CHAPTER 09

Novel Alternative Approaches for Treating Drug-Resistant Bacteria

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SUMMARY

Infectious diseases are the most prevalent cause of death in middle-income countries, and antibiotics have proven to be miracle cures. However, antimicrobial resistance (AMR) is a significant worldwide concern that needs to be handled cautiously and efficiently. Antibiotic therapy for evolving multidrug-resistant bacterial (e.g., tuberculosis, cholera) as well as fungal (e.g., candidiasis) illnesses remains very scarce, while there are several reasons and sources causing this type of resistance that continue to develop. Regarding the grave obstacles of emerging AMR, there should be a dire requirement for the recognition, improvement, assurance, and advancement of innovative approaches and techniques that are easy to implement in order to conquer this major problem. The chapter delves into novel approaches to control drug-resistant microorganisms. Antibiotic combination treatment is an intriguing approach for reducing the emergence of resistance and expanding the efficacy of antimicrobial drugs. Bacteriophage therapy additionally represents an innovative means of treatment to mitigate the progression of multidrug resistance (MDR). Moreover, CRISPR, an advanced genome modification tool, has an extensive number of uses for strengthening the host defenses and conquering diverse resistance problems. The cutting-edge approaches mentioned here, which include antibiotic combination therapy, nanotechnology, phage therapy, antimicrobial peptides, fecal microbiota transplantation, and CRISPR/Cas system, presented a brief summary of some of the newly developed approaches to be employed towards pathogenic microbes/microbial invasions, and they also provide sophisticated understanding regarding the several mechanisms of drug resistance that enable pathogenic microbes to acquire resistance against multiple antibiotics. As a consequence, recognizing innovative control plans/approaches as well as distinct mechanisms underlying drug resistance will assist in the effective discovery of prospective antibiotics and their specific targets, thereby contributing to alleviating the challenge of increased drug resistance among different infectious microbes.

INTRODUCTION

In the history of antimicrobial therapy, the emergence of antibiotic resistance was identified earlier, when penicillin was used in clinical trials (Harikumar & Krishanan 2022). Various methods, such as inactivation of the enzyme, (for example, by β-lactamases and aminoglycoside-modifying enzymes), modification or protection of the target (induced, for example, by an enzyme called topoisomerase targeting alteration by mutation), and evasion of the antibiotic target, can all lead to the development of antibiotic resistance. Unfortunately, not even last-resort drugs like colistin, and daptomycin, which affect the bacterial cell's outer membrane, have proved susceptible to resistance (Sharma et al., 2022). Simultaneously, the comprehensive contribution of antimicrobials as ancillary agents to critical life-saving therapeutics is particularly accentuated in regions characterized by resource constraints, where infection prevention measures and hygiene standards are notably suboptimal.

Since the beginning of the antibiotic era, antibiotic resistance has been a significant problem for the development of new antimicrobials (Bonomo & Rossolini 2008). A significant risk to the therapeutic application of antibacterial and

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subsequently, to the successful treatment of bacterial illnesses, is the emergence of bacteria that are resistant to numerous kinds of commonly used antibiotics. The recent investigation has revealed that innate resistance to antibiotics does not arise solely from the selective influence of antibiotic exposure; rather, it entails a complex interplay of genetic locus alterations. This phenomenon is not as simple as permeability barriers and efflux mechanisms but is intricately linked to the intricate network of genetic changes. The recent discovery highlighting the significant involvement of microorganisms in harboring resistance elements, coupled with the imminent medical threat posed by Gram-negative pathogens, has rendered the intrinsic resistance mechanisms of bacteria a captivating and distinctive therapeutic target. This avenue not only presents an intriguing prospect but also serves as a means to enhance our fundamental understanding of bacterial physiology (Cox & Wright 2013).

INTRINSIC MECHANISMS OF ANTIMICROBIAL RESISTANCE

Outer membrane permeability

The membrane in bacteria that helps to segregate and functions as a barrier to separate their cytoplasm from the outside environment is called an outer membrane (OM). Bilayers of lipids make the membranes, which makes them extensible self-sealing capsules. Fluidity is directly proportional to permeability. Bacteria that are Gram-positive, their peptidoglycan have a high permeability threshold and are open to molecules that range from 30-57 kDa. However, bacteria which are gram-negative are naturally resistant to a number of these different antibacterial drugs because they have an "outer membrane" (OM), a considerably fine molecular filter. Because of the OM's considerable impermeability, it is crucial that bacteria have other ways of absorbing vital nutrients.

