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Antimicrobial-Resistant Bacteria and Strategies to Overcome Antimicrobial Resistance

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ABSTRACT: Antibiotic resistance is an escalating global health crisis that threatens to undo the remarkable advancements made in modern medicine. The overuse and misuse of antibiotics have led to the evolution of resistant bacterial strains, which are increasingly difficult to treat. This paper explores the mechanisms behind antibiotic resistance, categorizing it into intrinsic and acquired resistance and discussing key resistant bacteria such as Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococci, and Carbapenem-resistant *Enterobacteriaceae*. This paper also inspects methods to combat antibiotic resistance, including antibiotic modification, combination therapy, the use of adjuvants, and the innovative use of bacteriophages. Finally, the potential for emerging technologies to address the crisis, such as CRISPR-Cas systems and antimicrobial peptides, is highlighted. Continued research and the development of new strategies are vital to overcoming the challenges posed by antibiotic-resistant bacterial infections and are required to safeguard the health of the public.

KEYWORDS: Microbiology, Antimicrobials and Antibiotics, Antibiotic Resistance, Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococci, Carbapenem-resistant *Enterobacteriaceae*.

Introduction

Bacterial infections remain a major global health concern, contributing significantly to morbidity and mortality worldwide. Each year, millions of deaths are attributed to bacterial infections, with lower respiratory infections and bloodstream infections contributing to the majority. The causative agents of these infections include a wide variety of bacteria, such as *Streptococcus pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*, each of which poses unique treatment challenges due to varying resistance patterns.[1]

The discovery of antibiotics in the early 20th century by Sir Alexander Fleming revolutionized medicine; his discovery of penicillin marked the beginning of the antibiotic era, transforming the treatment of bacterial infections that were once fatal. This breakthrough drastically reduced the mortality rates of infections, leading to significant improvements in public health and an increase in life expectancy.[2] Antibiotics, which target specific processes in bacterial cells,[3] have been vital in treating a wide range of bacterial infections. They have additionally allowed for many modern medical procedures such as cancer treatment, organ transplants, and open-heart surgery.[4]

However, the widespread and sometimes inappropriate use of antibiotics has led to a critical problem: antibiotic resistance.[4] Over time, bacteria evolve mechanisms to bypass the effects of antibiotics, rendering them ineffective. This phenomenon is driven by both intrinsic genetic factors and acquired resistance.[5]

Infections caused by multidrug-resistant organisms, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), and Carbapenem-resistant *Enterobacteriaceae* (CRE), have become more common, complicating treatment options and increasing mortality rates. These "superbugs" are resistant to multiple classes of antibiotics, making them particularly difficult to treat and posing a serious threat to public health.[6]

To address this growing crisis, there is an urgent need for innovative strategies to reduce the impact of antibiotic resistance and protect the efficacy of current and future antibiotics.[7] The current article focuses on antibiotic resistance, antibiotic-resistant bacteria, and different strategies to overcome antibiotic resistance.

Antibiotics:

Antibiotics are powerful medications used to treat bacterial infections by inhibiting the growth of or killing bacteria. Discovered in the early 20th century, they revolutionized medicine and consequently significantly extended the average human lifespan by over 23 years. The discovery of penicillin by Sir Alexander Fleming marked the beginning of the golden age of antibiotic discovery, which peaked in the mid-1950s. This age was followed by a decline in the discovery and development of new antibiotics, and, by 1962, all major classes of antibiotics had been discovered.[2] This led to the rise of antibiotic resistance in numerous bacterial pathogens, resulting in the current pressing crisis of antibiotic resistance.[4]

Bacterial cells have unique structures that aid in their ability to survive and reproduce. The components of a bacterial cell (present in most but not all) include a cell wall made of peptidoglycan, a compact and flexible cell membrane, a cytoplasm containing ribosomes and plasmids, a nucleoid usually consisting of a single chromosome, polysaccharide or polypeptide capsules that help the bacteria adhere to surfaces and escape the immune system of a body, pili, and a flagellum.[8] Bacterial cells act in multiple ways: they can multiply rapidly, overwhelming host tissues and disrupting normal function;

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kill cells and tissues; or secrete toxins that can paralyze and destroy cells' metabolic machinery, among many other methods of action.[9] Antibiotics take advantage of the bacterial cell's components and features, limiting or inhibiting its functions to effectively kill or hinder the cell.[3]

Antibiotics are classified into largely 5 types based on their mechanism of action.[10] Figure 1 illustrates 5 classes of antibiotics.

