HARNESSING COMBINATION THERAPY TO COMBAT DRUG-RESISTANT BACTERIA: TOWARD EFFECTIVE TREATMENTS

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons)

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled **"Harnessing Combination Therapy to Combat Drug-Resistant Bacteria: Toward Effective Treatments"** submitted by Bushra Shanaz (21146037) of Spring, 2025, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of School of Pharmacy on 21st August, 2025.

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Ethics Statement

The project does not involve any animal trials or clinical trials on humans.

Abstract

Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi, and parasites evolve to withstand drugs designed to eliminate them, making infections harder to treat. This global health threat is driven largely by antibiotic misuse and overuse in humans, animals, and agriculture. Combination therapy using two or more agents with different mechanisms offers a promising approach to improve treatment outcomes and slow resistance development. FDA-approved drugs examples include amoxicillin–clavulanate and piperacillin–tazobactam, both of which pair a β-lactam antibiotic with a β-lactamase inhibitor to restore drug activity. This review followed a structured literature search of studies published in the last 15 years from databases such as PubMed, ScienceDirect, Google Scholar, and Springer Nature, focusing on resistance mechanisms, clinical applications, and therapeutic outcomes. This review aims to highlight combination therapy's potential as a strategic tool against multidrug-resistant pathogens and to guide more effective, personalized treatment strategies in the era of rising AMR.

Keywords: Antibiotic resistance, Combination therapy, and Multidrug-resistant bacteria.

Dedication

This thesis is dedicated with all my heart to my Ma, whose love, prayers, and strength have been the backbone of everything I do. To my sisters Ritu, Ria, and Safa for always standing by me, cheering me on, and believing in me even when I doubted myself.

And to Warisul Haque, the one who has been my constant through every high and low, thank you for your endless support, patience, and love.

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List of Acronyms

LMICs	Low- and Middle-Income Countries
HICs	High-Income Countries
HGT	Horizontal Gene Transfer
PDR	Pan Drug-Resistant
AMR	Antimicrobial Resistance
ABR	Antibiotic Resistance
MRSA	Methicillin-Resistant Staphylococcus Aureus
HAIs	Healthcare-Associated Infections
CRE	Carbapenem-Resistant Enterobacteriaceae
ESBL	Extended-Spectrum Beta-Lactamases
VRE	Vancomycin-Resistant Enterococci
MDR	Multidrug-Resistant
XDR	Extensively Drug-Resistant
PBPs	Penicillin-Binding Proteins
KPC	Klebsiella Pneumoniae Carbapenemase
DHPS	Dihydropteroate synthase
PABA	Para-Aminobenzoic Acid
DHFR	Dihydrofolate Reductase
DHF	Dihydrofolate
THF	Tetrahydrofolate
MBL	Metallo-β-lactamases
MDROs	Multi-Drug Resistant Organisms
SSTIs	Skin & Soft tissue Infections
IV	Intravenous
UTI	Urinary Tract Infection
LPS	Lipopolysaccharide
PK/PD	Pharmacokinetics and Pharmacodynamics

1.0 Introduction

1.1 Introduction

Antibiotics are medications that are used to prevent and treat bacterial infections. They have saved many millions of lives and placed the majority of infectious diseases that afflict human history for many centuries under control. When antibiotics were first used in clinical settings in the 1940s, they were so effective at eliminating harmful bacteria that many people thought infectious illnesses would ultimately disappear from all human populations (Aminov et al., 2009). Antibiotics are one of the most significant drug discoveries in human history. Antibiotics pose a significant risk to humanity because people have developed a habit of overusing them, largely due to the influence of technology and evolution.

Antibiotic resistance happens when bacteria change in a way that makes antibiotics no longer effective against them. This means the medicines that used to cure infections stop working, making it harder to treat diseases. Antibiotic resistance has become one of the leading causes of death globally. In 2019, it directly led to 1.27 million deaths and contributed to 4.95 million more than diseases like AIDS and malaria (Smith et al., 2022). One of the main reasons for antibiotics resistance is the overuse and misuse of antibiotics in humans, animals, and plants. When antibiotics are used too often, for minor illnesses such as the common cold or fever. In 2011, around 70 billion doses of antibiotics were used worldwide, which means that on average, every person received 10 doses that year. Antibiotic use has been increasing globally, rising by 39% between 2000 and 2015. This increase was mainly seen in low- and middle-income countries (LMICs), where usage grew by 77%, while high-income countries (HICs) saw a small decrease of 4%. Additionally, children especially those in low-income areas receive the highest amounts of antibiotics. Many young children suffer from common colds

and respiratory infections, which are usually caused by viruses and do not require antibiotics. However, antibiotics are often prescribed unnecessarily. For example, in China, 78% of children with a common cold received antibiotics and in Turkey, nearly all children with flu-like symptoms were prescribed antibiotics. Even in the USA, despite a reduction in antibiotic prescriptions for children, unnecessary use remains high (Blaser et al., 2021). Also, when people stop taking antibiotics before finishing the whole course of treatment, some bacteria can survive and become resistant. These strong bacteria can then grow and spread, making the antibiotics less effective. Another reason is using antibiotics when they're not needed like for viral infections, which they can't treat. Antibiotics are misused to treat viral infections, even though they are only effective against bacteria. Viruses and bacteria are different; bacteria are living microorganisms that antibiotics can kill, while viruses need a host to survive and multiply, making antibiotics useless against them. Common viral infections such as colds, flu, COVID-19, and most sore throats do not respond to antibiotics (Blaser et al., 2021). However, many people still take them unnecessarily and believing it will help them recover faster. This misuse contributes to antibiotic resistance, where bacteria evolve and become stronger, making real bacterial infections harder to treat. Antibiotic resistance has become a serious danger to world health because it compromises vital medical operations like surgeries and cancer treatments that rely on efficient antibiotics to prevent infections. To address this worldwide epidemic, more responsible antibiotic usage, creative therapeutic approaches, and strong international cooperation are all needed. Combination therapy is a promising strategy that uses numerous medications to improve therapeutic effectiveness and slow the emergence of resistance.

1.2 History of Antibiotic

Antibiotics are substances produced by bacteria and fungi that kill and stop the growth of harmful microbes. The idea of using natural substances to fight infections days back to ancient Egypt, where moldy bread was used to treat wounds. However, it wasn't until 1928 that penicillin, the first true antibiotic, was discovered by Alexander Fleming, a bacteriology professor in London(The-Discovery-and-Development-of-Penicillin, 2011). In September 1928, Alexander Fleming made a groundbreaking discovery by accident. After returning from a two-week vacation, he noticed that a mold had grown on a Petri dish containing bacteria. Surprisingly, the area around the mold was clear, meaning the bacteria couldn’t grow there. Curious, Fleming studied the mold and found that it produced a substance that killed harmful bacteria like those causing pneumonia and meningitis. He named it "penicillin," and this discovery became a major breakthrough in medicine (Chhabra et al., 2024). Fleming and his team tried to isolate pure penicillin, but it was unstable, and they could only make crude preparations. In 1929, Fleming published his findings, mentioning it’s potential benefits for bacteriologists, but it seemed like it might only be useful in research at that time (The-Discovery-and-Development-of-Penicillin et al., 2011). Over the years, many antibiotics were discovered, such as streptomycin, tetracycline, and erythromycin, which helped cure diseases like tuberculosis and pneumonia. However, as people started using antibiotics too much, bacteria began to fight back and became resistant and making infections harder to treat.

Bacteria can also naturally change and sometimes these changes help them resist antibiotics. These resistant strains can subsequently spread within populations. Bacterial resistance refers to a bacterium's ability to survive exposure to antibiotics that would normally inhibit or kill it. Bacteria develop resistance to antibiotics mainly through mutations and horizontal gene transfer (HGT). Mutations are small changes in bacterial DNA that can occur due to antibiotic

exposure. Some of these mutations help bacteria survive by altering their target sites, reducing drug uptake, increasing the production of efflux pumps that remove antibiotics from the cell. HGT allows bacteria to share resistance genes with each other by three main methods-transformation, conjugation, and transduction. These processes help bacteria to quickly spread resistance, making infections harder to treat (Sandoval-Motta & Aldana et al., 2016). The misuse and overuse of antibiotics further speed up this process, increasing the risk of multidrug-resistant bacteria (Mittal et al., 2020). When the microorganism is no longer impacted by an antibiotic that would typically stop growth or kill it, this is known as bacterial resistance.

1.3 Challenges of Antibiotic resistance

Antibiotic resistance creates various problems that put the current treatments and public health at risk in the world. First of all, the main challenge is that single drug treatments are failing. When used properly, antibiotics are effective, but overuse and misuse can lead to bacterial resistance. However, a single antibiotic is used to treat a specific condition at the same time germs become resistant to the drug, which ends up a failure of the ongoing treatment, particularly for illnesses that are generally treatable. Along with this, it creates more difficulty in curing diseases that can formerly be readily healed under a single antibiotic. Moreover, the initial reason is the increase of pandrug-resistant (PDR) bacteria that have an extreme behavior to threat as existing antibiotics are being resisted by it. Furthermore, ‘superbugs’ are also known as one of the main reasons to create this emergence. As it is the misuse of widespread antibiotics and antibiotic overuse. With the alternation of drug targets, blocking the entrance, expelling the chemical from drug cells are resisted by bacteria. While here, *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa* are some of the most concerning PDR bacteria with their severe infection of the system of urinary, blood, and lung systems by preventing the usual setting of hospitals, notably in care units and surgical

wards, where patients are more susceptible. While this type of bacteria is medication-resistant and, with the lack of proper and necessary control of infection, is not followed, it would also survive on the surfaces, equipment of medical and healthcare workers. Many times, individuals who travel between hospitals or countries have inadvertently brought these superbugs with them, exposing them to unfamiliar medical settings. Transmission between patients can also result in quick epidemics. These infections have substantial death rates, ranging from 20% to 71%, which demonstrates their severity (Karakonstantis et al., 2020). The formation of PDR bacteria is also significantly influenced by practices like as administering antibiotics for viral diseases and not completing an entire course of antibiotics. Because of this, these viruses keep evolving, making diseases harder to cure. When infections occur, they often require more expensive and powerful medications that have a greater potential for adverse effects. Below mentioning the effects of PDR (Abat et al., 2018).