The opportunistic microbe *Pseudomonas aeruginosa* exhibits a very strong inherent resistance due to OM. The operational framework of the outer membrane (OM) in mitigating the intrinsic susceptibility of Gram-negative bacteria to particular antibacterial interventions is readily apparent. This membranous structure serves to attenuate the permeability of minute compounds, albeit without completely impeding their inward translocation. Consequently, it does not confer a significant peak of resistance to antimicrobial agents on its own.

MDR efflux pumps

All species, including those that don't manufacture antibiotics, contain efflux pumps, which indicates they may have formed goals other than avoiding antibiotics. In 1970, Tetracycline exhibited the first case of efflux-mediated antibiotic resistance. Efflux pumps may export a single molecule that is specific to the substrate or they may release classes of compounds that are diverse in structure. Due to their relative intrinsic resistance, some kinds of antibiotics are unable to cure infections caused by gram-negative microbes. Drug efflux is an "active" mechanism, it requires energy to transport substances up to a concentration gradient (Cox & Wright 2013).

Antibiotic sequestration

Drug-binding proteins have a role in sequestration, which keeps the antibiotic from reaching its intended target. They typically include several systems that function in conjunction to provide complete defense against the physiologically active substances they create. Remarkably the genetic factors that determine self-resistance typically are inevitably concentrated alongside the antibiotic-producing genes, thus their activity is co-regulated (Mak et al., 2014).

Target modification

Several types of antibiotics, such as β-lactams, glycopeptides, lincosamides, macrolides, streptogramins, and aminoglycosides, are susceptible to specific alteration as a self-resistance phenomenon. For antimicrobial agents, which link to the 50S ribosomal subunit, target alteration is also observed. 23S rRNA methyltransferases methylate 23S rRNA at position A-2058 as part of this process. Another form of protective resistance is provided by removing antibiotics from the target region. OtrA clears the ribosome of the antimicrobial oxytetracycline in *S. rimosus* (Peterson & Kaur 2018). To tackle these resistance mechanisms, scientists have been working on novel methods and techniques. Some of these are illustrated in the Fig 1.

NOVEL ALTERNATIVES TO TACKLE RESISTANT BACTERIA

Antibiotic combination therapy

Bacteria constantly stay one step ahead, as demonstrated by the fact that *Staphylococci* that are penicillin-resistant, they had already appeared a year before to penicillin's initial clinical application. Over the past 70 years, the discovery of monotherapy has largely influenced the advancement and consequent practical application of antibiotics for the treatment of bacterial illnesses. The utilization of combined antibiotic therapy has emerged as a notable exception to the established "code" of monotherapy, particularly evident in the management of microbial illnesses such as those induced by *Helicobacter pylori*, (Ford et al. in 2016), and Mycobacterium tuberculosis (Kerantzas & Jacobs, 2017). This paradigm shift towards prolonged combination antimicrobial therapy in the context of

bacterial infectious endocarditis underscores the pressing need to confront the intricate challenges posed by the infection, emphasizing a strategic departure from traditional therapeutic approaches to optimize efficacy and mitigate potential resistance dynamics. The treatment paradigm for the Human Immunodeficiency Virus (HIV) involves the application of combined therapies, yielding favorable outcomes. Similarly, combined chemotherapy has proven efficacious in the management of both solid cancers and hematological malignancies, exemplifying a therapeutic strategy applicable to non-contagious disorders (Coates et al., 2020). It is still debatable whether or not combined antimicrobial therapy for gram-negative bacterial infections provides any sort of defense against the emergence of resistance in humans (Eliopoulos & Eliopoulos 1988). Combination therapies have many advantages like

Synergy: By in vitro conditions, several antibiotics have increased their effectiveness against pathogens, when coupled with another antibiotic. Vancomycin's increased effectiveness towards *E. coli* whenever coupled with another drug called trimethoprim or nitrofurantoin is one example (Coates et al., 2020)**.**

Rejuvenation: The profound impact of synergistic combinations lies in their ability to rejuvenate the efficacy of obsolete medications against pathogens that have developed resistance, introducing a paradigm shift in "Antibiotic resistance". This innovative strategy advocates for the reuse of older antibiotics, challenging the conventional practice of discarding them upon the emergence of tolerance.

