A Critical Review of **Phage Therapy as an Emerging Antibiotic Alternative**

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ABSTRACT

In 2019, nearly 5 million deaths worldwide were attributed to antimicrobial resistance (AMR), underscoring its profound threat to global public health.[1] AMR causes traditional antibiotics to lose effectiveness against bacterial infections due to the emergence of resistant bacteria. Consequently, phage therapy, which employs bacteria viruses to combat bacterial infections, has become a potential solution.[2] Phages can enhance antibiotic sensitivity by targeting bacterial mutants, influencing the evolution of populations and impacting receptors responsible for antibiotic efflux from cells.[3]

Despite the advantages, several limitations to the use of bacteriophages exist. Phage-Antibiotic Synergy (PAS) may address these limitations. PAS describes the phenomenon of improved antimicrobial effect caused by stimulated phage replication in the presence of sublethal concentrations of antibiotics.[4] Phage-antibiotic therapy has effectively reduced the emergence of phage-resistant and antibiotic-resistant strains simultaneously.[3]

INTRODUCTION

Phage therapy, a century-old clinical approach first used by Felix d'Hérelle in 1919, has recently gained prominence as a potential alternative to antibiotics.[5,6] It involves utilizing bacteriophages, viruses that specifically infect bacteria, to combat bacterial infections.[2] Studies have shown promising results for phage therapy in treating bacterial infections in humans, animals, and plants, including multidrug-resistant pathogens such as *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Mycobacterium abscessus*.[4,6,7] Phage therapy mainly uses obligately lytic phages to kill their respective bacterial hosts while leaving human cells intact and reducing the broader impact on commensal bacteria that often results from antibiotic use.[6,7]

There remain challenges to overcome before phage therapy can be considered a practical alternative to antibiotics. These challenges include the safety, efficacy, and regulation of phage therapy, as well as the complexity of host-pathogen interactions.[5] However, novel phage strains and strategies have been developed to enhance the specificity and safety of phage therapy.[8] Additionally, investigations into the pharmacokinetics and pharmacodynamics of phages *in vivo* may provide valuable insights into the optimal dosing and administration routes for phage therapy.[9]

This critical review aims to provide a comprehensive exploration of phage therapy and its developments in case studies and clinical trials while also looking at the PAS which plays a crucial role in addressing AMR.[8,9] Subsequently, an evaluation is conducted on the limitations hindering the widespread adoption of phage therapy. Finally, we outline potential research directions that can pave the way for optimising phage therapy and PAS in the ongoing battle against AMR.

REVIEW FINDINGS: BACKGROUND

The viral replication cycle of a phage involves the infection of a bacterial host, replication of the phage genome, and the release of new phages to infect other bacterial hosts. Phages can have different replication cycles within the bacterial host, including lytic, lysogenic, and pseudolysogenic life cycles.[35] By integrating their genetic material into the host cell's genome, lysogenic phages can persist in the host and continue to produce new phages over time, potentially providing a longer-lasting treatment than lytic phages. Pseudolysogenic and chronic cycles are important areas of ongoing research, but their significance in phage therapy is not yet fully understood.[10]

