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Review

An Insight into Antibiotic Resistance Mechanisms: Microbiological Implications for Public Health

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Abstract

Antibiotics are antimicrobial agents that target bacteria to treat and prevent bacterial infections. They work by interfering with vital bacterial functions, such as the synthesis of nucleic acid, cell wall, and folate, but also disrupting the plasma membrane and ribosome function. Bacteriostatic antibiotics inhibit bacterial growth and those that are bactericidal kill bacteria. Unfortunately, over time, bacteria have become resistant to antibiotics. This resistance can occur through intrinsic or acquired mechanisms, and bacteria may contain more than one type of bacterial resistance. Bacteria can develop antibiotic resistance mechanisms by reducing intracellular antibiotic concentrations and inactivating or modifying antibiotics and their target sites. The misuse of antibiotics, including excessive use, unnecessary and incorrect prescription, and self-administration, is associated with antibiotic resistance and, therefore, a higher likelihood of extended hospital stays and a greater risk of mortality. To slow the spread of antibiotic resistance, careful antibiotic selection will be crucial, as will the development of new antibiotics and the chemical modification of existing antibiotics to withstand established resistance mechanisms of bacteria. This paper will provide insight into the mechanisms of antibiotics, their effect on bacteria, and the spread of antibiotic resistance. Additionally, this paper will look into the microbiological implications in public health by analyzing challenges regarding antibiotic resistance and potential ways to shed light and raise awareness of antibiotic resistance.

Keywords: antibiotic resistance, resistance mechanisms, antibiotic-resistant bacteria, resistance development, resistance prevention

Резюме

Антибиотиците са антимикробни средства, насочени към бактериите за лечение и предотвратяване на бактериални инфекции. Те увреждат жизненоважни бактериални функции, като синтеза на нуклеинова киселина, клетъчна стена и фолат, но също така нарушават плазмената мембрана и функцията на рибозомите. Бактериостатичните антибиотици инхибират бактериалния растеж, а тези, които са бактерицидни, убиват бактериите. За съжаление, с времето бактериите са станали резистентни към антибиотиците. Тази резистентност може да възникне чрез вътрешни или придобити механизми и бактериите могат да съдържат повече от един тип бактериална резистентност. Те могат да развият механизми за антибиотична резистентност чрез намаляване на вътреклетъчните концентрации на антибиотици и инактивиране или модифициране на антибиотиците и техните целеви места. Злоупотребата с антибиотици, включително прекомерна употреба, ненужно и неправилно предписване и самоприлагане, е свързано с антибиотична резистентност и следователно по-голяма вероятност от удължен болничен престой и по-голям риск от смъртност. За да се забави разпространението на резистентността към антибиотици, внимателният подбор на антибиотици ще бъде от решаващо значение, както и разработването на нови антибиотици и химическата модификация на съществуващи антибиотици, за да издържат на установените механизми на резистентност на бактериите. Тази статия ще даде представа за механизмите на антибиотиците, техния ефект върху бактериите и разпространението на антибиотичната резистентност. Освен това, този документ ще разгледа микробиологичните последици за общественото здраве чрез анализиране на предизвикателствата

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по отношение на антибиотичната резистентност и потенциалните начини за хвърляне на светлина и повишаване на осведомеността относно антибиотичната резистентност.