Resistance: The application of antibiotic combinations exhibits a mitigating effect on the progression of tolerance development. Notably, in the context of tuberculosis (TB), a synergistic combination of drugs enhances therapeutic outcomes by diminishing the emergence of resistance, in stark contrast to monotherapy, which induces resistance in the pathogenic response.

Spectrum: Empirical combination treatment expands the spectrum of targeted bacterial species for clinically diagnosed infections of unidentified origin, administered prior to the determination of culture sensitivity analysis. The treatment approach is exemplified in the management of community-acquired pneumonia, illustrating its applicability in instances where the causative agents are yet to be precisely identified.

Toxicity: Combinations of drugs can be used at lower doses of each to minimize toxicity while maximizing therapeutic benefit. For instance, amoxicillin with a lower dose for treating endocarditis caused by bacteria.

Duration: Some antibiotic combinations are the reason for the short duration of therapy (Coates et al., 2020).

NANOTECHNOLOGY

Overconsumption of Abs, their widespread implementation in the agricultural sector, and the lack of new antibiotics all contribute to the swift rise of antimicrobial resistance in microbes. Antimicrobial resistance is an imminent risk to the world's public health, accounting for at least 1.27 million deaths worldwide and around five million fatalities in 2019. To address this critical issue, nanotechnology and nanoparticles have been created in recent years to minimize as well as avoid bacterial resistance, multidrug-resistant (MDR), and even bacterial biofilm. Nanoparticles are manufactured nanostructures varying in dimension from 1 to 100 nanometers (nm). Because they not only act as carriers for naturally occurring drugs and antimicrobial agents but also proactively attack bacteria, Nanoparticles are the most effective means of combating MDR bacteria. Liposomes, micelles, solid lipid nanoparticles, carriers nano gels, nanocapsules, nanotubes, dendrimers, emulsions, nanostructured lipids, quantum dots, and polymeric NPs represent some of the current nanoparticulate antimicrobial systems (Hetta et al., 2023).

Antibiotic administration using Nanoparticles promotes absorption by improving solubility in water, lengthening resistance duration within the body, and focusing on particular regions of action. NMs can be inadvertent (as a consequence of commercial and organic processes), artificial, or natural, and can be derived from living animals and plants (Yayehrad et al., 2022). Nanoparticles (NPs) are able to reach pathogenic germs' cell membranes and disrupt critical metabolic pathways, resulting in novel antibiotic actions. NPs have shown synergy when combined with appropriate antibiotics and may contribute in addressing the global challenge of growing bacterial resistance. Multidrug-resistant organisms (MDROs) are posing an increasing global health threat, making many healthcare-associated diseases challenging to combat with antibiotics that are accessible. The administration of nanoparticles, also known as NPs, offers a viable method of controlling MDRO infections. Antibacterial NPs have the ability to inhibit or eradicate MDRO evolution (Lee et al., 2019).

Nanoparticles of silver (AgNps)

Because of their potent antibacterial activity, the AgNPs are expected to be the next line of therapeutics. AgNPs are at present one of the most widespread NPs accessible economically. Because of their small dimensions, AgNPs can infiltrate cells. Furthermore, they alter the layout and elasticity of the membrane, increasing penetration and leading to cell death. AgNPs' specific antibacterial impact has been connected

to the membrane of plasma and cell wall disintegration, as well as the generation of ROS (Brar et al., 2023).

AgNPs have two basic killing mechanisms: contact with the microbial membrane and impairment of membrane function. When positively charged AgNPs come into contact with negatively charged cell walls of bacteria, the cell wall changes, resulting in enhanced permeability of cells and death of cells. The antimicrobial efficacy of AgNPs at concentrations ranging from 30-100 mmol/L against erythromycin-resistant *E. coli*, *S. pyogenes*, and *P. aeruginosa* was demonstrated. Cylindrical AgNPs with a diameter of 30 nm displayed an excellent capacity to inhibit *P. aeruginosa* biofilm formation (Rabiee et al., 2022).