Table 1: Human deaths due to PDR bacterial strains

Country	**No. of deaths due to PDR infection**	**Mortality Rate**	**Underlying conditions**	**PDR bacterial species responsible for the death**	**Specimen**
Australia	2	6.7%	Cystic fibrosis	*Stenotrophomonas maltophilia* *Pseudomonas aeruginosa*	Sputum
USA & Canada	3	6.7%	Cystic fibrosis	*P. aeruginosa*	Sputum
Greece	8	33.3%	Various	*Acinetobacter baumannii* *K. pneumoniae*	Various
USA	1	NA	Hepatic abscess	*K. pneumoniae*	Blood

From the table, it's shown that two people in Australia lost their lives to PDR infections. 6.7% was the mortality rate. *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*, which were detected in sputum (lung mucus), were the microorganisms that caused the patients cystic fibrosis. Three people died in the United States and Canada, which likewise had a 6.7% mortality rate. The infection was caused by *P. aeruginosa*, which is also found in sputum, and the afflicted individuals also had cystic fibrosis. With a substantially higher fatality rate of 33.3% and eight deaths total, Greece recorded the greatest death toll. Both *K. pneumoniae* and *Acinetobacter baumannii* were detected in many samples from individuals with various illnesses. *K. pneumoniae* produced a hepatic abscess, or liver infection, that resulted in one death in the United States. Although a blood sample contained the bacterium, the fatality rate was not disclosed.

1.4 Aims and Objectives

The purpose of this study is to review new emerging strategies to combat antibiotic resistance through the use of combination therapy. It explores the root causes of resistance and thoroughly assesses the effectiveness of various combination treatments for drug-resistant bacterial infections. By referencing the latest studies, this review aims to identify and the most effective approaches to optimize combination therapy. Additionally, examining the transformative potential in personalized medicine, seems abrupt. Make a smoother transition how combination therapy directly contributes to personalized approaches.

1.5 Methodology

This study is based on a literature review, where existing research on antibiotic resistance and combination therapy was carefully analyzed. To gather relevant information, a thorough search was carried out in well-known scientific databases such as PubMed, Google Scholar,

ScienceDirect, and Springer Nature. The search used keywords like “antibiotic resistance,” “combination therapy,” and “multidrug-resistant bacteria” to find articles related to the topic.

Only studies published in the last 15 years were considered. The focus was mainly on how bacteria develop resistance and how different combinations of antibiotics are used to treat them. Important information was collected from the selected articles, including:

- Types of resistance mechanisms
- Treatment strategies that were applied
- Results regarding how effective the therapies were
- Limitations or challenges reported in the studies

Since this is a review of published research, no experiments were done, and no human or animal subjects were involved. All the references were organized using Mendeley Desktop (version 2.136), which helped ensure that proper citations were used throughout the paper.

2.0 Fundamental Mechanisms of Antimicrobial Resistance

2.1 Mechanisms of Antimicrobial Resistance

Bacteria can resist antibiotics through different mechanisms. Some bacteria are naturally resistant to certain types of antibiotics, means that all strains of that species are unaffected by those drugs. However, a more concerning issue is acquired resistance, where bacteria that were once sensitive to antibiotics become resistant over time, due to repeated exposure to those drugs. Despite this, microorganisms have developed effective defenses against antibiotics.

Firstly, they can pick up special genes that help them make enzyme like beta-lactamases that break down the antibiotic before it even has a chance to work. Some bacteria naturally possess genes that produce beta-lactamases enzymes that disable antibiotics like penicillin and cephalosporins by breaking down their structure before they can act; for example, *Enterobacter cloacae and Pseudomonas aeruginosa* have chromosomally encoded beta-lactamases integrated into their normal genetic makeup. This gives them a natural ability to fight off certain antibiotics without needing any outside help. On the other hand, acquired resistance happens when bacteria that didn't originally have beta-lactamase genes gain them from other bacteria, often through plasmids. These mobile genetic elements carry resistance genes from one bacterium to another. For example, the TEM, SHV, and CTX-M types of beta-lactamases are often acquired this way, and they can make bacteria resistant to a wide range of antibiotics, including strong third-generation cephalosporins (Bush & Bradford et al., 2016).

Secondly, they can develop efflux pumps that push the antibiotic out of the cell, so it never reaches the part it's supposed to attack. Efflux pumps play a critical role in both intrinsic and acquired antibiotic resistance. In case of intrinsic resistance, many bacteria naturally express efflux systems that actively expel a wide range of antimicrobial agents, contributing to baseline

resistance levels. For example, the AcrAB-TolC efflux system in Escherichia coli and MexAB-OprM in *Pseudomonas aeruginosa* are well-known multidrug efflux pumps that confer intrinsic resistance by reducing intracellular antibiotic concentrations. On the other hand, in acquired resistance, bacteria can enhance the expression of these efflux pumps or acquire new efflux genes through horizontal gene transfer (Li & Nikaido et al., 2009).

And thirdly, they can change intracellular targets by altering their cell walls or the pathways inside them, making it hard for the antibiotic to find and bind to its target. One example of this is reduced permeability, where bacteria make it harder for antibiotics to enter their cells. In intrinsic resistance, some bacteria naturally have an outer membrane that acts like a strong barrier. For example, Pseudomonas aeruginosa has fewer and more selective porin channels has tiny openings that let molecules in, so many antibiotics can't get through easily. In acquired resistance, some bacteria can modify or reduce the number of porins they produce, which further blocks antibiotics from entering. This is especially common in Gram-negative bacteria like *Klebsiella pneumoniae*, which can reduce porin expression in response to antibiotic pressure, making treatments less effective (Delcour et al., 2009). All these changes help bacteria survive, even when we try to kill them with potent medications.

2.2 Mechanistic Distinctions Between Intrinsic and Acquired Resistance

Intrinsic resistance refers to the natural defense mechanisms inherent to certain bacteria, which hinder antibiotic effectiveness. These bacteria are possess inherent structural defenses. For example, their thick and waxy cell walls which is unique and full of special fats called mycolic acid (Nasiri et al., 2017). These fats make the outer layer of the bacteria act like a shield, blocking many antibiotics and other harmful substances from getting inside. This makes it very hard for drugs to reach their targets within the bacteria. Scientists in this study tried turning off a specific gene called antigen 85C, which is involved in helping build that waxy wall. When

they did this, the bacteria lost about 40% of those protective fats, and suddenly, it became easier for certain molecules to get inside (Jackson et al., 2010). The researchers proved just how important that waxy outer layer is to the bacteria's built intrinsic resistance. Even though the bacteria are still able to grow normally, its permeability changes some substances could enter more easily, showing that this natural defense is a key reason why Mycobacterium tuberculosis (M. tuberculosis) bacteria are so hard to treat. However, this discovery opened new possibilities for scientists to find better ways to fight tuberculosis. If we can target the genes involved in making the waxy cell wall, we might be able to weaken the bacteria's natural defense and help antibiotics work better. In fact, some new drugs are now being developed to disrupt the formation of mycolic acids or inhibit the enzymes that help build the waxy wall. Intrinsic resistance comes from the bacteria's natural structure, like thick, waxy cell walls that act as strong barriers, making it hard for antibiotics to enter. These bacteria may also have fewer protective fats, which slightly allows drug entry but doesn't have a big effect on treatment (Figure1). It demonstrates how targeted gene disruption can enhance bacterial resistance by increasing membrane permeability, reducing protective lipid layers, and ultimately improving the effectiveness of medications (Stokes et al., 2020). The balance shown clearly indicates that overcoming innate resistance can significantly improve treatment outcomes.

Apart from this, acquired resistance which is a different antibiotic resistance that develops gradually and causes a change in bacterial genetics. Additionally, antibiotic-resistant bacteria arise with gene mutations, such as bacteria that can be resistant to quinolone antibiotics because of a mutation in the gene of gyrA (this is a gene encoding a subunit of DNA gyrase). Along with the alternation of these genetics that affects the target site of medication, decreases the ratio of drug that is absorbed by the cell, enhances the activity of efflux pumps that trigger the drug to be eliminated from the cells of bacteria (Sandner-Miranda et al., 2018).

