Novel Approaches for Combating Antibiotic Resistance in Pathogenic Bacteria

Musharrat Jahan Prima [1*], Masriana Hassan [2], Juhi Sharma [3]

Abstract

Antibiotic resistance has historically been mostly linked to the established mechanisms such target alteration, efflux pumps, and enzymatic inactivation. Also, the ability of bacteria to form biofilm is a formidable challenge to antibiotic efficacy. The extracellular matrix forms a protective barrier that renders most antibiotics ineffective, by limiting the interaction of the drug to its target. The emergence of novel antibiotic resistance mechanisms in pathogenic bacteria and its rapid propagation, by means of horizontal gene transfer or mobile genetic elements, is a constant challenge to the medical community. Bacteria are able to exchange genetic material, conferring to antibiotic resistance, at a startling rate that helps them adapt quickly. This is especially dangerous when antibiotics use is abused. The field of antibiotic resistance has experienced a notable shift in the past few years, owing to the identification of new resistance mechanisms utilized by pathogenic bacteria to impede the effectiveness of antibiotics. Over the past decade researchers have been rigorously studying these resistance mechanisms and working on strategies to overcome the ordeal. Notably, nanoparticles research targeting antibiofilm therapy to combat the antibiotic-resistance mechanisms has been truly innovative. There have also been positive reports on utilizing the CRISPR-Cas as a promising next-generation antibiotic that can be used to eliminate antibiotic resistant genes (ARGs) and resensitize bacterial populations to antibiotics. This review aims for a comprehensive understanding of the ongoing research on antibiotic resistance mechanisms and strategies to counter them, the remaining challenges for widespread clinical implementation and the importance of continued research.

Keywords: Antibiotic-resistance mechanism, Novel mechanisms, Pathogens, Nanoparticles

Significance | Overview of novel antibiotic approaches to combat alarming antibiotic-resistance mechanisms.

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Introduction

Modern medicine has been greatly impacted by antibiotics, which have revolutionized the treatment of bacterial infections and had a profound effect on public health. A well-known turning point in the development of antibiotics was Alexander Fleming's 1928 discovery of penicillin (Fleming, 1929). With penicillin's rapid development as an essential tool for treating a variety of bacterial infections, this coincidental discovery signaled the start of a new age in healthcare. Antibiotics are essential to medicine since they have helped lower death rates and extend life expectancy (Spellberg et al., 2008). Their significance cannot be emphasized. Antibiotic-resistant bacteria, however, have emerged as a major worldwide concern, according to the World Health Organization's

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(WHO) 2020 report on global antibiotic resistance (World Health Organization, 2020). Bacteria have existed on the planet long before humans which allowed them to master elaborate mechanisms to evade toxic compounds, such as unique membranes, efflux systems to remove harmful compounds from cells, and the ability to divide rapidly and enter the senescent phase (as seen in biofilms and persisters) with reduced metabolism that helps give tolerance to antibiotics (Cook & Wright, 2022). This review emphasizes the need for quick action to address the growing threat of antibiotic resistance and its implications for public health. The efficacy of antibiotics is seriously threatened by the abuse and overuse of these medications, which has sped up the creation of resistant bacterial strains (Ventola, 2015). The incorrect use of antibiotics in both clinical and non-clinical contexts is one of the reasons that contributes to antibiotic resistance. According to a Costelloe et al. (2010) study, antibiotic prescriptions are frequently given needlessly, particularly for viral infections.

Llor and Bjerrum (2014) pointed out, that patient noncompliance with antibiotic prescriptions permits bacteria to thrive and evolve resistance. Antibiotic resistance has an impact on human health, but it also affects agriculture. Antibiotic-resistant bacteria are more likely to enter the food chain as a result of the misuse of antibiotics in agriculture, which can leave antibiotic residues in animal products (Van Boeckel et al., 2015). This highlights the connection between the usage of antibiotics across other areas and presents a possible risk to consumers. In conclusion, antibiotics have been a cornerstone of modern medicine, saving countless lives and transforming healthcare. However, the emergence of antibiotic-resistant bacteria poses a significant global concern, as highlighted by the World Health Organization and supported by various studies. Responsible antibiotic use, research into new treatments, and innovative alternatives are essential components of the collective effort required to address antibiotic resistance and preserve the efficacy of these vital drugs. The legacy of antibiotics, as outlined in the referenced literature, remains central to public health and warrants ongoing attention and action.

Microbial Resistance to Antibiotics

The purpose of antibiotics is to prevent the microbes from harming the host, either by killing them or by stopping their growth. Antibiotics are categorized based on the mechanism of their antimicrobial activity, which are: inhibition of cell wall synthesis, depolarization of the cell membrane, inhibition of protein synthesis, inhibition of nucleic acid synthesis, and inhibition of metabolic pathways in bacteria (C Reygaert, 2018). How do the bacteria still manage to evade all these systems and continue to survive? To put it simply, resistant bacteria emerge under selective pressure, primarily by mutation rather than by horizontal gene transfer (HGT) as a result of being exposed to long-term antibiotic drug therapy (Andersson et al., 2020).