Currently, to target and eliminate pathogenic bacteria, phage therapy takes advantage of lytic phages. They are the preferred option for therapeutic purposes as they kill their bacterial hosts by attaching to their surface receptors.[10] Once attached, the phages inject their genetic material into the cell. The phage genetic material takes control of the bacterial cell's machinery and begins producing new phages at a rapid pace. The sheer volume of new phages causes the bacterial cell to burst open, releasing a swarm of new phages ready to infect other bacterial cells. In contrast, lysogenic phages do not outright kill the bacteria; rather they integrate their genome into the host cell, which may harbor AMR or toxin genes. Bioengineered lytic phages are used clinically to overcome the constraints of using naturally occurring phages, such as a limited host range and poor infectivity. To maximize the therapeutic impact, lytic phages are compiled into preparations called "phage cocktails", which consist of multiple phages proven to have *in vitro* efficacy against the target pathogen.[5,6] The administration methods include topical application, oral ingestion, and intravenous injection, dependant upon the type of infection and site specificity. Early successes include the treatment of a patient with a multidrug-resistant *Staphylococcus aureus* (MRSA) infection using a cocktail of phages without antibiotics.[11] Phage cocktails can target distinct types of bacteria, and through the use of multiple phages, provide a broad-spectrum approach to infection prevention.[10] Yet, the increased complexity from the additional phages also raises the risk of eventually compromising the efficacy. Overly complex cocktails can lead to dysbiosis, horizontal gene transfer, and even phage resistance.[12] An *in vitro* study conducted in 2022 demonstrated that a two-phage cocktail in combination with the antibiotic imipenem successfully resulted in the synergistic delay of the carbapenemase-producing *K. pneumoniae* growth.[13] However, it is also apparent that isolated applications of phage therapy independent of antibiotics have generally led to more negative results than positive. This includes Leitner et al. who failed to substantiate that phage therapy was more effective than antibiotics in treating cases of urinary tract infections.[14] Despite the failures, they provided insight into the administration routes for various types of infections, as well as the concentration of phages that avoids toxicity but still achieves a therapeutic effect.[15] A faster recovery rate with vastly reduced side effects can be

achieved using phages versus antibiotics, given higher precision in targeting solely harmful bacteria whilst sparing healthy ones.[11]

CASE STUDIES, CLINICAL TRIALS, AND ONGOING DEVELOPMENTS

Recent advancements in phage therapy unveil promising strides in diverse clinical applications. However, while there are plenty of reports on pre-clinical *in vitro* and *in vivo* use, there is a lack of rigorously-conducted clinical trials; most human findings are from individual cases or case series. One noteworthy progression in personalized medicine involves the targeted use of topical bacteriophage therapy for chronic non-healing wounds, as highlighted in the comprehensive study by Gupta et al. The research showcases substantial improvements in wound healing, revealing that seven patients achieved complete healing by day 21 through the application of a tailored bacteriophage cocktail.[16] These findings underline the potential of personalized phage therapy in addressing challenging cases of chronic non-healing wounds.

In addition, developments in personalized medicine concentrate on tailoring bacteriophage-based therapeutic cocktails to treat resistant bacterial infections. A case report conducted by Schooley et al. delineates the success of personalized bacteriophage therapy in treating a patient with a disseminated resistant *Acinetobacter baumannii* infection. The report demonstrates the efficacy of personalized approaches through significantly improved patient outcomes including a reduction in pressor requirements, coma recovery, and improved mental status and renal function.[17] This underscores the potential of personalized phage therapy as a valuable intervention in challenging scenarios of antibiotic-resistant infections.

Ryan et al. and Altamirano et al. shed light on the efficacy of PAS in eradicating bacterial biofilms. These studies emphasize the significant impact of PAS in overcoming challenges posed by biofilm formation and drug-resistant bacteria.[18,19] The findings suggest a promising strategy for enhancing treatment outcomes in cases where traditional therapies fail, providing valuable insights into the dynamic interplay between phage therapy and antibiotics in combating resilient bacterial populations.[18,19]

While preclinical evidence affirms the overall effectiveness of phage therapy in animal models, a review and meta-analysis by Gómez-Ochoa et al. bring attention to a crucial gap between research and clinical practice. The study demonstrates the need for future preclinical trials to align with clinical trials, emphasizing the need for informed translation of findings into clinical applications.[20] This recognition serves as a call to action for refining research methodologies to ensure a seamless transition to clinical use.