Cell Wall Synthesis Inhibitors:

The β-lactam antibiotics are a family of bactericidal drugs containing the β-lactam ring in their chemical structure. Classified in penicillins, cephalosporins, carbapenems, penems (also known as thiopenems), and monobactams, they are amongst the most commonly prescribed drugs.[11]

β-lactam antibiotics work by inhibiting penicillin-binding proteins (PBPs), enzymes that are vital to forming cross-links in the peptidoglycan layer of bacterial cell walls and ensuring cell wall stability. Their similarity to the peptidoglycan precursor (D-Ala-D-Ala) allows them to bind to PBPs and disrupt cell wall synthesis. This binding forms a stable enzyme–antibiotic complex that inactivates the enzyme and prevents the formation of the cell wall, leading to bacterial death.[12]

Glycopeptide antibiotics, like vancomycin, work by targeting lipid II, a molecule used to synthesize the cell wall in Gram-positive bacteria. Vancomycin binds to the D-Ala-D-Ala sequence of lipid II, furthermore blocking the action of PBPs. This prevents the proper formation of the cell wall, leading to bacterial cell death due to osmotic shock.[13]

Cell Membrane Integrity Disruptors:

Cyclic lipopeptide antibiotics such as daptomycin work by binding to the bacterial membrane in the presence of calcium ions. The Ca^{2+}–daptomycin complex forms micelles that penetrate the inner membrane, bind to negatively charged phosphatidylglycerol groups, and neutralize them. The Ca^{2+}–daptomycin complex is then inserted into the membrane and undergoes phosphatidylglycerol-dependent oligomerization. This causes the leakage of ions, mainly potassium and sodium, disrupting the cell's membrane function and ultimately leading to bacterial cell death.[10]

Polymyxins, such as colistin, primarily target the outer membrane of Gram-negative bacteria. They interact with lipid A, a component of lipopolysaccharide, displacing stabilizing ions and increasing membrane permeability. This allows the antibiotic to enter the cell and also causes damage to the inner membrane.[14] Polymyxins can cause the fusion of the inner and outer membranes. This promotes the exchange of phospholipids between the membranes, furthermore causing osmotic imbalance and cell lysis.[10]

Nucleic Acid Synthesis Inhibitors:

Quinolones work by targeting DNA gyrase and topoisomerase IV enzymes in bacteria. These enzymes regulate the topological state of DNA during replication and transcription by removing the accumulated positive supercoils. DNA gyrase maintains negative supercoils, while topoisomerase IV helps unlink newly synthesized DNA. Quinolones bind to the topoisomerase-DNA cleavage complex, stabilizing the DNA break and preventing the replication fork from moving.[15] This blockage halts DNA synthesis, preventing bacterial cell division and leading to cell death.[10]

Rifamycins inhibit bacterial RNA synthesis. The presence of a macrocyclic ring in its structure targets the β-subunit of prokaryotic DNA-dependent RNA polymerase near its catalytic center, blocking its ability to initiate transcription.[10] This inhibition prevents the production of RNA and, subsequently, protein synthesis, which results in bacterial cell death.[10]

Protein Synthesis Inhibitors Protein Synthesis Inhibitors:

Tetracyclines and aminoglycosides, along with other antibiotic classes such as macrolides, lincosamides, streptogramins B, and oxazolidinones, work by targeting bacterial protein synthesis.[10] These antibiotics specifically target bacterial, prokaryotic ribosomes, which are structurally different from eukaryotic ribosomes in terms of size, structure, and the number of RNA molecules they contain. This ensures no harm to human cells.[10]