Introduction

Antimicrobial resistance refers to the survival of microorganisms when exposed to antimicrobial agents that would normally kill or restrict their growth. Because of the emergence of multidrug-resistant strains of many bacterial species, this problem represents a significant threat to public health (Abushaheen *et al*., 2020; Francine, 2022). Antimicrobial agents can be classified as natural, semi-synthetic, or synthetic (Tenover, 2006; Zhou *et al*., 2015). The focus of this review paper is antibiotics, which by definition are antimicrobial agents that target bacteria to treat and eradicate bacterial infections (Patel *et al*., 2023). Antibiotics are frequently produced from mold or produced synthetically to kill bacteria (bactericidal) or stop their growth (bacteriostatic) (Nankervis *et al*., 2016). How antibiotics work includes: alterations in cell wall formation, protein synthesis, disruption of metabolic pathways, DNA replication, and translation (Tenover, 2006; Zhou *et al*., 2015; Abushaheen *et al*., 2020). While bacteriostatic antibiotics suppress bacterial growth, and bactericidal agents, by theory, ultimately kill bacteria, it does not exclude bacteriostatic antibiotics from killing bacteria too (Bernatová *et al*., 2013). Examples of bactericidal antibiotics that will be covered in this paper include β-Lactam (penicillins, cephalosporins, carbapenems), aminoglycosides, glycopeptides (vancomycin), fluoroquinolones, and nitroimidazoles along with the following antibiotics fall into bacteriostatic antibiotics: macrolides (like erythromycin), lincosamides (like clindamycin), sulfonamides, and tetracyclines (Patel *et al*., 2023). The division between bacteriocidal and bacteriostatic antibiotics is based on their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Pankey and Sabath, 2004). The MIC is the smallest concentration required to inhibit visible bacterial growth after 24 hours, whereas the MBC is the smallest dose required to kill bacteria (Patel *et al*., 2023). The MIC of a bacteria can be used to determine its susceptibility to an antibiotic and resistance. Bacterial resistance is achieved through either intrinsic or acquired mechanisms, often with different types of resistance. Different bacteria respond differently to the same antibiotic; which is based on the lack of target for a specific antibiotic - like the cell wall (Reygaert, 2018; Kowalska-Krochmal and Dudek-Wicher, 2021). Antibiotics obviously play a crucial role in healthcare and have been an inevitable part when it comes to the treatment of diseases caused by bacterial infections that were once deadly. However, the effectiveness of antibiotics has been weakened by the rapid increase in the development of resistance against them (Francine, 2022). Shortly after the discovery of the first antibiotic, penicillin, by Alexander Fleming in 1928, the battle against antibiotic resistance began. Penicillin was the first antibiotic to be used to treat a variety of infections, and with its excessive and widespread use came a decrease in its effectiveness as bacteria developed resistance mechanisms (Abushaheen *et al*., 2020; Giacomini *et al*., 2021; Francine, 2022). A *Staphylococcus* genus emerged in 1943 with acquired resistance against penicillin, even before this antibiotic became widely produced. This finding suggests that these bacteria may already have a gene for resistance in their genomes, and this type of resistance has likely evolved over the years. The emergence of bacterial resistance to antibiotics is a natural process that can take place without the involvement of humans (Hwang and Gums, 2016; Francine, 2022). However, since penicillin was first used to treat bacterial infections, the rate at which bacteria can acquire such resistance has been drastically accelerated. Bacteria can acquire resistance either from naturally resistant strains or more virulent ones (Fair and Tor, 2014; Hwang and Gums, 2016; Francine, 2022). In order to develop resistance, bacteria use two genetic strategies: vertical (endogenous) evolution and horizontal (exogenous) evolution. The formation of spontaneous mutations is part of vertical evolution. These mutations increase resistance, which often develops over time (Reygaert, 2018; Mancuso *et al*., 2021). Horizontal evolution is based on the transfer of a resistance-related gene from one susceptible bacterium to another (Burmeister, 2015). Three distinct mechanisms facilitate this transfer: conjugation, transduction, and transformation. Through a conjugative pilus, a resistant bacterium exchanges genetic material with another bacterium by transferring a resistance-plasmid containing antibiotic-resistance genes (Grohmann *et al*., 2003; Michaelis and Grohmann, 2023). The process of transformation involves modifying a bacterial genome by incorporating external DNA, contributing to the acquisition of resistance genes (Domingues *et al*., 2012). It has been discovered that enterococci carry plasmids that give them resistance to aminoglycosides and

ampicillin. Transposons, which may be incorporated into plasmids and chromosomes, are also found in bacteria (Neu and Gootz, 1996). Transduction involves the transfer of bacterial DNA via a viral vector. This transfer of resistance genes across bacterial species may have detrimental consequences since it could result in the spread of antibiotic-resistant strains that pose a more significant threat to public health (Thierauf *et al*., 2009; Mazaheri *et al*., 2011).

Types of resistance

Antibiotic resistance happens when bacteria evolve to bypass the effects of antibiotics. Bacteria can neutralize an antibiotic by modifying its components, or they may be able to export antibiotics out, and some may be able to change their exterior structure and receptors so that antibiotics cannot bind to them. These methods may result in certain bacteria surviving against antibiotic exposure, developing resistance by mutating their genetic material, and developing resistance that can spread to other bacteria. Antibiotic resistance mechanisms are classified as intrinsic and acquired resistance, genetic change, and DNA transfer (Habboush and Guzman, 2023).

• Intrinsic resistance: Specific functions that help bacteria develop resistance towards antibiotics where the antibiotics are no longer a target in these bacteria. Bacteria that lack a cell wall are not susceptible to antibiotics that damage the cell wall, such as β-lactams and glycopeptides. Export systems can also be found in bacteria on their outer membrane as a way to repel antibiotics, and some bacteria even produce enzymes that can neutralize antibiotics, such as AmpC β-lactamase in *Escherichia coli* (*E. coli*) (Abushaheen *et al*., 2020; Habboush and Guzman, 2023).

