenetic Conservation of Targets Across Species

Molecular phylogenies provide valuable insights into the evolution of various aspects of species. By examining the genetic relationships between different species, they can help identify clades, or groups of related species. When combined with data on allele frequencies, molecular phylogenies enable the identification of populations or regions that are significant in terms of evolutionary processes within a species. A comparison of historical and current data highlights significant changes in long-term trends and identifies populations that may require immediate attention. However, further theoretical and experimental studies are needed to demonstrate how molecular phylogenies can be effectively applied in conservation biology (Moritz, 1995).

Target Identification using in Silico Modeling

Most importantly, effective drug discovery requires the identification of new targets that maximize efficacy while minimizing side effects. To achieve this, big data and computational approaches can assist researchers in narrowing down potential targets. Both infectious and non-infectious diseases can be addressed using network-based approaches, although comparative genomics is primarily employed in the study of infectious diseases. While each approach has its own strengths and limitations, they can be combined to enhance the discovery of drug targets. The drug discovery process can be significantly facilitated by big data and computational methods, helping to reduce costs, shorten cycle times, and increase the likelihood of success (Zhang et al., 2022).

2. Antibiotic Discovery Strategies

There is an urgent need to research and develop new drugs to combat diseases caused by bacterial pathogens, as current therapies with available antibiotics are becoming ineffective. Actinomycetes are the source of many antibiotics used in human and animal therapy today. The genome of these organisms contains groups of genes known as biosynthetic gene clusters (BGCs), which encode the proteins required for the synthesis, resistance, transport, and other processes related to secondary metabolites, including antibiotics. Databases, protein family domain (Pfam) sequences, and bioinformatics tools have been developed to mine microbial genomes. These technologies facilitate the in silico detection of BGCs, enabling more efficient identification of potential antibiotic-producing genes (Ruetten et al., 2022).

High-throughput Screening (HTS) and Evolutionary-based Assays

High-throughput screening (HTS) techniques offer a reliable, time-efficient, and highly reproducible method for analyzing interactions between a large number of chemical compounds or biomolecules and specific targets. The development of smaller devices, more sensitive detection techniques, enhanced data analysis, and the automation of screening processes have significantly advanced HTS assays. As a result, HTS has found successful applications in various fields, including drug discovery, protein evolution, enzyme engineering, and the screening and identification of chemical probes, small compounds, and lipopeptides (Contreras-Llano & Tan, 2018). By combining HTS with evolutionary-based assays, researchers can create novel molecules (Smith et al., 2013).

Evolutionary-based assays are designed to guide the evolution of specific molecules. For example, a fluorescence-based HTS approach can be used to assess a library of sialyltransferase mutants, enabling the identification of variants with improved catalytic performance.

Natural Product-derived Antibiotics and Evolutionary Optimization

Natural compounds have proven effective in treating harmful bacterial infections; however, the rising prevalence of antibiotic-resistant infections necessitates the development of new antibiotics in healthcare. Natural antibiotics are particularly well-suited for evolutionary optimization due to their structural diversity and complexity (Figure 2). Over the past 80 years, readily accessible options have been exhausted, and the frequent rediscovery of existing compounds has become a costly and inefficient burden for screening initiatives (Table 1) (Kirst, 2013).

