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Review Article

EVOLUTION OF ANTIBIOTIC RESISTANCE IN BACTERIAL GENOMES: A COMPREHENSIVE REVIEW

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ABSTRACT. The persistent emergence of antibiotic resistance in bacterial populations poses a formidable challenge to global healthcare systems. Understanding the mechanisms and evolutionary dynamics underlying this phenomenon is of utmost importance. This study comprehensively explores the complex interplay between genomics and evolution within the context of antibiotic resistance in bacteria. It delves into the mechanisms by which bacteria develop resistance to antibiotics, including mutation and horizontal gene transfer, and highlights the pivotal role of natural selection and selective pressure in shaping genomic adaptations. Through the lens of genomics, we dissect case studies of specific bacterial species and strains that have evolved resistance, scrutinizing the genomic changes that underpin their survival. Long-term evolutionary trends and the genomic basis of resistance mechanisms are also examined, offering critical insights into the adaptive strategies employed by bacteria. Furthermore, the review addresses the challenges inherent in studying antibiotic resistance evolution and underscores the necessity for innovative research approaches. By bridging the domains of genomics, evolutionary biology, and disease ecology, this study contributes to a deeper understanding of the ongoing battle against antibiotic-resistant pathogens and provides a foundation for future research endeavors.

Keywords: *Antibiotic resistance, Genomics, Evolution, Resistance mechanisms, Multi-drug resistance (MDR)*

BAKTERİ GENOMLARINDA ANTİBİYOTİK DİRENCİNİN EVRİMİ: KAPSAMLI BİR İNCELEME

ÖZET. Bakteri popülasyonlarında antibiyotik direncinin ısrarcı bir şekilde ortaya çıkması, küresel sağlık sistemleri için büyük bir zorluk teşkil etmektedir. Bu olgunun altında yatan mekanizmaları ve evrimsel dinamikleri anlamak son derece önemlidir. Bu çalışma, bakterilerdeki antibiyotik direnci bağlamında genomik ve evrim arasındaki karmaşık etkileşimi kapsamlı bir şekilde araştırmaktadır. Mutasyon ve yatay gen aktarımı da dahil olmak üzere bakterilerin antibiyotiklere karşı direnç geliştirdiği mekanizmaları derinlemesine incelemekte ve genomik adaptasyonların şekillenmesinde doğal seçilim ile seçici baskının önemli rolünü vurgulamaktadır. Böylece biz genomik bir bakış açısıyla, direnç geliştiren spesifik bakteri türlerine ve suşlarına ilişkin vaka çalışmalarını inceliyor ve bunların hayatta kalmasını destekleyen genomik değişiklikleri inceliyoruz. Çalışmada uzun vadeli evrimsel eğilimler ve direnç mekanizmalarının genomik temeli de incelenerek, bakterilerin kullandığı adaptif stratejiler hakkında kritik bilgiler sunulmaktadır. Ayrıca bu derleme, antibiyotik direncinin evrimini incelemenin doğasında bulunan zorlukları ele almakta ve yenilikçi araştırma yaklaşımlarının gerekliliğinin altını çizmektedir. Genomik, evrimsel biyoloji ve hastalık ekolojisi alanları arasında köprü kuran bu çalışma, antibiyotiğe dirençli patojenlere karşı devam eden mücadelenin daha derinlemesine anlaşılmasına katkıda bulunmakta ve gelecekteki araştırma çabaları için bir temel sağlamaktadır.

Anahtar Kelimeler: *Antibiyotik direnci, Genomik, Evrim, Direnç mekanizmaları, Çoklu ilaç direnci (MDR)*

INTRODUCTION

Antibiotics have, without a doubt, transformed the landscape of modern medicine [1]. These remarkable drugs have revolutionized the treatment of bacterial infections [2], turning once-deadly diseases into manageable conditions and playing a pivotal role in extending human life expectancy [3]. The emergence of antibiotics marked a period where previously lethal infections became readily treatable, paving the way for advances in surgery, organ transplantation [4, 5], and cancer therapy [6]. However, beneath the surface of these remarkable achievements lies a sobering reality - the escalating global crisis of antibiotic resistance [7].

Antibiotic resistance is a biological phenomenon in which bacteria and other microorganisms evolve and adapt to withstand the effects of antibiotics, rendering these drugs less effective or entirely ineffective in treating infections. This resistance arises due to genetic changes within bacterial populations, driven primarily by the selective pressures imposed by antibiotics themselves. Antibiotic resistance is not a new phenomenon; it represents a fundamental characteristic of bacterial populations [8]. Bacteria have evolved mechanisms to resist the toxic effects of natural antimicrobial compounds long before the discovery of antibiotics by humans [9]. However, the rapid and widespread emergence of resistance to the antibiotics we rely on today is a cause for grave concern. It threatens to undo decades of progress in healthcare and presents a complex web of challenges that transcend medical, economic, and ecological domains [10].

At its core, the medical impact of antibiotic resistance is deeply unsettling. Infections that were once routinely treatable now pose a significant threat to public health [11]. Simple surgical procedures, such as cesarean sections [12] and appendectomies [13], become extremely risky when antibiotic-resistant bacteria are present. Immunocompromised individuals, including cancer patients and organ transplant recipients, face heightened risks of life-threatening infections for which treatment options are increasingly limited [14]. The possibility of entering a post-antibiotic era, in which common infections become untreatable, becomes increasingly imminent [15].

Beyond individual health, antibiotic resistance takes a toll on healthcare systems [16]. Patients suffering from infections caused by antibiotic-resistant bacteria often endure extended hospitalization periods, more complicated treatment regimens, and higher mortality rates [17]. The economic burden is staggering, with estimates suggesting that antibiotic-resistant infections cost healthcare systems billions of dollars annually [18]. Hospitals must allocate additional resources to control outbreaks, isolate patients, and develop alternative treatment strategies.

Antibiotic resistance extends its reach beyond the confines of healthcare institutions. Resistant bacteria, driven by selective pressures, can flourish in the environment, from soil and water to wildlife and livestock [19, 20]. This environmental reservoir of resistance genes poses ecological risks and can potentially lead to the contamination of food sources [21]. The consequences of antibiotic use in agriculture and veterinary medicine [22] further exacerbate the problem.

Economically, the fallout from antibiotic resistance is far-reaching. It has consequences on productivity, as individuals experiencing prolonged illnesses spend increased periods away from work, eventually exerting an influence on global economies [23]. The costs of engaging in research and the development of novel antibiotics are formidable, leading to a market failure where the economic incentives to create novel antibiotics are often insufficient [24].