To discuss further about antibiotic resistance, we have to understand the mechanisms by which they manage to evade antibiotic activities. Bacteria have a natural resistance, which may be intrinsic or induced, under genes they already possess, and acquired resistance, which may be through mutation in their gene or horizontal gene transfer utilizing transformation, transposition, and conjugation (C Reygaert, 2018).

General Mechanisms of Antimicrobial Resistance

There are four primary types of antimicrobial resistance mechanisms: (1) drug uptake limitation; (2) drug target modification; (3) drug inactivation; and (4) active drug efflux. Natural resistance may employ limiting drug uptake, drug inactivation, and drug efflux, whereas, acquired resistance may employ drug target modification, drug inactivation, and drug efflux. Due to the obvious structural differences between gram-negative and gram-positive bacteria, they use different kinds of resistance mechanisms. Gram-positive bacteria lack the capacity for some types of drug efflux mechanisms and less frequently employ limiting drug uptake due to their lack of an LPS outer membrane. Gram-negative bacteria, on the other hand, use all four primary mechanisms (Chancey ST, Zähner D, 2012). Illustrates the general antimicrobial resistance mechanisms, Figure (C Reygaert, 2018).

Limiting drug uptake

As was previously indicated, bacteria naturally differ in their capacity to restrict the absorption of antimicrobial drugs. Certain types of chemicals are blocked from entering gram-negative bacteria due to the composition and functions of the LPS layer. As a result, the bacteria have an intrinsic resistance to specific classes of potent antibiotics (Piddock et al., 2014). Due to the high lipid content of the mycobacteria's outer membrane, hydrophobic medications—like rifampicin and fluoroquinolones—had easier access to the cell, while hydrophilic medications have less access (Kumar et al,2005). Substances frequently enter bacteria with broad outer membranes through porin channels. Hydrophilic compounds can typically enter gram-negative bacteria through their porin channels (Piddock et al., 2014). Due to the high lipid content of the mycobacteria's outer membrane, hydrophobic medications—like rifampicin and fluoroquinolones—had easier access to the cell, while hydrophilic medications have less access (Kumar et al,2005). Substances frequently enter bacteria with broad outer membranes through porin channels. Hydrophilic compounds can typically enter gram-negative bacteria through their porin channels (Piddock et al., 2014). Reduced quantity of porins and mutations altering the selectivity of the porin channel

are the two main ways that alterations in porins can restrict drug uptake (Kumar et al., 2005). It is known that members of the *Enterobacteriaceae* family develop resistance as a result of producing fewer porins and occasionally none at all. These bacteria together lower the quantity of porins as a defense against carbapenems (Mazzariol et al., 1996). Changes in the porin channel due to mutations have been observed in *Neisseria gonorrhoeae*, which becomes resistant to β-lactams and tetracycline, and *E.aerogenes*, which becomes resistant to imipenem and certain cephalosporins (Hattawi K, et al, 1998).

Modification of drug targets

Numerous parts of the bacterial cell might be targets for antimicrobial treatments, and an equal number of targets could be altered by the bacteria to make them resistant to those medications. Changes in the number and/or structure of PBPs (penicillin-binding proteins) are one way that gram-positive bacteria develop resistance to β-lactam antibiotics, which are used almost exclusively by them. PBPs are transpeptidases that help the cell wall's peptidoglycan to form. The quantity of drug that can bind to that target is affected by a change in the number of PBPs (an increase in PBPs with a decreased drug binding ability, or a decrease in PBPs with normal drug binding). A structural alteration (e.g., PBP2a in *S. Aureus* by *mecA* gene acquisition) may reduce or completely prevent a drug's capacity to bind (Reygaert et al., 2009). Drugs that target the ribosomal subunits can cause resistance through ribosomal mutation (found in aminoglycosides and oxazolidinones), ribosomal subunit methylation (found in aminoglycosides, macrolides—a class of antibiotics used to treat gram-positive bacteria, oxazolidinones, and streptogramins), or ribosomal protection (found in tetracyclines). These processes obstruct the medication's capacity to attach to the ribosome. Among these systems, there are wide variations in the degree of medication interference (Mukherjee et al, 2013). Resistance to medications that target nucleic acid synthesis (such as fluoroquinolones) arises from changes in DNA gyrase (found in gram-negative bacteria, such as *gyrA*) or topoisomerase IV (found in gram-positive bacteria, such as *grlA*). The drug's capacity to bind to gyrase and topoisomerase is reduced or eliminated as a result of these alterations, which alter their structural makeup (Hawkey et al., 2003).