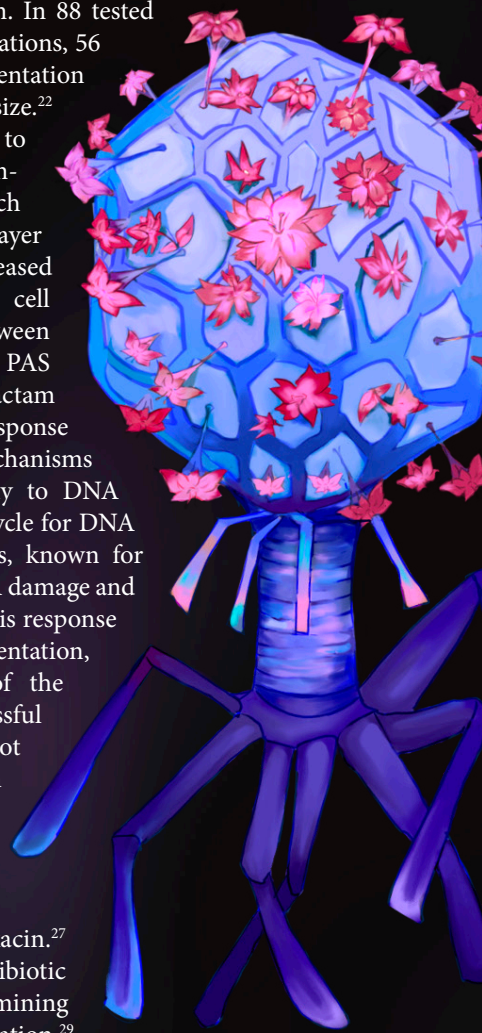
A study conducted by Borin et al. focuses on the λ phage and *Escherichia coli* model system and provides insights into the effectiveness of phage training. Phage training involves exposing bacteriophages to their target bacterial hosts over a period, allowing the phages to evolve and adapt to the bacteria's defenses.[22] While the results of this study are promising, the generalizability of phage training to diverse clinical scenarios with various bacterial strains and infections remains uncertain.

PHAGE–ANTIBIOTIC SYNERGY

PAS refers to the phenomenon where phage production increases in the presence of sublethal concentrations of certain antibiotics.[23,24] PAS allows enhanced bacterial suppression, increased biofilm penetration, and reduced capacity of bacteria to develop resistance against either therapy option. Employing low doses of antibiotics and phages can effectively manage antibiotic-resistant bacteria. While bacteria have developed defenses against antibiotics, they remain vulnerable to phages. This approach tips the balance in the coevolutionary "arms race" between phages and bacteria.[22] Furthermore, PAS also makes use of the mechanism of phage steering, wherein phages are able to specifically select resistive strains of bacteria, thereby reintroducing antibiotic sensitivity.[25] PAS was found to be evident in a variety of processes, including plaque assessments, bacteria filamentation, and clinical case experiments.[24,25]

Plaque assessments are one of the most known demonstrations of PAS. Comeau et al. found that phages stimulated with sublethal β-lactam and quinolone antibiotics increased plaque size.[24] By implementing antibiotics, there is a corresponding increase in plaque size and diameter through simulated phage activity due to increased bacteria inhibition.[25] An *in vitro* study conducted in 2021 observed that the production of progeny bacteriophages was heightened, which may eliminate more bacteria.[26]

Another PAS indicator is bacterial filamentation and elongation. In 88 tested phage and antibiotic combinations, 56 demonstrated bacterial filamentation and increased plaque size.[22] Filamentation is related to disturbances in penicillin-binding proteins (PBPs) which affect the peptidoglycan layer and cell wall, leading to increased phage attachment and cell lysis.[24] The correlation between bacterial filamentation and PAS can be justified through β-lactam antibiotics and SOS-response mechanisms.[27] SOS mechanisms in bacteria respond globally to DNA damage by halting the cell cycle for DNA repair.[28] β-lactam antibiotics, known for inhibiting PBPs, induce DNA damage and trigger the SOS response. This response causes bacterial filamentation, an abnormal elongation of the cell, promoting successful phage predation. While not universal, cell filamentation enhances susceptibility to phage infection, observed in filamentation-inducing antibiotics like ceftazidime, cephalexin, and ciprofloxacin.[27] The specific choice of antibiotic is also important in determining the level of filamentation.[29]