In this turbulent landscape, understanding the mechanisms and evolutionary dynamics pertaining to antibiotic resistance within bacterial populations is not merely an academic pursuit; it is an urgent imperative. Genomics, coupled with evolutionary biology, offers a lens through which we can dissect the genetic adaptations and selection pressures that underlie the development of resistance [25]. By unraveling the intricate interplay between genomics and evolution, we can identify vulnerabilities in bacterial populations and design strategies to combat resistance more effectively.

Mechanisms of Antibiotic Resistance

Genetic Mutations

Bacteria have the capacity to acquire resistance by means of spontaneous mutations in their DNA [26]. Point mutations encompass alterations in the DNA sequence capable of influencing the functionality of particular genes [27]. In the context of antibiotic resistance, point mutations often occur in genes that code for essential proteins, such as those involved in cell wall synthesis or DNA replication [28]. One common mutation-based mechanism is the modification of the antibiotic's target site. As an illustration, in the instance of antibiotic resistance in *Mycobacterium tuberculosis* (the causative agent of tuberculosis), mutations in the *rpoB* gene lead to changes in the RNA polymerase enzyme, making it less susceptible to rifampin, an antibiotic used in tuberculosis treatment [29]. Some mutations can lead to the overproduction or increased activity of efflux pumps. These pumps actively remove antibiotics from the bacterial cell, reducing the intracellular concentration of the drug and rendering it less effective [30].

Horizontal Gene Transfer (HGT)

Bacteria can swap genetic material through conjugation, a process in which two bacterial cells physically connect and transfer plasmids (small, circular DNA molecules) [31]. Plasmids often carry antibiotic-resistance genes. When a resistant bacterium conjugates with a susceptible one, the susceptible bacterium can acquire the resistance plasmid and become antibiotic-resistant [32]. In transformation, bacteria take up fragments of DNA from their environment. If this DNA contains antibiotic resistance genes, the recipient bacterium can incorporate these genes into its genome and become resistant. Transduction is a process in which bacteriophages (viruses that infect bacteria) transfer bacterial DNA from one cell to another [33]. If a phage carries antibiotic resistance genes from one bacterium to another, the recipient bacterium can acquire resistance.

Enzymatic Inactivation

Certain bacteria generate enzymes capable of chemically modify antibiotics, rendering them inactive. For example, β-lactamase enzymes can break down β-lactam antibiotics, such as penicillin, before they can manifest their antibacterial effects [34].

Alteration of Antibiotic Permeability

Bacteria can alter the permeability of their cell membranes to reduce the entry of antibiotics. This can be achieved by modifying the structure of porins, which serve as protein channels in the cell membrane that allow the passage of molecules, including antibiotics [35].

Biofilm Formation

In biofilms, bacteria form protective communities on surfaces, such as medical devices or tissues [36]. Within biofilms, bacteria can develop reduced susceptibility to antibiotics due to the physical barriers and altered metabolic states within the biofilm structure.

Persistence

Some bacteria can enter a dormant state known as persistence [37], making them less vulnerable to antibiotics. In this state, bacteria reduce their metabolic activity and become tolerant to antibiotics. Later, they can revive and cause recurrent infections.

Evolutionary Processes

Understanding the evolution of antibiotic resistance is essential for combatting this pressing global health challenge [38]. This evolution occurs via a sequence of complex processes that shape bacterial populations in response to the selective pressures imposed by antibiotics. In any population of bacteria, there is genetic variation. Some individual bacteria may carry genetic mutations or possess resistance genes due to horizontal gene transfer. This genetic diversity provides the raw material upon which natural selection acts. When antibiotics are introduced into an environment containing susceptible and resistant bacteria, they create a selective pressure. Antibiotics are formulated to either eradicate or impede the growth of bacteria by targeting specific cellular processes, such as cell wall synthesis or protein production [39].

Antibiotic-resistant bacteria, due to their genetic mutations or acquired resistance genes, are better equipped to survive the selective pressure of antibiotics. These resistant bacteria can continue to grow and reproduce even when the drug is present, while susceptible bacteria are killed or inhibited. Resistant bacteria, thanks to their survival advantage, have the opportunity to reproduce and pass on their resistance traits to their offspring or to other bacteria through horizontal gene transfer mechanisms like conjugation, transformation, or transduction [40].

Over time, as the antibiotic continues to exert selective pressure, the proportion of resistant bacteria in the population increases. This is because they have a higher fitness (survival and reproduction) compared to susceptible bacteria. As antibiotic-resistant bacteria become increasingly common, they may accumulate additional mutations or resistance genes, leading to the emergence of highly resistant strains [41]. These strains may be even more challenging to combat with antibiotics.

As the proportion of resistant bacteria within the population rises, the effectiveness of antibiotics diminishes. What was once a successful treatment for a

particular infection may become less reliable or entirely ineffective. Natural selection doesn't stop once resistance emerges. It continues to act as long as antibiotics are used. Bacteria can continue to evolve and adapt to new antibiotics or changes in treatment regimens, necessitating the development of novel drugs.

Genetic Basis of Antibiotic Resistance Mechanisms

Genomic data has played a crucial role in unraveling the genetic basis of antibiotic resistance mechanisms. Genomic data, obtained through techniques like whole-genome sequencing, allows researchers to analyze the complete genetic makeup of bacteria, including the presence of specific resistance genes [42]. Resistance genes, which can encode enzymes that modify antibiotics, efflux pumps that expel antibiotics, or altered drug targets, can be recognized through the comparison of genomic sequences with established databases of known resistance genes.

Genomic sequencing enables the identification and characterization of mutations in bacterial genes that confer resistance. Mutations can occur in genes encoding drug targets, leading to decreased drug binding affinity [43]. As an illustrative instance, in tuberculosis [44], mutations in the *rpoB* gene encoding the RNA polymerase beta subunit can be detected through genomic analysis, and these mutations confer resistance to rifampin. Genomic data offers insights into the specific mechanisms through which resistance genes or mutations operate. For instance, it can reveal how a resistance gene encodes an enzyme that chemically modifies an antibiotic, rendering it inactive. It can also explain how mutations alter the structure or function of drug targets, making antibiotics less effective. Genomic analysis enables the monitoring of the dissemination of resistance genes and resistant strains over time and across geographical regions. This information is vital for surveillance and epidemiological studies. For instance, it can reveal the movement of specific resistance genes between different bacterial populations and help identify emerging hotspots of resistance [45].

Genomic data can be used to predict the resistance phenotype of bacteria [46]. By analyzing the genetic content of a bacterial strain, researchers can predict whether it is likely to be resistant to specific antibiotics based on the presence of known resistance genes or mutations. Genomic insights into antibiotic resistance extend beyond current mechanisms. By studying the genomes of resistant bacteria, researchers can trace the evolutionary history of resistance mechanisms, offering insights into their evolutionary adaptation over time in response to antibiotic usage. Understanding the genetic basis of resistance can guide the formulation of novel treatment approaches. Researchers can develop medications tailored to address particular resistance mechanisms or identify vulnerabilities in resistant strains that can be exploited for therapeutic purposes.