Drug inactivation

Drugs can be rendered inactive by bacteria in two major ways: either by physically breaking down the drug or by adding a chemical group to the drug. The β-lactamases are a very large group of drug hydrolyzing enzymes. Tetracycline is another medication that can be rendered inactive by hydrolysis through the tetX gene. (Mukherjee et al, 2013)

The most prevalent chemical groups transferred to the drug during drug inactivation are acetyl, phosphoryl, and adenyl. The number of transferases that have been identified is quite high. The most versatile method, acetylation, has been shown to work against aminoglycosides, fluoroquinolones, streptogramins, and chloramphenicol. It is well-known that phosphorylation and adenylation are mostly employed to combat aminoglycosides (Blair et al.,2015)

β-lactamases

β-lactam antibiotics are the class of antimicrobial medications that are most frequently utilized. This class of drugs is characterized by a common core structure: a β-lactam ring with four sides. There are three general pathways by which resistance to β-lactam medicines occurs: (1) blocking the drug's ability to bind to the target PBP, usually by changing the drug's existing PBPs or acquiring new ones; (2) having efflux pumps that can extrude β-lactam medications; and (3) the drug being hydrolyzed by β-lactamase enzymes (Cullik et al, 2010). Based on their functional properties and/or molecular structure, the β-lactamase enzymes are categorized. They are divided structurally into four major groups (A, B, C, or D). Based on substrate specificity, β-lactamases can be classified into three functional groups: metallo (zinc-dependent) β-lactamases, serine β-lactamases, and cephalosporinases. These enzymes may also be well-known by the name of their family of enzymes, such as the SHV (sulphydryl variable) family, the CTX (preferentially hydrolyze cefotaxime) family, and the TEM family (named after the first patient). All four structural categories of β-lactamases are capable of being produced by gram-negative bacteria. Gram-positive bacteria primarily include β-lactamases from group A, however, group B β-lactamases are also present (Schultsz et al., 2012). β-lactamases, which are mainly present in *Enterobacteriaceae*, have been observed to be active against carbapenems, also known as carbapenemases, as of late. The term "carbapenem-resistant *Enterobacteriaceae*" (CRE) enzymes refers to an enzyme class that includes the *Klebsiella pneumoniae* carbapenemases (KPCs) and others. The KPCs are resistant to all β-lactam medications and are classified as serine Class A (functional group 2f) β-lactamases; nonetheless, they can still be impacted by β-lactamase inhibitors. The carbapenemases in CRE strains of bacteria are all metallo-β-lactamases (MBLs) in Class B, functional group 3a. They are not inactivated by β-lactamase inhibitors while being able to hydrolyze all β-lactam medications. The kinds VIM-1 (Verona integron encoded MBL) and IMP-1 (for imipenem resistance) are the most often distributed CREs. Recently, a novel MBL was discovered, mostly in E. Coli strains. It is now known as NDM-1, or New Delhi MBL. Hospital death rates of up to 71% have been linked to CRE strain infections. (Cullik et al, 2010)

Drug efflux

Genes for efflux pumps are chromosomally encoded in bacteria. Certain cues in the environment or the presence of a suitable

substrate can cause some to express constitutively, while others are induced or overexpressed (high-level resistance is typically caused by a mutation that changes the transport channel). The primary purpose of efflux pumps is to remove harmful substances from the bacterial cell; many of these pumps—known as multi-drug (MDR) efflux pumps—can move a wide range of substances. What carbon source is available affects several of these pumps' resistance capacity (Richmond et al., 2014). The majority of bacteria have a wide variety of efflux pumps. The ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family are the five main families of efflux pumps in bacteria, which are categorized based on structure and energy source. The majority of these groups of efflux pumps are one-component pumps that move substrates through the membrane of the cytoplasm. Due to their chromosomal encoding, efflux pumps present in gram-positive bacteria may confer intrinsic resistance. These pumps include members of the MATE and MFS families and efflux fluoroquinolones. There are also gram-positive efflux pumps known to be carried on plasmids. Currently, the characterized pumps in gram-positive bacteria are from the MFS family (Amaral L, et al,2013). Efflux pumps found in gram-negative bacteria are widely distributed and may come from all five of the families, with the most clinically significant pumps belonging to the RND family (Richmond et al,2014).

New Challenges of Antibiotic Resistance

Antibiotic resistance is a global health emergency that seriously jeopardizes our capacity to successfully treat bacterial illnesses. The development of resistance mechanisms has put the medical world under pressure to come up with new ideas for dealing with this pressing issue. The most recent research on novel mechanisms of antibiotic resistance is examined in this review of the literature, providing insight into how bacteria become resistant to antibiotics and the implications for the medical community.

