Using Viruses to our Advantage to Combat Antibiotic Resistance

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Abstract

Antibiotic resistance is one of the greatest public health threats we face. Approximately 2 million infections and 23,000 deaths are a result of antibiotic-resistant bacteria in the United States every year.[1] It occurs when bacteria acquire immunity to antibiotic treatments. In response, pharmaceutical companies usually develop new or improved antibiotics. However, as consumers overuse and misuse these treatments, multidrug-resistant bacteria with no effective treatment(s) emerge. Unfortunately, the proliferation of such microbes threatens to return society to pre-antibiotic era health conditions. Fortunately, phage therapy offers a potential solution. Phage therapy uses bacteriophages, viruses that exclusively infect bacteria, to target and kill antibiotic-resistant microbes at infection sites. This paper explores phage therapy and the challenges faced by researchers in developing it. It concludes with a discussion on the importance of phage therapy in the future on a global level.

Introduction

Traditionally associated with Paul Ehrlich's "magic bullet" and Alexander Fleming's discovery of penicillin, the start of the "antibiotic era" sparked a cascade of research and innovation in medicine, saving millions of lives from one of the leading causes of human morbidity prior to the twentieth century: bacterial infections.[2] As soon as antibiotics were introduced to the public; however, many bacterial species became resistant to the drugs they were once sensitive to. This means novel therapies are required, such as the discovery of new, novel antibiotics or the use of bacteriophage (phage) therapy.

Bacteriophages are a class of viruses that exclusively infect bacteria. There are two types – lytic and lysogenic – that largely differ in whether they kill the host during their replication cycle (lytic) or integrate themselves into the host's genome (lysogenic). In fact, a defining characteristic of bacteriophages is that they are host-specific and will only target bacteria that they can successfully infect. Phage therapy utilizes this principle by using targeted-lytic phages to kill antibiotic-resistant bacteria present in infections.[3] This paper will explore what bacteriophages are and how they can be utilized to treat antibiotic-resistant infections.

What is antibiotic resistance?

Antibiotic resistance is caused by the overuse and misuse of antibiotic treatments (Figure 1). Essentially, when bacteria are repeatedly exposed to antibiotics, selective pressures can emerge in which bacteria with antibiotic-resistance mutations and genes grow and proliferate in the environment.[4] These scenarios often occur when inappropriate antibiotics are prescribed or if patients fail to complete the recommended dosing regimens.[5, 6]

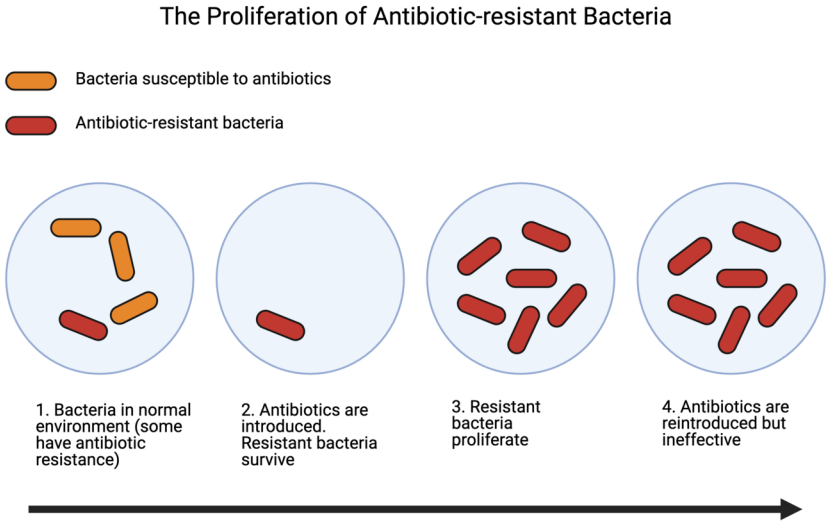

Figure 1: Bacteria are exposed to antibiotics which results in only antibiotic-resistant bacteria remaining. After resistant bacteria proliferate, they become the dominant strain in the environment.[5]

What are bacteriophages?