CASE STUDY

Vancomycin-Resistant Enterococcus (VRE)

Vancomycin-resistant *Enterococcus* (VRE) refers to strains of *Enterococcus faecalis* and *Enterococcus faecium* that have developed resistance to vancomycin, a glycopeptide antibiotic [47]. *Enterococci* are normally present in the human gastrointestinal tract and are considered opportunistic pathogens, typically afflicting individuals with weakened immune systems or those undergoing invasive medical procedures [48].

Mechanism of Resistance

The emergence of resistance in VRE is chiefly ascribed to the acquisition of *vanA* or *vanB* genes [49]. These genes modify the precursor of the cell wall structure, preventing vancomycin from binding effectively to its target, which is the cell wall of the bacteria [50]. This modification makes the bacteria less prone to the bactericidal effects of vancomycin [51].

Impact

Vancomycin is frequently regarded as the final option for managing severe infections resulting from Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and certain Enterococcus species [52]. The development of VRE limits treatment options, resulting in the use of less effective and potentially more toxic antibiotics. Infections caused by VRE are linked to extended hospitalizations because of the challenges in treating them effectively [53]. This increases healthcare costs and places a burden on both patients and healthcare facilities.

The utilization of alternative antibiotics, extended hospitalizations, and the necessity for supplementary infection control measures contribute to increased healthcare costs associated with VRE infections [54]. VRE can spread within healthcare settings, particularly in hospitals and long-term care facilities. Stringent infection control measures, including contact precautions, are essential to prevent its transmission [55]. Over time, VRE strains have evolved, resulting in the development of various resistance mechanisms, including resistance to other antibiotics like daptomycin, further complicating treatment options. VRE strains are hardy and can persist in the environment, making infection control measures more challenging [56].

Promoting responsible antibiotic use and avoiding unnecessary vancomycin prescriptions are crucial to prevent the emergence of resistance. Enforcing stringent infection control protocols, such as thorough hand hygiene, patient isolation, and environmental cleaning, plays a crucial role in containing the dissemination of VRE [57]. Ongoing research is focused on developing new antibiotics and treatment strategies to combat VRE infections effectively. VRE serves as a reminder of the ongoing challenges posed by antibiotic-resistant bacteria and highlights the importance of prudent antibiotic use and infection control strategies within healthcare settings.

Genomic Changes

VRE strains acquire *vanA* or *vanB* genes that modify the precursor of the cell wall structure targeted by vancomycin [58].

Mechanism

These genes are typically obtained via horizontal gene transfer through plasmids or transposons [59].

Impact

The genetic changes conferred by *vanA* or *vanB* genes reduce the affinity of vancomycin for its target, enabling VRE to withstand the antibiotic's action [60].

Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterial strain of *Staphylococcus aureus* that has acquired resistance to methicillin and other beta-lactam antibiotics. This resistance arose due to a series of genetic changes that allowed MRSA strains to circumvent the bactericidal effects of these antibiotics [61].

Methicillin, a synthetic beta-lactam antibiotic, was introduced in the 1960s as a substitute for penicillin to treat staphylococcal infections. Initially, *Staphylococcus aureus* was susceptible to methicillin and other beta-lactam antibiotics, which focus on inhibiting the synthesis of bacterial cell walls by binding to penicillin-binding proteins (PBPs). These proteins play a critical role in building and maintaining the bacterial cell wall [62].

Mechanism of Resistance

The key mechanism behind MRSA's resistance is the acquisition of the *mecA* gene. The *mecA* gene encodes for a modified penicillin-binding protein, *PBP2a* (also known as PBP2'), which has a reduced affinity for beta-lactam antibiotics. As a result, the antibiotics are less effective in inhibiting the synthesis of cell walls in MRSA, allowing the bacterium to survive and multiply even when exposed to these drugs [63].

Spread and Epidemiology

Initially, MRSA infections were linked with healthcare settings, such as hospitals and nursing homes (Healthcare-Associated MRSA or HA-MRSA). However, a concerning trend emerged in the late 20th century when MRSA infections started emerging within the community (Community-Associated MRSA or CA-MRSA). These CA-MRSA strains were often more virulent and possessed the capability to infect healthy individuals outside healthcare facilities [64].

Impact

MRSA infections are challenging to treat because many beta-lactam antibiotics, including methicillin, are no longer effective. This can lead to prolonged illnesses, increased healthcare costs, and higher mortality rates, particularly in instances of invasive MRSA infections like bloodstream infections or pneumonia [65].

MRSA has been a major concern in healthcare settings, where it can cause outbreaks and pose a risk to vulnerable patients, such as those undergoing surgery or with weakened immune systems. The emergence of CA-MRSA strains has raised concerns about MRSA infections occurring in healthy individuals in the community, often presenting as skin and soft tissue infections. These strains are renowned for their ability to spread rapidly. The rise of MRSA underscores the importance of antibiotic stewardship programs to promote responsible antibiotic use and limit the development of antibiotic resistance [66]. Stringent infection control measures, such as hand hygiene, isolation precautions, and environmental cleaning, are essential to prevent the transmission of MRSA in healthcare settings. MRSA research continues to focus on developing new antibiotics and alternative treatment strategies to combat these resistant infections.

MRSA serves as a poignant example of the ongoing battle against antibiotic-resistant pathogens emphasizing the significance of a multidisciplinary approach involving healthcare, research, and public health to mitigate its impact.

Genomic Changes

MRSA strains have acquired the *mecA* gene, responsible for encoding a modified penicillin-binding protein(PBP2a). This modified PBP2a exhibits diminished affinity for beta-lactam antibiotics like methicillin [67].

Mechanism

The acquisition of *mecA* involves horizontal gene transfer, wherein MRSA strains gained this resistance gene, likely from another species of *Staphylococcus* [66].

Impact

This genetic change allowed MRSA to withstand the action of methicillin and other beta-lactam antibiotics, leading to the development of resistance.

Multi-Drug Resistant Mycobacterium tuberculosis (MDR-TB)

Multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) is a form of tuberculosis (TB) caused by Mycobacterium tuberculosis strains that have developed resistance to multiple first-line drugs used to treat the disease. These first-line drugs include isoniazid and rifampin, which are the cornerstone of TB treatment. MDR-TB poses a severe challenge to global TB control efforts.

Mechanism of Resistance

The emergence of resistance in MDR-TB often arises due to spontaneous mutations in the bacterium's DNA. These mutations affect genes involved in the mechanism of action of isoniazid and rifampin [68]. Resistance to isoniazid often results from mutations in the *katG* gene or the inhA promoter region. K*atG* is responsible for activating isoniazid into its active form, which disrupts the mycobacterial cell wall [69]. Mutations in *katG* reduce this activation, rendering isoniazid less effective.

