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Addressing the rising threat of antibiotic resistance by optimizing phage therapy to combat antibiotic resistant bacteria

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ABSTRACT
Antibiotics have long been a cornerstone in the fight against virulent microbial infections, significantly improving public health outcomes. However, their widespread and persistent use has led to a serious threat: antimicrobial resistance (AMR). This growing issue thus raises a consequential threat to public health and the environment. Consequently, necessitating the search for an efficacious substitute is the need of the hour.

The resurgence of interest in phage therapy, one of the old remedies, is due to its preciseness in targeting the bacterial cells and causing lysis of the bacterial cell without causing harm to human or animal cells. Researchers worldwide are exploring the viability and efficiency of phage therapy as a means to address the challenge raised by the growing antibiotic resistance.

This review paper discusses the current progress on bacteriophages as a promising alternative to antibiotics in response to the growing issue of antibiotic resistance. It covers various aspects, including the history and structure of bacteriophages, methods of preparation, their current status in research and therapy, and their potential future applications.

(Bacteriophage therapy to combat antibiotic resistance: a brief review)

I. INTRODUCTION

Antimicrobial resistance (AMR) is a worldwide public health problem and is a threat to all humans and animals. The prevalence of antibiotics in the surrounding environment, due to the growing world economy, is the harbinger of antibiotic-resistant bacteria, which undermines the effectiveness of antibiotics in combating bacterial infections.

The World Health Organisation has also emphasised the upcoming threat of the post-antimicrobial era, wherein common infections will no longer have a cure. Conversely, the enthusiasm of the scientists towards exploring unique antimicrobial agents is lukewarm due to the antimicrobial market being less profitable than the other pharmaceutical products. The complications in the treatment of many antimicrobial infections have led scientists to review phage therapy and bacteriophage again. Phage therapy has the potential to become the most successful replacement of antibiotics to fight against antimicrobial infections. Phages are viruses that attack only the target site. This characteristic, along with the killing of the bacteria without harming humans and animals, makes it a likely substitution.

HISTORY

History in India:

The Sangam (intersection) of India's two major rivers—major waterways to be precise; namely, Ganga and Yamuna lie in the city of Allahabad. Ritualistic dips in these river waters are of great regard for their believed healing control against disease and contamination. These river waters carry legendary importance and mythological significance in Hinduism and other religions.

Nevertheless, these river waters also have a foundation in ancient microbiology. A British bacteriologist, Ernest Hanbury Hankin, in 1896 discovered the bacterial viruses, called bacteriophages, by observing their activity in the Ganga and Yamuna rivers on cholera. In 'Microbes,' an article published in the Annales de L'Institut Pasteur in 1896, Ernst Hanbury Hankin detailed an 'antiseptic substance present in the waters of the Ganges and

Jamuna rivers'. In his article, he specified that the Indian river waters (Ganges and Yamuna), when compared to European rivers, exhibited an extraordinary lack of microbes.

He gave a few clarifications for this scarcity of microbes: inadequately populated settlements near the river, absence of polluted factories, the source of rivers being the unadulterated melted snow of the Himalayas, or the self-filtering power of the air and water encircling it. In any case, other factors would have guaranteed pollution by cholera microbes: these waterways also received water from the drains of Agra, which indeed had cholera germs. Additionally, people also disposed of burnt cadavers into these rivers, which passed on cholera without proper filtration by fire. Bathing spots at the intersection of these rivers also showed the presence of cholera microbes.

Ultimately, Hankin interrogated as to why one has never seen an epidermis downstream of these rivers if cholera is a water-borne disease.

Hankin performed various experiments by collecting multiple water samples along the river and analysing the number of microbes per cubic centimetre. He concluded that the antiseptic present in the waters of Ganga and Yamuna 'had a powerful action on the cholera germ'. He asserted that the so-called antiseptic lost its dominance when the water was heated. He stated, **"It is seen that the unboiled water of the Ganges kills the cholera germ in less than 3 hours. The same water, when boiled, does not have the same effect. On the other hand, well, water is a good medium for this microbe, whether boiled or filtered."**

Hankin concluded that the cholera germ-curing novel antiseptic was formed 'in the river or in situ by the water'. A year before he published his article, in 1895, in a text that was published in Allahabad, he postulated that even though the cholera microbes were constantly introduced into these streams through the cholera-infected cadavers discarded into them, local people in the peripheral area never contracted cholera by drinking this river water.