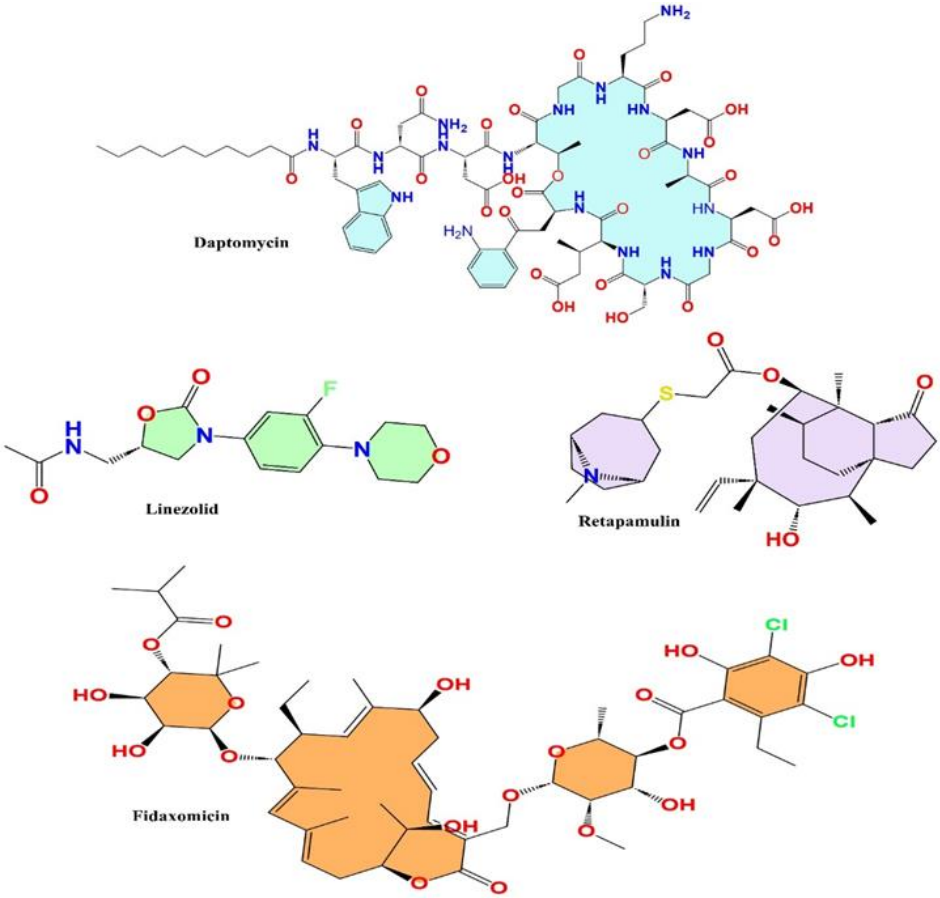

Fig. 2: 2D structures of Daptomycin, Linezolid, Retapamulin and Fidaxomicin (Chem Draw 22.0.032-bit)

3. Enhancement of Antibiotics Derived from Natural Products

Several methods by which antibiotics derived from natural products can be enhanced through evolution include:

- Identification of novel compounds
- Alteration of existing compounds
- Development of resistance mechanisms

To Identify new Antibiotics

Additional methods for identifying new antibiotics include:

- Bioprospecting
- Chemical derivatization
- Genome mining (Leeds et al., 2006).

Table 1: Examples of natural product-derived antibiotics in development

Antibiotic	Chemical Class	Stage of Development	Sponsoring organization	Reference
Tigecycline	Tetracycline	Launched	Wyeth Research	(Chopra, 2001).
Dalbavancin	Glycopeptides	Pre-registration	Pfizer	(Micheli et al., 2023)
Telavancin	Glycopeptides	Phase III	Theravance, Inc. and Astellas	(Pace & Judice 2005).
Tiacumicin B	Macrolide	Phase IIb	Optimer and Par	(Erb & Zhu 2013).
Retapamulin	Pleuromutilin	Pre-registration	GlaxoSmithKline	(Jones et al., 2006)

Resistance Mutation Rates and Antibiotics Potency

Antibiotics are a class of chemotherapeutic agents used in the treatment of diseases by either destroying pathogenic organisms or suppressing their growth at concentrations low enough to prevent adverse effects on the host organism (Dafale et al., 2016). The proposed strategy relies on the use of antibiotic concentrations that require the acquisition of two simultaneous resistance mutations for bacterial proliferation. This concentration is referred to as the "mutant prevention concentration" (MPC), as no resistant colonies are isolated, even when more than 10^{10} cells are inoculated (Zhao & Drlica, 2001).

The emergence of antibiotic-resistant mutant bacteria is believed to occur within a specific concentration range of the antimicrobial agent, known as the mutant selection window. This range spans from the minimum inhibitory concentration (MIC) of susceptible bacterial strains to the MIC of the least susceptible single-step bacterial mutants, which is referred to as the mutant prevention concentration (MPC). The MPCs for tobramycin, chloramphenicol, rifampicin, penicillin, vancomycin, and various fluoroquinolones have been determined through studies involving *Escherichia coli* and *Staphylococcus aureus* (Xilin & Drlica, 2002).

Resistance can be characterized in two distinct ways: A) Intrinsic resistance, in which microorganisms naturally lack target sites for antimicrobials, rendering the antimicrobial ineffective against them. B) Acquired resistance, which occurs when a microorganism that was initially susceptible to an antimicrobial develops mechanisms to protect itself from the drug's effects. These protective mechanisms may include the production of enzymes that neutralize the drug, alterations to the drug's target site, reduced drug absorption, or active efflux of the drug from the cell (Dowling et al., 2017).