Rifampin resistance is typically associated with mutations in the *rpoB* gene, which encodes the RNA polymerase subunit targeted by rifampin. Mutations in this gene prevent rifampin from binding effectively to its target, thereby reducing its bactericidal activity [70].

Impact

The impact of MDR-TB is substantial and extends to various aspects of public health and healthcare. MDR-TB complicates the treatment of tuberculosis. Patients with MDR-TB require longer and more complex treatment regimens, often involving a combination of second-line drugs. These drugs are typically less effective, more toxic, and more expensive than first-line drugs [71].

MDR-TB treatment can extend for 18 to 24 months or more, compared to the standard 6-month treatment for drug-susceptible TB [72]. This prolonged treatment duration heightens the probability of treatment interruption and non-adherence, which can lead to treatment failure and the development of extensively drug-resistant TB (XDR-TB).

MDR-TB imposes a significant economic burden on healthcare systems and patients due to the high cost of second-line drugs and extended hospital stays. MDR-TB strains can be more contagious than drug-susceptible strains, making them a greater threat in healthcare settings and communities. MDR-TB is considered a substantial global health threat, as it hinders TB control efforts and requires specialized expertise and resources for diagnosis and treatment [73]. In some cases, MDR-TB can further evolve into Extensively Drug-Resistant TB (XDR-TB), where resistance extends to additional second-line drugs. XDR-TB is even more challenging to treat and has limited treatment options [74].

Efforts to combat MDR-TB include improved diagnostic tools, the development of new drugs, and the implementation of effective infection control measures to prevent its spread. Tackling MDR-TB is a vital element of global TB control strategies.

Genomic Changes

MDR-TB strains exhibit spontaneous mutations in specific genes, such as *katG* and *rpoB*, that are involved in drug activation (*katG*) and the drug target (*rpoB*) [75].

Mechanism

These mutations result in altered protein structures or functions that reduce the effectiveness of isoniazid and rifampin [73].

Impact

These genetic changes confer resistance to multiple first-line TB drugs, making treatment more challenging [76].

Carbapenem-Resistant Enterobacteriaceae (CRE)

Carbapenem-resistant *Enterobacteriaceae* (CRE) represents a group of bacteria within the Enterobacteriaceae family that have developed resistance to carbapenem antibiotics. Carbapenems are a class of antibiotics often referred to as "last-resort" antibiotics because they are used to treat serious infections when other antibiotics have failed. CRE infections pose a significant threat to healthcare settings and patient health.

Mechanism of Resistance

The primary mechanism of carbapenem resistance in CRE is the creation of carbapenemase enzymes [77]. These enzymes can break down carbapenem antibiotics, rendering them ineffective. There are several types of carbapenemases, including the KPC (Klebsiella pneumoniae carbapenemase), NDM (New Delhi metallo-beta-lactamase), and OXA-type carbapenemases, among others [78].

Impact

CRE infections have several significant impacts on healthcare and public health. The resistance of CRE to carbapenem antibiotics significantly restricts the available treatment choices for infections triggered by these bacteria. This leads to the use of less effective and often more toxic antibiotics, which might be at an elevated risk of treatment failure [79]. CRE infections are linked to elevated mortality rates, especially among vulnerable patient populations, such as those in intensive care units or with compromised immune systems [80]. CRE can spread easily within healthcare settings, posing a particular risk to patients in hospitals and long-term care facilities. Effective infection control measures are crucial to prevent its transmission. CRE has become a global concern, with reported cases in various countries. The potential for international transmission raises concerns about the global spread of resistance.

Managing CRE infections requires a multidisciplinary approach, including the judicious use of antibiotics, active surveillance, and the development of novel treatment strategies. Combination therapy with multiple antibiotics may be necessary.

CRE underscores the importance of antibiotic stewardship programs to hinder the development and dissemination of antibiotic-resistant bacteria. These programs aim to promote responsible antibiotic use and minimize unnecessary antibiotic prescriptions.

Genomic Changes

CRE strains often harbour genes encoding carbapenemase enzymes, such as KPC, NDM, or OXA enzymes, which can break down carbapenem antibiotics [81].

Mechanism

These resistance genes are typically gained through horizontal gene transfer via plasmids or other mobile genetic elements [82].

Impact

The acquisition of these carbapenemase genes provides CRE with the ability to inactivate carbapenem antibiotics, rendering them ineffective. In all of these cases, the genomic changes involve the acquisition or mutation of specific genes that affect the target of antibiotics or the antibiotic itself. These changes provide the bacteria with a selective advantage in the presence of antibiotics, allowing them to survive and multiply despite antibiotic treatment.

Understanding these genomic changes is crucial for developing strategies to address antibiotic resistance, like the progression of new antibiotics, the implementation of antibiotic stewardship programs, and the enhancement of infection control measures to inhibit the transmission of resistant strains. It also highlights the ongoing evolutionary battle between bacteria and antibiotics, emphasizing the need for responsible antibiotic use and innovative approaches to address antibiotic resistance.

LONG TERM IMPACTS

Long-term evolutionary trends in antibiotic resistance are of significant concern, as they reflect the dynamic and adaptive nature of bacteria in response to selective pressures imposed by antibiotic usage.

Emergence of Multi-Drug Resistance (MDR)

One of the most concerning long-term trends in antibiotic resistance is the emergence of MDR bacteria [83]. MDR strains have developed resistance to multiple classes of antibiotics, rendering them highly resistant to treatment options. MDR can result from the accumulation of multiple resistance mechanisms, such as the uptake of genes conferring resistance through horizontal gene transfer, mutations in target genes, and efflux pumps that actively expel antibiotics from bacterial cells. MDR bacteria often require complex and resource-intensive treatment regimens, involving combinations of antibiotics that are typically less effective, more toxic, and costly.

Persistence of Resistance Mechanisms

Long-term trends in resistance evolution involve the persistence of resistance mechanisms within bacterial populations. Once resistance genes or mutations arise, they can persist in bacterial genomes, even in the absence of antibiotic selection pressure [84]. This persistence occurs because resistance mechanisms can provide a fitness advantage in specific ecological niches or under conditions where antibiotics occur naturally in other microorganisms.

Continuous Evolutionary Arms Race

Antibiotic resistance represents an ongoing evolutionary arms race between bacteria and antibiotics. Bacteria have evolved diverse strategies to elude the effects of antibiotics, including modification of drug targets, drug inactivation, and efflux pumps that expel antibiotics [85].