• Acquired resistance: This happens when bacteria that were initially susceptible to antibiotics manage to develop resistance. They do this by picking up genetic material from resistant bacteria (Urban-Chmiel *et al*., 2022; Habboush and Guzman, 2023).

• Genetic change: Bacterial DNA can modify and change protein synthesis, resulting in components and receptors that are not recognized by the antibiotics (Urban-Chmiel *et al*., 2022; Habboush and Guzman, 2023).

• DNA transfer: Horizontal gene transfer allows bacteria to share genetic material with other bacteria. Bacteria typically receive external genetic material through transformation, transduction, or conjugation (Urban-Chmiel *et al*., 2022; Habboush and Guzman, 2023).

Mechanisms of antimicrobial action

An antimicrobial agent is a natural or synthetic substance that can either completely kill or inhibit the growth of a microorganism. For example, antifungals are used against fungi, while antibiotics are used against bacteria (Burnett-Boothroyd and McCarthy, 2011). In this sense, antimicrobial agents target essential microbial functions, like the synthesis of cell walls, nucleic acids, and folate, but also hinder plasma membrane integrity and ribosomal function (Neu and Gootz, 1996). Table 1 provides a summarized overview of various mechanisms of antimicrobial action along with examples of associated antibiotics.

• Inhibition of bacterial cell wall synthesis

The bacterial cell wall is essential for keeping the intact shape of bacterial cells and protecting bacteria from their surrounding environment (Neu and Gootz, 1996; Abushaheen *et al*., 2020). ß-lactam antibiotics like penicillins and cephalosporins, which inhibit the development of peptidoglycan, and vancomycin, which bind to its structural components, both affect cell wall synthesis (Neu and Gootz, 1996). Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall and an outer membrane with lipopolysaccharides.

While Gram-positive bacteria lack an outer membrane, they are surrounded by much thicker peptidoglycan layers than Gram-negative bacteria (Silhavy *et al*., 2010). The peptidoglycan layer is a good target because damage to the peptidoglycan layer results in the destruction of the cell wall, eventually leading to cell death (Neu and Gootz, 1996). Since peptidoglycan makes up most of the bacterial cell wall, its structure is made up of long glycan chains that are joined together by enzymes called transpeptidase and carboxypeptidase, which are also known as penicillin-binding proteins (PBPs). Antibiotics target the physical integrity of the cell wall by binding with PBPs and glycopeptides, interfering with transpeptidase activity, and disrupting cell wall structure (Abushaheen *et al*., 2020).

• Disruption of the bacterial cytoplasmic membrane

The cytoplasmic membrane acts as a transport system and is a diffusion barrier for water, ions, and nutrients to pass into the cell. Polymyxin antibiotics have a positive charge that attracts negatively charged bacteria. Negatively charged lipopolysaccharides in Gram-negative bacteria give their cell wall an overall negative charge, which makes them the perfect target for polymyxin anti-

Table 1. Summary of antimicrobial mechanisms

Mechanism of action	Examples of antibiotics	Targeted Structures/ Processes	References
Inhibition of Bacterial Cell Wall Synthesis	ß-lactam Vancomycin	Peptidoglycan layer in cell wall; disruption of cell wall synthesis	(Neu and Gootz, 1996; Silhavy *et al*., 2010; Abushaheen *et al.*, 2020)
Disruption of the Bacterial Cytoplasmic Membrane	Polymyxin	Bacterial cytoplasmic membrane; changes in membrane shape, permeability, and leakage	(Neu and Gootz, 1996; Abushaheen *et al*., 2020)
Inhibiting Protein Synthesis/Ribosome Function	Aminoglycosides Tetracycline Chloramphenicol Lincosamides (Clindamycin) Macrolides (Erythromycin) Lincinoids	Bacterial ribosomes; interference with protein synthesis	(Serio *et al*., 2018; Tereshchenkov *et al*., 2018; Abushaheen *et al.*, 2020; Patel *et al*., 2023)
Inhibiting Synthesis of Nucleic Acid	Fluoroquinolone Quinolones Nitroimidazoles Rifampin	DNA gyrase/Topoisomerase II (Gram-negative) Topoisomerase IV (Gram-positive) DNA supercoiling; inhibition of DNA replication and RNA synthesis	(Kohanski *et al*., 2010; Redgrave *et al*., 2014; Hooper and Jacoby, 2016; Abushaheen *et al*., 2020)
Inhibition of Metabolic Pathways/ Bacterial Enzymes	Sulfonamides Trimethoprim	Folate synthesis pathway; disruption of folate metabolism, blocking tetrahydrofolate production	(Neu and Gootz, 1996; Kohanski *et al*., 2010; Trevor *et al*., 2013; Abushaheen *et al*., 2020; Ovung and Bhattacharyya, 2021)

biotics (Abushaheen *et al*., 2020). Polymyxin binds on the cell membrane, causing a change in shape and becoming more permeable. In this way, polymyxins disrupt the integrity of the membrane, causing leakage and allowing nucleic acids and cations to escape, causing the cell to die (Neu and Gootz, 1996).