Fluoroquinolones (FQs) are potent antibiotics that have been utilized in healthcare settings for over 30 years. Their clinical applications have been well-established for more than three decades, and they are now integral components of therapeutic interventions. The action of FQs is mediated through their specific targeting of bacterial DNA gyrase and DNA topoisomerase IV, where they stabilize a covalent complex between the enzyme and DNA, leading to the cleavage of both DNA strands. The ultimate result of this mechanism has contributed to the development of an extremely effective strategy for eradicating bacterial pathogens (Bush et al., 2020).

4. Pharmacocokineti /pharmacodynamics (PK /PD) and Evolutionary Modeling

Pharmacokinetic/pharmacodynamic (PK/PD) modeling establishes a relationship between drug concentration (PK) and its effect (PD), enabling the elucidation and prediction of the temporal changes in pharmacological effects following a specified dosing regimen (Derendorf & Meibohm, 1999). PK/PD modeling methodologies can be fundamentally categorized based on four principal characteristics. The first characteristic delineates the relationship between quantified drug concentration and the response system, distinguishing between a direct and an indirect link. The second examines how the response system correlates effect site concentration with the observable outcome, differentiating between a direct and an indirect response. The third characteristic concerns the nature of the data—whether clinically or experimentally obtained—that is used to establish the connection between concentration and effect, contrasting a hard link with a soft link. Finally, the fourth characteristic addresses the temporal dependency of pharmacodynamic model parameters, distinguishing between time-variant and time-invariant characteristics (Derendorf & Meibohm, 1999).

Six pharmacological agents—benzylpenicillin, cefuroxime, erythromycin, gentamicin, moxifloxacin, and vancomycin—encompassing a diverse range of mechanistic pathways and pharmacokinetic (PK) and pharmacodynamic (PD) properties, were analyzed. A dose fractionation analysis was meticulously simulated, employing a broad spectrum of total daily doses. The temporal dynamics of drug concentration (PK model) were assessed alongside the bacterial response to the administered drug exposure using an in vitro PK/PD model (Nielsen et al., 2011). Quantitative data on antibiotic pharmacokinetics and pharmacodynamics (PK/PD) is often underutilized, limiting its applicability for optimal dosing strategies and study cohorts. In vitro investigations provide critical insights into the temporal dynamics of antibiotic activity, which underpin diverse PK/PD models that vary in the mechanisms through which antibiotic exposure drives the proliferation of resistant bacterial strains (Nielsen & Friberg, 2013).

Dosing Strategies to Minimum Resistance Selection

The global rise in antibiotic resistance presents a major public health challenge. Increasingly, it is recognized that the selection of dosage and treatment duration can influence the emergence of antibiotic-resistant mutants. As a result, there has been a growing body of research utilizing pharmacodynamic models to elucidate drug exposure parameters and pharmacodynamic breakpoints essential for mitigating and predicting the onset of resistance (Olofsson & Cars, 2007).

The type of resistance plays a significant role in determining the appropriate dosage to delay the emergence of resistant populations. High

doses of a toxin can select for monogenic resistance, while lower doses may lead to a gradual increase in resistance levels. A mathematical model suggests that alternating between low doses and a higher dose could delay the evolution of resistance longer than applying a constant dose throughout the treatment (Gardner et al., 1998). When designing a dosing regimen, applying knowledge of an antibiotic's pharmacokinetic/pharmacodynamic profile enhances the likelihood of achieving optimal exposure, even against Gram-negative organisms with higher MICs (Monogue et al., 2016).

Gentamicin, an aminoglycoside antibiotic, exhibits strong bactericidal action against aerobic Gram-negative bacteria, making it an effective treatment for a variety of severe infections. Due to its poor absorption through the digestive system, gentamicin is administered parenterally in topical, ophthalmic, and systemic (intramuscular and intravenous) forms (Karunarathna et al., 2024). To experimentally investigate how nutrients and antibiotic dosages interact to promote antibiotic survival, we cultivated *Escherichia coli* populations under daily 5-hour treatments with varying levels of amikacin and nutrients, interspersed with growth periods in a nutrient-rich, antibiotic-free medium. Antibiotic-resistant mutants can proliferate at antibiotic doses higher than the minimum inhibitory concentration (MIC) of the susceptible strain, whereas resistant cells are typically non-dividing in the presence of antibiotics. Since persistence is associated with a growth deficit, we hypothesized that the availability of nutrients during antibiotic treatment would promote resistance more than persistence, based on this critical distinction (Karunarathna et al., 2024),