In response, antibiotics have been developed or modified to overcome resistance mechanisms. However, bacteria can adapt to these new antibiotics over time, leading to the emergence of resistance to novel drugs.

Environmental Reservoirs

Long-term trends in antibiotic resistance also involve the existence of environmental reservoirs of resistance genes. Antibiotic resistance genes can be present in environmental bacteria, including those found in soil and water [86]. These environmental reservoirs serve as a source of resistance genes that can be transferred to human pathogens through horizontal gene transfer mechanisms, contributing to the spread of resistance.

Antibiotic Use and Misuse

The selective pressure exerted by the widespread use and misuse of antibiotics is a primary driver of long-term resistance evolution. The overuse of antibiotics in human medicine, agriculture, and livestock production accelerates the emergence and spread of resistant bacteria [87].

Inadequate adherence to antibiotic courses, self-medication, and the availability of antibiotics without prescription can further exacerbate resistance trends.

Evolutionary Trade-Offs

Bacterial resistance mechanisms often come with trade-offs. For instance, acquiring resistance may reduce bacterial fitness in the absence of antibiotics [88]. Long-term trends may involve the balancing act between maintaining resistance and maintaining overall bacterial fitness.

CHALLENGES AND FUTURE DISCUSSIONS

Studying antibiotic resistance evolution and genomics presents several challenges due to the complex and dynamic nature of bacteria, their rapid adaptability, and the diverse environments in which they evolve.

Rapid Evolution

Bacteria can undergo rapid evolutionary changes, including the acquisition of genes associated with resistance, as well as the development of mutations that confer resistance [89]. This pace of evolution can make it challenging to keep up with emerging resistance mechanisms.

Genetic Heterogeneity

Bacterial populations are genetically diverse. Within a single population, there can be numerous genetic variants, some of which may have different resistance profiles [90]. This heterogeneity can complicate the identification of resistance mechanisms.

Horizontal Gene Transfer

Resistance genes can be easily transferred between bacteria through horizontal gene transfer mechanisms like conjugation, transformation, and transduction [91]. This transfer can lead to the rapid dissemination of resistance genes across different species and genera.

Diversity of Resistance Mechanisms

Resistance mechanisms are diverse and can involve a wide range of genetic changes, including point mutations, gene amplifications, efflux pumps, and lateral gene transfer. Investigating all these mechanisms requires diverse research approaches [92].

Data Integration

Integrating genomic data with clinical and epidemiological data is essential for understanding the spread and impact of antibiotic-resistant bacteria [93]. However, data integration can be challenging due to privacy concerns, data silos, and compatibility issues.

Ethical and Regulatory Considerations

Studying antibiotic resistance in clinical settings often involves ethical considerations related to patient privacy and informed consent. Regulatory requirements can also pose challenges for collecting and sharing clinical and genomic data [94].

Bias in Data Collection

Data collection for genomic studies of antibiotic resistance can be biased towards clinically relevant or easily cultured strains, potentially missing insights from environmental or less-studied bacterial populations [95]. Despite these challenges, ongoing research efforts, advances in genomic technologies, and international collaborations have made significant strides in understanding antibiotic resistance evolution and genomics. These efforts are crucial for guiding strategies to combat antibiotic resistance and improve patient outcomes. The future of research in antibiotic resistance evolution and genomics holds great promise, with several key directions aimed at addressing the challenges posed by antibiotic-resistant bacteria.

Development of Novel Antibiotics

Research will continue to focus on the exploration and creation of novel antibiotics with novel mechanisms of action. Targeting specific resistance mechanisms, such as efflux pumps or resistance enzymes, will be a priority.

Precision Medicine for Antibiotic Treatment

Personalized approaches to antibiotic treatment based on the genomic profile of infecting bacteria and the host will become more common [96]. This can optimize antibiotic selection, dosing, and treatment duration.

Antibiotic Stewardship

Expanding antibiotic stewardship programs to promote responsible antibiotic use in both clinical and agricultural settings [97].

Targeting Persistent Infections

Developing strategies to target persistent bacterial infections, which are frequently linked to antibiotic resistance [98].

Therapeutic Adjuvants

Exploring the use of adjuvants or potentiators that can enhance the activity of existing antibiotics, potentially overcoming resistance [99].

Education and Public Awareness

Continuing public education efforts to raise awareness about responsible antibiotic use and the consequences of antibiotic resistance.

Future research in antibiotic resistance evolution and genomics will require interdisciplinary collaboration, innovative technologies, and a global commitment to combat this critical public health challenge. By addressing these research directions, we can hope to better understand, prevent, and treat antibiotic-resistant infections, ultimately preserving the effectiveness of antibiotics for future generations.

CONCLUSION

The evolution of antibiotic resistance presents a dynamic and pressing challenge in the realm of public health. Bacteria have demonstrated remarkable adaptability, developing diverse mechanisms to withstand the selective pressures exerted by antibiotics. Multi-drug resistance (MDR) is an alarming trend, restricting treatment options and increasing the complexity of healthcare interventions. Genomics has emerged as an indispensable tool, shedding light on the genetic basis of resistance mechanisms. Through genomics, researchers can identify resistance genes, dissect the function of mutations, and comprehend the molecular intricacies of resistance. Importantly, genomic epidemiology and surveillance efforts enable the tracking of resistant strains and resistance genes, facilitating outbreak investigations and the implementation of targeted interventions.

However, the study of antibiotic resistance evolution and genomics is not without its challenges. Bacteria evolve rapidly, genetic variation within populations can be immense, and the acquisition and transfer of resistance genes pose hurdles to understanding and control. The cost and computational demands of genomic sequencing, along with the integration of clinical and epidemiological data, are additional complexities. Despite these challenges, the future holds promise in the form of novel therapies, precision medicine approaches, and enhanced surveillance techniques. A One Health approach that considers human, animal, and environmental factors is essential, as is the continued promotion of responsible antibiotic use and stewardship. Furthermore, public awareness remains paramount in ensuring a collective effort to address this critical global health concern.

REFERENCES

[1] Aminov, R. (2017): History of antimicrobial drug discovery: Major classes and health impact. Biochemical pharmacology, 133, 4-19.

[2] Coates, A. R. M., Hu, Y. (2007): Novel approaches to developing new antibiotics for bacterial infections. British journal of pharmacology, 152(8), 1147-1154.

[3] Keeney, K. M., Yurist-Doutsch, S., Arrieta, M. C., Finlay, B. B. (2014): Effects of antibiotics on human microbiota and subsequent disease. Annual review of microbiology, 68, 217-235.

[4] Anesi, J. A., Blumberg, E. A., Abbo, L. M. (2018): Perioperative antibiotic prophylaxis to prevent surgical site infections in solid organ transplantation. Transplantation, 102(1): 21-34.