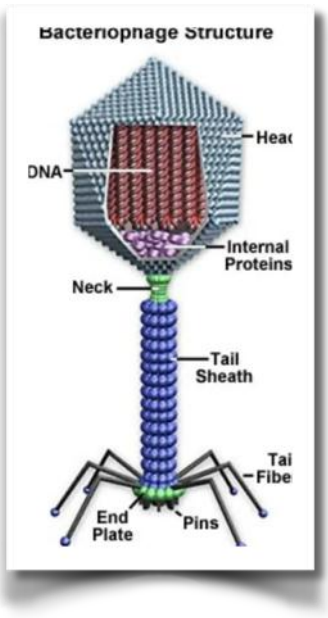
These were the rudimentary analyses made years before bacteriophage activities were again discovered and studied by Frederick Twort and Félix d'Herelle in 1915 and 1917.

(https://royalsocietypublishing.org/doi/full/10.1098/rsnr.2020.0019)

History in Europe and Soviet Union:

Frederick Twort was the first to rediscover phages in England in 1915 and Felix d'Herelle in France in 1917. Bacteriophages were named so by Felix d'Herelle. Phages were used to treat bacterial infections in the 1920s-1930s around World War I in Europe and the Soviet Union, but their usage decreased with the introduction of penicillin as an antimicrobial agent.

Felix d'Herelle developed a phage preparation during the time of World War I to treat the soldiers affected by dysentery. Bacteriophages (viruses) were seen by scientists worldwide as a promising solution for treating bacterial infections, as they had no harmful effects on humans or animals. Felix d'Herelle suggested that bacteriophages be used as antimicrobial agents to treat microbial infections. However, this approach was not embraced by the West. Following the discovery of antibiotics in the 1940s, the idea of using phages for treating humans and animals was largely disregarded. In contrast, phage therapy gained significant attention in the Soviet Union, where Felix d'Herelle collaborated closely with his Georgian colleagues to advance the research.



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What are bacteriophages?

Bacteriophages essentially means bacteria eater. Phages are strong controllers of bacterial infections and are omnipresent; they are found in water, soil, plants, and even in the human microbiota. Phages are viruses that infect bacteria but are different from plant and animal viruses.

STRUCTURE

Structures of bacteriophages share common characteristics but vary in their make-up. Phages vary in size from approximately 2 to 200 kilobases per strand of nucleic acid. Various types of nucleic acids are found in the phages, such as ds DNA, ds RNA, ss DNA, and ss RNA.

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Phages vary in size from approximately 2 to 200 kilobases per strand of nucleic acid. Various types of nucleic acids are found in the phages, such as dsDNA, dsRNA, ssDNA, and ssRNA. The majority of the bacteriophages contain dsDNA genomes. The head of the bacteriophage t4 is attached to the helical tail through the neck. Multiple tail fibres and tail pins are joined to the tail at the end. These tail fibres are syringe-like structures that bind to the receptors on the host cell.

Preparation of phages:

REPLICATION:

The phages replicate in two ways: lytic infection or lysogenic infection.

Lytic infection is a process wherein the cells of the bacteria let the bacteriophage replicate. The phages reproduce new phages, which burst the bacterial cell, thereby killing it.

Lysogenic infection is a process in which the phage is injected into the bacterial cell; the virus hides in the dormant DNA, and it does not show any activity unless activated by something. It follows the process of lytic infections after becoming activated.

The lytic phage is most suitable for phage therapy since it quickly replicates within the host cell and lyses the bacteria, growing exponentially in the process. Each "parent" phage can produce on average approximately 200 "daughters" per lytic cycle, depending on the species and the favourable conditions. If each daughter infects and kills a host bacterium, there will be 40,000 progeny at the end of the 2nd cycle, 8 million at the end of the 3rd cycle, 1.6 billion at the end of the 4th cycle, and so on.