The structure of Tetracyclines consists of four flat aromatic hydrocarbon rings, and they work by binding to the 30S subunit of the bacterial ribosome. This binding prevents the transfer of amino acids during protein synthesis by blocking the attachment of aminoacyl-tRNA to the A-site of the ribosome. As a result, the bacterial cell is unable to build the proteins it needs to survive.[16]

Aminoglycosides, such as gentamicin and amikacin, also bind to the 30S subunit of bacterial ribosomes. This binding leads to the misreading of mRNA, causing the wrong amino acids to be incorporated into the protein chain. This results in faulty proteins which can disrupt the function of bacterial membranes, allowing more aminoglycosides to enter the cell and kill the bacteria. Some aminoglycosides also inhibit the formation of the initiation complex or block the movement of tRNA during protein synthesis.[10]

Metabolic Pathway Disruptors:

Sulfonamides, like sulfamethoxazole, are bacteriostatic antibiotics that inhibit bacterial growth by interfering with folic acid synthesis, a substance necessary for the synthesis of nucleic acids and proteins. They act by competing with para-aminobenzoic acid (PABA) for the active site of the enzyme dihydropteroate synthase (DHPS). DHPS is involved in converting PABA to dihydropteric acid, a precursor to folic acid.[17]

Trimethoprim also disrupts folic acid synthesis by inhibiting Dihydrofolate reductase (DHFR). DHFR converts dihydrofolate to tetrahydrofolate, an essential cofactor for nucleotide synthesis. By blocking this conversion, trimethoprim disrupts DNA and protein synthesis in bacteria.[18]

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Antibiotic Resistance:

Antibiotic resistance is a phenomenon that occurs when microorganisms evolve to resist the effects of medications designed to kill or inhibit them. This phenomenon arises from the overuse and misuse of antibiotics, both in human medicine and agriculture, leading to the emergence of "superbugs"–microorganisms, particularly bacteria, that have developed resistance to multiple antibiotics–that are harder to treat.[19] A growing health concern, most organizations consider this a serious problem.[20] However, despite the billions of dollars that have been put into research and solutions, the development of antibiotic resistance has proven to be relentless.[21] Antibiotic resistance falls largely into two categories: intrinsic resistance and acquired resistance.[22]

Intrinsic Resistance:

Intrinsic resistance is a natural ability found in certain bacteria that allows them to withstand the effects of antibiotics. This resistance is due to the bacteria's genetic makeup and exists independently of any exposure to antibiotics. Gram-negative bacteria exhibit a multidrug-resistant phenotype,[23] attributed to the presence of an additional outer membrane rich in lipopolysaccharides in their cell wall[13] that acts as a barrier and prevents many antibiotics from entering the cell while simultaneously aiding their ability to acquire resistance mechanisms from their surroundings.[23] Additionally, gram-negative bacteria have efflux pumps, which can actively push antibiotics out from inside their cells, making these drugs less effective.[24] Gram-positive bacteria, as well, often possess certain features that contribute to intrinsic resistance. Enterococci, for example, can exhibit resistance to penicillin through the overproduction of a low-affinity penicillin-binding protein (PBP5), which is capable of maintaining cell wall synthesis despite antibiotic pressure. In Enterococcus faecium, specific amino acid substitutions in PBP5 further reduce penicillin affinity, enhancing resistance to β-lactam antibiotics such as ampicillin.[25]

There is significant concern regarding intrinsic resistance. As infections caused by these resistant bacteria rise, treatment options become limited, leading to challenges in managing what were once treatable infections. This situation is especially significant due to the increasing frequency of infections caused by Gram-negative pathogens, which can be particularly hard to treat.[24] Environmental bacteria, such as those found in soil, are intrinsically resistant to many classes of antibiotics, with resistance that predates the clinical use of antibiotics. While these bacteria may not be direct threats, they carry along with them the added risk of transferring their resistant traits to pathogens, which further complicates the fight against antibiotic resistance.[24]

Acquired Resistance:

Acquired resistance refers to the ability of bacteria to develop resistance to antibiotics that were previously effective against them, typically through genetic mutations or the acquisition of resistance genes from other microorganisms. This can happen through selection pressure that causes mutations that give certain bacteria an edge in survival or through horizontal gene transfer (HGT). Additionally, the overuse and misuse of antibiotics in healthcare and agriculture create pressure that favors the growth of resistant strains. As a result, acquired resistance is a growing concern, especially for treating infections caused by multidrug-resistant Gram-negative bacteria, making effective treatment increasingly challenging.[26]

HGT is one method of acquired resistance in which bacteria can share genetic material with one another and, therefore, adopt each other's traits and abilities. This was first demonstrated by Frederick Griffith in 1928 when he discovered that harmless pneumococcus bacteria could become dangerous by taking up DNA from virulent strains. There are a few key ways this happens: transformation, where bacteria absorb free DNA from their surroundings; conjugation, which involves a one-way transfer of DNA through a structure called a sexual pilus; and transduction, where viruses that infect bacteria move DNA between cells. Once inside, this new DNA can either be degraded, remain as independent pieces, or integrate into the host's genome, furthermore possibly changing the host's characteristics.[27]

Along with HGT, there are a multitude of methods by which bacteria can acquire resistance. Bacteria can produce enzymes that degrade or chemically modify antibiotics, rendering them ineffective.[28] They can alter the target sites of antibiotics, preventing effective binding, which can allow the bacteria to develop certain resistance mutations.[28] The presence of antibiotics additionally creates a selective pressure, encouraging mutation as well as favoring the survival of resistant strains.[28]

Antibiotic-Resistant Bacteria:

Antibiotic resistance has become a challenging problem in the past few decades.[29] There are hundreds of different antibiotic-resistant bacteria today.[21] The current review article aims to focus on the most common antibiotic-resistant bacteria, namely Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), and Carbapenem-Resistant *Enterobacteriaceae* (CRE). There are certain genes, either acquired or intrinsic, that allow for antibiotic resistance. The resistance can escalate to a serious threat, especially as a single gene is often able to render a multitude of different types of antibiotics useless against certain bacteria.[6] Prolonged hospital stays, higher treatment costs, and greater mortality rates are common consequences of infections involving antibiotic-resistant bacteria, highlighting the urgent need for improved infection control practices, careful use of antibiotics, and the development of new treatments to manage these dangerous infections.[20]

Methicillin-resistant Staphylococcus aureus (MRSA):

The Gram-positive Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat in healthcare settings due to its ability to resist β-lactam antibiotics. This resistance is mainly due to the mecA gene, which encodes the PBP2a protein that allows cell wall synthesis to continue even in the presence of β-lactams.[30] The *mecA* gene is located on the staphylococcal cassette chromosome (SCCmec),[31] facilitating horizontal gene

transfer between different bacterial strains. MRSA is naturally found, asymptomatically, on the skin, skin glands, and mucous membranes, including the nose and gut. However, when it breaches these barriers, it can cause a range of infections, from mild skin conditions to life-threatening conditions like pneumonia and bloodstream infections. Its ability to evolve further complicates treatment, with some MRSA strains acquiring additional resistance to other antibiotics. As a result, MRSA infections require alternative therapies, often involving the use of non-β-lactam antibiotics or combination treatments.[30]

Vancomycin-resistant Enterococci (VRE):

Out of the fifty-eight *Enterococcus* species that have been described to date, *Enterococcus faecalis* and *Enterococcus faecium* are responsible for the majority of human infections. Frequently found as normal members of the gastrointestinal microbiota, they sometimes become opportunistic pathogens, especially in critically ill and immunocompromised patients. They are known to cause a variety of infections, including skin and soft tissue infections, urinary tract infections, and bloodstream infections.[32]

The Gram-positive Vancomycin-resistant *Enterococci* (VRE) develop resistance through genetic changes, specifically by obtaining *vanA* and *vanB* genes. These genes alter the bacterial cell wall's peptidoglycan layer, which vancomycin typically targets to prevent bacterial growth. The *vanA* gene results in high-level resistance by replacing D-alanine-D-alanine,[30] a dipeptide located at the terminus of the pentapeptide stem in peptidoglycan precursors,[33] with D-alanine-D-lactate, a change that prevents vancomycin from binding. The *vanB* gene also alters the peptidoglycan, though its effects are more variable. Due to these genetic modifications, VRE can resist vancomycin's ability to disrupt their cell walls, furthermore making them much harder to treat. VRE are often found in the gastrointestinal tract and can spread to cause severe infections, especially in hospital settings.[32]