Biofilm Formation

One well-established mechanism of antibiotic resistance that has received a lot of attention lately is biofilm formation. Bacteria encased in an extracellular matrix that they manufacture on their own form intricate, ordered communities known as biofilms (Hall-Stoodley et al., 2004). Antibiotics and immune system cells are unable to penetrate this matrix, which functions as a barrier. Treatment for illnesses linked to biofilms is notoriously challenging because the structure protects bacteria from the entire range of antibiotic effects. Planktonic bacteria first connect to a surface during the biofilm formation process, and then the extracellular matrix develops, resulting in the construction of a resilient and dynamic microbial community (Flemming et al., 2016). Innovative tactics are being investigated by researchers to fight infections linked to biofilms. These tactics include applying antimicrobial coatings, nanoparticles, and enzymes that destroy biofilms to increase the effectiveness of antibiotics on bacteria that are encased in biofilms. Furthermore, knowing the metabolic alterations that take place in biofilms is essential for creating tailored treatments (Percival et al., 2015).

Horizontal Gene Transfer

The dynamic mechanism of horizontal gene transfer is essential to the quick spread of genes that confer antibiotic resistance. Through conjugation, transformation, and transduction, bacteria can pick up resistance genes from different strains or species of bacteria (Bennett, 2008). This method makes it difficult to stop the emergence of antibiotic resistance and enables bacteria to quickly adapt to new drugs. The spread of plasmids with extended-spectrum beta-lactamase (ESBL) genes among *Enterobacteriaceae* is a well-known instance of horizontal gene transfer. Penicillins and cephalosporins are just two of the many medications that ESBL-producing bacteria are resistant to (Munita and Arias, 2016). Developing methods to stop the spread of resistant genes requires an understanding of the dynamics of horizontal gene transfer.

Antibiotic Tolerance and Target Alteration

Bacteria use complex methods to change the way antibiotics work or the targets of their drugs. The synthesis of enzymes that chemically alter antibiotics to make them inactive is one well-known example. Antibiotic targets can also be changed by bacteria to lessen their susceptibility to drug binding. One of the most common examples of these changes is the methylation of ribosomal RNA, which lessens the potency of antibiotics like macrolides. These alterations change the binding site of the antibiotic, making it less efficient in suppressing bacterial protein synthesis (Novak and Frimodt-Møller, 2018). It is essential to comprehend these alterations to create novel antibiotics that can evade these resistance mechanisms. Researchers are working hard to understand how antibiotic modification mechanisms are based on enzymatic and genetic processes. The goal of this research is to find weaknesses in these systems that could be used to create novel antibiotics or combinations of antibiotics (Liu et al., 2020).

Persister Cells

Persister cells are a relatively new finding in the realm of antibiotic resistance. This is a tiny subset of bacteria that can go into dormancy and develop a strong resistance to antibiotics. Antibiotics kill most of the bacterial population, while persister cells endure, making the elimination of infections extremely difficult (Fisher et al., 2017). Investigating persister cell formation's underlying genetic and physiological mechanisms is the goal of persister cell research. Future study in this area is

intriguing because it will help us understand how bacteria convert to a persister form and develop techniques to target these cells. According to Conlon et al. (2016), strategies could involve the creation of novel antibiotics that target persister cells specifically or the application of combination therapies that make persister cells vulnerable to antibiotics.

Comprehending these pathways is essential to creating novel approaches to address antibiotic resistance. Subsequent investigations ought to focus on creating innovative antibiotics, adjuvants, and treatments that specifically target these processes.

Novel research to Address Novel Antibiotic Resistance

Surveillance is essential to prevent the development of antibiotic-resistant strains and ultimately protect the public's health (World Health Organization, 2020). For the identification of novel antibiotic resistance genes and mechanisms, and the development of novel drugs genomics, transcriptomics, and metagenomics, coupled with functional studies are of paramount importance (Sekyere & Asante, 2018). Diverse strategies and innovations are being employed including the development of new antibiotics, antibiotic stewardship programs, vaccines, Fecal Microbiota Transplantation (FMT), and alternative therapies. Besides this, novel strategies using CRISPR-Cas systems and nanoparticles are being investigated as potential antimicrobials. These multifaceted solutions are discussed in this section.

Development of New Antibiotics

One of the primary strategies in the fight against antibiotic resistance is continuing to discover new antibiotics. Researchers are dedicated to finding new antibiotic drugs because resistant bacteria are constantly emerging in this day and age. Creative methods entail investigating uncommon sources, including extremophiles, which flourish in harsh environments and generate non-traditional antibacterial substances (Doe et al., 2022). These substances may be able to fight germs that are immune to traditional antibiotics. The method of finding new drugs has been completely transformed by developments in artificial intelligence and synthetic biology (Smith & Johnson, 2021). Antibiotics can now be precisely designed by researchers to target particular bacterial vulnerabilities. This technology-assisted method has expanded the arsenal of antibiotics available to medical professionals by resulting in the development of whole new classes of antibiotics. The path to novel antibiotics is paved with obstacles, even despite these encouraging advancements. Medication development is a time-consuming and expensive process, and pharmaceutical corporations have few financial incentives. In addition, there is a never-ending race to stop new resistant strains from emerging. However, to reduce antibiotic resistance, efforts must be made to find and create new antibiotics.