• Inhibiting protein synthesis/ribosome function

The structural differences in bacterial and eukaryotic ribosomes are the perfect target for antibiotics that interfere with protein synthesis. Bacterial ribosomes include the 50S and 30S subunits. Antibiotics interfere with the subunits in bacterial ribosomes, which together form the 70S ribosome crucial for bacterial protein synthesis (Abushaheen *et al*., 2020; Patel *et al*., 2023). Ribosome function is disrupted by aminoglycosides, tetracycline, chloramphenicol, erythromycin, and clindamycin (Patel *et al*., 2023). Streptomycin (first discovered aminoglycoside antibiotic), nowadays used to treat tuberculosis, binds to the S12 protein of the 30S ribosomal subunit, causing the genetic code to be misread (Neu and Gootz, 1996; Serio *et al*., 2018). The binding of other aminoglycosides to the S12 protein of the 30S ribosome and partially the L6 protein of the 50S ribosome is key for causing resistance of bacteria to aminoglycosides. They may also bind other 30S ribosome sites, forming abnormal, nonfunctional complexes and misreading, which can cause bacterial death. Tetracyclines also bind to 30S ribosomes. Three classes inhibit the 50S ribosomal subunit: chloramphenicol, macrolides, and lincinoids (Neu and Gootz, 1996). Chloramphenicol inhibits Gram-positive as well as Gram-negative bacteria by attaching to a peptidyltransferase enzyme on the 50S ribosome and blocking the synthesis of peptide bonds (Tereshchenkov *et al*., 2018; Patel *et al*., 2023). Similar to erythromycin, macrolides attach to 50S ribosomes, disrupting the peptidyltransferase reaction and/or translocation. Erythromycin is effective against Gram-positive bacteria and some Gram-negatives like *Haemophilus*, *Mycoplasma*, *Chlamydia*, and *Legionella*.

Lincinoids, especially clindamycin, work similarly to macrolides by blocking the formation of peptide chains (Patel *et al*., 2023).

• Inhibiting synthesis of nucleic acid

Fluoroquinolones work by preventing bacterial DNA replication. These antibiotics block the DNA gyrase enzyme, a type II topoisomerase required to start DNA replication in Gram-negative bacteria (Hooper and Jacoby, 2016). Meanwhile, gram-positive bacteria, target the topoisomerase IV enzyme, which is responsible for daughter cell segregation (Kohanski *et al*., 2010; Redgrave *et al*., 2014; Abushaheen *et al*., 2020).

• Inhibition of metabolic pathways/bacterial enzymes

Bacterial DNA replication requires folate, which is inhibited by trimethoprim and sulfonamides. They work by disrupting folate metabolism by preventing the production of tetrahydrofolate, which is required for the synthesis of DNA, RNA, and bacterial cell wall proteins (Neu and Gootz, 1996; Trevor *et al*., 2013). The folate biosynthesis pathway serves as an ideal antibiotic target. In the folate synthesis pathway, the enzyme dihydropteroate synthase requires para-aminobenzoic acid (PABA). Sulphonamides, which structurally resemble PABA, act as competitive inhibitors, and in this way, they lower folate availability (Kohanski *et al*., 2010; Abushaheen *et al*., 2020; Ovung and Bhattacharyya, 2021).

Specific mechanisms of resistance to certain antibiotics

Bacterial resistance mechanisms to antibiotics fall into the 3 types (Laws *et al*., 2019).

Reducing intracellular antibiotic concentrations (increased efflux/decreased influx)

Bacteria have two tactics for limiting the buildup of antibiotics within their cells: reduced influx and enhanced efflux (Gaurav *et al*., 2023). Porins, which are outer membrane proteins, serve as gateways for antibiotics like tetracyclines and β-lactams to enter bacteria. When porin genes are downregulated, structurally altered, or even deleted, it decreases the influx of antibiotics (Abushaheen *et al*., 2020). Increased efflux works to actively pump antibiotics out of bacterial cells. This is accomplished by multidrug transporters, such as those found in the resistance-nodulation-cell division family of gram-negative bacteria (Abushaheen *et al*., 2020; Gaurav *et al*., 2023).