(https://citeseerx.ist.psu.edu/doc
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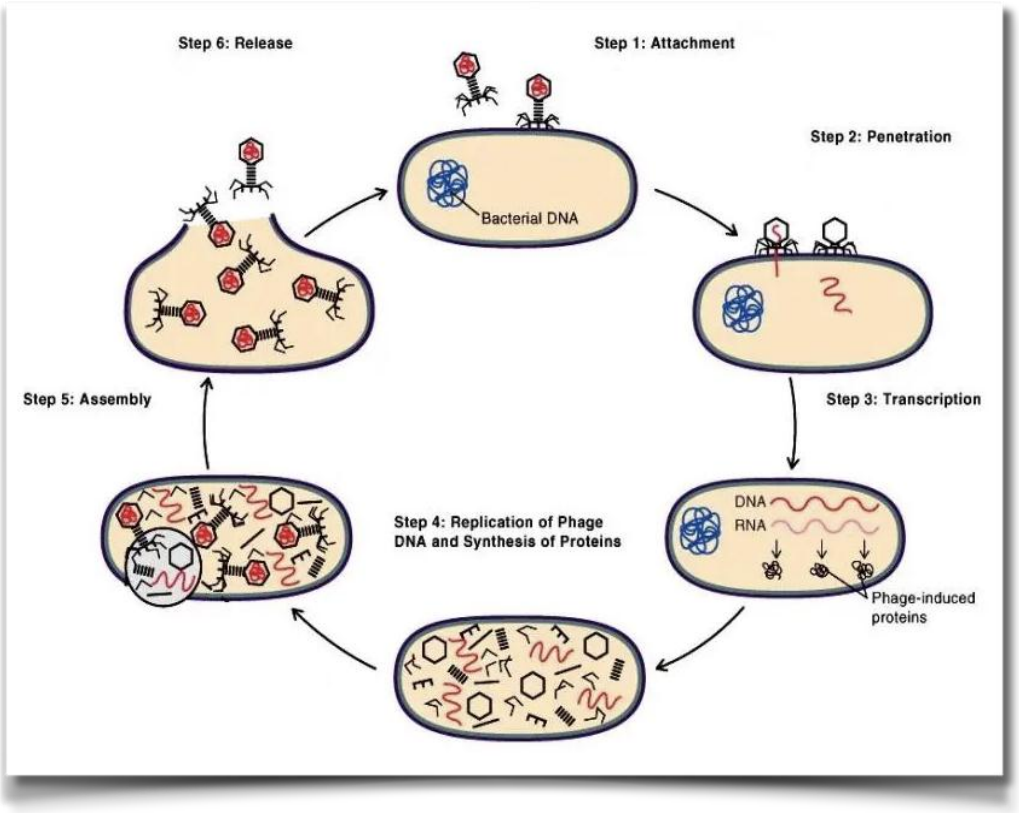


(https://microbeonline.com/bacteriophage-structure-replication-use/)

ATTACHMENT:

Osmosis of bacteriophage is the initial step to infect the bacteria. The proteins located on the tail of the bacteriophage bind with the cell wall of bacteria and recognise specific receptors. Both the bacteriophage and bacterial cell undergo morphological changes that assist in the penetration of the phage within the host cell.

PENETERATION:

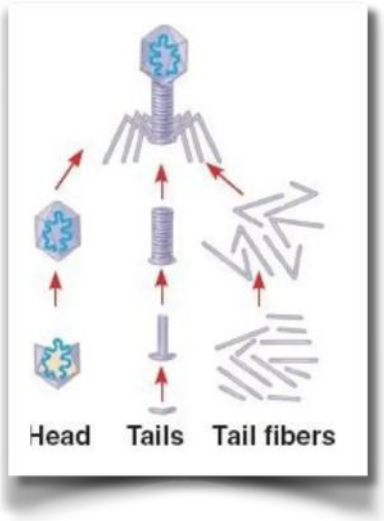
Enzymes like lysozyme are found in the tail of bacteriophages, which weakens the cell wall of the host cell. The tail sheath contracts and penetrates a hollow tube within the weakened cell wall and comes into contact with the cell membrane.