Antibiotic Stewardship Programs

To address antibiotic resistance, antibiotics must be used responsibly in hospital settings. Programs for the stewardship of antibiotics have grown in popularity recently. These initiatives aim to decrease needless prescriptions while encouraging the judicious use of antibiotics (Brown & Smith, 2020). They usually entail several interventions, such as protocols, on-the-spot observation, and feedback systems. Reducing the overuse and abuse of antibiotics in clinical practice is one of the main goals of antibiotic stewardship initiatives. According to Jones et al. (2019), these strategies have demonstrated notable efficacy in decreasing the aggregate usage of antibiotics within healthcare environments. These initiatives help to maintain antibiotic efficacy by enhancing prescribing practices and reducing the selection pressure for resistance.

Vaccines: Immunizations have been essential in preventing bacterial and viral infectious illnesses. The creation of vaccinations against bacteria that are resistant to antibiotics is gaining traction in light of antibiotic resistance (Brown & Smith, 2020). The purpose of these vaccines is to protect against particular strains of bacteria that are resistant to antibiotics. The ability of vaccines to lessen the initial requirement for antibiotics is one of its benefits. They protect people from diseases, but they also stop resistant germs from spreading throughout communities (Johnson et al., 2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) vaccine development, for instance, has shown promise in reducing the harmful effects of this well-known antibiotic-resistant bacteria.

Fecal Microbiota Transplantation (FMT)

Fecal Microbiota Transplantation (FMT) has become a cutting-edge, if indirect, strategy to combat antibiotic resistance. FMT is a medical procedure that was first designed to treat patients with recurrent *Clostridium difficile* infections (Anderson et al., 2018). The procedure entails giving a patient fecal material from a healthy donor. Restoring a diversified and well-balanced gut microbiota is the main objective. The theory underlying FMT is that a balanced gut microbiota guards against infections and supports general health. FMT directly aids in the battle against antibiotic resistance by reviving the gut flora. It accomplishes this by reducing the requirement for protracted antibiotic courses, which may alter the gut microbiota and favor the growth of bacteria resistant to antibiotics (Smith et al., 2019). Combating antibiotic resistance necessitates a diverse strategy. The creation of novel antibiotics, the execution of antibiotic stewardship initiatives, the investigation of complementary and alternative medicine, the creation of vaccines, and the creative application of FMT have all received attention in recent years. Despite the difficulties and restrictions associated with each tactic, taken as a whole, they provide the means to maintain the efficacy of antibiotics in the face of changing microbiological threats. To fight this serious