Antibiotic inactivation/modification

Antibiotics and enzymes can both be chemically altered by Gram-positive and Gram-negative bacteria. By doing this, the antibiotic cannot bind to their target sites. In enzymatic inactivation, bacterial enzymes directly bind to antibiotics and break them down, often through hydrolytic cleavage actions. For example, β-lactamases can disintegrate penicillins and cephalosporins. This enzymatic interference is frequently found in Gram-negative bacteria as well as Gram-positive bacteria (Abushaheen *et al*., 2020; Breijyeh *et al*., 2020).

Modifications at the target sites

1. Mutations in the Quinolone-Resistance-Determining Region

These mutations occur in both Gram-negative and Gram-positive bacteria and impact DNA gyrase/topoisomerase II and topoisomerase IV, resulting in fluoroquinolone resistance (Minarini and Darini, 2012).

2. Chemical modification via methylation

Methylation modification is accomplished by Erm methylases and is highly successful in inducing resistance to macrolide and lincosamide antibiotics, which is present in both Gram-positive and Gram-negative bacteria because of cfr gene methylation (Maravic, 2004; Vester, 2013).

3. Replacement of sensitive targets

Some bacteria replace drug-sensitive targets with drug-resistant ones, as observed in trimethoprim and sulphonamide resistance. Genes such as sul1, sul2, and sul3 in Gram-negative bacteria code for dihydropteroate synthases, resulting in sulphonamide resistance. Another example are alternative penicillin-binding proteins encoded in mecA and mecC genes in *Staphylococcus* (Sköld, 2001; Abushaheen *et al*., 2020).

β-Lactam antibiotics

Bacteria employ two strategies to resist β-lactams. One involves changing the structure of PBPs to weaken the attraction between antibiotics and PBPs. The other strategy is the production of penicillinase enzymes. These enzymes also target cephalosporins, carbapenems, and monobactams, making the term „β-lactamases“ more appropriate (Mora-Ochomogo and Lohans, 2021). Since these antibiotics work by binding to PBPs, which prevents cell wall formation, bacteria produce β-lactamase enzymes that break the amide bond in the antibiotic, making them ineffective. These enzymes are excreted outside the cell in Gram-positive bacteria and in the periplasmic space in Gram-negative bacteria, rendering the antibiotic useless before it reaches the PBPs (Abushaheen *et al*., 2020; Pan-

dey and Cascella, 2023). Penicillin-binding efficiency is decreased in *Streptococcus pneumoniae* strains resistant to penicillin G due to modifications in their PBPs. The resistance shown in *Staphylococcus aureus* methicillin-resistant strains, is also caused by changes in PBPs. PBP2a is a new penicillin-binding protein synthesized in response to β-lactams, and it does not bind with any β-lactam antibiotic (Neu and Gootz, 1996). A plasmid-mediated β-lactamase was discovered in *Haemophilus influenzae*, and the TnA transposon has contributed to increasing resistance to penicillin G and ampicillin among *Haemophilus* species (Tristram *et al*., 2007). This β-lactamase, or TEM enzyme, is the most prevalent plasmid β-lactamase. *Citrobacter*, *Pseudomonas*, *Citrobacter*, *Enterobacter*, and *Proteus-Providencia* are among the many species that have chromosomally mediated β-lactamases. In the case of *Klebsiella* species, all carry a chromosomally mediated β-lactamase that primarily functions as a penicillinase (Neu and Gootz, 1996).

Aminoglycoside antibiotics

Bacteria employ various methods to develop resistance against aminoglycoside antibiotics, namely: Ribosomal mutations and modifications, Aminoglycoside-Modifying Enzymes (AMEs), and efflux pumps. One common resistance mechanism involves changing the antibiotic‘s target, which is the ribosome. This alteration can occur through mutations or enzyme modifications. Some mutations, like those in the rrs gene affecting the 16S region of the ribosome‘s A-site, can be lethal to bacteria (Garneau-Tsodikova and Labby, 2016). Ribosomal alteration in the aminoglycoside binding site can also occur via 16S ribosomal RNA methyltransferases (RMTases). RMTases can be passed on to other bacteria using plasmids containing the RMTase gene (Garneau-Tsodikova and Labby, 2016; Abushaheen *et al*., 2020). AMEs chemically modify aminoglycosides, making them ineffective. Another way of defense includes aminoglycosides being actively removed from bacterial cells via efflux pumps. However, because of the complex structure of aminoglycosides, only a few efflux pumps are efficient. AcrAD, which is found mostly in gram-negative bacteria, is one such pump (Abushaheen *et al*., 2020). Porin channels in *Enterobacteriaceae* and *Pseudomonas* species allow aminoglycosides to pass through the cell wall. When they reach ribosomes, they exclusively attach to those that are actively participating in protein synthesis. Some bacteria possess enzymes that modify aminoglycosides, like *S. aureus*. Some Enterobacteriaceae and *Pseudomonas aeruginosa* may have resistance due to altered porin channels, preventing drug uptake without aminoglycoside-inactivating enzymes (Neu and Gootz, 1996).