PAS has also been successfully implemented in clinical experiments. For instance, a study in 2020 reported a case of a patient with a urinary tract infection (UTI) of *Klebsiella pneumoniae* that resisted all the tested antibiotics, excluding tigecycline and polymyxin B.[30] After tigecycline failed to treat the UTI, the patient participated in a successful phage therapy with a phage cocktail III and trimethoprim-sulfamethoxazole (SMZ-TMP) antibiotic combination. In particular, from a series of common antibiotics used for UTI treatment, SMZ-TMP was selected specifically due to its strong synergistic effect with phage cocktail III. When the phage cocktail III was applied on its own, it only inhibited growth for 12 hours. SMZ-TMP applied individually was unable to result in any sign of inhibition of bacterial growth. However, the SMZ-TMP and phage cocktail III combination resulted in complete recovery. This case demonstrated major potential for treatment with the application of PAS antibiotic and bacteriophage synergism as a phage therapy strategy.[30]

LIMITATIONS

Despite recent advances, there are still several limitations to phage therapy such as the narrow host range of phages and the lysogenic phenomenon.[31] If the bacteria community is stable, presence of the lysogenic phage may cause harm to the host by strengthening the community.[31] While obligately lytic phages are the predominant choice in current trials, there are instances where the usage of temperate phages may be crucial. This need arises from the fact that obligately lytic phages may not always be available, as exemplified in the study by Derick et al.[6] In this study, engineered phages were employed to address a disseminated *Mycobacterium abscessus* infection, emphasizing the potential significance of incorporating temperate phages in certain therapeutic contexts.

A fundamental limitation of using self-replicating entities such as phages is the potential for rapid evolution and genetic mutations. Phages can mutate quickly to adapt to changing conditions, including the development of resistance.[2] This adaptability can make it challenging to predict and control the effectiveness of phage therapy over time. Additionally, the complex interactions between phages and bacteria in the bodily environment may introduce uncertainties in treatment outcomes.

Pharmacological limitations are also a concern for phage therapy.[32] Clinical trial results of phage treatment of bacterial infections show a low to moderate efficacy, and the variation in infection clearance between subjects within studies is often large.[32] *In vivo*, the pharmacokinetics and pharmacodynamics of phages are virtually unknown, and there is a lack of standardization due to the great variation of phages, bacteria, and infections.[32]

Another limitation of phage therapy is the bacteria mutation dynamics that can outpace phages.[27] Bacteria can evolve resistance to phages by developing mutations that prevent phages from attaching to their surface receptors.[33] In addition, phages can select for small colony variants (SCVs) that are resistant to antibiotics.[34] SCVs are slow-growing bacteria that can evade the immune system and persist in chronic infections.[34] However, recent research has shown that combining phage therapy with antibiotics can address this limitation.[34] By following the administration of phages with antibiotics, the limitation of SCVs can be addressed.[33,34]

Finally, the high cost of phage therapy can be prohibitive, costing thousands of dollars for a single treatment.[36] The personalized approach of finding or engineering the correct phage is time-consuming and expensive. As phage therapy is developed, the cost may decrease, but new infections and the need for multiple rounds of treatment pose additional challenges.

CONCLUSION

In conclusion, the promise of PAS in addressing AMR is evident, yet it is crucial to acknowledge the persistent challenges and complexities of phage therapy. Intricate interactions between phages and bacteria reveal gaps in our understanding, particularly in instances where bacterial strains are unknown and complex. Phage training and steering, while intriguing, cannot be guaranteed universally, adding a layer of uncertainty to the applicability of phage therapy. Moreover, the efficacy of phage therapy proves to be variable across different infections, emphasizing the need for a nuanced approach that is tailored to the specific characteristics of each bacterial strain. Additional complexities surrounding phage therapy include limitations such as narrow host range, the lysogenic phenomenon, and pharmacological uncertainties. Despite intriguing developments in laboratory settings and ongoing research showcasing PAS, the journey towards establishing phage therapy as a widely applicable solution is still in its early stages. The lack of definitive references to successful clinical trials raises questions about the current practicality of phage therapy in real-world scenarios. As we navigate these challenges, further research to uncover the mechanisms involved is imperative to determine the true potential and limitations of phage therapy in the ongoing battle against AMR.

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