REPLICATION:

Genes of bacteriophage take control over the metabolic machinery of the host cell. It governs the host cell to produce only viral products. Bacterial DNA is uptake by the phage and disrupted, and their nucleotides are used to produce new phage. DNA of the phage is transcribed to mRNA using the machinery of the host cell.

ASSEMBLY:

Different parts of the phage are assembled after their individual development in the host cell. Phage tails are formed from newly formed base plates, sheaths, and collars. Each head is attached to a tail after being packed with DNA. After their maturation, tail fibres, head, and tail are integrated, and a fully formed infective phage is developed.



LYSIS AND RELEASE:

The bacterial cell wall is then lysed by the lysozyme by breaking the bacterial cell wall. The newly formed phage infects other potential bacteria, and the process of lysis of the host cell and replication of the phage is repeated.

Dosage and Administration:

Bacteriophages are infected with the bacteria by a process wherein the phage solution is poured into the petri dish containing the bacteria. The phages soon form plaques. Plaques are the clearing areas appearing on the dish, which is caused by the lysis of the bacteria by the phage. Bacteriophages can be prepared and amplified to high titer (>10^12 PFU/ml) at a research laboratory scale, which is sufficient for hundreds or even thousands of doses depending on dosage and stability. Phages need a sterile condition while forming plaques. The plaque solution should also contain low endotoxin [typically lipopolysaccharide (LPS)] levels, lower than the level approved by the FDA. When phages are prepared on gram-negative hosts, more precautions need to be taken by the scientists since they contain a higher endotoxin layer and have a thick polysaccharide layer. Non-LPS-containing hosts are free from endotoxin. Phages have a narrow spectrum, which also adds to their efficacy to treat infections. Each phage is specific for a particular infection and requires a unique medium to sustain. Phages will also need to be prepared using good manufacturing practices for clinical and commercial use. This increases the cost, maintenance, and challenges. Favourable environmental conditions and concentrations (>10^10) are necessary for retaining the viability of phage preparations. (4–10°C) temperature is also required to maintain its viability.

Like antibiotics, phage is also used to treat microbial infections, but both of them show different mechanisms for killing the pathogen. Phage pharmacokinetics is associated with phage amplification, so the number of phages in the body differs from patient to patient depending on when the page infection occurs and what its target

site within the body is. A potential dosage of 10^9 PFU/dose, twice a day, has been used in several passionate-use cases; nonetheless, the dosage and regimens may be very different in different patients.

A similar dosage, instead of being given as phage cultures, is given as a nebulizer. However, the choice of nebuliser is critical since it can reduce the effectiveness of the dose by a million times by damaging the phage particles.

Phages are used in the form of a nebuliser in Pulmonary infections like cystic fibrosis and used for skin treatment and injected into the joints. High dosages of antibiotics might harm the body; however, the chances of hurting the body are comparatively less.

The current status on bacteriophages:

CLINICAL TRIALS ON PHAGE THERAPY

1. PhagoBurn Trial :A clinical trail called the PhageBurn Trail was launched in 2013. Under this trial, 27 patients who were suffering from burn wound infections were recruited from hospitals located in France and Belgium to be treated with phage therapy (a cocktail of 12 lytic phages) or 1% sulfadiazine silver emulsion cream to compare the efficiency of both treatments in patients with wounds infected by P. aeruginosa (Jault et al. 2019). The effect of both treatments was administered, and the overall aftereffect of the phage cocktail was found to be positive. The phage cocktail was able to decrease the burn wounds, but the process was slower than in the standard treatment. On the optimistic side, no negative effects were found in the phage-treated group. The limited efficacy of the phage cocktail is believed to be caused by a significant drop of the phage titer after GMP manufacturing, which led the patients to receive a concentration that was much lower than had been previously estimated. Additionally, in the in the bodies of patients in whom the phage treatment failed, the targeted bacteria were later found to be resistant to low phage doses.