Macrolide antibiotics

Resistance is achieved through three enzymatic modifications: ereA or ereB genes hydrolyze the antibiotic structure, triggering esteratic ring breakage. The mgt gene induces glycosylation in macrolides, whereas the mphA, mphB, and mphB genes cause macrolide phosphorylation. Resistance to macrolide-lincomycin in *Staphylococci* and *Streptococci* is plasmid-mediated and encoded on transposons. Resistance varies by species, with erythromycin being a more powerful inducer in most Gram-positive bacteria when compared to clindamycin (Neu and Gootz, 1996; Abushaheen *et al*., 2020).

Fluoroquinolone antibiotics

The most significant resistance to newer fluoroquinolones often arises from chromosomal mutations, leading to amino acid substitutions in DNA gyrase‘s A subunit. DNA gyrase subunit mutations, particularly the GyrA and GyrB subunits, cluster in the Quinolone resistance region, lowering antibiotic affinity. Some bacteria may exhibit multiple resistance mechanisms, resulting in higher fluoroquinolone resistance (Neu and Gootz, 1996; Ma *et al*., 2022). Resistance is enhanced in gram-negative bacteria due to reduced drug diffusion caused by fewer protein porins in the outer membrane (Abushaheen *et al*., 2020; Solano-Gálvez *et al*., 2021)

Rifampin antibiotics

Rifampin resistance occurs when bacteria develop mutations in the rpoB gene (Abushaheen *et al*., 2020). Rifampin inhibits bacterial RNA polymerase, and bacterial resistance to rifampin results from a single amino acid change, leading to reduced rifampin binding (Campbell *et al*., 2001). Resistance is related to the degree of enzyme alteration. Natural selection can lead to the development of this resistance during antibiotic treatments since it is naturally present in bacterial populations. The *Enterobacteriaceae* family quickly developed rifampin resistance in antibiotics used for urinary tract infections. In cases like *Neisseria meningitidis*, rifampin resistance emerged in confined military settings where rifampin was used for prophylaxis (Neu and Gootz, 1996).

Sulphonamide and trimethoprim antibiotics

Sulfonamides can become ineffective if dihydropteroic synthetase changes, resulting in a de-

creased affinity for sulfonamides and a higher affinity for p-aminobenzoic acid. This resistance can result from a single point mutation or via a plasmid that causes the changed enzyme to be produced (Neu and Gootz, 1996; Sköld, 2001). What is a more concerning issue is the increased resistance to trimethoprim, mediated by plasmids and transposons, resulting from an altered dihydrofolate reductase with significantly reduced trimethoprim affinity (Neu and Gootz, 1996). Trimethoprim resistance in bacteria such as *S. aureus* and *S. pneumoniae* is likely due to a single amino acid substitution in the dhfr gene. Sulphonamide resistance in *S. pneumoniae* is caused by the duplication of two amino acids in the folP gene (Neu and Gootz, 1996; Sköld, 2001; Abushaheen *et al*., 2020).

Tetracycline antibiotics

Tetracycline resistance involves reduced drug accumulation, influenced by decreased uptake and increased efflux. It is prevalent in Gram-negative as well as Gram-positive bacteria, and it is often plasmid-encoded (Chopra and Roberts, 2001; Grossman, 2016). Tetracycline resistance is common in enteric bacteria, with TetB being the most common, and it has been detected in *H. influenzae*. Resistance in *S. aureus* is generally due to plasmids, and chromosomal resistance is uncommon. Resistance in other bacteria differs depending on where it is found, for instance, on the chromosome of some streptococci, *C. difficile*, *S. agalactiae*, *S. pneumoniae*, and on nonconjugative plasmids in *S. faecalis* (Neu and Gootz, 1996; McCarthy and Lindsay, 2012). Tetracycline resistance can spread between Enterobacteriaceae members via plasmids, and these plasmids have also transferred resistance to various *Staphylococcus* and *Streptococcus* species (Neu and Gootz, 1996).

Vancomycin antibiotics

Some transposable genetic elements carry genes for unique cell wall-synthesizing enzymes, altering the D-Ala-D-Ala side chain in the peptidoglycan pathway. This change prevents vancomycin binding. High levels of resistance to glycopeptides can arise depending on the specific vancomycin resistance gene. So far, this resistance type has been observed in enterococci (Neu and Gootz, 1996; Cetinkaya *et al*., 2000; Abushaheen *et al*., 2020).