Nanoparticles of gold (AuNps)

AuNPs possess a range of forms, including triangles, sphere-shaped, hexagonal shapes, and rod-like configurations. Triangular-shaped AuNPs have been shown to have a stronger antibacterial effect than spherically-shaped AuNPs against a variety of microbes. AuNPs adhere to the membrane by electrostatic interaction, undermining transmembrane permeability. Ampicillin coupled with AuNPs proved extremely effective towards ampicillin-resistant *E. coli*, *P. aeruginosa*, MRSA, and *Enterobacteraero-genes.*

AgNPs have been widely employed for antibacterial applications in conjunction with various metallic types known to generate nanocomposites. Anti-infective effectiveness of AgNPs versus a variety of microorganisms including MRSA, was demonstrated (Vanamala et al., 2021). The antimicrobial effectiveness of AuNPs and drug-attached AuNPs against oral infections was investigated. Ionic gold has the potential to be employed as an alternative antimicrobial agent against AMR *P. aeruginosa.* When AuNPs undergo modifications, their antibacterial capabilities improve (Rabiee et al., 2022).

Nanoparticles of titanium oxide (TiO2NPs)

TiO2Np interferes with the process of oxidative phosphorylation and disrupts membranes of cells through the emission of ROS. It also delays the formation and degradation of heme groups (Foster et al., 2011). In a disc-diffusion assay, their use with various antimicrobial combinations like cephalosporins, glycopeptides, and azalides showed anti-MRSA action. TiO2 NPs generate free radicals when exposed to UV light, which enhances their ability to destroy MRSA (Vanamala et al., 2021).

Nanoparticles with mesopores

Mesoporous nanoparticles are honeycomb materials that are porous with diameters ranging from 2 to 50nm. Recent research found that the pores of mesoporous nanoparticles might influence AMP accumulation as well as discharge, along with antibacterial action. The wider the pore diameters, the more rapid the flow rates, and thus the stronger and greater the antibacterial effect (Dizaj et al., 2022).

Liposomes

Liposomes are sphere-shaped vesicles with bilayers of lipids that are widely employed in drug delivery investigations. Liposomes have been demonstrated to increase medicine half-life, biological degradation, and/or biological compatibility, and reduce toxic effects, rendering them particularly appealing alternatives for biomedical research (Allahou et al., 2021). The liposome-based formulation performed better against *Listeria monocytogenes* (Dizaj et al., 2022).

Nanoparticles made of polymers

Poly (lactic-co-glycolic acid) (PLGA) is the polymer that has received the greatest attention and research. Vancomycin has also been delivered using hydroxyapatite-based hollow NPs against MRSA linked to osteomyelitis that is persistent. Another investigation found that daptomycin-loaded PCL microparticles had specific action toward the biofilm formation by methicillin-resistant staphylococcus aureus and also prevented biofilm reformation. The use of NO-based Nanoparticles reduced the bacterial load in MRSA-infected exposed lesions on the skin. The antibiotics were delivered directly into the cells after the nanoparticles bonded with the double layer of lipids formed by the bacterial cells (Vanamala et al., 2021).

PEPTIDES WITH ANTIMICROBIAL PROPERTIES

Antibacterial peptides also referred to as host-defense peptides, are an integral part of many lifeforms' inherent immune responses. They have substantial antimicrobial, antifungal antiparasitic, and antivirus action. These peptides are typically constituted of 10-50 amino-acid repeats that are grouped in distinct categories based on the amino-acid layout, dimension, as well as configuration. These destroy microorganisms quickly, which prevents resistance from arising, even though only some cases of resistance have been discovered. They depicted a broad range of efficacy towards both gram-positive and negative bacteria, mycobacterial (including *Mycobacterium tuberculosis*), encapsulated organisms such as viruses and fungi, as well as altered cells that are malignant (Kaur, 2016). Antimicrobial peptides operate through a variety of mechanisms

The neutralization process or segmentation of lipopolysaccharides

In gram-negative microorganisms, lipopolysaccharides are essential elements of the outermost flap of the outer protective membrane. LPSs make great antibacterial peptide targets. They are capable of directly suppressing multidrug-resistant bacterial development. They counteract the impact of secreted Lipopolysaccharide by boosting immune system cells. PMAP-23 is a porcine myeloid antimicrobial peptide of 23 amino acids that exhibited damaging capability towards a wide range of microbiological species, carboxyl terminal inhibited *E. coli* growth by coming into contact with the outer layer harboring Lipopolysaccharide (Park et al., 2011).

Permeation of membrane enhancement

They are thought to trigger the outflow of materials within the cell by breaking the membrane that surrounds the cytoplasm. Pore generation via a bucket-stave or a toroidal porous process, or by means of a nonpore floorboard-like process.