2. Oral phage therapy of acute bacterial diarrhoea with two coliphage preparations: Nestlé (Switzerland) also performed a phase I/II trial in collaboration with the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (Sarker et al. 2016). This randomised trial was conducted to determine the safety and efficacy of the oral administration of T4-like Phage cocktail in children hospitalised due to acute diarrhea. No effective benefits were observed from the treatment; the phages did not show replication even though the oral coliphages could reach the intestine. On that point, the authors concluded the need for higher oral phage doses and that due to the low host range of the phage cocktail, the trail did not show any outcome for the diarrhoeal infection (Sarker et al. 2016). The oral intake of the phages without their encapsulation to be protected against factors like the acids in the stomach reduces the phage numbers reaching the intestine, which might be insufficient to cure the infection and show visible therapeutic effects. Nonetheless, it was found that the main cause of the acute diarrhoea was not E. coli, and therefore the phage treatment for E. coli did not show any results in response to the acute bacterial diarrhoea (Satter et al. 2017; Nelson et al. 2018). This clinical trail highlights the importance of identifying the accurate etiologic agent(s) causing infection.

3. A clinical trial on phage therapy for chronic rhinosinusitis: Recently, Ooi et al. conducted a clinical trial to test the safety and effectiveness of a phage cocktail made of three lytic phages, applied intranasally to patients with stubborn chronic rhinosinusitis (CRS) caused by S. aureus (Ooi et al. 2019). The treatment, given twice daily for 14 days, was safe and well-tolerated by all nine patients, with no reported side effects. Although two patients showed promising results with the infection being cleared, the authors emphasised the need for a larger, randomised trial to find the best dosage and confirm the phage cocktail's effectiveness (Ooi et al. 2019). The high safety of phage therapy has also been reported in other studies from Poland (Międzybrodzki et al. 2012; Rogóż et al. 2019).

The Potential of Bacteriophages:

Phages produce several proteins, some of which have antibacterial properties. These include enzymes like endolysins, virion-associated lysins (VALs), and polysaccharide depolymerases, as well as receptor-binding proteins (RBPs) that help phages attach to host cells. Other important proteins involved in phage therapy include holins, pinholins, and spanins. Phages also contain genes with unknown functions. For instance, Shapiro and Putonti (2020) discovered a filamentous phage, UPϕ901, that had two or more genes with unknown roles. Notably, some of these mysterious genes are located near regions of the genome that are important for host behaviour and virulence (Maiprochnow et al., 2015). Additionally, phages can distinguish between living and dead cells.

Advatages:

a. Phages are very specific. They identify, attack, and kill the host bacteria through their ability to bind to the target sites found on the host cell.

b. They perform lysis of the gram-positive or gram-negative bacterial cells (Allen et al., 2014; Briers and Lavigne, 2015; Love et al., 2018). Although there are some exceptional cases wherein the phage lysis both the gram-positive and gram-negative bacteria. One such example is given by Wang et al. (2020ab), where the phage-derived protein.

c. Phage cocktail employs the use of multiple phages. A polyphase can be made up of a combination of several proteins. Genetically bioengineered phages are mostly used in cocktails. Phage Cocktail containing multiple phages will more effectively kill the host since they will have a greater host range as compared to a single phage or phage enzyme (Ross et al., 2016).

d. Phages can be bioengineered for diverse therapeutic activities and have also shown promising results against drug-resist pathogens. Moreover, they have shown bacteriolytic activity against various high-burden pathogens, especially ESKAPE.

Phage anti CRISPR proteins could be an efficient therapeutic biomaterial against drug resistant pathogens:

A major issue in the success of phage therapy was that of the cas systems widely referred to as the clustered regularly interspaced short palindromic repeat (CRISPR), which acts as the bacterium's memory cellist and allows the bacteria to recognise and degenerate the foreign DNA (Labrie et al., 2010). The host cell bacteria can either modify its receptors to prevent the entry of the foreign viral DNA during phage adsorption or can also employ a restriction enzyme to lyse the foreign DNA, thereby protecting the bacteria cell from its effects. CRISPR is a bacterial defence mechanism that can be adapted into a genome-editing tool (Binnie et al., 2021) since it provides promising treatment therapy for combating drug-resistant bacteria (Gholizadeh et al., 2020).

The CRISPR-Cas system can be delivered to the bacteria in several ways, out of which phage-based vector is the most common reported approach (Kim et al., 2019; Yeh et al., 2022). Due to its ability to remove the target gene of interest (Hau et al., 2014) and specificity, phage-based vector is an optimistic approach. Additionally, it can also be a target for removing or editing some specific bacterial genes (Nath et al., 2022).