Carbapenem-resistant Enterobacteriaceae (CRE):

The Gram-negative Enterobacteriaceae, which includes common bacteria like *E. coli* and *K. pneumoniae*, have developed resistance to carbapenem antibiotics through several mechanisms, furthermore making them difficult to treat. The primary method is the production of carbapenemase enzymes, such as KPC, MBLs, and OXA-48-like enzymes, which degrade carbapenems and prevent them from working.[34] These enzymes are often plasmid-mediated, so they can transfer between bacteria and therefore spread resistance.[35] In addition to carbapenemase production, these bacteria use efflux pumps that actively remove antibiotics from the bacterial cell, and they may alter porin proteins, which act as entry points for the drugs. When these changes occur together, bacteria become resistant to multiple drugs, complicating treatment options. Efflux pump systems such as AcrAB-TolC, which is particularly common in *E. coli*, and CusABC both contribute to multidrug resistance.[36] Changes in porin synthesis or expression further block the ability of carbapenems to reach their targets inside the bacterial cell. This mechanism is particularly evident in *K. pneumoniae*, where carbapenem resistance is linked to both the production of carbapenemases and altered porins. While these resistance mechanisms do not typically spread easily in the community, hospital conditions are ideal for these bacteria to reproduce rapidly. The growing prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) is a major concern for public health.[36]

Strategies to Overcome Antibiotic Resistance:

Antibiotic resistance is directly linked to antibiotic consumption, which has been steadily increasing since the discovery of antibiotics. In terms of DDD (defined daily doses), global antibiotic consumption increased by 65% in a span of 15 years between 2000 and 2015 (21.1–34.8 billion DDDs), while the rate of consumption increased by 39% (11.3–15.7 DDDs per 1,000 inhabitants per day).[37] Reducing consumption is, therefore, the easiest, cheapest, and most effective method to prevent the outbreak of new resistant strains. The COVID-19 pandemic has additionally contributed to the increase in consumption of antibiotics. Despite COVID-19 being viral, many patients were treated for bacterial infections, causing overuse and misuse of antibiotics.[38] Secondary infections were a common consequence of the virus, and due to healthcare systems being overwhelmed by the pandemic, the infection control measures were relatively weak.[38] Extended ICU stays and delays in accurate diagnostics furthered the spread of resistant bacteria.[38]

New drug development is extremely expensive,[39] and not always possible, so the following strategies are commonly used or under research and have the potential to be commonly used in the future instead.

Researchers have been exploring and have found many promising approaches to combating the antibiotic resistance crisis. 19 promising approaches have been identified, 10 of which show good clinical potential.[40] These include targeting quorum sensing, a bacterial communication system, antimicrobial peptides, which disrupt bacterial membranes, among others.[41] CRISPR-Cas systems have also been experimented with, and they hold substantial promise in the fight against antimicrobial resistance.[42] It operates by guiding Cas nucleases to specific resistance genes, either on plasmids or chromosomal DNA, thereby inactivating or eliminating them. Studies have demonstrated the successful use of CRISPR-Cas9 to remove resistance genes such as *mecA*, *blaNDM*, and *mcr-1*, resensitizing pathogens like *E. coli*, *K. pneumoniae*, and *S. aureus* to last-line antibiotics.[43] The most common strategies employed are described as follows:

Structural Modifications:

One way to combat antibiotic resistance is to structurally modify currently available antibiotics to make them more effective against resistant strains. Research has been conducted to modify the peripheral structure of the antibiotic Vancomycin, which includes the resistant strain Vancomycin-Resistant Enterococcus (VRE).[41] These modifications have been conducted on the binding pocket and have additionally induced bacterial cell membrane permeability. Due to these changes, Vancomycin is now 6000 times more potent against VRE.[41]