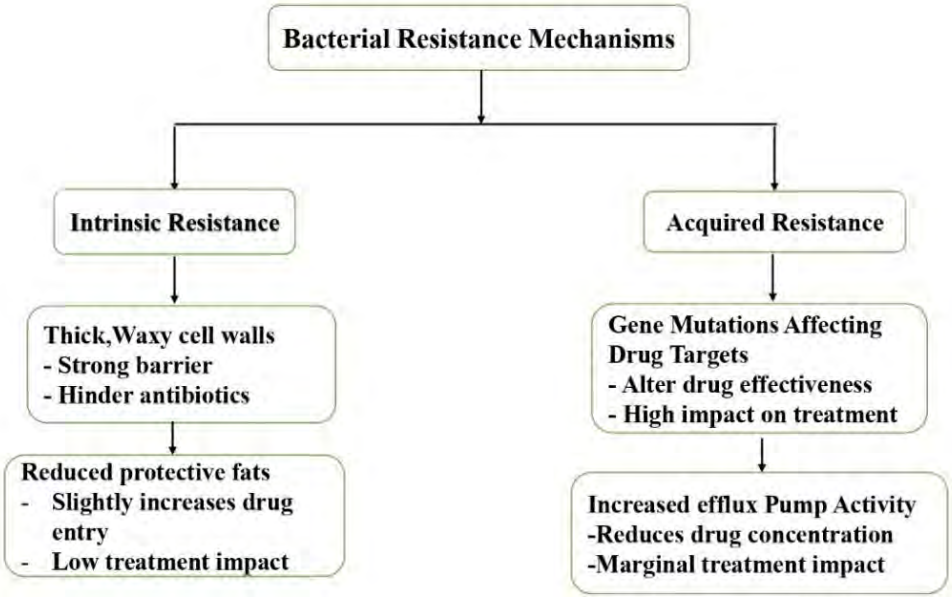

Figure 1: *Mechanistic Distinctions Between Intrinsic and Acquired Resistance [Created with PowerPoint]*

Acquisition of resistance of bacteria by several means HGT is a key mechanism through which bacteria acquire resistance. The gene transfer occurs through an element of mobile genetics, including integrons, transposons, or plasmids. This type of exchange of genes is commonly seen in the surroundings, like hospitals. The place where antibiotics are used widely and prompts bacteria to adapt faster. Here, conjugation is one of the greatest methods of prevention for HGT that connects bacteria physically and transmits plasmid that creates resistance. Followed by another method that is known as bacteriophages, which relates to viruses that involves a process that is considered as transduction for transferring the material of genetics within the cells of bacteria. Moreover, free DNA can be taken by bacteria from the surroundings, that allows for genetic makeup alternation along with a mechanism of resistance genes spread rapidly even between unrelated bacterial species. That also makes it critical for infection treatment, as well as causing severe difficulties for up-to-date medical practice (Von Wintersdorff et al., 2016). Bacteria can acquire resistance to multiple antibiotics as mobile genetic components carry several genes of resistance at a single time (Sandner-Miranda et al.,

2018). The overuse of antibiotics in hospitals leads to a significant increase in the development of antibiotic resistance. In this type of structure, bacteria can adapt fast and share the traits of resistance to a continuous pressure of survival.

3.0 Epidemiology of Global Antimicrobial Resistance

3.1 Global Burden of AMR

Antimicrobial resistance (AMR) happens when germs like bacteria, fungi, parasites, and viruses change over time and become resistant to the medicines which are designed to kill them, such as antibiotics. As a result, these treatments become less effective. AMR has become a major global concern in the 21st century because resistant infections are spreading rapidly, while the development of new antimicrobial drugs has slowed down, making it harder to fight these infections (Prestinaci et al., 2015a). One of the major contributors to the rise of antimicrobial resistance is the overuse and misuse of antibiotics in different areas, especially in medical treatments, animal healthcare, and the food industry. Although AMR, also known as the "Silent Pandemic," initially shows no symptoms, it has major long-term consequences and need quick, coordinated intervention. AMR has long been recognized by the World Health Organization (WHO) as a major global health threat; in 2001, WHO introduced a framework to curb the spread of resistant microorganisms, which was later expanded in the 2012 publication The Evolving Threat of Antimicrobial Resistance, outlining strategies such as strengthening healthcare systems, promoting responsible antibiotic use, preventing infections, developing new drugs and vaccines, and securing strong political commitment. Furthermore, WHO collected data from many nations and organizations to publish its first worldwide report on AMR surveillance in April 2014. However, many parts of the world still lack proper surveillance, making it difficult to fully understand how widespread and severe AMR is (Prestinaci et al., 2015).

3.2 Statistics on morbidity, mortality of AMR worldwide

4.95 million of deaths were attributed to AMR in 2019. AMR is now one of the leading causes of death worldwide. AMR is most prevalent in low-resource areas, particularly in South Asia and Sub-Saharan Africa, where poor access to high-quality medical care and potent antibiotics makes the problem worse. Using information from systematic reviews, microbiological monitoring, and hospital records, 23 bacterial pathogens and 88 pathogen–drug combinations from 204 countries and territories were analyzed (Mittal et al., 2020). Lower respiratory infections accounted for over 1.5 million AMR-related deaths, followed by bloodstream and intra-abdominal infections. The six deadliest drug-resistant pathogens were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa*, responsible for 3.57 million AMR-related deaths. Among pathogen–drug combinations, *methicillin-resistant Staphylococcus aureus (MRSA)* was the leading cause of deaths, followed by multidrug-resistant tuberculosis and third-generation cephalosporin-resistant E. coli (Murray et al., 2022).

3.3 Economic impact of AMR worldwide

AMR causes serious financial pressure on healthcare systems around the world. When antibiotics fail to work, infections become harder and more expensive to treat. Patients often need stronger, more costly medicines and longer hospital stays, which increases overall healthcare expenses. For example, in the United States, over $4.6 billion is spent every year just to treat six major types of drug-resistant infections (Antibiotic Resistance Threats in the United States et al., 2019). In 34 European nations, drug-resistant infections cost more than €26.5 billion a year. Just two common resistant diseases cost hospitals more than $11 million annually, even in Australia.

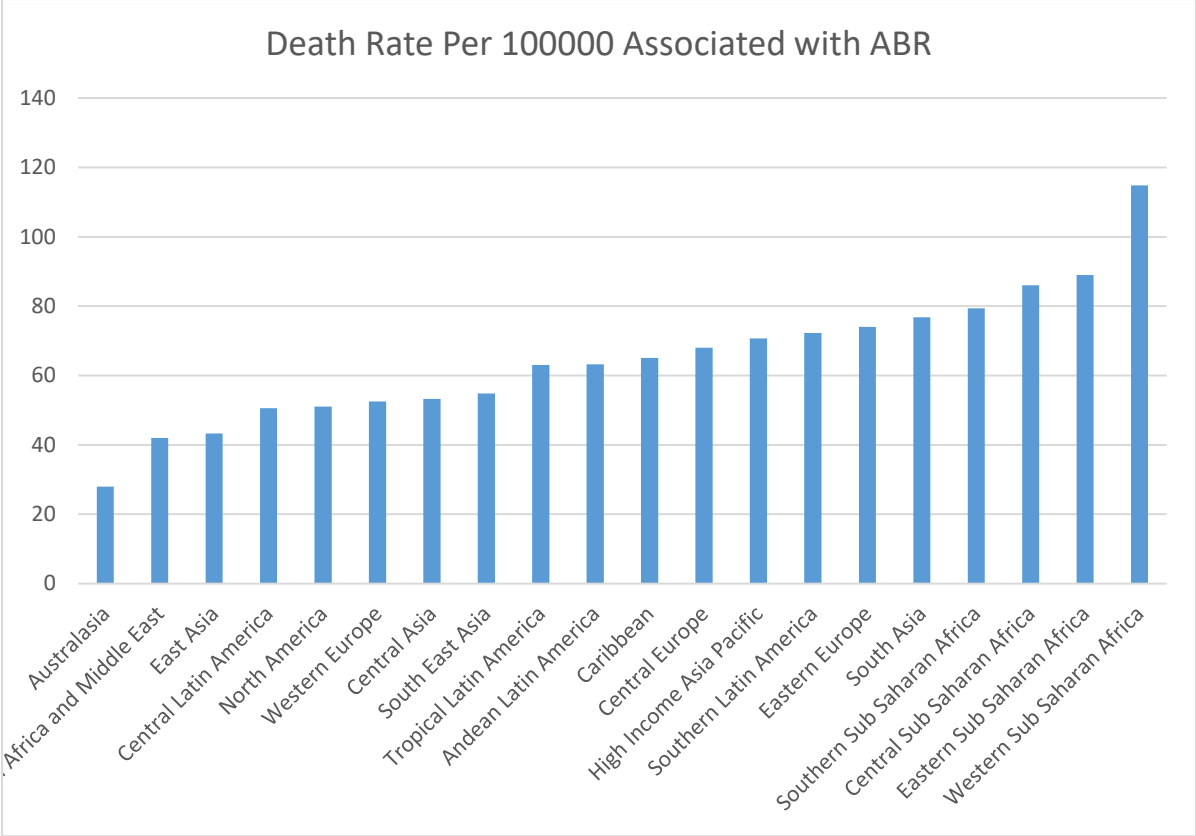

Figure 2: *Regional Death Rates from Antibiotic-Resistant Bacteria*

(The bar chart illustrates regional variations in death rates per 100,000 people associated with antibiotic-resistant bacteria (ABR). Western Sub-Saharan Africa exhibits the highest death rate, while Australasia has the lowest. The Y-axis shows the death rate per 100,000 due to ABR, while the X-axis represents different global regions) (Smith et al., 2022) [Created with Microsoft excel]

The bar chart shows in Figure 2 how many people per 100,000 died in different regions of the world due to infections caused by antibiotic-resistant bacteria (ABR). The Y-axis shows the death rate, while the X-axis lists various global regions from lowest to highest death rates. From the chart, it's clear that Australasia has the lowest death rate, with fewer than 30 deaths per 100,000 people. Other regions with relatively low rates include Africa and the Middle East, East Asia, and Central Latin America, all falling under 50 deaths per 100,000. In the middle range, we see regions like Western Europe, South East Asia, Andean Latin America, and

Southern Latin America, where death rates hover around 60–70 per 100,000. These areas still face significant challenges, but not as severe as some others. As we move to the right side of the chart, the death rates rise noticeably. Eastern Europe, South Asia, and all parts of Sub-Saharan Africa report higher death rates. Western Sub-Saharan Africa stands out with the highest rate over 120 deaths per 100,000, nearly five times that of Australasia. This visual clearly shows that regions with fewer healthcare resources and limited access to effective antibiotics tend to suffer more from ABR-related deaths. It emphasizes the urgent need for better infection control, antibiotic stewardship, and global efforts to reduce resistance, especially in vulnerable areas.

It is also essential to emphasize that AMR presents considerable economic issues in addition to being a major health concern. Depending on the severity of the crisis, AMR could cause a 1.1% to 3.8% reduction in global GDP by 2050, according to the World Bank. Increased healthcare costs, lower worker productivity, and the need for more costly treatments are the main cause of this financial burden (Institute for Health Metrics and Evaluation (IHME) et al., 2022).