However, bacteriophages encode anti-CRISPR proteins against the bacterial CRISPR-Cas defence system. These act as inhibitors and obstruct the bacteria's CRISPR-Cas defence system, which results in the lysis of the bacteria. Hence, this inhibitor protein is a good alternative for drug-resistant pathogens (Vyas and Harish, 2022). Apart from bacteriophages, the discovery of the inhibitor of the bacterial CRISPR system has also been regarded as an effective alternative against drug-resist pathogens.

All in all, subsequent studies should consider using a phage-based vector to deliver the CRISPR-Cas system as pivotal to combat the antimicrobial-resistant crisis. Additionally, the subject of anti-CRISPR protein is still in its infancy and needs to be investigated to properly understand its utility and mechanisms of action. However, for our better understanding of animal models, experiments and clinical trials are needed. Despite still being in its nascentstages,s studies on phage-mediated delivery ofthe CRISPR-Cass systemholds immense potential.

(https://www.sciencedirect.com/science/article/pii/S0944501322001951)

Phage Antibiotic Synergy:

Combining phages clinically with antibiotics is another feasible and probable use to be used against the antimicrobial infections (13). The combination of the phage and antibiotics can provide multiple results. They may act additively; that is, their combinatorial efficacy will be equal to their individual effects. They can also provide synergistic results by providing the total efficacy much higher than their individual effects. Another case may be that it will yield no effect due to the inactivity of each individual component. And yet, there may also be antagonism whereby the action of either of the agents could affect the action of the other. This recent awareness of antagonism has led to some in vitro assessments of phage-antibiotic action prior to the treatment of selecting the

combinations, and this approach has led to satisfactory results (15, 16). Several studies have examined the combinations of phages and antibiotics. However, phage-antibiotic synergy is often tested with one other two antibiotic doses, which is not enough to predict the most effective combinations for the treatment. And because of this reason, we were able to study how well phages kill bacteria by considering the presence of a resistance gene, the chances of developing resistance, and how environmental conditions also effect the effectiveness of phage-antibiotic synergy.

Studies were conducted using a known myovirus (ΦHP3) that targets a highly virulent strain of ExPEC (strain JJ2528). The findings suggest that for phage-antibiotic combination therapy, clinicians should consider three things:

I. choosing antibiotics that don't interfere with the phage's ability to function within the bacterial cell.
II. The balance between the amounts of phage and antibiotic used.
III. How the host environment might impact treatment effectiveness.

CASE STUDY

Steffanie Strathdee's TEDx talk, "How Sewage Saved My Husband's Life from a Superbug," is a gripping narrative that revolves around her husband, Tom Patterson, who was stricken by a deadly superbug and saved by an innovative treatment using bacteriophages.

The Onset of Crisis

Tom Patterson and Steffanie Strathdee, both accomplished scientists, were enjoying a vacation in Egypt when Tom fell severely ill. Initially misdiagnosed with pancreatitis, his condition deteriorated rapidly, revealing an underlying and more sinister cause: an infection with Acinetobacter baumannii. This bacterium is notorious for its resistance to antibiotics, earning the moniker "Iraqibacter" due to its prevalence among soldiers wounded in Iraq. Despite the best efforts of medical professionals in Egypt and subsequently in Germany, where he was transferred for advanced care, Tom's condition continued to worsen.

Desperation and Discovery

Back in the United States, Tom was airlifted to the University of California, San Diego (UCSD), where Steffanie worked as an epidemiologist. The infection, however, proved unyielding to all known antibiotics. Tom was in a coma, and the prognosis was grim. It was during this desperate time that Steffanie, leveraging her background in infectious diseases, began exploring alternative treatments.

In her quest, Steffanie came across phage therapy, an old and largely forgotten medical treatment using bacteriophages—viruses that infect and kill bacteria. Phage therapy had been discovered over a century ago but had fallen out of favour in the West with the advent of antibiotics. However, it continued to be used in some parts of the world, particularly in Eastern Europe and the former Soviet Union, with notable success.