Bacteriophages are naturally occurring bacterial-specific viruses that are integral parts of the ecosystem. Structurally, most have three major components: nucleic acids, an icosahedral protein capsid, and a tail (Figure 2). The nucleic acids contain genetic information to transcribe, translate, and ultimately replicate the phage; the capsid encases and protects the nucleic acids, and the tail recognizes and attaches the bacteriophage to the host.[7]

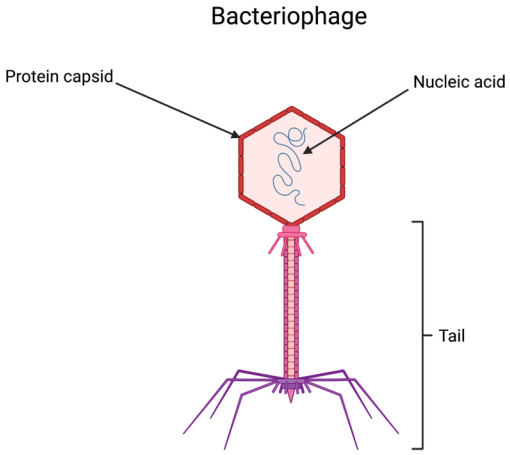

Figure 2: General schematic of a bacteriophage. Shown are nucleic acid (genetic material of the phage), protein capsid (encompasses the nucleic acid), and tail (identifies and attaches to the host bacterium).[7]

Each bacteriophage follows one of two replication cycles: lytic and/or lysogenic. During the lytic cycle (Figure 3), the tail of the bacteriophage docks onto receptors on the surface of the host bacteria. Next, the tail contracts and injects the nucleic acid into the host. Finally, the nucleic acids are transcribed (DNA is copied to RNA by matching the DNA bases with complementary RNA bases) and translated (proteins are produced from the transcribed RNA at ribosomes) by the bacteria's own machinery to replicate the phage. The subsequent stage depends on the nucleic acid of the bacteriophage. If the nucleic acid in the bacteriophage is DNA, the DNA utilizes the host's RNA polymerase and ribosomes to perform transcription and translation in order to produce the icosahedral capsids and tails. This creates protein "shells" of bacteriophages because the nucleic acid has not yet been added. If the nucleic acid is RNA, only translation is needed in order to produce the same product.

After biosynthesis, maturation begins. Maturation involves the replication of the nucleic acid and the insertion of that molecule into the protein "shells," creating mature phages. Depending on the nucleic acid of the bacteriophage, the phage utilizes different enzymes in the bacteria host to replicate the genetic material. Once the bacteriophages have matured, they breach the cell wall, lysing the bacteria, and are released into the environment.[8]