4.0 Factors contributing to AMR rise

Because of the use of anti-infective medication for human and animal both in the manner of appropriate and inappropriate along with production of food, AMR is rising as a serious concern for public health. It also spreads because of inadequate infection control techniques.

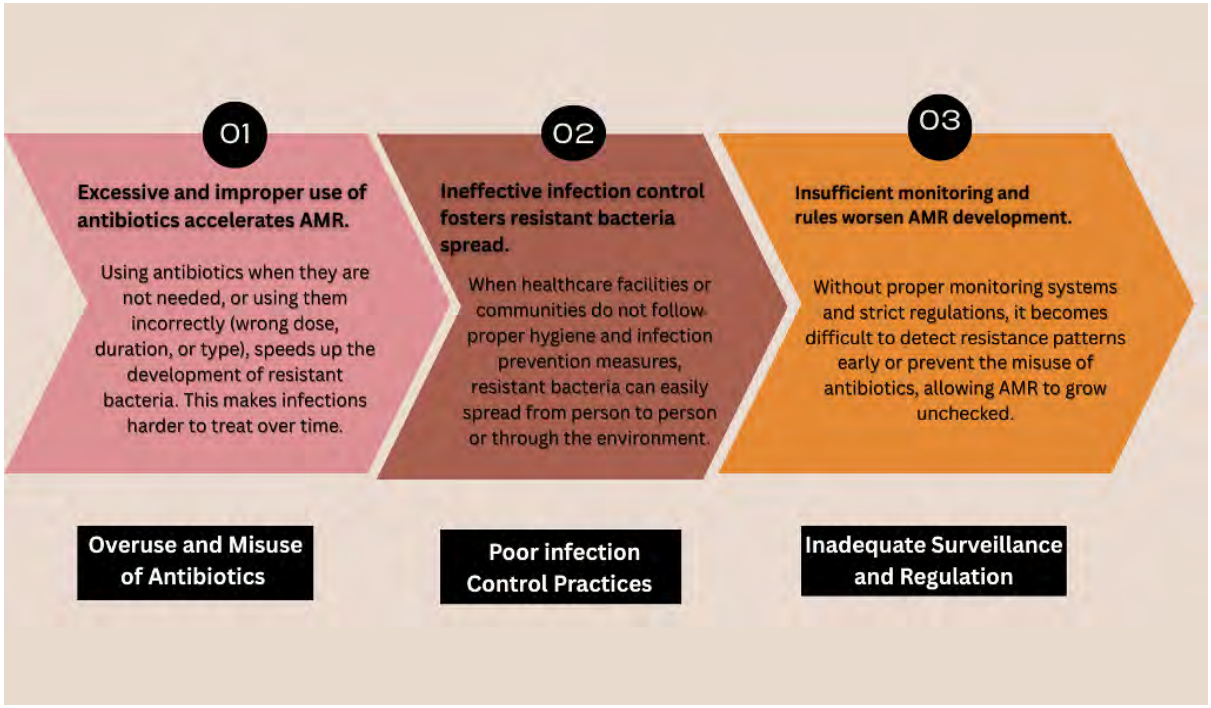

Figure 3: *Unveiling the Roots of Antimicrobial Resistance [Created with Canva]*

Recognizing AMR's serious public health threats, multiple nations and international agencies have adopted policies across healthcare, agriculture, and food production to address it. Many common infections are made more difficult and expensive to cure by AMR, which frequently causes treatment delays or, in extreme situations, makes it impossible to provide effective treatments. Numerous modern medical treatments, including organ transplants and chemotherapy for cancer, significantly depend on potent anti-infective medicines. Resistance increases mortality, causes more complications, lengthens recovery periods, and raises sickness rates. AMR has a financial impact by increasing costs for diagnosis and treatment as well as

decreasing productivity (Fletcher et al., 2015). Due to insufficient data in many countries, it is still difficult to precisely measure the major health and financial implications.

AMR is not just a biological issue; human action, especially the abuse and overuse of antibiotics across a range of disciplines, is a major contributing factor. The issue is also accelerated by poor healthcare practices, insufficient system of regulation, and low public awareness of appropriate antibiotic use and infection prevention. The main causes of the origin and global spread of AMR are addressed in this chapter.

4.1 Overuse and misuse of antibiotics in Human medicine, Veterinary medicine & Agriculture

4.1.1 Reasons for overprescription of antibiotics in healthcare

Healthcare professionals' overprescription of antibiotics, especially when they are ineffective, is a major contributor to antibiotic resistance. Medicines can treat viral infections majority of sore throats, the common cold, influenza, and other common diseases are caused by viruses, which medications cannot treat (Vikesland et al., 2019). Nevertheless, doctors still commonly prescribe antibiotics because of

- Patient pressure: Antibiotics are often prescribed without a reason because patients think antibiotics to relieve their symptoms.
- Diagnostic uncertainty: Physicians may administer antibiotics "just in case" when bacterial and viral illnesses exhibit similar symptoms.

Antibiotics are widely abused in many LMICs because they are easily accessible without a prescription (Do et al., 2021). The following factors are responsible for this phenomenon:

- Weak enforcement of rules: Strict laws governing the selling of antibiotics are absent from several nations.
- Self-medication practices: Patients purchase antibiotics based on previous experience or advice from pharmacists rather than medical consultation.
- Economic and healthcare barriers: When healthcare is difficult to access, people take care of themselves to save money (Vikesland et al., 2019).

4.1.2 Veterinary Medicine: Abuse of Antibiotics in the Production of Livestock and Poultry

Antibiotics are frequently utilized in veterinary medicine for three primary reasons: prophylactic prevention of diseases, treatment of infections, and animal growth stimulation. Because of their role in the development of resistant bacterial strains, the latter two applications in particular have given rise to significant public health concerns. Animals kept in crowded, unclean environments are particularly vulnerable to diseases in many intensive farming methods (Cattaneo et al., 2022). In order to counteract this, antibiotics are frequently given on a regular basis, even to healthy animals, either as a preventative precaution or to encourage quicker development and greater feed efficiency. Because of the ongoing selection pressure this non-therapeutic usage places on bacteria, resistant strains are more likely to survive and proliferate.

A widely cited example is the frequent use of colistin an antibiotic considered a last-resort treatment in human medicine in livestock across several countries. With the invention of the gene of colistin-resistant mcr-1 is primarily identified in the pigs of China and after that in humans globally. That indicates the inappropriate antibiotic use in animals can seriously undermine important treatment options for human health care or treatments (Liakopoulos et al., 2016).

4.1.3 In Agriculture

With the antibiotic use in the production of agriculture it contributes to the AMR development. Unfortunately, the issue gets fewer attention rather than the antibiotic use in livestock. Antibiotics, including streptomycin and oxytetracycline that are usually applicable for fruit trees such as apple trees and pear trees for combating diseases such as fire blight. While these kinds of antibiotics are effective in treating diseases of plants that create stresses on environmental microbes that lead to developed resistance. However, insecticides that contain antibiotics and fertilizers derived from feces of animals are able to get their way to soil, river, as well as on water subsurface while being used in farms. Contaminated soil and water provide ideal conditions for bacteria in the environment to develop resistance. HGT is a natural method by which these bacteria can share resistance genes with other bacteria (Heuer et al., 2011).

Studies reveal that the soil around antibiotic-using farms frequently harbors multi-drug-resistant bacteria. The fact that these resistant bacteria might persist in the environment after antibiotic usage has ceased is concerning. This implies that resistant germs can proliferate among plants, animals, and even people since the environment turns into a storage space for resistance (X. X. Zhang et al., 2010).

4.2 Poor infection control practices

One of the main causes of the growing number of microbes resistant to antibiotics in hospital settings is inadequate infection control procedures. Overpopulation in hospital wards, inadequate hand hygiene, and inappropriate sterilization of medical equipment are important contributing causes. One of the most important aspects of infection prevention is hand cleanliness. Healthcare professionals' compliance, however, is frequently below ideal. According to studies, doctors are less likely than nurses to follow proper hand hygiene procedures, which are used in only 25% to 50% of situations. The danger of healthcare-

associated infections (HAIs), which are frequently brought on by resistant microorganisms, is greatly increased by this gap (Wester et al., 2002). The issue is made worse by hospital overcrowding and understaffing. High patient-to-staff ratios may lead to neglected infection control procedures, allowing resistant pathogens to spread (Clements et al., 2008). However, when hospital surfaces aren't cleaned properly and medical tools aren't sterilized correctly, it creates a favorable environment for harmful, drug-resistant germs to thrive.

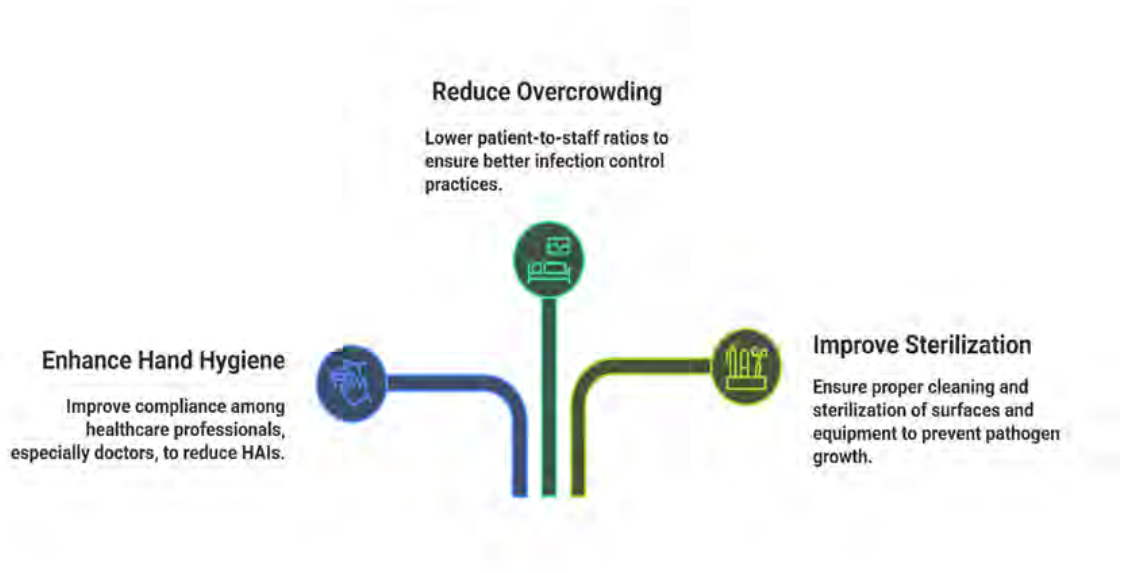

Figure 4: *Poor infection control* [Created with Canva]

If cleaning products aren't used as the manufacturer recommends, or if the disinfectants chosen aren't effective against certain bacteria, the cleaning process may not eliminate these dangerous microbes. Additionally, using cleaning wipes with insufficient antimicrobial properties can leave surfaces inadequately disinfected, allowing pathogens to survive and spread to patients and healthcare workers (Boyce et al., 2016).