Mobilising a Scientific Community

Determined to save her husband, Steffanie reached out to phage researchers and clinicians globally. She connected with Dr. Robert Schooley, an infectious disease specialist at UCSD, who shared her interest in phage therapy. Together, they formed a multidisciplinary team, including experts from Texas A&M University and the Navy Medical Research Center. This collaborative effort aimed to identify and purify bacteriophages that could target Tom's specific strain of Acinetobacter baumannii.

Regulatory Hurdles and Breakthroughs

One of the significant challenges was obtaining regulatory approval for the experimental treatment. Given the urgency of Tom's condition, the team sought and received emergency investigational new drug (IND) approval from the U.S. Food and Drug Administration (FDA) in a remarkably short period. This expedited process underscored the severity of Tom's situation and the potential of phage therapy as a life-saving measure.

The Treatment and Recovery

The phages were administered intravenously, a novel approach that had rarely been tried before. The treatment was risky and experimental, but the results were nothing short of miraculous. Tom's condition began to improve within days of the phage administration. His organs started to recover, and the bacterial load diminished. Over time, he emerged from the coma and began a long, arduous recovery process. The successful use of phage therapy in Tom's case was groundbreaking, providing a compelling proof-of-concept for this century-old treatment method.

Implications and Future Directions

Steffanie Strathdee's TEDx talk is not just a personal story of resilience and hope; it also highlights critical issues in modern medicine. The rise of antibiotic-resistant bacteria is a looming crisis, threatening to render many of our current treatments ineffective. Phage therapy offers a promising alternative, one that needs renewed scientific focus and investment.

Phages have several advantages over traditional antibiotics. They are highly specific, targeting only the harmful bacteria without affecting beneficial microbiota. This specificity reduces the collateral damage often seen with broad-spectrum antibiotics, which can disrupt the body's natural microbial balance and lead to secondary infections. Moreover, phages can evolve alongside bacteria, potentially circumventing the issue of resistance.

However, the development and implementation of phage therapy face significant challenges. Each phage must be matched to its bacterial target, requiring personalised approaches to treatment. This specificity demands robust diagnostic capabilities and a more complex regulatory framework to ensure safety and efficacy. Additionally, there is a need for large-scale clinical trials to establish standardised protocols and validate the effectiveness of phage therapy across different types of infections and patient populations.

Advocacy and Awareness

Strathdee's advocacy extends beyond her TEDx talk. She has become a prominent voice in promoting phage therapy research and awareness. Her work has helped catalyse a renewed interest in this field, leading to increased funding opportunities, research initiatives, and the establishment of phage therapy centres.

Her story also emphasises the importance of scientific collaboration. The rapid mobilisation of a global network of researchers and clinicians was crucial in saving Tom's life. This collaborative model could serve as a blueprint for addressing other complex medical challenges, demonstrating the power of interdisciplinary and cross-institutional cooperation.

Conclusion for the case study

Steffanie Strathdee's account of how phage therapy saved her husband's life is a poignant reminder of the ever-present battle against infectious diseases and the innovative solutions that lie within our grasp. It is a call to action for the medical community to explore and invest in alternative treatments like phage therapy, which hold the promise of overcoming the growing threat of antibiotic resistance. Her story is a testament to the power of love, determination, and the relentless pursuit of scientific advancement, offering hope to millions facing similar battles against superbugs.

II. CONCLUSION

The increasingly observed acquisition of antibiotic resistance by bacteria necessitates new strategies for combating drug-resistant bacteria. The results of research on bacteriophages, indicating that they can be an alternative means of eliminating pathogens posing a threat to humans and animals, justify its continuation, particularly in view of increasing drug resistance in bacteria and restrictions on the use of antibiotics. The development of adequate phage preparations may in the future prove to be one of the most effective methods for fighting bacteria that are pathogenic for humans and animals and will also make it possible to obtain products that are safe and free of antibiotics.

Our results concluded that bacteriophage is a useful alternative to antibiotics, especially for preventing and treating multidrug resistance infections. It is also concluded that the combination between the antibiotic and bacteriophage therapy reduces and rationalises the levels of antibiotics used in treating bacterial diseases.

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