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Combination Therapy:

Two or more different antibiotics can also be used in combination to increase their efficacy. Combination therapy, albeit dangerous due to the possibility of interaction between the drugs, is a common and necessary practice. The therapy is key to many cancer and HIV treatments and is used almost exclusively for the treatment of Mycobacterium tuberculosis infections, with combinations of up to four typical drugs. Combination therapy can be chosen to (1) inhibit targets in different pathways, like the combination of isoniazid, rifampicin, ethambutol, and pyrazinamide that is used to treat M. tuberculosis infections, (2) inhibit different targets in the same pathway, like the combination of sulfamethoxazole and trimethoprim that inhibits successive steps in the folic acid biosynthetic pathway (3) inhibit the same target with multiple drugs with, for example, streptogramins. Another example of inhibiting different targets in the same pathway is tunicamycin, which inhibits teichoic acid synthesis and works synergistically with β-lactam antibiotics to significantly lower the concentration needed for bacterial inhibition. Similarly, ticlopidine enhances the effectiveness of cefuroxime,[44] an antibiotic frequently utilized for empirical therapy in community-acquired infections,[45] by disrupting specific bacterial processes. Other inhibitors of early cell wall synthesis, such as fosfomycin and vancomycin, also exhibit synergy with β-lactams. These combinations reduce resistance and improve efficacy, showing great potential in overcoming resistant bacterial strains.[44]

Adjuvants:

Adjuvants are substances that, when administered in conjunction with vaccine antigens, enhance the immune response to the antigen by increasing its immunogenicity.[46] Adjuvants can also be used to enhance the effectiveness of antibiotics without directly killing bacteria. A well-known example is Augmentin®, which combines amoxicillin with clavulanic acid to inhibit β-lactamase enzymes that degrade the antibiotic. Additionally, avibactam, when combined with ceftazidime, significantly boosts its effectiveness against *P. aeruginosa* by inhibiting AmpC β-lactamase.[41] Researchers are also repurposing non-antibiotic drugs, such as antihistamines and anti-inflammatory agents, which have shown potential to enhance antibiotic activity against resistant bacteria like MRSA.[41]

Bacteriophages:

Bacteriophages, also known as phages, are viruses that target bacteria. They are also being developed, with treatments like Phico Therapeutics' SASPject™ showing rapid bacterial destruction without harming normal flora.[41] Phages have even been seen used in combination with antibiotics. The concept of "phage-antibiotic synergy" (PAS), coined by Comeau *et al.* in 2007, refers to the enhanced effectiveness of phages when combined with sublethal concentrations of antibiotics.[47] Studies have shown that antibiotics, such as β-lactams and quinolones, increase phage plaque size and replication efficiency by reducing the latent period and enhancing phage adsorption.[47] PAS has been observed in various bacteria, including Pseudomonas aeruginosa and *Escherichia coli*. This synergy can be particularly useful in treating drug- and phage-resistant bacteria. PAS has proven effective in reducing bacterial density and virulence factor production, even in resistant strains.[47] For example, a combination of ciprofloxacin and phage *ECA2* reduced *E. coli* colony counts significantly,[48] while phage-antibiotic combinations have been shown to prevent phage resistance in *P. aeruginosa*.[49] However, interactions between phages and antibiotics can vary, with some combinations showing antagonistic effects, such as when rifampicin inhibits phage replication.[47]

■ Conclusion

Antibiotic resistance represents a significant and escalating global challenge, posing serious risks to public health, healthcare systems, and economies. Strains such as MRSA, VRE, and CRE have developed mechanisms to resist conventional antibiotic treatments, complicating the management of infections. These bacteria evolve through genetic mutations that enable them to survive despite the presence of antibiotics. While traditional treatments are increasingly ineffective, innovative solutions such as drug combinations, modifications to existing antibiotics, and emerging therapies like bacteriophages and CRISPR-Cas offer promising alternatives. CRISPR-Cas especially stands out for its remarkable precision and transformative potential. Though it remains under development, it holds significant promise for future applications. These strategies have the potential to restore the effectiveness of antibiotics.

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