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Medical application of Bacteriophage as a potential solution to antibiotic resistance: A literature review

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INTRODUCTION

Broad-spectrum antibiotics are highly desirable because they can be used against a broad variety of bacteria without the need to identify the agent causing the infection. This advantage has led to widespread misuse and overuse of antibiotics, which has contributed to the emergence of antibiotic resistance.[1] Due to the spread of resistance, antibiotic treatment for bacterial infections has become less effective. Particularly, ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) have caused life-threatening infections in humans and pose a significant threat to global health due to their high level of antibiotic resistance.[2] In addition, numerous antibiotic-resistant microorganisms (including the ESKAPE species) and antibiotic resistance genes (ARGs) have been identified in beach sand and intertidal beach water, industrial and municipal wastewater systems, soils impacted by human activities and dumpsites, vegetables, raw or ready-to-eat foods, irrigation water, groundwater, and surface water systems, including drinking water systems. Approximately 46.4% of bacteria obtained from hospitals, sewage treatment facilities, and pharmaceutical factories are resistant to multiple antibiotics.[3] Using bacteriophages as antimicrobial agents is one of the alternatives to antibiotics that is considered. In the current era of drug-resistant pathogens, phage therapy is a vital alternative to antibiotics. Since 1966, bacteriophages have been utilized as antibacterial agents, playing a significant role in the development of molecular biology.[4] Novel strategies are investigated, such as using genetically engineered bacteriophages, antibiotic-phage synergy and phage-based vaccines.

WHY CHOOSES BACTERIOPHAGE?

Bacteriophages are extremely varied organisms that are capable of infecting virtually all bacteria on the planet.[5] Every phage can be viewed as a delivery vehicle. Any genetic material packaged within their capsid can be delivered to the phage-specific host and initiate the development of progeny phages and cell lysis or participate in recombination, transcription, or other processes that result in a phenotypic change or cell death.[6]

Bacteriophage therapy utilizes strictly lytic phage particles as an alternative in the antimicrobial treatment of resistant bacterial infections and is being rediscovered as a safe method because these biological entities devoid of metabolic machinery have

no affinity for eukaryotic cells.[7] In addition, although bacteria can develop phage resistance, phage resistance is not nearly as concerning as antibiotic resistance. Similarly to bacteria, phages undergo mutation and can therefore evolve to combat phage-resistant bacteria. Furthermore, the development of phage resistance can be prevented entirely if phages are administered in cocktails (preparations containing numerous types of phages) and/or in combination with antibiotics.[4] These characteristics allow phages to become a potential alternative of conventional antibiotics rather than other chemical compounds.

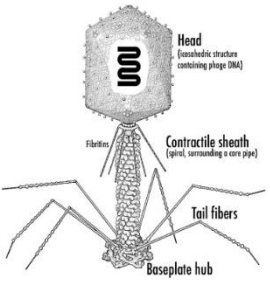

Figure 1: General structure of a phage. [7]

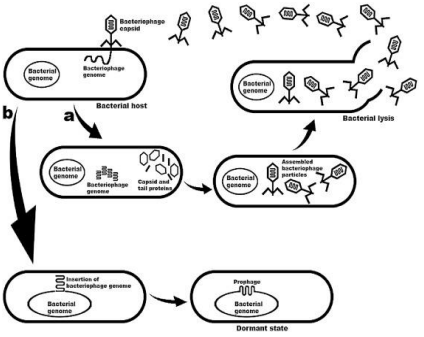

Figure 2: How phages infect bacteria. [7]

POSSIBLE CLINICAL STRATEGIES:

1. Antibiotic-bacteriophage synergy

Combining phages with clinically-used antibiotics is one of the most appealing and feasible uses for them. Scientists have started the research of finding the probability of this strategy many years ago. After the discovery of penicillin in 1928, a number of scientists assessed the efficacy of combining this antibiotic with phages for the treatment of bacterial infections. In 1945, Himmelweit et al. cultured 130 million Staphylococci/mL in the broth to which they added (a) penicillin, (b) phages, or (c) both. Complete lysis occurred in 6 hours and three minutes for penicillin alone (a), 1 hour and fifty-five minutes for phages alone (b), and 1 hour and twenty-five minutes for the phage–penicillin combination (c).[8] This experiment clearly indicates that certain combinations may shortens the length of time for the medicine to take effect. The use of phage and antibiotics together may result in a variety of outcomes. Some antibiotics stimulate the production of phages by a bacterial host and result in the formation of larger plaques. The application of phages and antibiotics is recommended for the treatment of Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Enterococcus strains, according to research.[9] Scientists believe that synergistic and antagonistic interactions are highly dependent on the mechanism of bacterial inhibition by the class of antibiotic coupled to the phage, and when synergism is observed, it inhibits the emergence of resistant cells.[10] The reason is that combinations of phages and antibiotics inhibit bacterial proliferation and antibiotic resistance by targeting distinct bacterial receptors. Due to the increasing difficulty for bacteria to develop resistance when they are invaded via multiple routes. This mechanism is similar to that observed with phage cocktails, which reduce the likelihood of the emergence of multi-drug resistance by attacking multiple bacterial receptors simultaneously.[11] The possible types of phage-antibiotic interactions