5. Case Studies

Beta-lactam antibiotics and beta-lactamase evolution

β-Lactamases are ancient enzymes that predate the development of therapeutic antibiotics, with estimates suggesting they are over 2 billion years old. The first identification of a bacterial enzyme capable of inactivating penicillin was reported by Abraham and Chain in 1940. β-Lactamases have been discovered in isolated environmental sites, including caves, permafrost sediments, and ancient bone samples, indicating their evolutionary role in protecting bacteria from naturally occurring β-lactams (Figure 3). These enzymes have been identified in samples from glaciers, fungi, and various bacteria, although some organisms, such as *S. pneumoniae* and *H. pylori*, lack these enzymes. The emergence of β-lactamases is closely linked to the evolution of β-lactam-based antibacterial agents, which were initially discovered in natural sources. These enzymes have been detected in a variety of environmental settings, including soil and water samples (Bush, 2018).

Discovery of Benzylpenicillin

The discovery of benzylpenicillin by Sir Alexander Fleming in 1928 marked the beginning of antibiotics as essential life-saving medications. Today, various classes of antibiotics are employed to combat a wide range of bacterial infections, with β-lactam antibiotics being the most commonly used and prescribed. Over the past decade, β-lactams have accounted for over 60% of all antibiotic prescriptions. However, the increasing use of antibiotics has heightened the risk of antimicrobial resistance (AMR) among bacterial pathogens. AMR is typically acquired through horizontal gene transfer or spontaneous mutations in essential target genes. Human infectious diseases are often linked to those caused by ESKAPE pathogens, including *Enterococcus spp.*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which are frequently responsible for the spread of resistance. DTR pathogens, resistant to multiple first-line antibiotics, have been reported globally, and with the increasing threat of AMR, it is speculated that bacterial infections may eventually become incurable with existing antibiotics (Narendrakumar et al., 2023).

Classification and Mechanism

The increasing resistance to β-lactam antibiotics, particularly among Gram-negative bacteria, has been largely attributed to the emergence of extended-spectrum β-lactamases (ESBLs) and carbapenemases. Elevated mortality rates have been associated with the CTX-M family, a major contributor to the development of multidrug-resistant characteristics. The global spread of carbapenemases has been facilitated by international travel and commerce. New β-lactamases have emerged in response to both naturally occurring β-lactams and the excessive use of β-lactam antibiotics, which act as selective pressures. β-Lactamase genes remain stable in mixed-species communities, even in the absence of antibiotic pressure. The contamination of public water supplies with β-lactamase-producing, antibiotic-resistant pathogens has become an increasing public health concern. New β-lactamase inhibitors, such as avibactam and vaborbactam, have been approved for use in combination with ceftazidime or meropenem. A phase 3 clinical trial is currently underway for a combination of avibactam and aztreonam to target Gram-negative bacterial infections. Additionally, fluoroquinolones (FQs) have also faced resistance, with 1.33% of 1581 *M. leprae* isolates demonstrating resistance to ofloxacin (Chauffour et al., 2021).

DNA gyrase and topoisomerase IV regulate the architecture of bacterial DNA by splitting the DNA strands, allowing the duplex DNA to flow through the break, and then sealing the breach. The fluoroquinolone antibiotic family forms drug-enzyme-DNA complexes in which the DNA is cleaved, and these antibiotics can reversibly interfere with this process. In these complexes, the C-7 ring of the fluoroquinolone is oriented toward the GyrB/ParE subunits, and these structures, known as cleaved complexes, have been crystallized due to the existence of DNA breaks. Thiol-reactive C-7-modified chloroacetyl derivatives of ciprofloxacin (Cip-AcCl) create cross-linked cleaved complexes with the mutant GyrB-Cys466 gyrase, a process confirmed by resistance to both EDTA and heat treatment. This result aligns with findings from X-ray crystallography. Interestingly, complexes containing the mutant GyrA-G81C gyrase also exhibited detectable cross-linking, suggesting a novel drug-gyrase interaction not previously observed in crystal structures. Cip-AcCl demonstrated potent bacteriostatic effects against a quinolone-resistant strain of *Escherichia coli* (GyrA-G81C) and its analog in *Mycobacterium smegmatis* (GyrA-G89C) when the fluoroquinolone was cross-linked with GyrA-G81C gyrase. The existence of a GyrA-drug cross-link was further confirmed by evidence of Cip-AcCl's permanent suppression of DNA replication. These findings support interactions between GyrA, GyrB, and the fluoroquinolone's C-7 ring. The crystal structure of the cleaved complexes reveals that the GyrA-Gly81 and GyrB-Glu466 residues are 17 Å apart, suggesting the presence of two distinct quinolone binding mechanisms (Figure 4). The potential for the formation of different quinolone-enzyme-DNA complexes based on these two binding mechanisms opens new avenues for research and applications related to the relationship between drug structure and activity in type II DNA

topoisomerases (Mustaev et al., 2014).