[5] Adamu, B., Abdu, A., Abba, A. A., Borodo, M. M., Tleyjeh, I. M. (2014): Antibiotic prophylaxis for preventing post solid organ transplant tuberculosis. Cochrane Database of Systematic Reviews, (3).

[6] Bhattacharya, B., Mukherjee, S. (2015): Cancer therapy using antibiotics. Journal of Cancer Therapy, 6(10): 849.

[7] Andersson, D. I., Hughes, D. (2011): Persistence of antibiotic resistance in bacterial populations. FEMS microbiology reviews, 35(5): 901-911.

[8] Varela, M. F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L. M., Hernandez, A.J., Parwatti, A., Kumar, S. H. (2021): Bacterial resistance to antimicrobial agents. Antibiotics, 10(5):593.

[9] Overton, K., Fortané, N., Broom, A., Raymond, S., Gradmann, C., Orubu, E. S. F., Podolsky, S.H.,Van Katwyk S.R., Zaman, M.H., Kirchhelle, C. (2021): Waves of attention: patterns and themes of international antimicrobial resistance reports, 1945–2020. BMJ global health, 6(11): e006909.

[10] Friedman, N. D., Temkin, E., Carmeli, Y. (2016): The negative impact of antibiotic resistance. Clinical Microbiology and Infection, 22(5): 416-422.

[11] Hofmeyr, G. J., Smaill, F. M., & Cochrane Pregnancy and Childbirth Group. (1996): Antibiotic prophylaxis for cesarean section. Cochrane Database of systematic reviews, 2009(4).

[12] Hu, A., Li, J., Vacek, J., Bouchard, M., Ingram, M. C., McMahon, M., Mithal, L.B., Raval, M.V, Reynolds, M., Goldstein, S. (2022): Antibiotic resistance is common in the cultures of intraabdominal abscess drainage after appendectomy. Journal of pediatric surgery, 57(9), 102-106.

[13] Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., Cheng, Z. (2019): Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology advances, 37(1): 177-192.

[14] Alanis, A. J. (2005): Resistance to antibiotics: are we in the post-antibiotic era? Archives of medical research, 36(6): 697-705.

[15] Ventola, C. L. (2015): The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40(4): 277.

[16] Gandra, S., Barter, D. M., Laxminarayan, R. (2014): Economic burden of antibiotic resistance: how much do we really know? Clinical microbiology and infection, 20(10): 973-980.

[17] Lee, S., Fan, P., Liu, T., Yang, A., Boughton, R. K., Pepin, K. M., Miller, R.S., Jeong, K. C. (2022): Transmission of antibiotic resistance at the wildlife-livestock interface. Communications Biology, 5(1), 585.

[18] Ma, Z., Lee, S., Jeong, K. C. (2019): Mitigating antibiotic resistance at the livestock-environment interface: a review.

[19] Van, T. T. H., Moutafis, G., Tran, L. T., Coloe, P. J. (2007): Antibiotic resistance in food-borne bacterial contaminants in Vietnam. Applied and environmental microbiology, 73(24): 7906-7911.

[20] Teuber, M. (2001): Veterinary use and antibiotic resistance. Current opinion in microbiology, 4(5): 493-499.
[21] Jit, M., Ng, D. H. L., Luangasanatip, N., Sandmann, F., Atkins, K. E., Robotham, J. V., Pouwels, K. B. (2020). Quantifying the economic cost of antibiotic resistance and the impact of related interventions: rapid methodological review, conceptual framework and recommendations for future studies. BMC medicine, 18(1),:1-14.
[22] Charles, P. G., Grayson, M. L. (2004): The dearth of new antibiotic development: why we should be worried and what we can do about it. Medical Journal of Australia, 181(10): 549-553.
[23] Gillings, M. R., Paulsen, I. T., Tetu, S. G. (2017): Genomics and the evolution of antibiotic resistance. Annals of the New York Academy of Sciences, 1388(1): 92-107.
[24] Ibargüen-Mondragón, E., Mosquera, S., Cerón, M., Burbano-Rosero, E. M., Hidalgo-Bonilla, S. P., Esteva, L., Romero-Leitón, J. P. (2014): Mathematical modeling on bacterial resistance to multiple antibiotics caused by spontaneous mutations. Biosystems, 117: 60-67.
[25] Maquat, L. E. (2001): The power of point mutations. Nature genetics, 27(1): 5-6.
[26] Negishi, T., Ohya, Y. (2010): The cell wall integrity checkpoint: coordination between cell wall synthesis and the cell cycle. Yeast, 27(8):513-519.
[27] Palomino, J. C., Martin, A. (2014): Drug resistance mechanisms in Mycobacterium tuberculosis. Antibiotics, 3(3): 317-340.
[28] Amaral, L., Martins, M., Viveiros, M. (2007): Enhanced killing of intracellular multidrug-resistant Mycobacterium tuberculosis by compounds that affect the activity of efflux pumps. Journal of antimicrobial chemotherapy, 59(6): 1237-1246.
[29] Goñi-Moreno, A., Amos, M., de la Cruz, F. (2013): Multicellular computing using conjugation for wiring. PLoS One, 8(6): e65986.
[30] Soucy, S. M., Huang, J., Gogarten, J. P. (2015): Horizontal gene transfer: building the web of life. Nature Reviews Genetics, 16(8): 472-482.
[31] Balcázar, J. L. (2018): How do bacteriophages promote antibiotic resistance in the environment? Clinical Microbiology and Infection, 24(5): 447-449.
[32] Sandanayaka, V. P., & Prashad, A. S. (2002): Resistance to β-lactam antibiotics: structure and mechanism based design of β-lactamase inhibitors. Current medicinal chemistry, 9(12): 1145-1165.
[33] Pagès, J. M., James, C. E., Winterhalter, M. (2008): The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nature Reviews Microbiology, 6(12): 893-903.
[34] Pires, D. P., Melo, L. D., Boas, D. V., Sillankorva, S., Azeredo, J. (2017): Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Current opinion in microbiology, 39: 48-56.
[35] Martins, P. M., Merfa, M. V., Takita, M. A., De Souza, A. A. (2018): Persistence in phytopathogenic bacteria: do we know enough? Frontiers in microbiology, 9: 1099.
[36] Davies, J., Davies, D. (2010): Origins and evolution of antibiotic resistance. Microbiology and molecular biology reviews, 74(3): 417-433.
[37] Kohanski, M. A., Dwyer, D. J., Collins, J. J. (2010): How antibiotics kill bacteria: from targets to networks. Nature Reviews Microbiology, 8(6): 423-435.
[38] Scheel, D. (1998). Resistance response physiology and signal transduction. Current opinion in plant biology, 1(4): 305-310.
[39] Andersson, D. I., Hughes, D. (2012): Evolution of antibiotic resistance at non-lethal drug concentrations. Drug resistance updates, 15(3): 162-172.
[40] Punina, N. V., Makridakis, N. M., Remnev, M. A., Topunov, A. F. (2015): Whole-genome sequencing targets drug-resistant bacterial infections. Human genomics, 9: 1-20.
[41] Hopkins, A. L., Groom, C. R. (2002): The druggable genome. Nature reviews Drug discovery, 1(9): 727-730.