4.3 Inadequate surveillance and regulation

Effective surveillance systems are essential for tracking developments regarding AMR and directing the proper use of antibiotics. However, there are significant gaps and challenges in integrated surveillance for AMR, as shown in Figure 5. In many areas, especially in low-income countries, reliable surveillance facilities are lacking. This limitation hinders the implementation of targeted interventions, such as infection control measures or antibiotic stewardship programs, and makes it more difficult to detect emerging resistance trends in a timely manner. Numerous structural issues in LMICs make the issue worse. There isn’t enough money to invest in new and advanced tools for diagnosing diseases. Also, testing for antibiotic resistance isn't always accurate or reliable because many labs don’t have modern equipment, enough supplies, or trained staff. Useful information about AMR is also hard to get because microbiology services are not used enough often because doctors don’t know much about them or rely too much on guesswork when choosing treatments (Global, Regional, and National Disability-Adjusted A Systematic Analysis for the Global Burden of Disease Study et al., 2018).

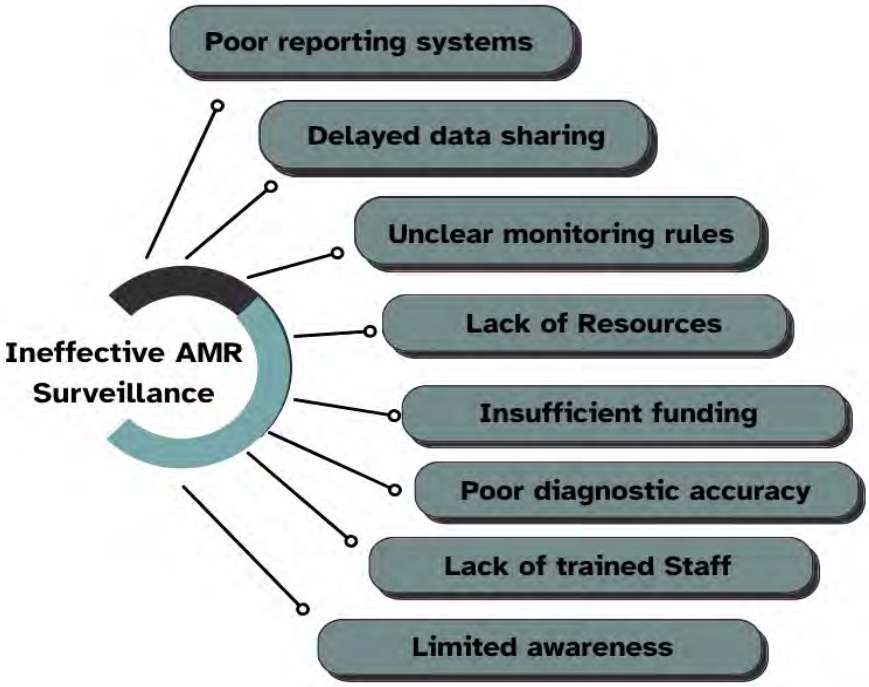

Figure 5: *Challenges in Antimicrobial Resistance Surveillance* [Created with Canva]

Another reason behind this is often shared late or not at all both within countries and across the world is because reporting systems are not well connected and there are no clear rules for monitoring. For example, many LMICs struggle to include AMR tracking into their existing disease reporting systems, like those for HIV or TB. Because of this, public health officials don't get timely and reliable information to track the spread of drug-resistant bacteria such as MRSA and Carbapenem-Resistant Enterobacteriaceae (CRE) (Cabral et al., 2024). This makes it harder to stop these infections from spreading in hospitals and communities.

5.0 Role of Combination Antibiotic Therapy

Combination therapy means using two or more antimicrobial medicines together to treat an infection. This approach can work better because it targets the bacteria in more than one way. For example, the drugs sulfamethoxazole and trimethoprim block different steps in the production of folate, which bacteria need to grow. It can also help treat infections caused by different types of bacteria at once and lower the chances of the bacteria becoming resistant to the medicine this is especially important in serious diseases like HIV or tuberculosis (treated with drugs like isoniazid, rifampin, and pyrazinamide) (A. Ahmed et al., 2014). Some combinations can even make resistant bacteria easier to kill. For instance, amoxicillin works better when combined with clavulanic acid, which blocks enzymes (β-lactamases) that some bacteria produce to resist antibiotics.

However, using combination therapy the wrong way can cause more side effects or make resistance worse. Combination therapy can be life-saving in serious or complicated infections like sepsis, infections with drug-resistant bacteria, or in patients with weak immune systems. But if a single, narrow-spectrum antibiotic can treat the infection well, it's usually better, as it causes fewer side effects and less resistance (Paul et al., 2004). To get the best results, treatment plans should be adjusted based on lab test results and follow guidelines for responsible antibiotic use.

5.1 Synergistic effects

A well-known example of synergistic antimicrobial treatment is the combination of aminoglycosides with β-lactam antibiotics. Penicillin and cephalosporins are examples of β-lactams that disrupt with the formation of bacterial cell walls, resulting in holes that facilitate the absorption of aminoglycosides like amikacin or gentamicin. Aminoglycosides attach to the

30S ribosomal subunit inside the bacterial cell, preventing protein synthesis and producing rapid bactericidal action. Theoretically, this dual method kills germs more quickly and especially in cases of severe infections like endocarditis or sepsis (Tamma et al., 2012). This synergy is regularly shown in vitro experiments, especially when it comes to Gram-negative bacteria like Enterococcus species and Pseudomonas aeruginosa.

5.1.1 Integrative Analysis of Synergistic Mechanisms: Laboratory Findings Versus Clinical Realities

When used together, β-lactams (such ceftazidime and piperacillin-tazobactam) and aminoglycosides (like tobramycin and gentamicin) clearly exhibit synergistic lethality in controlled laboratory conditions. By interfering with the formation of bacterial cell walls, β-lactams produce holes that enhance membrane permeability. Normally unable to pass through the membranes of Gram-negative bacteria, aminoglycosides are able to enter cells more readily. Once inside, aminoglycosides bind to ribosomes, disrupt protein synthesis, and exert rapid bactericidal effects. For example, the combination of ceftazidime and tobramycin has been shown in laboratory studies to eliminate *Pseudomonas aeruginosa* 10 to 100 times more effectively than either antibiotic used individually (Paul et al., 2004).

However, clinical data shows that this synergy frequently does not transfer into better survival or cure rates when treating sepsis, even if lab research suggest that combining aminoglycosides with β-lactams might promote bacterial death. A significant factor is the pharmacokinetic mismatch: β-lactams are most effective when maintained at consistent levels over time, whereas aminoglycosides require high peak concentrations to be effective (Tamma et al., 2012).This discrepancy makes it challenging to synchronize dosing schedules in clinical settings, potentially diminishing the intended synergistic effect. Additionally, patient-specific

factors, such as impaired kidney function common in critically ill individuals, can alter aminoglycoside clearance and increase the risk of nephrotoxicity.

Moreover, aminoglycosides have limited tissue penetration, making them less effective in treating infections in certain areas like lung abscesses. Importantly, unlike combination therapies in diseases like tuberculosis or HIV, the β-lactam and aminoglycoside combination does not consistently prevent the development of antibiotic resistance in sepsis cases (A. Ahmed et al., 2014).

5.2 Broadened spectrum activity

A broadened resistance profile refers to the ability of a microorganism, particularly bacteria, to resist numerous antibiotics from different classes because of its chemical and functional diversity. These organisms resist not only initial antibiotics but also antimicrobial agents from multiple unrelated classes. Resistance frequently occurs as a result of genetic mutations or acquisition of resistance genes through horizontal gene transfer that results in MDR (Multidrug-Resistant) or extensively drug-resistant (XDR) strains (Vestergaard et al., 2016). This has proven to be a huge problem in the clinical setting where it hampers treatment options and the risk of stubborn infections raises the worldwide threat of AMR.

5.2.1 Mechanism of Broadened Spectrum Activity

The combination of MK-0826's (ertapenem) high-affinity binding to Penicillin-Binding Proteins (PBPs), structural resistance to β-lactamases, and sustained effectiveness under high bacterial inoculum results in its broad-spectrum behavior.

High-Affinity Binding to PBPs

- PBPs are bacterial enzymes that synthesize and remodel the peptidoglycan layer.
- MK-0826 binds irreversibly to PBP 2 (IC_{50} = 0.01 µg/mL) and PBP 3, disrupting:

- Transpeptidation (cross-linking of peptidoglycan chains).
- Cell wall elongation & septation (leading to defective division) (Papp-Wallace et al., 2011).

- Comparison with Other β-Lactams:
  - Imipenem (carbapenem): Similar PBP 2 binding (IC_{50} ~0.01 μg/mL).
  - Cefepime (4th-gen cephalosporin): Weaker PBP 2 binding (IC_{50} ~0.5 μg/mL).
  - Ceftriaxone (3rd-gen cephalosporin): Much weaker (IC_{50} >1 μg/mL) (Kohler et al., 2011).