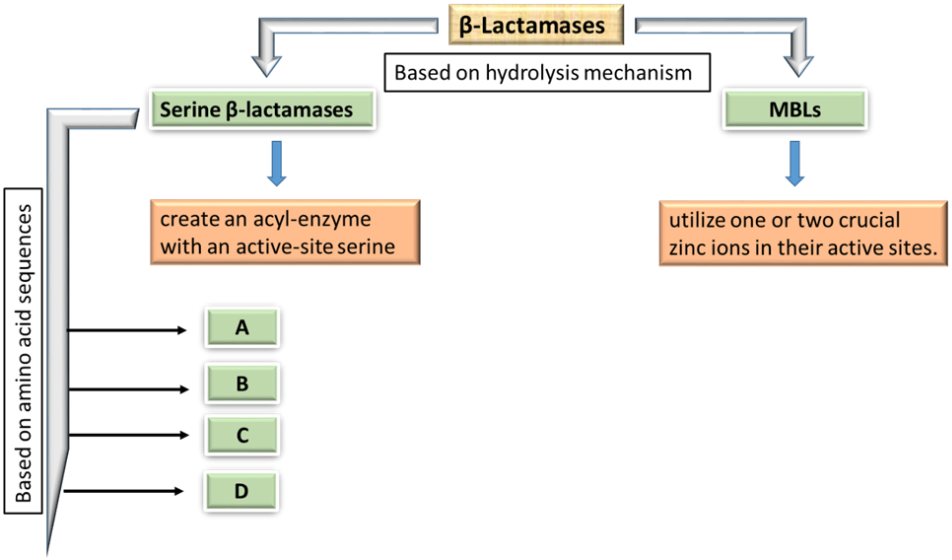

Fig. 3: Mechanism of AMR

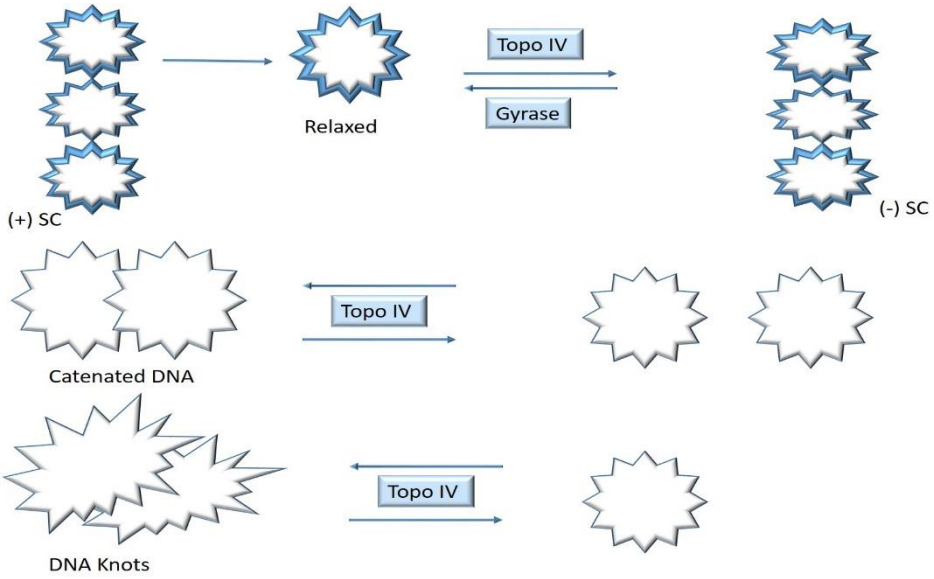

Fig. 4: The activities of gyrase and topoisomerase IV regarding DNA strand passage are essential.