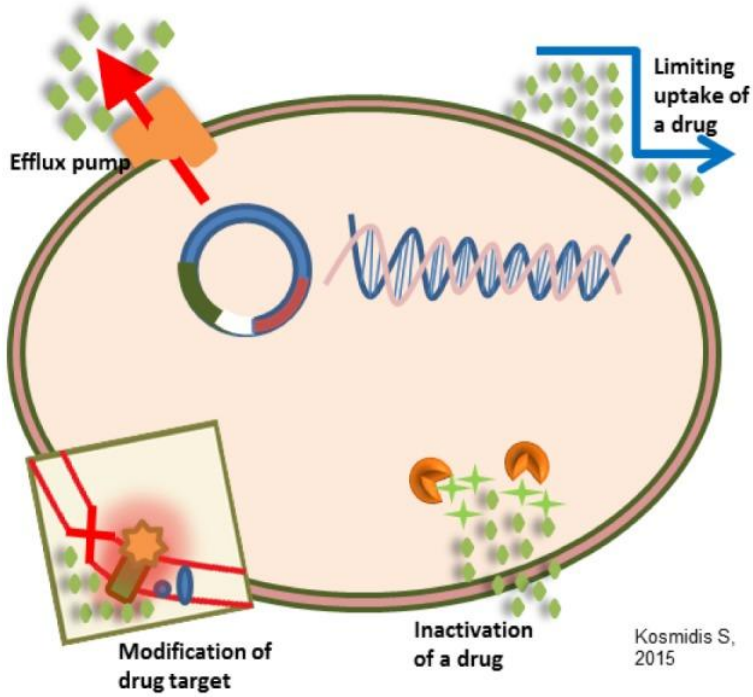

Figure 1. Mechanism of Resistance

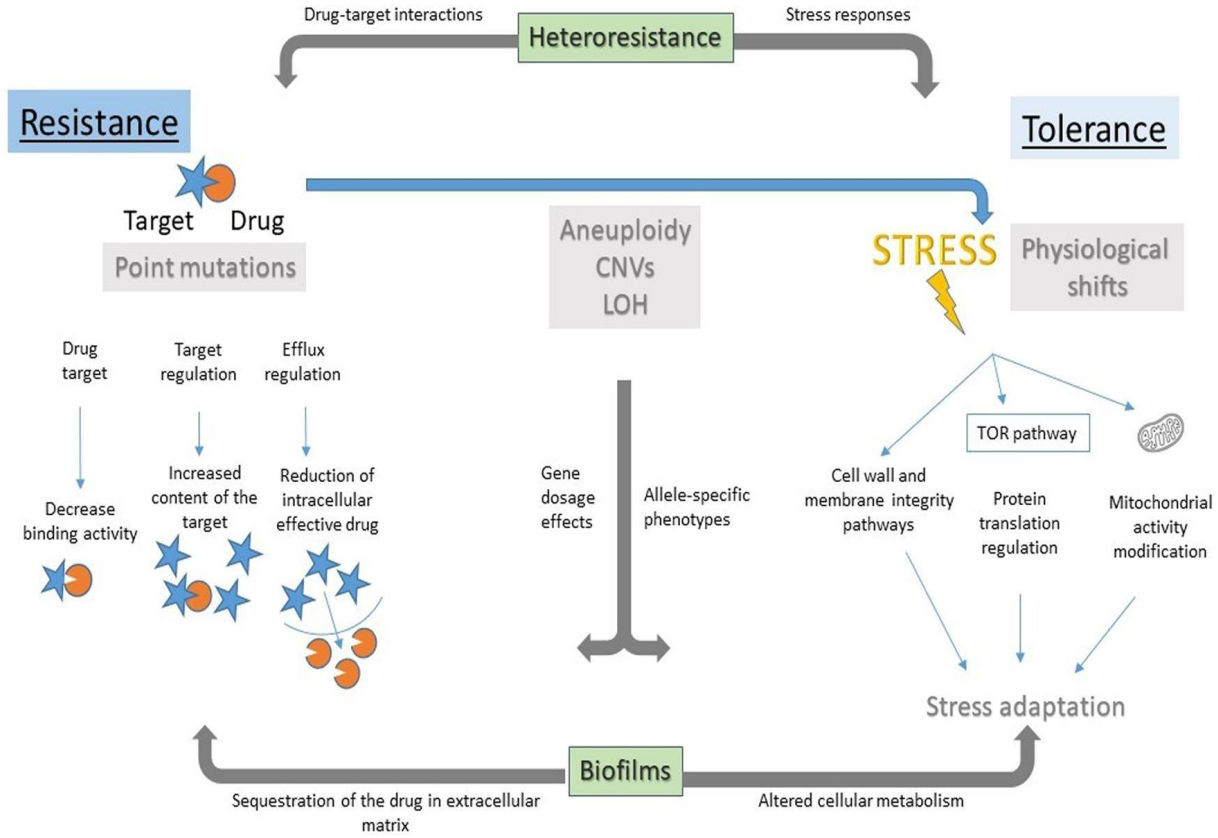

Figure 2. Antibiotics Resistance

global health issue, academics, medical professionals, and politicians must keep up their efforts.

Alternative Therapies

Antibiotic resistance may be addressed by alternative medicines, which are becoming more and more viable. In particular, phage therapy has drawn interest as a substitute for conventional antibiotics. Bacteriophages, viruses that infect and destroy particular strains of bacteria, are used in this process. Because phage therapy may precisely target pathogens, it is very attractive (Garcia & Martinez, 2021). Phages, in contrast to broad-spectrum antibiotics, only kill the bacteria they are designed to target, sparing helpful microbes. Scholars are currently investigating bacteriocins and antimicrobial peptides as potential alternatives to phage therapy (**White & Lee, 2022**). Naturally occurring proteins called bacteriocins are made by bacteria to stop the growth of closely related strains. They present an opportunity to treat bacterial infections with the possibility of preventing widespread antibiotic resistance. Antimicrobial peptides, on the other hand, are naturally occurring molecules with broad-spectrum antibacterial properties.

CRISPR-Cas systems

The clustered regularly interspaced short palindromic repeats (CRISPR) CRISPR-associated protein (Cas) system is a novel and promising tool against the growing concern of multidrug resistance prevalence (Bikard & Barrangou, 2017). CRISPR/Cas9 has high specificity to precisely destroy the drug/antibiotic resistance gene in bacteria and resensitize them to antibiotics. This gene-editing tool in combination with nanotechnology may be the solution to overcome the hurdles of traditional drug delivery methods. While this strategy shows promise, it has its limitations, hence continued dedicated research is needed. Successful compatible customized nanoparticle delivery systems for the CRISPR/Cas9 components are essential for their efficacy and safety, which can then be considered for marketing (Wan et al., 2021). The principle of the CRISPR-Cas gene editing system is that the RNA-based spacer will guide the Cas proteins to target and break DNA that complements the spacers. The CRISPR-Cas9 system can be used to neutralize antibiotic resistance genes in the selected bacterial population without killing the beneficial bacteria in wild-type populations, by designing guide RNAs against specific antibiotic resistance genes (Kim et al., 2016). Some notable studies on CRISPR/Cas systems and their antimicrobial activities are briefly mentioned in this section.