Fosfomycin antibiotics

Fosfomycin antibiotics penetrate bacteria by glucose-6-phosphate or glycerol-phosphate transport systems, and these antibiotics act by preventing the formation of cell walls. Gram-positive bacteria with poorly established transport systems are resistant and can therefore successfully synthesize cell walls (Zheng *et al*., 2022). This type of resistance is typically encoded in the chromosome. Resistance in Gram-negative bacteria stems mostly from bacteria that can function in the absence of the transport system. Plasmids and transposons have been identified in bacteria such as *Serratia marcescens* that spread fosfomycin resistance (Neu and Gootz, 1996).

Chloramphenicol antibiotics

Because of the presence of the enzyme chloramphenicol transacetylase, both Gram-positive and Gram-negative bacteria can be resistant to chloramphenicol. This enzyme acetylates hydroxyl groups on chloramphenicol, resulting in reduced binding to the 50S ribosome (Neu and Gootz, 1996; Kapoor *et al*., 2017).

Consequences of antibiotic resistance

Antibiotic resistance causes long-term infections, and because treatment choices are limited, patients with these infections may require stronger therapies, prolonged hospital stays, treatment failures, and increases in healthcare costs (Dadgostar, 2019). The improper use of antibiotics has been observed both among the general population and within healthcare settings. This misuse is primarily attributed to the excessive use of antibiotics and inappropriate prescriptions, as well as the use of antibiotics without consulting with a healthcare professional. Incorrect dosage and prolonged treatment periods are key contributors to antibiotic resistance (Awad and Aboud, 2015; Dache *et al*., 2021). To address these issues, „antibiotic-de-escalation“ has emerged as a potential solution. De-escalation involves starting with broad-spectrum antibiotics and then switching to narrower options. If no infection is confirmed, the antibiotic treatment is stopped. Additionally, de-escalation includes using fewer antibiotics and optimizing the dose and how the antibiotics are given. In terms of long-term antibiotic treatment consequences, antimicrobial agents can be toxic, impact the toxicity of other agents, alter microbial flora, and trigger allergic reactions (Giacomini *et al*., 2021). Almost all antibiotics can cause *Clostridium difficile* overgrowth by altering the gut flora. *C. difficile* toxins cause diarrhea and other inflammatory gut issues, such as pseudomembranous colitis. As a result of the gut dysbiosis, *Candida* can overgrow in the mouth, vagina, or gastrointestinal tract (Johanesen *et al*., 2015; Giacomini *et al*., 2021). Given that antibiotics are processed in the

Table 2. Mechanisms of Antibiotic Resistance in Bacteria

Antibiotic class	Mechanism of resistance	Examples of resistant bacteria	References
β-Lactams	Changes in PBPs structure Production of β-lactamase enzymes	Enterococci *Mycobacterium tuberculosis* (*M. tuberculosis*) *S. pneumoniae* *S. aureus* Methicillin-resistant *S. aureus* (MRSA) *H. influenzae* *Klebsiella* spp. *P. aeruginosa*	(Abushaheen *et al.*, 2020: Neu and Gootz, 1996; Fisher and Mobashery, 2016; Pandey and Cascella, 2023)
Aminoglycosides	Ribosomal mutation Aminoglycoside-Modifying Enzymes (AMEs) Efflux pumps	*Enterobacteriaceae* spp *Pseudomonas* spp *S. pyogenes* *S. aureus* *S. faecalis* *Bacteroides*	(Garneau-Tsodikova and Labby, 2016; Abushaheen *et al.*, 2020; Neu and Gootz, 1996)
Macrolides	Enzymatic modifications; Hydrolysis, glycosylation, and phosphorylation of macrolides	*Staphylococcus* spp. *Enterococcus* spp. *Bacillus* spp. *E. coli* *Proteus vulgaris*	(Abushaheen *et al.*, 2020; Neu and Gootz, 1996)
Quinolones and fluoroquinolones	Alterations in enzymes and drug-access; Mutations in DNA gyrase (GyrA and GyrB subunits); Expression of MDR efflux pumps;	*S. pneumoniae* *Klebsiella pneumoniae* (*K. pneumoniae*) *E. coli* *Neisseria gonorrhoeae* *S. aureus*	(Abushaheen *et al.*, 2020; Neu and Gootz, 1996; Ma *et al.*, 2022; Georgina Solano-Gálvez *et al.*, 2021)
Rifampin	Mutation in DNA-directed RNA polymerase	Enterobacteriaceae N. meningitidis M. tuberculosis	(Campbell *et al.*, 2001; Abushaheen *et al.*, 2020; Neu and Gootz, 1996)
Sulphonamide and Trimethoprim Antibiotics	Changes in enzyme affinity; Altered dihydropteroic synthetase and dihydrofolate reductase	*S. aureus* (Trimethoprim resistance) *S. pneumoniae* (Trimethoprim and sulphonamides resistance)	(Abushaheen *et al.*, 2020; Sköld, 2001; Neu and Gootz, 1996)
Tetracycline	Can be plasmid-encoded Reduced drug accumulation through decreased uptake and increased efflux	*Proteus* spp. *Enterobacteriaceae* *H. influenzae* *S. aureus* *S. faecalis* *S. pneumoniae* *S. agalactiae* *Clostridium* spp	(Neu and Gootz, 1996; Grossman, 2016; Chopra and Roberts, 2001; McCarthy and Lindsay, 2012)
Fosfomycin	Inhibit cell wall synthesis	P. aeruginosa S. marcescens Enterobacterales	(Neu and Gootz, 1996; Zheng *et al.*, 2022)
Vancomycin	Alteration of D-Ala-D-Ala side chain; Prevention of vancomycin binding	Enterococci Vancomycin-resistant *S. aureus* (VRSA)	(Abushaheen *et al.*, 2020; Neu and Gootz, 1996; Cetinkaya et al., 2000)
Chloramphenicol	Enzyme-mediated resistance; Acetylation of chloramphenicol by chloramphenicol transacetylase	H. influenzae	(Neu and Gootz, 1996; Kapoor *et al.*, 2017)