6. Challenges and Solutions

Resource limitations, particularly in low-income countries, significantly hinder the development of antibiotics and diagnostic tools. Disparities in healthcare systems and policies further impede integration and coordination, resulting in a lack of standardized adherence to stewardship measures. The rise of antibiotic resistance has reduced the efficacy of current drugs, underscoring the urgent need for new antibiotics. Traditional methods of antibiotic development are costly, time-consuming, and often yield insufficient results. Emerging technologies such as CRISPR and artificial intelligence (AI) offer promising solutions, enabling more efficient discovery and targeting of immune-resistant pathogens. These technologies have the potential to enhance the accuracy, efficiency, and cost-effectiveness of developing new antibiotics (Muteeb et al., 2023).

CRISPR in the Development of Antibiotics

CRISPR is a technique that allows for precise modification of an organism's DNA, making it particularly beneficial for studying bacterial genes and their resistance mechanisms. It can be used to eliminate antibiotic-related genes present in pathogens. By manipulating bacterial genomes, researchers have been able to identify new potential targets for the development of novel antibiotics (Geinoro et al., 2024).

7. Artificial Intelligence/AI in Antibiotic Discovery

AI plays a pivotal role in antibiotic discovery, particularly through high-throughput screening, which enables the identification of compounds with bacterial-killing properties. Machine learning algorithms can analyze vast datasets, allowing AI to virtually screen millions of

compounds and identify potential antibiotics without the need for actual laboratory testing. AI has also been utilized to uncover new applications for existing drugs, such as Halicin, an antibiotic effective against resistant bacteria. Other emerging technologies, including high-throughput screening, robotics, and metagenomics, are accelerating the process of testing thousands of compounds and discovering new antibiotics from environmental sources. However, challenges remain, including issues related to pricing, legal concerns, and the ethical use of CRISPR technology. Future progress may depend on enhanced knowledge exchange among academia, industry, and government to support the discovery and development of new antibiotics (Melo et al., 2021).

Conclusion

AMR (antimicrobial resistance) poses a significant threat to the medical advancements we have made over the years and necessitates the development of new, innovative solutions. This study explores how evolutionary processes, including natural selection and genetic variation, help us understand the development of resistance. The rapid rise of resistance is largely driven by the misuse of antibiotics, so using them responsibly and in the correct dosages plays a crucial role in alleviating the pressure that drives this issue. Emerging technologies, including genome mining, CRISPR, and artificial intelligence, are opening new avenues for antibiotic discovery. These technologies enable researchers to identify novel drug targets, enhance naturally occurring substances used in medicine, and accelerate the testing of potential treatments. The resilience of certain pathogens, which resist antibiotics like β-lactams and fluoroquinolones, serves as a clear indicator of the urgent need for interdisciplinary collaboration in pharmacy. Countries must unite in the global fight against AMR. It is essential that programs promoting responsible antibiotic use, improving monitoring, and consolidating research efforts continue to ensure the effectiveness of these drugs. By leveraging insights from evolutionary biology and the latest technological advances, we can combat AMR and protect ourselves from the growing burden of infections driven by drug-resistant pathogens.

References

Ahmad, S., Aqib, A. I., Ghafoor, M., Shoaib, M., ul Haq, S., Ataya, F. S., & Jianxi, L. (2024). Drug resistance modulation of dairy MRSA through Berberine. Artesunate and Quercetin in Combination with β-Lactams. *Pak Vet J*, *44*(2), 510-516.

Alanis, A. J. (2005). Resistance to antibiotics: are we in the post-antibiotic era? *Archives of Medical Research*, *36*(6), 697-705.

Alm, R. A., McLaughlin, R. E., Kos, V. N., Sader, H. S., Iaconis, J. P., & Lahiri, S. D. (2014). Analysis of Staphylococcus aureus clinical isolates with reduced susceptibility to ceftaroline: an epidemiological and structural perspective. *Journal of Antimicrobial Chemotherapy*, *69*(8), 2065-2075.

Barrett, R. D., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*(1), 38-44.

Brooks, B. D., & Brooks, A. E. (2014). Therapeutic strategies to combat antibiotic resistance. *Advanced Drug Delivery Reviews*, *78*, 14-27.

Bush, K. (2018). Past and present perspectives on β-lactamases. *Antimicrobial agents and chemotherapy*, *62*(10), 10.1128/aac. 01076-01018.

Bush, N. G., Diez-Santos, I., Abbott, L. R., & Maxwell, A. (2020). Quinolones: mechanism, lethality and their contributions to antibiotic resistance. *Molecules*, *25*(23), 5662.