Resistance to Hydrolysis by β-Lactamases

- Shield-like Protection: MK-0826 contains a unique feature called the 1-β-methyl group, which acts like a tiny "shield." This shield helps protect the drug's important ring structure from being attacked and broken down by certain enzymes. These enzymes, known as β-lactamases, are responsible for destroying many common antibiotics like penicillins and cephalosporins. However, because of this protective group, MK-0826 can avoid being broken down as easily.
- Works Against Many Enzymes (but not all): MK-0826 is able to resist damage from several common β-lactamase enzymes, such as TEM, SHV, CTX-M, and AmpC. These enzymes are usually involved in breaking down antibiotics, but MK-0826 can stand strong against them. Its ability to block these enzymes helps preserve the activity of β-lactam antibiotics. This strength mainly comes from the 1-β-methyl group, which plays a key role in stopping these enzymes from working. However, MK-0826 isn't perfect. It doesn't work as well against some stronger enzymes, like KPC (Klebsiella pneumoniae carbapenemase), which are found in more dangerous, drug-resistant bacteria. So, while MK-0826 is effective against many resistant bacteria, it may not work against the most extreme "superbugs" (Zerdan et al., 2022).

5.3 Reduced emergence of resistance

Using a combination of antibiotics is a powerful way to fight infections and prevent bacteria from becoming resistant. When two or more antibiotics with different ways of attacking bacteria are used together, it makes it much harder for the bacteria to survive and develop resistance. For defending oneself, bacteria need a number of ways in a single time that is tough for them to do (Vestergaard et al., 2016). The method is used without a single antibiotic that works as a barrier against resistance.

- Here, clavulanic acid and amoxicillin combination is a relevant example. Where Clavulanic acid protects the antibiotic by enzymes inhibition of bacteria that decreases it. On the other hand, amoxicillin works by disrupting the wall of a bacterial cell. The collaboration not only mitigates the resistance development risk but also increases treatment effectiveness (Cantón & Morosini et al., 2011). That keeps us one step ahead of resistant bacteria and treatments for a longer period.
- On the other side, another great innovation is how the treatment of dual antibiotics successfully ignores the resistance, which is the co-trimoxazole combination of trimethoprim and sulfamethoxazole. Trimethoprim inhibits dihydrofolate reductase, while sulfamethoxazole prevents dihydropteroate synthase, targeting two stages of sequential in the process of bacterial folic acid production. As folic acid is needed for developing bacteria, but it is hard for germs to be resistant for both medications, and when it is synthesis at several phases (Tyers & Wright et al., 2019). In terms of mitigating obstinate bacteria that frequently exhibit resistance to individual antibiotics, including *Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus,* the strategy is quite effective.

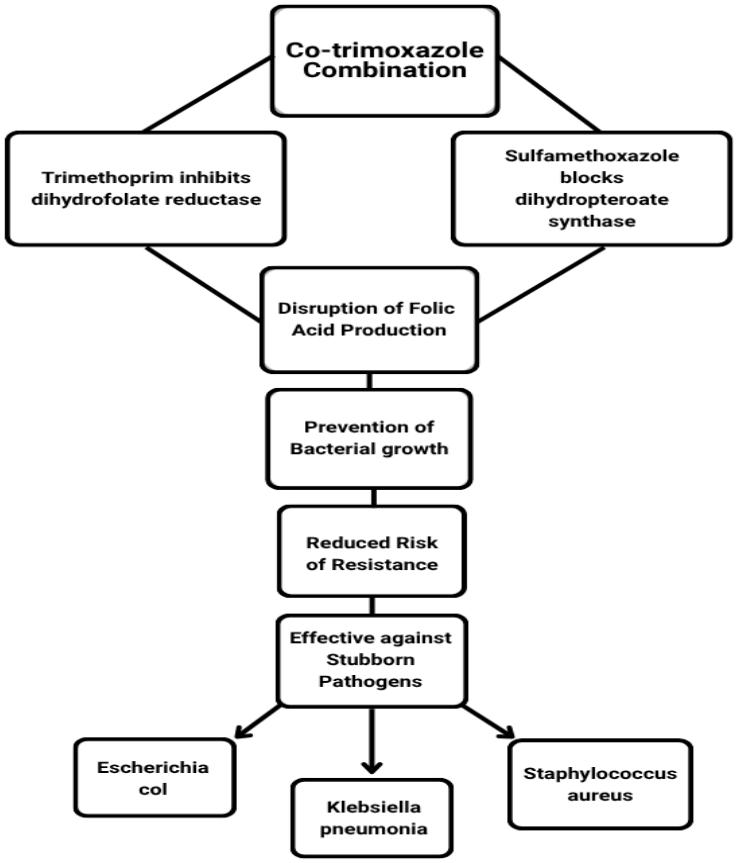

Figure 6: *Co-trimoxazole Combination* [Created with Canva]

6.0 Understanding the Mechanisms of Resistance Suppression via Combination Therapy

AMR is a serious global health threat that reduces the effectiveness of existing antibiotics, making common infections harder to treat and jeopardizing procedures like surgeries, transplants, and cancer therapies that rely on infection control. While the fast growth of resistance to drugs germs has exceeded the development of new antibiotics, it's a critical need for novel techniques to preserve the effectiveness of presently available drugs (Chevereau et al., 2015).

Combination treatment, which treats an infection by giving two or more antimicrobial medicines at the same time, is one strategy that is becoming progressively more common. Compared to monotherapy, this approach has a number of benefits. Targeting different bacterial species or stages of bacterial growth increases the treatment's breadth and also increases the possibility that the disease will be successfully eliminated. Using many medications lowers the possibility that the virus will be able to survive with only one mutation or resistance mechanism (Tyers & Wright et al., 2019).

6.1 Blocking the pathways

Combination therapy prevents antibiotic resistance by simultaneously targeting many important bacterial pathways, which prevents bacteria from developing resistance to all drugs at once. Below are important instances and mechanisms-

- The combination of trimethoprim and sulfamethoxazole is a well-established example of how targeting multiple steps within a single metabolic pathway can effectively hinder bacterial resistance. Sulfamethoxazole works by inhibiting the enzyme dihydropteroate synthase (DHPS), thereby blocking the conversion of para-

aminobenzoic acid (PABA) into dihydropteroate, an essential early step in bacterial folic acid synthesis. Trimethoprim complements this action by inhibiting dihydrofolate reductase (DHFR), the enzyme responsible for converting dihydrofolate (DHF) into tetrahydrofolate (THF), which is crucial for DNA and nucleotide synthesis. Together, these drugs disrupt the folic acid pathway at two critical points, making it more difficult for bacteria to develop resistance. The folate pathway is essentially stopped at two crucial locations by the sequential or "double blockade" that is produced by this dual targeting. Because mutation probabilities are multiplicative, simultaneous mutations in the folA gene and the folP gene would be necessary for resistance to arise. This is a statistically uncommon occurrence. This synergy makes the combination both clinically effective and evolutionarily resilient by strengthening the bactericidal action and dramatically reducing the likelihood of resistance formation (Wormser et al., 2016).

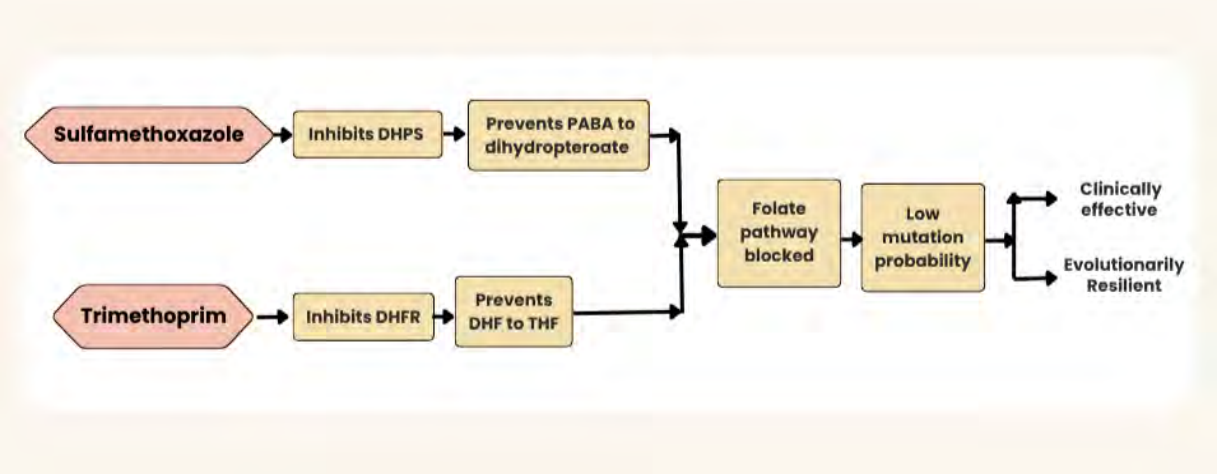

Figure 7: *Mechanism of Action for Sulfamethoxazole &Trimethoprim*

(The figure illustrates how sulfamethoxazole and trimethoprim synergistically inhibit DHPS and DHFR, blocking folate synthesis in bacteria. This dual action reduces mutation rates, enhancing clinical effectiveness and resistance prevention) [Created with Canva]

- Gentamicin and vancomycin work best together to demonstrate dual-pathway targeting's capacity to stop bacterial resistance and enhance treatment outcomes. Vancomycin disrupts the cell wall synthesis pathway by binding to the D-alanyl-D-

alanine termini of peptidoglycan precursors. This stops the transpeptidation and transglycosylation processes that are required for the development of bacterial cell walls. The cell wall's structural integrity is weakened as a result. Concurrently, gentamicin affects the protein synthesis process by permanently attaching itself to the 30S ribosomal subunit's A-site, which results in mRNA misreading and the creation of proteins that are misfolded or inoperable. The synergy occurs because gentamicin further inhibits the bacteria's ability to repair their damaged cell walls, while vancomycin-induced cell wall degradation promotes increased gentamicin absorption (Watanakunakorn & Bakie et al., 2014) .