(additivism, synergism, antagonism, and neutrality) were primarily determined by the class of antibiotics used in therapy. It is also affected by host physiological environment changes and types of bacteria targeting.[12] Although this amazing combination of previous and novel technology seems to have found a solution to multiple diseases, the dosage ratio needed to be clearly investigated in clinical trials.

2. Genetically engineered bacteriophages

Although the sheer abundance of phage in the environment facilitates the use of naturally occurring bacterial viruses in a variety of therapeutic approaches, phages can be improved through genetic engineering.

For instance, some modifications to the phage DNA may be intended to modify the phage capsid so that it acquires affinity for certain bacterial or eukaryotic cells that are not the normal targets of the parental phage.[6]

One of the most common techniques for phage engineering is homologous recombination, in which a heterologous segment of DNA is recombined with the phage genome at sites of homology within a bacterial host. To prevent the potential toxicity of phage replication on the bacterial host, yeast-based or in vitro phage genome assembly technologies have been developed. Since yeast cells have an efficient recombination system and phage genes are not toxic to yeast, Saccharomyces cerevisiae can facilitate the assembly of synthetic genomes through transformation associated recombination (TAR) cloning. The assembled genomes can then be electroporated into a permissive bacterial host for rebooting.[13]

Multiple proteins within phage capsids can be targeted for modification without necessarily affecting the functions of the capsid and phages can carry genetic information. These two characteristics enable scientists to modify phages in order to increase their affinity for targeted bacteria hosts or to insert the genetic information they want.

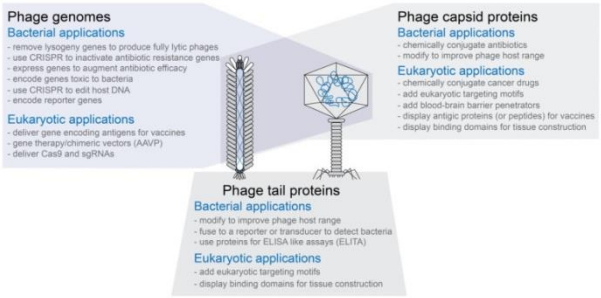

Figure 3: Multiple proteins on phages readily to be modified.[14]

Certain clinical techniques can be utilized to aid phages in eradicating targeted goals:

Encapsulation

Phages can be swiftly inactivated by enzymes *in vivo*, the host immune system, or pH at wound infection sites and in the stomach. To overcome these issues with stability, phages are encapsulated in nanoparticles composed of lipids or polymers. Infected intracellular pathogens, such as Mycobacterium tuberculosis, Staphylococcus sp., and Chlamydia sp., can gain easier access to phagocytic cells due to phage encapsulation.[15]

Inhalational delivery

Antibacterial agents, such as bacteriophages and antibiotics, can be administered locally to the lung tissues as aerosols for respiratory tract infections. This allows for higher concentrations at the site of infection, preventing the dispersion of antibacterial agents in other sites where such activity is not required, thereby significantly increasing their activity *in situ* and reducing potential side effects.[7]

As vaccines

The purpose of vaccine development is to induce safe, durable, and selective immunity to a specific antigen. Increasingly, bacteriophages play crucial roles in the development of vaccines, particularly in peptide selection and antibody production. Additionally, engineered phages have been designed to directly stimulate immune responses.[16]

Vaccines are one of the most significant bioproducts in medicine. Since the invention of the smallpox vaccine in 1796, a variety of vaccines for numerous diseases have been developed. However, certain vaccines have limitations, such as high cost and inadequate immune responses.[17]

To surmount the limitations of conventional vaccines, bacteriophage-based vaccines are considered as a potent alternative. Utilizing the inherent properties of bacteriophages, this method enhances the stability and immunogenicity of displayed antigens.[18]

Phages are utilized in numerous applications, including drug delivery, phage therapy, the development of biosensors, and as vaccine delivery systems. The development of phage display technology, which is based on the manipulation of bacteriophages to display antigens on their surface, has made many of these applications feasible. Bacteriophages are a specific class of viruses that infect and replicate within bacteria and archaea but are incapable of infecting eukaryotes. These viral particles are composed of genetic material, either DNA or RNA, encapsulated in protein structures known as capsids that can be either simple or complex.