liver, they can induce liver damage, notably isoniazid, which is used to treat tuberculosis. Aminoglycosides can lead to kidney damage, and harm the auditory or vestibular system. As for the effect of combination therapy, combining antibiotics may create a stronger effect than what each one does alone, or it can make them work against each other if one antibiotic hinders the other (Neu and Gootz, 1996). Because of their high resistance levels, the World Health Organization has identified high-priority pathogens, the „ESKAPE“ pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and Enterobacter species) (Singh *et al*., 2017; Giacomini *et al*., 2021). Effective treatments are currently unavailable for resistant strains of several of these bacteria, most notably *A. baumannii*. When there are no safe and suitable antibiotics for treatment, it leads to the use of antibiotics with a low therapeutic index as a last resort for specific infections. New antibiotics with novel mechanisms of action or a distinct chemical structure that enables new target interactions while keeping a proven mode of action are desperately needed (Singh *et al*., 2017).

Discussion

Antimicrobial agents work by disrupting essential processes for the growth and division of microorganisms. They fall into different groups based on how they inhibit cell walls, cytoplasmic membranes, nucleic acid synthesis, and ribosome function. Table 1 highlights the diverse strategies of antimicrobial agents, like antibiotics, to combat bacteria and includes examples of well-known antibiotics associated with each mechanism. These agents can either kill bacteria - bactericidal or slow down their growth - bacteriostatic. Over time, bacteria developed resistance to antibiotics whose resistance factors may be found on plasmids or the chromosome. This results in reduced drug entry, changes in the antibiotic target receptor, or the inactivation of the drug through metabolic processes. Out of these mechanisms, only one or a combination more may be present at the same time. Table 2 shows antibiotic classes, resistance mechanisms, and examples of resistant bacteria. In order to fight and slow down antibiotic resistance proper antibiotic selection will be fundamental alongside the discovery of new antibiotics, as well as the chemical modification of existing antibiotics to produce compounds that can resist established resistance mechanisms. A new approach that could help is antibiotic de-escalation, which is becoming increasingly important in the fight against antibiotic resistance. Inappropriate antibiotic use is widely acknowledged as the primary cause of antibiotic resistance. This misuse encompasses various behaviors, such as excessive and often unnecessary antibiotic consumption, including overuse, inappropriate prescriptions, self-medication, careless administration, and incorrect dosages or treatment durations. The patient‘s mindset is equally important in this situation. Antibiotic prescriptions must be more careful and thoughtful. Over-prescription can be troublesome and is one cause of therapeutic inertia, in which antibiotics are taken even when a bacterial infection does not apparently cause symptoms. The goal is to optimize prescription practices to ensure antibiotics are used appropriately and effectively.

Conclusion

In conclusion, antibiotic resistance is a rising public health concern that largely resulted from antibiotic misuse and has led to the emergence of resistant bacteria. Developing new antibiotics and modifying existing ones is necessary to slow down and fight antibiotic resistance. Additionally, promoting responsible antibiotic use and raising public awareness are the beginning steps essential to tackle this problem.

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