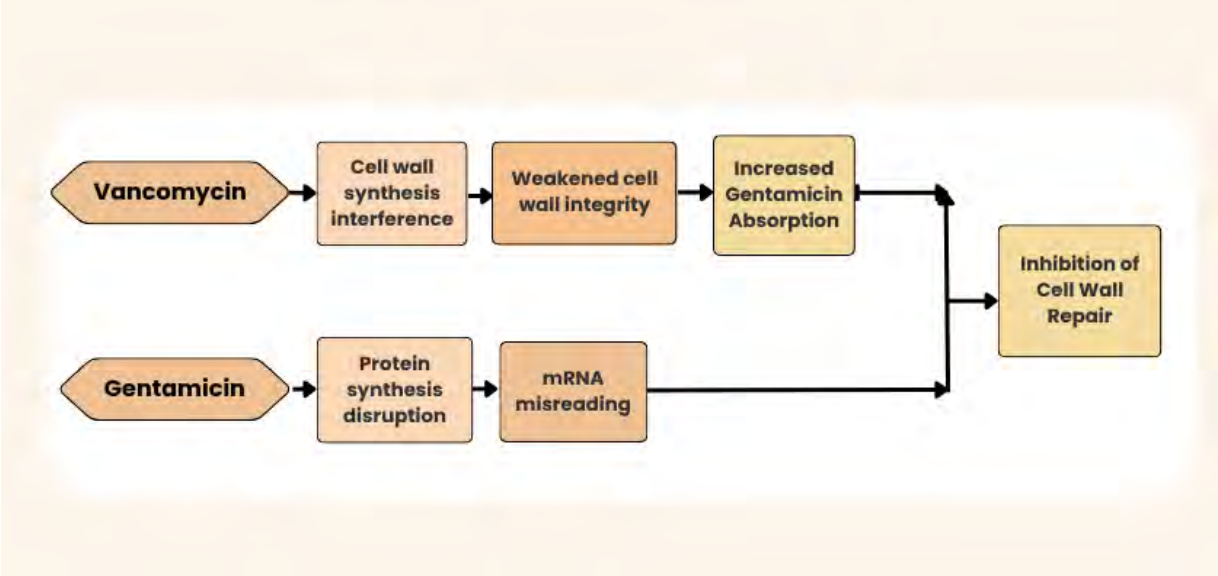

Figure 8: *Synergistic Action of Vancomycin and Gentamicin*

(Vancomycin weakens the bacterial cell wall, allowing more Gentamicin to enter and disrupt protein synthesis through mRNA misreading. Together, they block cell wall repair, leading to enhanced bacterial killing through dual-targeted action) [Created with Canva]

6.2 Blocking resistance mechanisms by Enzyme Inhibition

Reversible Acylation and Metallo-β-Lactamase Inhibition Mechanisms: In contrast to irreversible (suicidal) inhibitors, reversible acylation involves the formation of a covalent but reversible bond between the inhibitor and the enzyme. This allows for sustained inhibition

without permanently deactivating the enzyme. This method is demonstrated by avibactam, which targets serine β-lactamases and reversibly acetylation the enzyme's active site, temporarily deactivating its action. What makes avibactam particularly effective is its ability to undergo recyclization, a process that allows the molecule to detach and rebind repeatedly, thereby prolonging its inhibitory activity (Choi et al., 2016) This non-suicidal mechanism enhances its reusability and efficiency within the enzymatic cycle.

In contrast, metallo-β-lactamases (MBLs) such as NDM-1 present a different challenge, as they utilize zinc ions at their active site rather than serine residues. Traditional β-lactamase inhibitors are ineffective against MBLs. However, taniborbactam addresses this issue through a metal-chelating mechanism, binding directly to the zinc ion and thereby inhibiting the enzyme’s ability to hydrolyze carbapenems, an essential class of last-resort antibiotics (Sharma et al., 2020).

7.0 Clinical Applications of Combination Therapy

In the context of drug-resistant infections, combination antimicrobial treatment involves the simultaneous administration of two or more antimicrobial medicines. With the goals of increasing treatment efficacy, preventing the emergence of new resistance, and eventually improving patient outcomes, this therapeutic approach is commonly used in clinical practice when monotherapy proves ineffective (M. Zhou et al., 2024).

7.1 Use in the Management of Multidrug-Resistant Organisms

The phrase “multi-drug resistant” organisms (MDROs) describe illnesses, primarily caused by bacteria, that have developed resistance to numerous antibiotic classes, making treatment difficult. The most prevalent kinds are MRSA, which is resistant to beta-lactam antibiotics, including methicillin; VRE (Vancomycin-Resistant Enterococci), which are resistant to the antibiotic used as a last resort; and ESBL (Extended-Spectrum Beta-Lactamases) producing Enterobacteriaceae, which include Escherichia coli and Klebsiella pneumoniae, which can degrade a range of penicillin and cephalosporins. Of the subgroups of bacteria that are resistant to antibiotics, CRE are particularly concerning (Abat et al., 2018). This is because they are resistant to carbapenems, which are among the most potent antibiotics available.

- First, the term "superbug" is frequently used to describe MRSA, a strain of Staphylococcus aureus that has developed resistance to many antibiotics. Antibiotics such as penicillin, oxacillin, and methicillin usually function by attacking a protein that is necessary for the formation of bacterial cell walls (Vikesland et al., 2019). However, MRSA can manufacture a variant form of this protein called PBP2a because it possesses certain genes called mecA or mecC. Because of this changed protein's ineffective binding to certain antibiotics, MRSA is able to withstand therapy. The fact that MRSA

is resistant to more than simply methicillin makes it very deadly (Lee et al., 2018). Many strains of MRSA are also resistant to other antibiotic classes, including macrolides, which are used to treat skin and respiratory infections, and fluoroquinolones, which are commonly used to treat urinary tract infections. MRSA poses a significant risk in hospital environments due to its highly contagious traits and ability to spread through direct contact, contaminated surfaces, and medical equipment. Because MRSA may spread fast to individuals with surgical wounds, invasive equipment (like catheters), or compromised immune systems, hospitals and clinics are particularly vulnerable to MRSA outbreaks. MRSA infections can range in severity from simple skin and soft tissue infections (SSTIs) like boils and abscesses to more serious invasive diseases including bacteremia, osteomyelitis, necrotizing pneumonia, and endocarditis. Bloodstream infections have a mortality rate of about 20%, and if left untreated, these infections can develop into sepsis.

- Secondly, the bacteria known as VRE have developed the ability to withstand vancomycin, one of the most potent and final drugs that doctors use when other treatments fail. *Enterococcus faecalis and Enterococcus* faecium are two prevalent forms of VRE. These bacteria develop resistance due to genetic alterations (e.g., vanA or vanB) that enable them to modify their cell wall in a way that prevents vancomycin from attaching and killing them (M. O. Ahmed & Baptiste et al., 2018). VRE is mostly seen in hospitals, where it can be readily transmitted by contaminated surfaces, unclean medical equipment, or the hands of healthcare personnel. Patients who are already very weak, such as those undergoing chemotherapy, organ transplants, or long-term catheter users, are particularly at risk. As an illustration, consider a patient recovering after a kidney transplant at a hospital. They have a catheter in place and a weakened immune system (Cetinkaya et al., 2010). Dangerous diseases that is resistant to standard

antibiotics might result from VRE being transferred to them through a nurse's unwashed hands or a discarded medical instrument.

- Thirdly, Escherichia coli and Klebsiella pneumoniae are examples of bacteria that produce ESBLs, which are harmful enzymes. Penicillin, cephalosporins (like ceftriaxone), monobactams (like aztreonam), and other common antibiotics are broken down by these enzymes, making it very difficult to treat the bacterium (Ramatla et al., 2023). Because the genes that make ESBL are commonly carried on plasmids, which are small DNA fragments that may travel across bacteria, resistance can spread quickly among several bacterial species. Furthermore, among other medications, these bacteria commonly show resistance to aminoglycosides, trimethoprim-sulfamethoxazole, and fluoroquinolones (Mou et al., 2024). As a result, doctors are frequently forced to use carbapenems, which are strong antibiotics usually saved for extreme cases (Abera et al., 2024).
- Lastly, the CRE category includes *Escherichia coli and Klebsiella pneumoniae,* two prominent bacteria that have become resistant to carbapenems, which are frequently used as a last option to treat acute infections. The main cause of this resistance is the synthesis of enzymes known as carbapenemases, which degrade and render carbapenem antibiotics inefficient. Because they can cause potentially deadly diseases such bloodstream infections, pneumonia, and urinary tract infections, especially in immunocompromised people, CRE infections are a serious risk, especially in hospital settings (Park et al., 2024). Treating CRE infections is challenging due to a lack of effective medications. Even though drugs like colistin and tigecycline have been used in the past, their applicability has been limited because of rising resistance and toxicity issues. Recent studies have shown that ceftazidime-avibactam and other new antibiotics work better (Morrill et al., 2015).

For example, those who received ceftazidime-avibactam had a lower probability of dying within 14 days, according to studies comparing treatment results. CRE infections that are moderate to severe can now be treated with novel β-lactam/β-lactamase inhibitor combinations, including imipenem-cilastatin-relebactam, meropenem-vaborbactam, and ceftazidime-avibactam as first-line treatments (Zheng et al., 2023).

7.2 Recent Combination Therapy

A. **Piperacillin-tazobactam:** A potent antibiotic combination called piperacillin-tazobactam is used to treat severe bacterial infections. It functions as a team, with each medication playing a distinct role. The primary antibiotic that targets bacteria by damaging their cell walls is piperacillin. Piperacillin prevents bacteria from constructing or repairing their walls, which leads to their rupture and death. Because of this, it works extremely well against a wide variety of bacteria, particularly those that develop fast (El-Haffaf et al., 2021).