[42] Kapur, V., Li, L. L., Iordanescu, S., Hamrick, M. R., Wanger, A., Kreiswirth, B. N., & Musser, J. M. (1994): Characterization by automated DNA sequencing of mutations in the gene (rpoB) encoding the RNA polymerase beta subunit in rifampin-resistant Mycobacterium tuberculosis strains from New York City and Texas. Journal of clinical microbiology, 32(4): 1095-1098.
[43] Gillings, M. R., Paulsen, I. T., Tetu, S. G. (2017): Genomics and the evolution of antibiotic resistance. Annals of the New York Academy of Sciences, 1388(1): 92-107.
[44] Su, M., Satola, S. W., & Read, T. D. (2019): Genome-based prediction of bacterial antibiotic resistance. Journal of clinical microbiology, 57(3): 10-1128.
[45] Ghanem, G., Hachem, R., Jiang, Y., Chemaly, R. F., Raad, I. (2007): Outcomes for and risk factors associated with vancomycin-resistant Enterococcus faecalis and vancomycin-resistant Enterococcus faecium bacteremia in cancer patients. Infection Control & Hospital Epidemiology, 28(9): 1054-1059.
[46] Krawczyk, B., Wityk, P., Gałęcka, M., Michalik, M. (2021): The many faces of Enterococcus spp.—commensal, probiotic and opportunistic pathogen. Microorganisms, 9(9): 1900.
[47] McManus, M. C. (1997): Mechanisms of bacterial resistance to antimicrobial agents. American Journal of Health-System Pharmacy, 54(12): 1420-1433.
[48] Pfeltz, R. F., Wilkinson, B. J. (2004): The escalating challenge of vancomycin resistance in Staphylococcus aureus. Current Drug Targets-Infectious Disorders, 4(4): 273-294.
[49] Mühlberg, E., Umstätter, F., Kleist, C., Domhan, C., Mier, W., Uhl, P. (2020): Renaissance of vancomycin: Approaches for breaking antibiotic resistance in multidrug-resistant bacteria. Canadian journal of microbiology, 66(1): 11-16.
[50] Rice, L. B. (2006): Antimicrobial resistance in gram-positive bacteria. American journal of infection control, 34(5): S11-S19.
[51] Kollef, M. H. (2001): Optimizing antibiotic therapy in the intensive care unit setting. Critical care, 5: 1-7.
[52] Ang, J. Y., Ezike, E., Asmar, B. I. (2004): Antibacterial resistance. The Indian Journal of Pediatrics, 71: 229-239.
[53] Muto, C. A., Jernigan, J. A., Ostrowsky, B. E., Richet, H. M., Jarvis, W. R., Boyce, J. M., Farr, B. M. (2003): SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and enterococcus. Infection Control & Hospital Epidemiology, 24(5): 362-386.
[54] Hall, A. D., Steed, M. E., Arias, C. A., Murray, B. E., Rybak, M. J. (2012): Evaluation of standard-and high-dose daptomycin versus linezolid against vancomycin-resistant Enterococcus isolates in an in vitro pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations. Antimicrobial agents and chemotherapy, 56(6): 3174-3180.
[55] Gopal Rao, G. (1998): Risk factors for the spread of antibiotic-resistant bacteria. Drugs, 55: 323-330.
[56] Kang, H. K., Park, Y. (2015): Glycopeptide antibiotics: Structure and mechanisms of action. Journal of Bacteriology and Virology, 45(2): 67-78.
[57] Heuer, H., Smalla, K. (2007): Horizontal gene transfer between bacteria. Environmental biosafety research, 6(1-2): 3-13.
[58] Lambert, P. A. (2005): Bacterial resistance to antibiotics: modified target sites. Advanced drug delivery reviews, 57(10): 1471-1485.
[59] Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., ... & Holden, M. T. (2017): Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genome biology, 18(1): 1-11.
[60] Chambers, H. F. (1997): Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clinical microbiology reviews, 10(4): 781-791.

[61] Khorvash, F., Mostafavizadeh, K., Mobasherizadeh, S. (2008): Frequency of mecA gene and borderline oxacillin resistant Staphylococcus aureus in nosocomial acquired methicillin resistance Staphylococcus aureus infections. Pak J Biol Sci, 11(9): 1282-85.
[62] Millar, B. C., Loughrey, A., Elborn, J. S., Moore, J. E. (2007): Proposed definitions of community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA). Journal of Hospital Infection, 67(2): 109-113.
[63] Batabyal, B., Kundu, G. K., Biswas, S. (2012): Methicillin-resistant *Staphylococcus aureus*: A brief review. International research journal of biological sciences, 1(7):65-71.
[64] Wu, S. W., De Lencastre, H., Tomasz, A. (2001): Recruitment of the mecA gene homologue of *Staphylococcus sciuri* into a resistance determinant and expression of the resistant phenotype in *Staphylococcus aureus*. Journal of bacteriology, 183(8):2417-2424.
[65] Sarhan, S. R., Hashim, H. O., Al-Shuhaib, M. B. S. (2019): The Gly152Val mutation possibly confers resistance to beta-lactam antibiotics in ovine Staphylococcus aureus isolates. Open Veterinary Journal, 9(4): 339-348.
[66] Barlow, M. (2009): What antimicrobial resistance has taught us about horizontal gene transfer. Horizontal Gene Transfer: Genomes in Flux, 397-411. NOOO
[67] Müller, B., Borrell, S., Rose, G., Gagneux, S. (2013): The heterogeneous evolution of multidrug-resistant Mycobacterium tuberculosis. Trends in Genetics, 29(3): 160-169.
[68] Rattan, A., Kalia, A., Ahmad, N. (1998): Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerging infectious diseases, 4(2): 195.
[69] Koch, A., Mizrahi, V., Warner, D. F. (2014): The impact of drug resistance on Mycobacterium tuberculosis physiology: what can we learn from rifampicin? Emerging microbes & infections, 3(1): 1-11.
[70] Seung, K. J., Keshavjee, S., Rich, M. L. (2015): Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harbor perspectives in medicine, 5(9).
[71] Podewils, L. J., Gler, M. T. S., Quelapio, M. I., Chen, M. P. (2013): Patterns of treatment interruption among patients with multidrug-resistant TB (MDR TB) and association with interim and final treatment outcomes. PLoS One, 8(7): e70064.
[72] Lönnroth, K., Migliori, G. B., Abubakar, I., D'Ambrosio, L., De Vries, G., Diel, R., ... & Raviglione, M. C. (2015): Towards tuberculosis elimination: an action framework for low-incidence countries. European Respiratory Journal, 45(4): 928-952.
[73] Isakova, J., Sovkhozova, N., Vinnikov, D., Goncharova, Z., Talaibekova, E., Aldasheva, N., Aldashev, A. (2018): Mutations of rpoB, katG, inhA and ahp genes in rifampicin and isoniazid-resistant Mycobacterium tuberculosis in Kyrgyz Republic. BMC microbiology, 18(1): 1-8.
[74] Louw, G. E., Warren, R. M., Gey van Pittius, N. C., McEvoy, C. R. E., Van Helden, P. D., Victor, T. C. (2009): A balancing act: efflux/influx in mycobacterial drug resistance. Antimicrobial agents and chemotherapy, 53(8): 3181-3189.
[75] Everett, M., Sprynski, N., Coelho, A., Castandet, J., Bayet, M., Bougnon, J., ... & Lemonnier, M. (2018): Discovery of a novel metallo-β-lactamase inhibitor that potentiates meropenem activity against carbapenem-resistant Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 62(5):10-1128.
[76] Grundmann, H., Livermore, D. M., Giske, C. G., Canton, R., Rossolini, G. M., Campos, J., Vatopoulos, A., Gniadkowski, M., Toth, A., Pfeifer, Y., Jarlier, V., Carmeli, Y., the CNSE Working Group, C. (2010): Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Eurosurveillance, 15(46).
[77] De Angelis, G., Del Giacomo, P., Posteraro, B., Sanguinetti, M., Tumbarello, M. (2020): Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in Enterobacteriaceae. International journal of molecular sciences, 21(14):5090.