Division of cells and sustainability-related proteins from the cytoplasm are restriction

Though the majority of antibacterial peptides serve largely to membrane disruption, several antibacterial proteins can permeate the cytoplasm of bacteria via a flip-flop pathway or a membrane's outer protein creating passage (Park et al., 2011).

THE IMPLANTATION OF FECAL MICROBIAL COMMUNITY

Fecal Microbiota Transplantation (FMT), frequently referred to as fecal bacteriotherapy or stool transplantation, is a viable strategy that has lately been acknowledged. The method entails transferring excrement from a donor who is in good health to a recipient. Because antimicrobial targets are non-specific, they destroy both pathogenic microorganisms and the bacteria in the gut. This type of treatment restores intestinal bacteria in the recipient's colon by transplanting it from the feces of a healthy benefactor. suppressing the specific development of aggressive variants of *Clostridium difficile* microbe, resulting in a reduction in their quantity and, ultimately, ending the pattern of repeated CDI. That eliminates the necessity for repetitive drug administration, lowering the likelihood of drug-linked resistance (Kaur, 2016).

PHAGE THERAPY

The term "virus" refers to infectious agents that require a living host to replicate. Viruses infect humans and other animals, plants, fungi, protists, and bacteria. Bacteriophages (commonly called phages) are specific viruses that are hosted by bacteria. Considering their number (around 10^{31}-10^{32}), phages are the most prevalent biological entities on Earth that regulate bacterial communities, especially in the marine environment (Suttle, 2007). Phages were discovered independently by Frederick Twort in 1915 (Twort, 1915), and Félix d'Hérelle in 1917; Félix d'Hérelle coined the term "bacteriophage" (meaning "bacteria eater") (d'Herelle, 1917). About a decade before the discovery of penicillin, phage therapy was being developed as a viable treatment option for bacterial infections. In 1919, phages were used to treat shigellosis caused by *Shigella dysenteriae* [*S. dysenteriae* (Chanishvili, 2012)]. Since at that time, they could not be visualized due to the absence of advanced microscopy techniques, their presence was controversial and proved to be a hindrance in early documentation and research (Ackermann, 2011). Due to these obstacles, phage therapy was abandoned and researchers focused on the development of antibiotics.

The Discovery of antibiotics and their efficacy in bacterial infection treatment revolutionized the world. The role of proper sanitation was highlighted and diseases that were incurable at that time became curable due to antibiotics (Yoshikawa, 2002). However, prolonged and extensive use of antibiotics has led to the development of antimicrobial resistance (AMR) in pathogenic bacteria. Antibiotic resistance genes (ARGs) could be transferred among bacteria and these genes now appear to be proliferating in the environment. With the rapid spread of AMR, admonitions about the return of the "pre-antibiotic era" are on the rise. Centers for Disease Control (CDC) and the World Health Organization (WHO) have marked AMR as a global threat to bacterial infection treatment and human health (Lin et al., 2017).

Carbapenems are considered the last resort treatment option against multi-drug resistant (MDR) pathogens because of their relatively high side effects. The emergence of Carbapenem-resistant *Klebsiella pneumoniae* has caused a significant increase in the mortality rate of infected individuals (Lin et al., 2017). At this rate, soon there will be no antibiotics left to treat infections, and the "pre-antibiotic era" will return. To tackle this problem, phage therapy is being considered the most preferred novel method due to its specificity and less toxicity to humans (Bourdin et al., 2014). Phage therapy has gained attention in the rt years because of the emergence of MDR pathogens, but this practice has been around for nearly a century, even before the discovery of antibiotics.

Mode of action

Phages are non-living biological entities, made up of genetic material (DNA or RNA) enclosed by a capsid, that require a bacterial host to replicate. Phages bind to specific receptors on the surface of bacteria to either enter bacterial cells themselves or to insert genetic material into the bacteria. Genetic material then incorporates itself into the bacterial genome to initiate

either the lytic cell (lytic phages) or lysogenic cycle (temperate phages). Temperate phages reproduce vertically as bacteria replicate from mother to daughter cells.

Lytic phages replicate inside host bacteria and cause lysis of their host after a critical number of progeny phages has been obtained (Delbrück, 1940). Conventional phage therapy relies solely on lytic phages due to their ability to lyse/kill bacteria. Phage cocktails, consisting of multiple phages having *in vitro* activity against the target pathogen, are made for treatment purposes (Lin et al., 2017).

