Bacteriophage Therapy: A Resurgence in Combating Multidrug-Resistant Bacterial Infections.

Abstract

The emergence of multidrug-resistant (MDR) bacterial pathogens is a pressing issue that requires the creation of new therapeutic approaches that differ from the conventional antibiotic mode of action, like phage therapy.This paper offers a critical review of bacteriophage therapy, the fundamental principles of phage biology and how they interact with bacterial hosts. Specifically the challenges presented by biofilm formation and bacterial defense mechanisms. The review also delves into the modern methods of overcoming such obstacles in the form of synergistic therapeutic models and sophisticated bioengineering strategies. Analysis of current clinical data favors the contention that phage therapies are a promising and versatile approach to addressing the global challenge of MDR bacterial disease.

Keywords

Multidrug resistant bacteria, Bacteriophage,Antimicrobialresistance,Biofilm Disruption

Introduction

Bacteriophages- derived from “bacteria” and the Greek word “phagein”, meaning “to devour”- are viruses that specifically infect and destroy certain bacterial cells.[1] Bacteriophages were first observed in 1915 by William Twort, although their potential to kill bacterial cells and their classification as viral agents wasn’t done till 2 years later, in 1917, by Felix d'Herelle.[2] He suggested the active use of laboratory-generated phages for both the prevention and treatment of bacterial infections.[3] This viewpoint, however, challenged the common consensus at the time, namely that bacteriophages were bacterially produced lytic enzymes, causing d’Herelle’s theory to be met with hostility.[3] The use of phage therapy itself was then undermined by mainly 2 key aspects, the discovery of antibiotics, which were broad spectrum, and the outbreak of the second world war.[3] The electron microscope provided the first images of the bacteriophage, confirming d’Herelle’s theory of bacteriophages being viral agents.[3] These images were produced in Germany, which was viewed as a hostile nation at the time to say the least.

Due to this, bacteriophage therapy never caught on in the west after the pre antibiotics era. Though it became and remained prevalent in places like the former USSR, and is still widely employed for therapeutic purposes in these regions. Phage therapy is used mostly on compassionate grounds in the rest of the world today due to a lack of clinical trials and research.

As MDR bacteria become increasingly prevalent, however, more and more of our antibiotics are rendered useless, and the creation of new antibiotics to combat these resistant strains has slowed drastically. This has become a major health concern across the globe. According to a report by the World Health Organisation (WHO), published in 2019, an estimated 7,00,000 people die from drug resistant infections yearly, and this number is predicted to increase drastically by 2030 if nothing is done to solve this problem. In light of this, bacteriophages are becoming an increasingly promising tool in the fight against bacteria.

Modern antibiotics function by targeting either processes unique to bacteria—such as cell wall or folic acid synthesis—or bacterial-specific components within shared biological pathways like protein and DNA replication. [4] When bacterial cells become immune to these drugs in doses that should have been fatal, they are said to have become resistant. Similarly multidrug resistance (MDR) can be defined as the insensitivity to fatal doses of multiple, structurally different, drugs and chemicals in a cell.[5][6]

There are four principal mechanisms by which antimicrobial resistance occurs: reduced uptake of the drug, alteration of its target, enzymatic inactivation, and active efflux from the cell.[7] As a result of structural differences (namely the presence of lipopolysaccharides, amongst others), Gram-negative bacteria employ all four major resistance mechanisms, while Gram-positive bacteria are less likely to rely on reduced drug uptake as a strategy.[7] Resistance is not be confused with persistence in bacterial cell populations.

Persistence is the phenomenon where bacterial cells are not impacted by the presence of an antibiotic, or survive its presence, without the genes that code for resistance mechanisms to said antibiotic.[7] What differentiates these cells from regular cells is the fact that they do not grow and divide when exposed to antibiotics.[8] As a result, the mechanisms that are targeted by the administered antibiotic are paused, rendering the antibiotic ineffective, even when the cell isn't using any of the mechanisms that cause resistance. The phenotype for persistence arises through a spontaneous and reversible transition from normal cells to persister cells.[9] These persister cells only account for a very, very small fraction of bacterial populations, their exact number tends to depend on the specific strain of bacteria.

Mechanism of bacteriophages

Like any other virus, phages rely on host cells (bacteria) to reproduce. Phages utilize either the lytic cycle or the lysogenic cycle. They work by attaching themselves to the host cell (bacterium cell in this case) by utilizing distinctive tail-like structures to attach to the surface of the host cell

and inject their genetic information from the virion head (the viral cell's DNA or RNA enclosed in a protein coating) into the host cell.[10] The process post this differs. In the lytic cycle, once the viral DNA enters the host bacterium cell, It exploits the host's cellular machinery to synthesize virally encoded proteins necessary for replicating its genetic material. The mechanisms present in the cell allow for the assembly of other bacteriophages within the host cell.[11] Once enough phages have accumulated in the cell, it bursts, releasing the phages to infect other bacterial cells.