Phage display is a powerful technology that involves the expression of peptides or proteins fused to the surface coat proteins of a phage.[17]

Phage display is an effective method for the development of novel vaccine delivery vehicles. Using this method, phage display or phage DNA vaccines can be developed. A hybrid phage vaccine has also been proposed. Compared to standard vaccines, phage vaccines elicit a more powerful and less variable immune response. It is anticipated that the hybrid phage vaccine will induce an efficacious humoral and cellular response. The employed phage not only expresses antigen, but also a targeting molecule; consequently, it appears to have a potentially significant advantage for target-directed therapy.[19] A vaccine carrier should ideally be simple to mass-produce, secure, and able to effectively present antigens to the immune system in order to elicit the desired immune response. Although phage particles have been demonstrated to be safe for animal and human use, phages administered orally have the potential to infect intestinal bacteria, resulting in dysbiosis. Infection and further replication of phages may trigger the release of endotoxins from infected bacteria, thereby increasing the risk of host injury.[20]

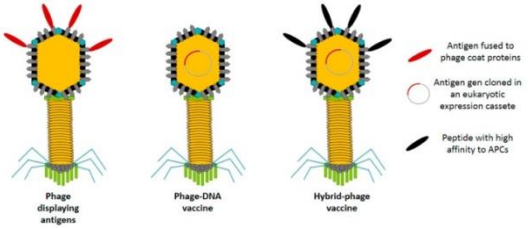

Figure 4: Structures of phage-based vaccines. [21]

Ethical problems and drawbacks of bacteriophage therapy

Bacteriophage therapy is still under discovery and testing, so this treatment is still immature since it faces a wide range of ethical as well as technological challenges.

Assuming that phage therapy is ultimately approved for routine use by physicians, surgeons, and infectious disease specialists in hospitals, patients will be required to consent to being infected with 'live' viruses in order to cure or prevent bacterial infections. Antibiotics cannot target infections as precisely as phages can. Patients treated with phage therapy can recover from infection with minimal adverse effects and risks if they are correctly diagnosed and dosed. Compared to phage, broad-spectrum antibiotics frequently modify or compromise a patient's microbiome. For instance, an increasing number of patients develop clostridium difficile-related complications after taking antibiotics. Clostridium difficile is an opportunistic pathogen that swiftly multiplies when a patient's use of antibiotics eliminates competing bacteria. C. diff can cause chronic damage to digestion and intestinal health.[22] It has also been reported that bacteriophages have both beneficial and detrimental effects on disease management in the context of the crisis of multidrug-resistant bacteria. Bacteriophages (phages) have significant ecological and evolutionary effects on their bacterial hosts and have been linked to the therapeutic use of killing bacterial pathogens, but they can also contribute to the transmission of antibiotic resistance.[23]

CONCLUSION

Bacteriophage therapy has a lengthy history; the Book of Kings describes how the prophet Elisha cured the disease of the general Naaman by commanding him to bathe seven times in the Jordan River. Since ancient times, it has been reported that river water can cure infectious diseases such as leprosy.[4] Literature on the use of phages and phage-derived proteins in the treatment of bacterial infections, particularly those caused by multidrug-resistant bacteria, indicates a promising future for phage therapy as either an alternative to or a complement to antibiotics. Before implementing phage therapy on a large scale, it is imperative that scientists gain a deeper understanding of the interaction between phage, microbiome, and the human host, as recent findings on immunomodulatory effects, host range, and the potential for horizontal gene transfer are inconsistent. In addition to their reduced immunological potential, phage lysins may be a more practicable therapeutic agent due to their simplicity of production, purification, and storage. [21] Scientists have suggested several possible clinical therapies, for instance, when genetically engineered phages are applied to eradicate certain targeted bacteria or when antibiotics are used in conjunction with specific phages. Certain techniques like encapsulation or inhalational delivery all helps the phages to take effect under specific conditions. These strategies all provide an

increasing efficacy intreating antibiotic-resistant bacteria and brought human beings the dawn of bacteriophage therapy. Undoubtedly, the usage of phages has implied us a potential pathway for preventing antibiotic resistance, meanwhile, further research is anticipated to be done in order to complete this technology.

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