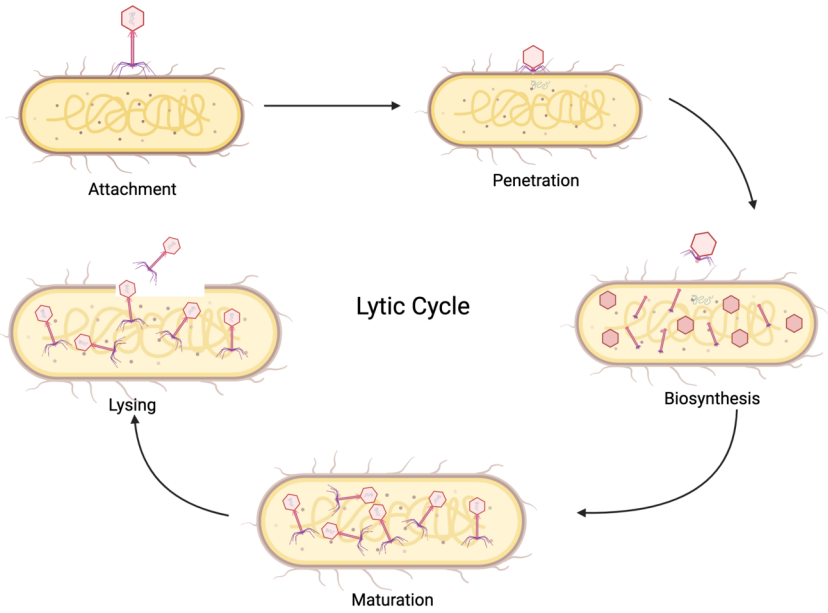

Figure 3: There are five major stages to the lytic cycle: attachment (phage docks to the bacteria), penetration (injects nucleic acid), biosynthesis (phage proteins are assembled), maturation (full phages are formed), and lysing (phages exit bacteria by rupturing the cell wall).[8]

The lysogenic cycle can be considered as an "add-on" to the lytic cycle for certain bacteriophages. Essentially, following nucleic acid injection, the viral genome integrates itself into the bacterial genome as a prophage and remains dormant. As a result, when the host bacterium undergoes a reproductive cycle, it also replicates the viral prophage, creating numerous copies of the viral genome. When optimum bacteriophage replication conditions occur, the prophage exits the lysogenic cycle and resumes the lytic cycle. A key difference between both cycles is timing; while the lytic cycle can be fulfilled in one generation of bacteria, the lysogenic cycle can last through multiple generations of bacterial replication.[9]

Treating infections caused by antibiotic-resistant bacteria with bacteriophage therapy

Bacteriophages were first discovered by Felix d'Herelle when he first speculated that phages were responsible for recovery from diarrheal illnesses caused by bacterial infections.[10] To test his hypothesis, d'Herelle proposed a potential treatment against avian typhosis (Salmonella gallinarum) that utilized laboratory-produced phages as therapeutic agents and successfully administered the treatment in February 1919.[11] This treatment is the first field trial for phage therapy, where phage suspensions were administered orally, through injections, or directly on the surface of an infection.

Interest in phage therapy has recently increased due to the increased demand for alternative therapies to antibiotics. Additionally, unlike antibiotics, the host-specificity of phages ensures that the gut microbiome remains healthy as specific bacterial populations can be targeted and killed, leaving the endogenous community alone. A key player in this research field is UC San Diego's Center of Innovative Phage Applications and Therapeutics (IPATH). In 2019, the FDA approved IPATH's first clinical trial with phage therapy. The trial involves using a bacteriophage cocktail to treat Staphylococcus aureus-infected ventricular assist devices in patients. With approximately 10 participants, this study is currently evaluating the efficacy and tolerability of phage therapy combined with antibiotic treatments.[12]

Challenges/Roadblocks of Phage Therapy

While phage therapy has the potential of saving tens of millions of lives as an alternative to antibiotics,[13] there are many obstacles that researchers must overcome in order to administer the treatment to the general public. These include preventing phage-resistance and finding adequate sources of funding to support new research.

Phage-resistance can arise in many ways, including the CRISPR-Cas system in bacteria. With CRISPR-Cas, phage-derived sequences are integrated into the bacterial genome as CRISPR loci that can be used by Cas proteins to recognize and cleave viral nucleic acid when present (Figure 4).[14] If a phage is repeatedly introduced to the bacterial colony, the chances of that phage's genome being derived into a CRISPR loci increases, leading to phage resistance in bacteria. Beyond CRISPR, researchers have discovered more than 45,000 bacterial genomes that contain phage-resistant characteristics and are yet to fully understand the mechanisms behind them.[15] Luckily, scientists have observed a 'trade-off' in bacteria between phage-resistance and antibiotic resistance. In essence, bacteria with high antibiotic resistance tend to have low phage resistance and vice versa.[16] However, certain studies have also observed cases of pleiotropy which allows for the co-expression of phage-resistance and antibiotic-resistance.[17]