In the lysogenic cycle, however, instead of immediately destroying the host, the phage genome may integrate into the bacterial chromosome or persist as an episomal element, in either case being replicated and inherited by daughter cells. Such integrated genomes are called prophages, and the bacteria harboring them are known as lysogens. Prophages can later switch to the lytic cycle, leading to the death of the host cell.[12]

There are 2 types of phages, virulent phages (phages that exclusively use the lytic cycle) and temperate phages (employ both the lytic and the lysogenic cycle). As the lytic cycle is the one that results in the immediate death of the bacterial cells, virulent phages are utilised for phage therapy. Another issue associated with using temperate phages is that they typically induce lysogenic transformation, resulting in changes to the bacterial phenotype as the viral DNA is integrated into the cell. The expression of the combination of these genes can often lead to these host cells (and their daughter cells) to display increased toxicity to humans.[13] Hence, temperate phages aren't utilised for therapeutic applications in the form of phage therapy.

So far, we have discussed phage therapy in the context of planktonic bacterial cells: freely floating, singular bacterial cells. However, a key mechanism of bacterial (or other pathogenic microbial) cells in minimizing the impacts of antibiotics on their populations is the formation of bacterial biofilms. Biofilms are communities of microorganisms enclosed within a self-produced extracellular polymeric substances (EPSs).[14] These colonies of bacteria are sessile in nature, i.e, they are attached to a biotic or abiotic surface.[15] Biofilms formed by pathogenic bacteria can withstand antimicrobial agents at levels hundreds of times higher than planktonic cells, which makes infections involving biofilms highly challenging to treat even with high doses of antibiotics.[15]

Extracellular polymeric substances (EPS) are composed of diverse biomolecules, including polysaccharides, proteins, lipids, nucleic acids such as extracellular DNA and RNA, along with various other components.[15] Extracellular polymeric substances (EPSs) function as a protective barrier, providing resilience against hostile conditions, including exposure to antibiotics.[16] Bacteriophages act on these biofilms in a similar fashion as when they target bacterial cells. The most important mechanism they employ against biofilms is the usage of enzymes like depolymerases and lysins that act on and breakdown EPSs in which the bacterial

cells are encompassed.[15] The breakdown of EPSs causes the release of all the pathogenic microbes (primarily bacteria) that were present in the biofilm. This allows the phages to then act on and destroy the bacterial cells embedded within these biofilms, an aspect where antibiotics are almost entirely ineffective.

Case Example

In August 2022, an 83-year-old man with acute respiratory failure, admitted at the Southern University of Science and Technology Hospital, was diagnosed with an MDR Pseudomonas aeruginosa (pneumonia) infection. Standard antibiotics such as meropenem, amikacin, and colistin, naturally failed to suppress the infection. As a last resort, a phage cocktail was administered. Within the next few weeks, the patient's inflammatory and oxygenation levels gradually normalized, with significant clinical stabilization and no adverse effects. [17]

Bacterial resistance to phages

A challenge in the context of phage therapy is phage resistance in bacterial cells. Phage resistance is by no means a recent phenomenon. Phage resistance has existed since the earliest interactions between bacterial cells and bacteriophages. Phage resistance occurs through 2 main mechanisms: receptor adaptations that reduce or prevent attachment, and host defense pathways that block infection. [18]

Receptor adaptations that reduce or prevent attachment

As phages need to identify and attach to bacterial cells in order to infect, one of the mechanisms of resistance against phages is blocking phage adsorption by limiting the availability of binding receptors. Mutations in bacterial DNA can cause a change in or reduced expression, or modification of the structure of these receptors. As a result, point mutations (a mutation where a single nucleotide base is changed) in the bacterial genome represent one of the simplest mechanisms by which bacteria can achieve complete resistance to phages.[18]

Host defense pathways

Bacteria have developed certain mechanisms against pathogens such as phages in response to evolutionary pressure, referred to as host defense pathways. The defense mechanisms in bacteria heavily depend on their ability to distinguish between their own genome and that of the phage. Bacterial cells often employ the restriction-modification system (RM system) to label their own genome via methylation (the addition of a methyl group to DNA molecules), consequently, the unmarked DNA is destroyed.[19] A phenotype identified in phage resistant bacterial strains is the phage growth limitation (Pgl) phenotype. Strains carrying this phenotype allow for a single

cycle of phage replication, but the resulting phages are unable to infect new host cells. [19] A reverse RM phenomenon is observed here, where the phage DNA is marked via methylation, and other bacteria in the colony activate the Pgl system in order to prevent phage growth. [19]