Chauffour, A., Morel, F., Reibel, F., Petrella, S., Mayer, C., Cambau, E., & Aubry, A. (2021). A systematic review of Mycobacterium leprae DNA gyrase mutations and their impact on fluoroquinolone resistance. *Clinical Microbiology and Infection*, *27*(11), 1601-1612.

Chen, L., Zhang, Y. H., Wang, S., Zhang, Y., Huang, T., & Cai, Y. D. (2017). Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. *PloS One*, *12*(9), e0184129.

Chopra, I. (2001). Glycylcyclines: third-generation tetracycline antibiotics. *Current Opinion in Pharmacology*, *1*(5), 464-469.

Contreras-Llano, L. E., & Tan, C. (2018). High-throughput screening of biomolecules using cell-free gene expression systems. *Synthetic Biology*, *3*(1), ysy012.

Dafale, N. A., Semwal, U. P., Rajput, R. K., & Singh, G. (2016). Selection of appropriate analytical tools to determine the potency and bioactivity of antibiotics and antibiotic resistance. *Journal of Pharmaceutical Analysis*, *6*(4), 207-213.

Derendorf, H., & Meibohm, B. (1999). Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: concepts and perspectives. *Pharmaceutical Research*, *16*, 176-185.

Dowling, A., O'Dwyer, J., & Adley, C. (2017). Antibiotics: mode of action and mechanisms of resistance. *Antimicrobial Research: Novel Bioknowledge and Educational Programs*, *1*, 536-545.

Durand, G. A., Raoult, D., & Dubourg, G. (2019). Antibiotic discovery: history, methods and perspectives. *International Journal of Antimicrobial Agents*, *53*(4), 371-382.

Erb, W., & Zhu, J. (2013). From natural product to marketed drug: the tiacumicin odyssey. *Natural Product Reports*, *30*(1), 161-174.

Gardner, S. N., Gressel, J., & Mangel, M. (1998). A revolving dose strategy to delay the evolution of both quantitative vs major monogene resistances to pesticides and drugs. *International Journal of Pest Management*, *44*(3), 161-180.Geinoro, T., Chala, G., & Mulugeta, W. (2024). Exploiting Crispr-Cas System to Combat Antibiotic Resistance: A Review. *Global Veterinaria*, *26*, 25-36.

González-Candelas, F., Comas, I., Martínez, J. L., Galán, J. C., & Baquero, F. (2011). The evolution of antibiotic resistance. In *Genetics and Evolution of Infectious Disease* (pp. 305-337). Elsevier.

Jones, R. N., Fritsche, T. R., Sader, H. S., & Ross, J. E. (2006). Activity of retapamulin (SB-275833), a novel pleuromutilin, against selected resistant gram-positive cocci. *Antimicrobial Agents and Chemotherapy*, *50*(7), 2583-2586.

Johnson, N. B., Hayes, L. D., Brown, K., Hoo, E. C., Ethier, K. A., Control, C. f. D., & Prevention. (2014). CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors--United States, 2005-2013. *Morbidity and Mortality Weekly Report Supplements*, *63*(4), 3-27.

Karunarathna, I., Gunasena, P., Gunathilake, S., & De Alvis, K. (2024). Antibiotic resistance and gentamicin: Current trends and clinical

implications. (pp.152-163). *Uva-Clinical Pharmacology*. ISBN 978-624-93721-0-8

Kirst, H. A. (2013). Developing new antibacterials through natural product research. *Expert Opinion on Drug Discovery*, *8*(5), 479-493.

Leeds, J. A., Schmitt, E. K., & Krastel, P. (2006). Recent developments in antibacterial drug discovery: microbe-derived natural products–from collection to the clinic. *Expert Opinion on Investigational Drugs*, *15*(3), 211-226.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., & Olsson-Liljequist, B. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268-281.

Melo, M. C., Maasch, J. R., & de la Fuente-Nunez, C. (2021). Accelerating antibiotic discovery through artificial intelligence. *Communications Biology*, *4*(1), 1050.

Micheli, G., Chiuchiarelli, M., Taccari, F., & Fantoni, M. (2023). The role of long-acting antibiotics in the clinical practice: a narrative review. *Le Infezioni in Medicina*, *31*(4), 449.

Mohr, K. I. (2016). History of antibiotics research. *How to Overcome the Antibiotic Crisis: Facts, Challenges, Technologies and Future Perspectives*, 237-272.

Monogue, M. L., Kuti, J. L., & Nicolau, D. P. (2016). Optimizing antibiotic dosing strategies for the treatment of gram-negative infections in the era of resistance. *Expert Review of Clinical Pharmacology*, *9*(3), 459-476.