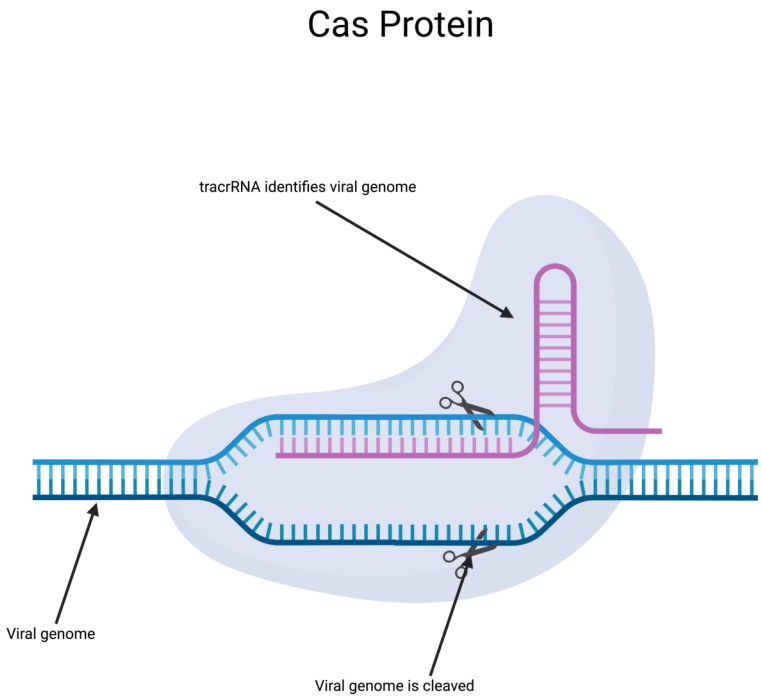

Figure 4: Cas Protein contains tracrRNA which identifies viral genetic material. Once identified, the protein cleaves it.[14]

There is a notable lack of investment in clinical trials with phage therapy by pharmaceutical companies.[18] One reason for this is that pharmaceutical companies have chosen to invest more in improving current

treatments instead of creating novel therapies. This allows them to continue innovating while also saving the costs of bringing a new treatment into the market.[18] Since there is no licensed phage product for human therapy in the Western market, its costs are unknown. Unfortunately, the journey to bring phage therapy into the public market will remain an uphill battle as phage products are not yet profitable enough in the context of human use. [19] That said, this hurdle is common to researchers developing new antibiotics. Even if the antibiotic were to be profitable, pharmaceutical companies would prefer to improve existing drugs to maximize profits. A second reason is that investors are aware that the antibiotic field is not profitable.[20] This was exemplified in the recent bankruptcy of companies such as Achaogen. Plazomicin, their leading FDA-approved antibiotic used to treat infections caused by multidrug-resistant E. coli, struggled tremendously once in the market, earning less than $1 million in sales.[21] The choice of profitability over innovation poses a significant ethical concern regarding what should be prioritized by pharmaceutical companies: money or research. The current trend of choosing money may benefit pharmaceutical companies in the short term. Still, with the rise of antibiotic-resistance, the costs of deprioritizing research will eventually overshadow pharmaceutical profits as 10 million lives will be lost annually due to infections induced by antibiotic-resistant bacteria by 2050, emphasizing the importance of pursuing phage research and alternative therapies.[13]

Conclusion

Phage therapy provides a unique and innovative treatment against bacterial infections in two ways. First, the host-specificity of the bacteriophages used in phage therapy allows for only infectious bacteria to be targeted and killed at a site of infection, allowing the normal state of the gut microbiome to be maintained as the pathogenic bacteria are removed. Secondly, phage therapy is a potential solution to antibiotic resistance itself. Antibiotic resistance is caused by the overuse and misuse of antibiotics. As bacteria evolve to acquire multidrug resistance, fewer viable treatments will become available. With phage therapy, bacteriophages that target multidrug-resistant bacteria will be able to target and kill them with a higher success rate compared to antibiotics alone. While a major hurdle for the field includes low confidence from the pharmaceutical industry driven by recent failures in new antibiotics and the push to improve existing drugs instead of finding new ones, the full potential of phage therapy may be a promising avenue once obstacles like funding and knowledge gaps are addressed.

Endnotes

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Additional Note

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