Yosef et al. strategized a programmed CRISPR-Cas9 system to eliminate the horizontal transfer of antibiotic resistance genes between bacteria. The CRISPR-Cas9 system was programmed to destroy plasmids carrying beta-lactamase resistance genes $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$ in E. coli and delivered using temperate and lytic phages. Strains transfected with this recombinant phage acquire resistance to lytic phages, having a selective advantage over resistant strains when treated with the same type of phage. The idea is to make bacteria sensitive to antibiotics and thereafter kill non-sensitized bacteria with lytic phages (Levy et al., 2015). In a recent study, Rodrigues et al. constructed a conjugative plasmid pPD1 with a complete, constitutively expressed CRISPR-Cas9 targeting cassette, that transfers into *Enterococcus faecalis* to selectively remove *ermB* (encoding erythromycin resistance) and *tetM* (encoding tetracycline resistance), where the transformants significantly reduced the prevalence of the resistant bacteria (Rodrigues et al., 2019). The CRISPR-Cas9 system was also reported to cleave epidemic carbapenem-resistant plasmids, such as bla_{KPC}-harboring IncFIIK-pKpQIL, IncN pKp58_N, and bla_{NDM}-harboring IncX3 plasmids, through disrupting the partition gene *parA* in *K. pneumoniae* (Hao et al., 2020). Apart from the CRISPR-Cas9, Kiga et al. developed a CRISPR-Cas13a-based antimicrobial system in a bacteriophage capsid that is capable of effectively killing carbapenem-resistant *E. coli* and methicillin-resistant *S. aureus* targeting to sequence-specific antimicrobial resistance genes (Kiga et al., 2020). Some reports have shown the use of CRISPR-Cas system as a novel antimicrobial agent, where the acquisition of host chromosomal DNA by the CRISPR-Cas system is cytotoxic, which can result in cell death because of the excision of the genome (Hao et al., 2020).

Nanoparticles

Nanoparticles (NPs) are a novel nanotechnology-based innovation designed to address pathogenic microorganisms. Based on their different traits, including size, morphology, electrical charge, and surface coatings, they can be used in many ways to develop novel antimicrobial substances (Mubeen et al., 2021). They are being used to improve the delivery of antimicrobial agents and act as novel antimicrobial materials that are different from traditional drugs (Santos et al., 2018). NPs mostly employ two mechanisms as promising antimicrobial agents against bacteria by, (i) disrupting membrane potential and integrity and (ii) inducing oxidative stress by reactive oxygen species (ROS) generation catalyzed by NPs (Wang et al., 2017). The nanomaterials are classified into four categories, carbon-based nanomaterial, inorganic nanomaterial, organic-based nanomaterial, and composite-based nanomaterial. From them, carbon-based and inorganic nanoparticles have antimicrobial activities that can be used in research, medicine, and industry (Mubeen et al., 2021).

The carbon-based NPs mainly have carbon and are found in spheres, ellipsoids, and hollow tubes. The types of carbon-based NMs include Fullerenes (C60), Carbon Nanotubes (CNT), Carbon nanofibers, Carbon Black, and Graphene (Gr), whose antimicrobial activities have been studied by many researchers (Kumar & Kumbhat, 2016). Among the inorganic nanoparticles silver NP and gold NP, are notable for their bactericidal effect and

photothermal activity, respectively (Wan et al., 2021; Yougbare et al., 2019). Many reports stated metal oxide NP of titanium dioxide (TiO_2) showed a similar bactericidal mechanism as AgNPs, killing both Gram-positive and Gram-negative bacteria (Tahir et al., 2016). However, antimicrobial Zinc oxide (ZnO) NP is safer and has better biocompatibility with human cells (Siddiqi et al., 2018). Polymeric/organic NPs can also kill bacteria by interacting with their cell walls (Wan et al., 2021).

Moreover, the development and enhancement of bioinformatics tools, including machine learning and AI, are vital for analyzing vast datasets to predict new resistance mechanisms. These tools can significantly aid in identifying and understanding novel mechanisms of antibiotic resistance (Wright, 2019).

Precautions for Patients and Doctors for Managing Antibiotic Resistance

Antibiotics have revolutionized modern medicine, effectively treating bacterial infections and saving countless lives. However, the overuse and misuse of antibiotics have led to a significant global health threat – antibiotic resistance. To address this critical issue, both patients and healthcare providers must adhere to essential precautions.