Phage Countermeasures and Applications in Combination Therapy

In response to these mechanisms, phages can alter their host specificity by mutating their receptor-binding proteins (RBPs) in response to changes in bacterial surface receptors, with the genes coding for these proteins experiencing the highest rate of mutations in the phage genome.[18] Some phages have evolved to recognize and degrade bacterial capsules and extracellular layers by binding to them and employing depolymerases.[18] Phages employ several mechanisms to evade the RM systems of bacterial cells, these include: Mutation of restriction sites (Phages can remove or alter restriction sites in their genome, preventing recognition by restriction enzymes),
Modification of target sequences (Phages can chemically modify the DNA sequences recognized by restriction enzymes, such as substituting cytosine with modified bases),
Altering site arrangement (By changing the distance or orientation of restriction sites, phages can prevent enzymes that require specific spacing or orientation from cutting),
Occlusion by proteins (Phages can coat restriction sites with proteins that are delivered along with the genome, blocking enzyme access), Sequestration of restriction enzymes (Some phage proteins mimic DNA structure to bind and inhibit restriction enzymes), and Genome methylation (Phages can acquire or stimulate methyltransferases to modify their DNA, rendering it resistant to restriction enzymes) [18]

In bacteria, surface factors are commonly referred to as virulence factors or antibiotic resistance mechanisms, since they facilitate host attachment and damage as well as antibiotic efflux. [20] These surface factors are critical in the presence of disease phenotypes in bacterial cells. As these surface factors are targeted by phages in order to identify bacterial strains, it is only natural for mutations to occur in them. These mutations cause modifications in structures like antibiotic efflux channels, and other defensive measures against antibiotics, potentially compromising them. As a result, a trade off is created, wherein the bacterial strain can either be immune to antibiotics, or to phages. It is to exploit this fundamental weakness in bacterial cells that combination therapy (usage of both phages and antibiotics together) can be used to treat MDR infections. Additionally, the potential for reduced virulence towards the host of bacterial cells has also been observed as a result of the mutations in the bacterial strain targeted by certain phages. [20] This approach is known as phage steering.

The mutations in bacterial cells to resist infections via phages are unpredictable and random. As a result, it has also been observed that virulence in bacterial cells increases post phage exposure. In order to mitigate this risk, amongst others things, scientists have turned to the genetic engineering of bacteriophages as a solution.

Genetic engineering of phages

Homologous recombination

Among the most prevalent techniques when it comes to engineering phages is homologous recombination, a technique in which foreign DNA is integrated into the phage genome in regions on said genome where the foreign DNA and the phage DNA have matching sequences, within a bacterial host cell.[21] Recombination of phage genomes, however, is highly inefficient, making screening for phages that can turn into recombinant phages or are recombinant phages necessary.[21] Additionally, the bacterial hosts that are vital to facilitate this process run the risk of lysing before the desired result is reached. To overcome this limitation, yeast-based and in vitro phage genome assembly approaches have been developed.

Yeast based replication

Owing to their efficient recombination machinery and tolerance to phage genes, *Saccharomyces cerevisiae* (a species of yeast) can assemble synthetic phage genomes through transformation-associated recombination (TAR) cloning, the process of inserting desirable fragments of phage DNA into the host cell to be stitched together by the host cell's machinery to produce the target genome, after which the completed genomes are introduced into a bacterial host via electroporation in order to produce the recombinant phages. [21]

In vitro replication

In vitro phage replication, also known as phage rebooting, is the creation of a phage genome with the desired sequences in the absence of a host cell. Phage rebooting involves amplifying the phage genome in smaller PCR fragments, introducing desired modifications, and subsequently reassembling these fragments into a complete genome.[22] The reconstructed genome is then injected into a favourable bacterial cell, enabling the production of phages containing the engineered sequence.

Conclusion

In conclusion, understanding the defense mechanisms of bacteria and corresponding phage counter-strategies has been pivotal in the advancement of the therapeutic use of bacteriophages. While mutations and new immune-like systems continue to enable bacteria to resist phage

infections; combination therapy and genetic engineering of phages represent promising ways to overcome these barriers. Integration of synthetic biology tools, alternative host systems, and rational design strategies now enables the precise manipulation of phage genomes with very low safety risks but the highest efficacy possible. Together, these aspects further strengthen the position of phage therapy as a dynamic and flexible approach against a wide range of multidrug-resistant bacterial infections.

Ethics Statement

The authors have nothing to report.

Conflict of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose. The authors have no conflicts of interest to declare that are relevant to the content of this article

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