Clinical Significance

The majority of the research involving phages has been carried out using animal models. At the time, no phage therapy products were available for human use; however, clinical studies prove its effectiveness and possible uses for future application of human disease treatment. Orally administered phages have been successfully used to save about 67% of mice from gut-derived bacteremia caused by *Pseudomonas aeruginosa* while all the mice in the control group died (Watanabe et al., 2007). Phages administered along with *Clostridium difficile (C. difficile)* were sufficiently effective in preventing infection in a hamster model. Post-administration of phages after the infection saved 11 out of 12 mice while the control group mice died (Ramesh et al., 1999). Phage cocktails reduced the *C. difficile* growth and proliferation in both *in vitro* and *in vivo* studies (Nale et al., 2016).

Mice models showed a 100% survival rate upon single strain inoculation of phage in mice infected with vancomycin-resistant *Enterococcus faecium* (Biswas et al., 2002), and *ESBL-producing E. coli* (Wang et al., 2006). Phage cocktails have been successfully administered to treat various infections caused by antimicrobial-resistant *P. aeruginosa* in animal models (Soothill, 1992; Watanabe et al., 2007). Studies also show that phage therapy can successfully cure infections caused by the multidrug-resistant pathogen *E. coli* O25:H4-ST131 (Pouillot et al., 2012). There is a possibility that antibiotic sensitivity could be restored in multidrug-resistant *P. aeruginosa* by using phages, which may open a new frontier towards AMR treatment (Chan et al., 2016).

Human trials have also been conducted for over a century in various institutes in several countries. The Eliava Institute has used phages for the treatment of infections caused by many pathogens such as *E. coli, S. dysenteriae, P. aeruginosa,* etc. (Kutateladze & Adamia 2008). In the 1938 clinical trial, 219 patients were administered phage cocktails targeting multiple pathogens and 74% of patients showed improvement or were completely cured (Chanishvili & Sharp 2008). During the 1974 typhoid epidemic, typhoid phages were used in a prophylactic trial and 18577 children were inoculated with these phages. Phage therapy reduced the typhoid incidence five-fold compared to placebo (Kutateladze & Adamia 2008). Phages work more efficiently when administered in combination with antimicrobials and this approach needs to be investigated in humans to prevent the rise in AMR (Abedon et al., 2011).

PHAGE THERAPY VS. ANTIBIOTICS

Safety

Antibiotics cause several well-documented side effects on human health such as allergic reactions, hepatotoxicity, nephrotoxicity, neurotoxicity, gastrointestinal problems, and many more (Granowitz & Brown 2008). The use of phage therapy is relatively new, so its safety profile is also new. However, it has been reported that oral administration of phages is safe, especially the translocation of phage from intestinal epithelium to blood downregulates host immune response against indigenous gut microbiome antigens by suppressing the production of interleukin-2, gamma-interferon, and tumor necrosis factor (TNF) (Górski et al., 2006). While the advantages of phage therapy outweigh the disadvantages in the case of non-immunocompromised individuals, hypothetically the enhanced immune response in immunocompromised individuals may pose a health risk. However, some researchers argue against this possibility (Borysowski & Górski 2008). Most of the research involving phage therapy is focused on animal trials. So to establish an elaborate safety profile, human clinical trials need to be focused.

Specificity

Phages are both species and strain-specific in their mode of action while antibiotics target and kill beneficial gut bacteria as well in addition to pathogens, which may lead to complications such as antibiotic-associated diarrhea caused by *C. difficile* infection, increased risk of asthma, obesity, and diabetes(Metsälä et al., 2015; Cox & Blaser 2015; Rea et al., 2016; Mikkelsen et al., 2016). However, this specificity may limit their use in infection treatment, where more than one pathogen is involved such as in infected burn wounds, a distinct advantage of Broad-spectrum antibiotics.

Biofilm penetration

Antibiotics are unable to degrade extracellular polymeric substance which makes up the EPS matrix surrounding the biofilm, limiting their efficacy in biofilm-based bacterial infections. In contrast, phages possess enzymes such as EPS depolymerase that degrade the EPS matrix and expose bacteria embedded within the biofilm. Phages complete the lytic cycle and propagate to degrade further layers of biofilm (Abedon,

2015). Low doses of antibiotics do not have any significant effect on the integrity of dense biofilms; therefore, we need high doses to inhibit bacterial propagation, and even then, it may not result in the complete eradication of biofilm. High doses cause severe side effects (Anwar et al., 1992; Amorena et al., 1999). Phage therapy solves the problem of persistent infections involving biofilm formation in implanted medical devices by degrading biofilms formed by *P. aeruginosa, L. monocytogenes,* and *S. epidermidis* (Labrie et al., 2010).