   Some bacteria can produce β-lactamase enzymes that degrade antibiotics, such as piperacillin, preventing them from working effectively. Tazobactam is useful in this situation. It works as a barrier, preventing these harmful enzymes and preserving piperacillin's effectiveness. In the absence of tazobactam, resistant bacteria may survive and carryon infecting people. Compared to piperacillin alone, the two medications together cover a far greater spectrum of microorganisms. Many Gram-negative bacteria (such as *E. Coli, Pseudomonas, and Klebsiella*), Gram-positive bacteria (such as *Streptococcus* and some *Staphylococcus*), and even anaerobes can be effectively prevented with this combination. In hospitals, where infections are more likely to be resistant to standard antibiotics, piperacillin-tazobactam is particularly helpful (Pacifici et al., 2023). In severe situations, it is administered intravenously (IV) for rapid effect.

Despite being potent, it is typically well tolerated, yet like all antibiotics, it occasionally has adverse effects such allergic reactions or diarrhea.

B. Amoxicillin-clavulanate: One common penicillin-type antibiotic that targets the bacterial cell wall is amoxicillin. Amoxicillin disrupts the formation of the peptidoglycan outer wall, which is essential for bacterial survival. By attaching itself to PBPs, which are crucial enzymes responsible for the development and repair of the bacterial cell wall, this process functions. Inhibiting these enzymes compromises the cell wall's structural integrity, especially while the bacteria are growing and dividing. Consequently, cell lysis brought on by the compromised cell wall results in bacterial mortality (Veeraraghavan et al., 2021).

Some bacteria create β-lactamase enzymes as a defense mechanism, which can break down amoxicillin before it can have an antibiotic impact. Clavulanate, also known as clavulanic acid, is essential in several situations. Despite having little antimicrobial action by itself, clavulanate protects amoxicillin by binding irreversibly to β-lactamase enzymes. Because of this inhibition, amoxicillin is unable to be broken down by enzymes, which keeps it effective against bacterial strains that might otherwise show resistance (J. Zhang et al., 2023). Both Gram-positive and Gram-negative bacteria are among the many pathogens that the amoxicillin/clavulanate combination effectively fights. It is active against the following Gram-positive pathogens: Streptococcus pneumoniae, which causes pneumonia and otitis media; Streptococcus pyogenes, which causes strep throat and skin infections; Enterococcus faecalis, which frequently causes urinary tract and abdominal infections; and β-lactamase-producing strains of Staphylococcus aureus, which do not include MRSA.

When it comes to Gram-negative bacteria, it works against Moraxella catarrhalis, which is linked to sinusitis and bronchitis, Escherichia coli, which is often involved in UTIs

(Urinary Tract Infection), though some resistant strains may avoid treatment, Klebsiella species, which can cause pneumonia and urinary tract infections, and haemophilus influenzae, which is a common cause of ear, lung, and sinus infections (Veeraraghavan et al., 2021).

C. **Polymyxins-carbapenems:** When treating severe infections brought on by MDR Gram-negative bacteria, especially carbapenem-resistant Pseudomonas aeruginosa and CRE, the combination of polymyxins and carbapenems (like meropenem, imipenem, or doripenem) is an essential last resort treatment. Clinicians have very few alternatives for treating these illnesses since they are resistant to almost all existing antibiotics, posing a severe danger to world health. Combining polymyxins and carbapenems is an essential strategy in treating life-threatening infections such as sepsis, ventilator-associated pneumonia, and complex intra-abdominal infections because of their synergistic interaction, which aids in overcoming resistance mechanisms (Samal et al., 2021).

   Polymyxins (e.g., colistin) exert their antibacterial activity by binding to lipid A, a critical component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria. Bacterial cell death results from this interaction because it weakens the integrity of the membrane, increasing permeability and allowing cytoplasmic contents to leak out. Additionally, by weakening the outer membrane barrier that would normally prevent drug penetration, polymyxins also make it easier for other antibiotics, including carbapenems, to enter the body (Y. Zhou et al., 2024). Carbapenems are β-lactam antibiotics that irreversibly bind to PBPs, blocking the cross-linking of peptidoglycan and thereby disrupting bacterial cell wall synthesis, leading to structural instability and cell lysis. However, many MDR Gram-negative bacteria produce carbapenemases enzymes such as KPC, and OXA-48 that degrade carbapenems and

neutralize their activity. Polymyxins, by disrupting the outer membrane, enhance the ability of carbapenems to bypass porin-related resistance and penetrate bacterial cells more effectively, even in the presence of some carbapenemases. This cooperative mechanism explains the synergistic effect of polymyxin–carbapenem combination therapy (Chen et al., 2024).

8.0 Challenges and Limitations

Combination antibiotic treatment has the potential to treat resistant infections, but it also has significant drawbacks, including complicated pharmacokinetics and antagonistic interactions. When some antibiotics, such as particular beta-lactams, interfere with one another's efficacy, antagonism may result. Sometimes, combination therapy may trigger beta-lactamase production, reducing the overall effectiveness of the antibiotics (Mehta et al., 2014). Pharmacokinetic challenges can significantly impact patient care, particularly in critically ill individuals. Alterations in physiological processes can disrupt how drugs are absorbed, transported, metabolized, or eliminated from the body. As a consequence, patients may receive inadequate levels of antibiotics, undermining the effectiveness of their treatment and heightening the risk of dangerous drug interactions. In critical care settings, these issues are not merely academic; they pose serious threats to patient safety and can lead to detrimental therapeutic outcomes. Addressing these challenges is vital for ensuring optimal care and improving patient recovery (Cattaneo et al., 2022).

Although combination antibiotics may provide some benefits, they also present serious risks that cannot be overlooked, with the elevated risk of toxicity being the most alarming. For instance, adolescents receiving combination treatment for gram-negative bacteremia face a significantly higher likelihood of nephrotoxicity compared to those on monotherapy, highlighted by an adjusted odds ratio of 2.15. This stark difference underscores the importance of carefully weighing the advantages against the potential dangers of combination therapy (Tamma et al., 2013). When comparing treatment methods, the data reveals a striking contrast: monotherapy results in an acute kidney injury rate of only 6.2%, while combination treatment nearly quadruples this risk, leading to an alarming incidence of 23.4% in patients with Staphylococcus aureus bacteremia (Ye et al., 2021).

The cost and availability of combination antibiotic treatments present formidable challenges, particularly in resource-limited settings. These therapies often rely on multiple newer antibiotics, which tend to be prohibitively expensive and difficult to procure. In LMIC, where access to advanced pharmaceuticals and essential healthcare resources is severely restricted, combination therapy frequently proves to be economically unviable. Alarmingly, these very regions also bear the brunt of the highest rates of antibiotic resistance, driven by misuse, insufficient regulations, and a lack of diagnostic capabilities. As a result, despite the significant advantages that combination therapy could offer, it remains an impractical option in many high-need areas, necessitating urgent action to enhance access and affordability (Lee Ventola et al., 2019).

9.0 Future directions

New combination drugs are being developed to address the growing threat of AMR. These strategies involve using two or more medications that work together to improve treatment outcomes, reduce the emergence of resistance, and enhance the effectiveness of bacterial elimination. The combinations may include approved antibiotics or alternative agents, such as antimicrobial peptides, β-lactamase inhibitors, efflux pump inhibitors, or bacteriophage-derived drugs. For example, the combination of colistin and rifampicin is effective due to synergistic action, where colistin disrupts the outer membrane of *Acinetobacter baumannii*, enhancing permeability and allowing rifampicin to enter and inhibit RNA synthesis. This synergy improves activity against multidrug-resistant strains. *A. baumannii* is a critical hospital-acquired pathogen known for its resistance to multiple antibiotics, ability to survive in harsh environments, and involvement in severe infections such as pneumonia and bloodstream infections, making it a significant global health threat (Huttner et al., 2015). These therapies not only enhance access to vital healthcare but also have the potential to revive the effectiveness of antibiotics previously thought to be ineffective. To ensure their successful implementation in clinical settings, it is imperative to develop a deep understanding of their PK/PD, possible toxicities, and mechanisms of interaction. By doing so, we can maximize their benefits and transform patient outcomes.

Researchers are discovering and developing new drug combinations to combat antibiotic resistance using artificial intelligence (AI) and machine learning (ML). AI and ML are emerging as powerful tools in combating antibiotic resistance by enabling faster and more accurate discovery of new antibiotics and synergistic drug combinations. These technologies analyze large datasets including chemical structures, microbial genomics, resistance mechanisms, and drug–drug interaction data to predict effective antibiotic pairs that may not

be obvious through traditional methods. One key contribution is in identifying synergistic combinations (Stokes et al., 2020). AI models can simulate bacterial responses to multiple antibiotics, predict interactions, and recommend optimal pairings (Elalouf et al., 2025). For instance, a deep learning model developed by MIT researchers successfully identified that the combination of halicin (a newly discovered antibiotic) and other non-antibiotic compounds had strong activity against *E. coli*. This would have been extremely time-consuming using conventional lab screening methods (Smith et al., 2020).

Another practical example involves colistin and meropenem: ML algorithms, by analyzing thousands of bacterial growth and resistance profiles, predicted this combination would be highly effective against carbapenem-resistant *Klebsiella pneumoniae*. Lab validation later confirmed their synergistic killing effect. A specific model type used in this field is Multiple Instance Learning (MIL). MIL is valuable when precise labels for individual data points (e.g., molecular features or bacterial responses) are unknown, but broader group labels are available (Blasse et al., 2017). MIL algorithms can then infer which specific features within a group contribute to the observed synergy, enabling more targeted and explainable predictions for drug combinations. In summary, AI and ML, including models like MIL, are revolutionizing antibiotic discovery by accelerating the identification of synergistic therapies, helping overcome resistance, and guiding precision medicine approaches in infectious disease treatment.

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