Moritz, C. (1995). Uses of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *349*(1327), 113-118.

Mustaev, A., Malik, M., Zhao, X., Kurepina, N., Luan, G., Oppegard, L. M., Hiasa, H., Marks, K. R., Kerns, R. J., & Berger, J. M. (2014). Fluoroquinolone-gyrase-DNA complexes: two modes of drug binding. *Journal of Biological Chemistry*, *289*(18), 12300-12312.

Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, *16*(11), 1615.

Narendrakumar, L., Chakraborty, M., Kumari, S., Paul, D., & Das, B. (2023). β-Lactam potentiators to re-sensitize resistant pathogens: Discovery, development, clinical use and the way forward. *Frontiers in Microbiology*, *13*, 1092556.

Nielsen, E. I., Cars, O., & Friberg, L. E. (2011). Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. *Antimicrobial Agents and Chemotherapy*, *55*(10), 4619-4630.

Nielsen, E. I., & Friberg, L. E. (2013). Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacological Reviews*, *65*(3), 1053-1090.

Olofsson, S. K., & Cars, O. (2007). Optimizing drug exposure to minimize selection of antibiotic resistance. *Clinical Infectious Diseases*, *45*(2), S129-S136.

Pace, J. L., & Judice, J. K. (2005). Telavancin (Theravance). *Current Opinion in Investigational Drugs (London, England: 2000)*, *6*(2), 216-225.

Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science*, *293*(5536), 1786-1790.

Penesyan, A., Gillings, M., & Paulsen, I. T. (2015). Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. *Molecules*, *20*(4), 5286-5298.

Perron, G. G., Whyte, L., Turnbaugh, P. J., Goordial, J., Hanage, W. P., Dantas, G., & Desai, M. M. (2015). Functional characterization of bacteria isolated from ancient arctic soil exposes diverse resistance mechanisms to modern antibiotics. *PloS One*, *10*(3), e0069533.

Podolsky, S. H. (2018). The evolving response to antibiotic resistance (1945–2018). *Palgrave Communications*, *4*(1).

Pontes, D. S., de Araujo, R. S., Dantas, N., Scotti, L., Scotti, M. T., de Moura, R. O., & Mendonca-Junior, F. J. B. (2018). Genetic mechanisms of antibiotic resistance and the role of antibiotic adjuvants. *Current Topics in Medicinal Chemistry*, *18*(1), 42-74.

European Centre for Disease Prevention and Control. (2017). Antimicrobial resistance surveillance in Europe 2015. *Annual report of the European antimicrobial resistance surveillance network (EARS-net)*. https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net

Ruetten, A., Kirchner, T., & Musiol-Kroll, E. M. (2022). Overview on strategies and assays for antibiotic discovery. *Pharmaceuticals*, *15*(10), 1302.

Satlin, M. J., Soave, R., Racanelli, A. C., Shore, T. B., Van Besien, K., Jenkins, S. G., & Walsh, T. J. (2014). The emergence of vancomycin-resistant enterococcal bacteremia in hematopoietic stem cell transplant recipients. *Leukemia & Lymphoma*, *55*(12), 2858-2865.

Smith, R. E., Tran, K., & Vocque, R. H. (2013). Network medicine and high throughput screening. *Current Drug Discovery Technologies*, *10*(3), 182-194.

Townsend, J. P., Nielsen, K. M., Fisher, D. S., & Hartl, D. L. (2003). Horizontal acquisition of divergent chromosomal DNA in bacteria: effects of mutator phenotypes. *Genetics*, *164*(1), 13-21.

Varela, M. F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L. M., Hernandez, A. J., Parvathi, A., & Kumar, S. H. (2021). Bacterial Resistance to Antimicrobial Agents. *Antibiotics*, *10*(5), 593. https://www.mdpi.com/2079-6382/10/5/593

Xilin, Z., & Drlica, K. (2002). Restricting the selection of antibiotic-resistant mutant bacteria: measurement and potential use of the mutant selection window. *The Journal of Infectious Diseases*, *185*(4), 561-565.

Zhang, X., Wu, F., Yang, N., Zhan, X., Liao, J., Mai, S., & Huang, Z. (2022). In silico methods for identification of potential therapeutic targets. *Interdisciplinary Sciences: Computational Life Sciences*, 1-26.

Zhao, X., & Drlica, K. (2001). Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clinical Infectious Diseases*, *33*(Supplement_3), S147-S156.