Limitations

There are a few limitations associated with the use of phage therapy. As discussed before, in the case of mixed bacterial infection, broad-spectrum antibiotics are the preferred treatment option. For phage therapy to be successful in the case of mixed infection, we need a cocktail of different bacteriophages that could target multiple bacteria at the same time. Another limitation is the rapid clearance of phages from the body, but this can be dealt with by modifying bacteriophage structural characteristics (Merril et al., 1996). The public may also feel reluctant towards this approach because of their negative perception of viruses (Verbeken et al., 2007). Several regulatory issues must also be addressed for the successful implementation of phage therapy.

PHAGE-DERIVED LYTIC PROTEINS

Phages encode lytic enzymes that work similarly to eukaryotic lysozymes. These enzymes are expressed by the bacterial host at the end of the phage replication cycle and help in the hydrolysis of the bacterial cell wall and release of phage progeny for further propagation. Most of the phages use two protein classes for the completion of the lytic cycle. One of them is called holin, a transmembrane protein that creates holes in the bacterial cell membrane. Another one is called endolysin, a hydrolase enzyme that degrades peptidoglycan in the cell wall of bacteria after holins create an opening. Both these proteins work in conjunction with each other. A single phage can code for multiple variants of holins and endolysins (Roach & Donovan, 2015).

Endolysins can cause bacterial cell lysis on their own while holins can't; therefore, endolysins have been investigated for their potential as antimicrobial agents. These proteins are safe to use, as they are inactive against eukaryotic cells. Endolysins were successfully used to treat mice infected with MDR pathogens such as *Acinetobacter baumanii* (Lood et al., 2015), methicillin-resistant *Staphylococcus aureus* (Schmelcher et al., 2015), and *Streptococcus pneumoniae* (Witzenrath et al., 2009). As endolysins target peptidoglycan in the bacterial cell wall, bacteria are less likely to develop resistance against them, increasing their potential for use as antibacterial agents (Roach & Donovan, 2015).

CRISPR-CAS SYSTEM

A bacterial adaptive immune mechanism called CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR Associated) protects the organism from foreign genetic material including plasmids and phages. Guide RNAs (gRNAs) and Cas (CRISPR-associated) proteins are two of the system's main building blocks. While the gRNA directs the Cas protein to the target location, the Cas protein functions as a molecular scissor to split the foreign DNA (Brouns *et al.,* 2008).

The worldwide health concern of antibiotic resistance (AMR) has increased mortality and morbidity rates along with large economic losses (O'Neill, 2016). Antimicrobial agents' effectiveness has decreased due to the evolution of resistant bacterial strains, necessitating the development of novel therapeutic approaches. CRISPR-Cas systems are one such approach.

The CRISPR-Cas system stands as a versatile tool for the development of novel antimicrobial therapies, as demonstrated by scientists who harnessed its potential to selectively target and deactivate specific genes in antibiotic-resistant bacteria. This strategic intervention enabled the reactivation of previously ineffective antibiotics, exemplifying a transformative approach to combat antibiotic resistance (Gootenberg, 2018)

Clinical samples can also be used to quickly and precisely identify antimicrobial resistance genes using the CRISPR-Cas system. In order to help doctors, make well-informed treatment decisions, researchers have created CRISPR-based diagnostic tools that can detect the presence of particular AMR genes in a sample. These tools provide timely information on the pathogen's level of resistance (Arroyo-Mendoza, 2021).

Using CRISPR-Cas gene drives, it may be possible to prevent the spread of antibiotic resistance genes in bacterial populations. Gene drives are genetic mechanisms that rapidly disseminate particular genes among populations. They may be used to disseminate genes that make bacteria resistant to antibiotics or to stop bacteria from sharing resistance genes with the next generation. (Noble,2018).

Phages, which are viruses that infect bacteria, can be employed as a natural antibiotic substitute and bacterial predator. Phages with improved specificity and efficacy against antibiotic-resistant bacteria can be created using the CRISPR-Cas system, enabling the targeted and exact elimination of the resistant strains (Goren, 2021).

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