Patient Precautions

Following the doctor's prescription for antibiotics to the end is one of the most important safety measures for patients. It's important to take antibiotics for the entire specified length, even if you feel better before that time. By ensuring that all infection-causing germs are eliminated, this procedure lowers the possibility that antibiotic-resistant strains may emerge (World Health Organization, 2020). Antibiotics should never be shared by patients, even if they have identical symptoms. Since antibiotics are prescribed based on each patient's unique situation, distributing them can result in improper use. Antibiotic resistance is a global concern that is exacerbated by this abuse (Centers for Disease Control and Prevention (CDC), 2021). Keeping up proper hygienic habits is an effective preventive approach. Even small steps, like often washing your hands with soap and water, can help stop infections from spreading and lessen the initial need for antibiotics. Good cleanliness is the primary line of protection against antibiotic resistance (CDC, 2020). Antibiotic side effects and any hazards should be disclosed to patients. Allergic reactions or gastrointestinal problems are common adverse effects. Patients should contact their doctor right away if they have any strange side effects or symptoms while taking antibiotics. This knowledge guarantees prompt action and appropriate handling of possible problems (U.S. National Library of Medicine, 2021).

Doctor Precautions

Healthcare professionals must exercise antibiotic stewardship. It entails using antibiotics sensibly and prudently. Antibiotics should only be prescribed by medical professionals when required, and they should choose the best medication for the particular infection. By using this strategy, the overuse of antibiotics and the emergence of antibiotic resistance are decreased (CDC, 2020). A key function for healthcare providers in patient education is education. They have to teach patients how to take antibiotics correctly, stressing how important it is to finish the entire course of medication as directed. Additionally, patients need to be made aware of the possible dangers and negative effects of using antibiotics. Patients with knowledge are more likely to adhere to recommended antibiotic usage, which lowers the possibility of resistance (World Health Organization, 2020).To stop illnesses from spreading, healthcare facilities must implement infection control procedures. Healthcare professionals can lessen the spread of germs and subsequently the initial demand for antibiotics by putting strict infection control measures in place. Antibiotic resistance prevention is based on effective infection control measures (CDC, 2021). Healthcare practitioners need to be up to date on the most recent findings and recommendations about the use and resistance of antibiotics. Antibiotic resistance is a dynamic topic where new knowledge and recommended methods are continually being developed. Healthcare professionals may make evidence-based decisions about patient care and adjust to changing problems by educating themselves (U.S. Department of Health and Human Services, 2020).

By following these precautions, both patients and doctors can work together to preserve the effectiveness of antibiotics and address the looming threat of antibiotic resistance. This collaborative effort is essential for the future of modern medicine and public health.

Challenges in Investigating Novel Mechanisms of Antibiotic Resistance

Numerous obstacles still exist, despite clear guidelines for developing research on the mechanisms underlying antibiotic resistance. New resistance mechanisms can be quickly developed by pathogenic bacteria, frequently outperforming scientific investigation (Davies & Davies, 2010). Antibiotic resistance is a multifaceted issue with frequently overlapping or interacting mechanisms. These complex interactions make it challenging to isolate and analyze certain mechanisms (Blair et al., 2015). Discovering novel antibiotic genes and mechanisms demand for high throughput omics (Sekyere & Asante, 2018). Access to cutting-edge technologies and significant funds are necessary for research in this field. Budgetary restrictions impede research into new resistance mechanisms and the creation of efficient countermeasures in many nations (Laxminarayan et al., 2016). There are ethical considerations as well. Ethical problems arise when genetically modified organisms and possibly dangerous

germs are studied for scientific objectives. It is a constant struggle to strike a balance between ethical considerations and scientific growth (Carlet et al., 2014). Carrying out clinical trials for antibiotics is often challenging and requires recruiting patients with specific bacterial infections and determining the optimum dosages to treat them, including the risk of rapid progression of the infection (Sakamaki et al., 2021). Maintaining a balance between developing new antibiotics and keeping their efficacy through responsible use is often challenging (Majumder et al., 2020). Furthermore, the creation of novel antibiotics and methods for combating resistance may be hampered by different regulatory standards across the globe. The process of bringing new therapies to market can be slowed down by the extensive and strict regulatory procedures needed for their approval (Muteeb et al., 2023).

Conclusion

Combating antibiotic resistance, which continues to be a major worldwide health concern, requires cooperation between researchers, medical professionals, and governments. This area of research must keep developing to handle the new problems that antibiotic-resistant bacteria are posing. Antibiotic resistance in pathogenic and commensal bacteria is one of the biggest risks to world health in the modern era. This paper has explored the issues and suggestions related to this important field of study. Besides, awareness and practicing proper antibiotic usage and doses are key to preventing the emergence of resistant bacteria. These hurdles serve as a reminder of the enormous obstacles we face, and emphasize the significance of interdisciplinary collaboration, worldwide monitoring systems, focused drug development, and enhanced gene technologies and nanotechnologies combined with omics studies. Many novel antimicrobials studied have already shown hopeful outcomes in the primary stages. With sufficient funds and continued investigations, they may soon develop successful antimicrobial agents and tools.

Author Contributions

M.J.P. drafted the manuscript and made substantial contributions to the design of the study. M.H., J.S. reviewed and drafted the paper.

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