[78] De Angelis, G., Del Giacomo, P., Posteraro, B., Sanguinetti, M., Tumbarello, M. (2020): Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in Enterobacteriaceae. International journal of molecular sciences, 21(14): 5090.
[79] Friedman, N. D., Temkin, E., Carmeli, Y. (2016): The negative impact of antibiotic resistance. Clinical Microbiology and Infection, 22(5): 416-422.
[80] Hansen, G. T. (2021): Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infectious diseases and therapy, 10: 75-92.
[81] Nordmann, P., Dortet, L., Poirel, L. (2012): Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends in molecular medicine, 18(5): 263-272.
[82] Obolski, U., Stein, G. Y., Hadany, L. (2015): Antibiotic restriction might facilitate the emergence of multi-drug resistance. PLoS computational biology, 11(6): e1004340.
[83] Rahman, S., Kesselheim, A. S., Hollis, A. (2023): Persistence of resistance: A panel data analysis of the effect of antibiotic usage on the prevalence of resistance. The Journal of Antibiotics, 76(5): 270-278.
[84] Lorusso, A. B., Carrara, J. A., Barroso, C. D. N., Tuon, F. F., Faoro, H. (2022): Role of efflux pumps on antimicrobial resistance in Pseudomonas aeruginosa. International Journal of Molecular Sciences, 23(24): 15779.
[85] Burch, T. R., Newton, R. J., Kimbell, L. K., LaMartina, E. L., O'Malley, K., Thomson, S. M., Marshall, C.W., McNamara, P. J. (2022): Targeting current and future threats: recent methodological trends in environmental antimicrobial resistance research and their relationships to risk assessment. Environmental Science: Water Research & Technology, 8(9): 1787-1802.
[86] Tagoe, D. N. A., Attah, C. (2010): A Study of Antibiotic Use and Abuse in Ghana: a case study of the Cape Coast Metropolis. The Internet Journal of Health, 11(2): 1-5. NOOO
[87] Robinson, T. P., Wertheim, H. F., Kakkar, M., Kariuki, S., Bu, D., Price, L. B. (2016): Animal production and antimicrobial resistance in the clinic. The Lancet, 387(10014): e1-e3.
[88] Pál, C., Papp, B., Lázár, V. (2015): Collateral sensitivity of antibiotic-resistant microbes. Trends in microbiology, 23(7): 401-407.
[89] McAdams, H. H., Srinivasan, B., Arkin, A. P. (2004): The evolution of genetic regulatory systems in bacteria. Nature Reviews Genetics, 5(3): 169-178. NOOO
[90] Finley, R. L., Collignon, P., Larsson, D. J., McEwen, S. A., Li, X. Z., Gaze, W. H.,Reid-Smith, R., Timinouni, M., Graham, D.W., Topp, E. (2013): The scourge of antibiotic resistance: the important role of the environment. Clinical infectious diseases, 57(5): 704-710.
[91] Mariam, S. H., Werngren, J., Aronsson, J., Hoffner, S., Andersson, D. I. (2011): Dynamics of antibiotic resistant Mycobacterium tuberculosis during long-term infection and antibiotic treatment. PloS one, 6(6): e21147.
[92] Sun, D., Jeannot, K., Xiao, Y., Knapp, C. W. (2019): Horizontal gene transfer mediated bacterial antibiotic resistance. Frontiers in microbiology, 10: 1933.
[93] Baquero, F., Blázquez, J. (1997): Evolution of antibiotic resistance. Trends in ecology & evolution, 12(12): 482-487.
[94] Moradigaravand, D., Palm, M., Farewell, A., Mustonen, V., Warringer, J., Parts, L. (2018): Prediction of antibiotic resistance in Escherichia coli from large-scale pan-genome data. PLoS computational biology, 14(12): e1006258.
[95] Parsonage, B., Hagglund, P. K., Keogh, L., Wheelhouse, N., Brown, R. E., Dancer, S. J. (2017): Control of antimicrobial resistance requires an ethical approach. Frontiers in microbiology, 8:2124.
[96] Gaynes, R. (1995): Surveillance of antibiotic resistance: learning to live with bias. Infection Control & Hospital Epidemiology, 16(11): 623-626.

[97] Merker, M., Tueffers, L., Vallier, M., Groth, E. E., Sonnenkalb, L., Unterweger, D., Baines J.F., Niemann, S., Schulenburg, H. (2020): Evolutionary approaches to combat antibiotic resistance: opportunities and challenges for precision medicine. Frontiers in Immunology, 11, 568485.
[98] Tamma, P. D., Cosgrove, S. E. (2011): Antimicrobial stewardship. Infectious Disease Clinics, 25(1): 245-260.
[99] Dhanda, G., Acharya, Y., Haldar, J. (2023): Antibiotic Adjuvants: A Versatile Approach to Combat Antibiotic Resistance. ACS omega, 8(12): 10757-10783.