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Exploring Gut Phages as a Defense Mechanism Against Drug-Resistant Bacterial Infections

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Abstract

The human gut microbiota is a complex and dynamic ecosystem consisting of various microorganisms, including bacteria, viruses, fungi, and protozoa, that contribute to overall health. Among these, bacteriophages (phages) are viruses that specifically target and infect bacteria, playing a critical role in regulating microbial balance within the gut. In recent years, the rise of antibiotic-resistant bacterial infections has become a major global health challenge, largely attributed to the overuse and misuse of antibiotics. As a result, there has been growing interest in phages as a promising alternative to conventional antibiotics, particularly in combating drug-resistant infections. Phages offer several advantages, including their ability to selectively target specific bacteria, their rapid replication in the presence of host bacteria, and their potential to minimize disruption to the beneficial gut microbiota. Furthermore, phages may serve as a natural mechanism to restore microbial equilibrium in the gut, providing a novel strategy for treating infections that are difficult to manage with antibiotics. This manuscript examines recent research on gut phages, exploring their potential therapeutic applications, the mechanisms behind their bactericidal action, and their broader implications for human health, particularly in the context of rising antimicrobial resistance.

Keywords Gut microbiota; Bacteriophages; Antibiotic resistance; Microbial balance; Therapeutic applications

1. Introduction

The human gut hosts a vast array of microorganisms, including bacteria, viruses, fungi, and protozoa, all of which interact closely to maintain a balanced ecosystem essential for health. Among these organisms, bacteria and viruses are the most numerous within the intestinal microenvironment (1). Bacteriophages are prominent type of virus in the gut, responsible for helping regulate bacterial populations and thereby supporting microbial balance (2). In recent years, as antibiotic usage has expanded globally, so has bacterial resistance to these drugs. Consequently, approaches focusing on the gut microbiota have gained attention for managing health. Identifying the types of phages within the intestines and understanding their roles is now a pivotal area in microbial ecology and human health preservation (3). The gut harbors a bacterial population exceeding 10^{14}, predominantly composed of *Firmicutes*, *Bacteroides*, *Proteobacteria*, and *Actinobacteria*, making up over 90% of the intestinal microbiota (4). Phages, which make up a significant portion of the intestinal virome, are abundant, with concentrations reaching up to 10^{8} virus-like particles (VLPs) per milliliter of fecal filtrate (5). Phages can be classified as lytic or temperate (lysogenic) based on their infection cycles (6). Morphologically, phages can be tailed, tailless, or filamentous and belong to families such as *Siphoviridae*, *Myoviridae*, and *Podoviridae* (7).

During infection, lytic phages follow a series of steps: attachment, invasion, replication, assembly, maturation, and host lysis. After binding to the host bacterial surface,

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phages use tail-associated enzymes to breach the peptidoglycan layer and inner membrane, injecting their genetic material into the bacterial cell. This genetic material then integrates with the host genome or replicates independently, leading to the production of new phage particles. These progeny phages eventually cause the host cell to burst, releasing more phages that can infect neighboring bacteria. Due to their ability to replicate and destroy bacterial cells, lytic phages are widely considered as potential agents for treating bacterial infections (8). On the other hand, lysogenic phages incorporate their DNA into the host genome, replicating along with the bacterial DNA during cell division. This process, known as lysogenic conversion, does not typically result in host cell lysis, allowing the phage DNA to persist across bacterial generations (9). However, under certain conditions, these phages may revert to the lytic cycle, resume phage assembly, and lyse the host cells. Lysogenic phages are prevalent in the human intestine, with crAss-like phages being some of the most widespread (10). Studies have found several core and common phages among healthy individuals worldwide, with over 23 core phages (shared by more than 50% of individuals) and 132 common phages (shared by 20-50% of individuals) identified in various populations (11).

Recent research has developed a classification system based on genome-wide nucleotide identity, using thresholds of 85% sequence coverage and 95% average nucleotide identity (ANI), combined with phylogenetic trees and gene-sharing networks, as proposed by the International Committee on Taxonomy of Viruses (ICTV). These efforts aim to better understand the connections between phages and human health, providing insight into their roles in disease prevention and treatment (12). Phages identify and attach to bacterial hosts through a specific match between the protein structures on their tails and molecules on the bacterial surface. Tailed phages produce lytic enzymes that degrade the bacterial peptidoglycan layer, enabling progeny phages to escape (13). Phages utilize different enzyme types to break down biofilms exolytic enzymes allow genome entry early in infection, while endolytic enzymes facilitate bacterial degradation to release progeny phages at the end of the cycle (14). After phages lyse bacteria, they leave unique CRISPR spacer sequences on bacterial genomes, offering a record of past bacterial infections in both humans and animals (15). This "predation" effect plays a vital role in maintaining the stability of the gut microbiota (16). Phage infection often targets specific bacterial species; for instance, certain phages only infect *Faecalibacterium prausnitzii*, sparing other gut bacteria (17). However, broader host-range phages also exist in the human gut, as shown by macrogenomic analysis of CRISPR spacer sequences. This variation underscores the complexity of phage-bacterial interactions and highlights the potential of phages as precision tools in microbiome management (18).

2. Methods

This research synthesizes recent findings on the potential use of gut bacteriophages in addressing antibiotic-resistant bacterial infections. Research materials were sourced from recognized academic databases, including PubMed, Google Scholar, Scopus, and Web of Science, focusing on high-quality peer-reviewed studies, meta-analyses, randomized controlled trials, and relevant articles. To ensure contemporary relevance, the search was limited to literature published within the last ten years, highlighting recent advancements in the field.

Studies were included based on criteria emphasizing (i) the structural diversity and functional roles of phages in the gut microbiome, (ii) phage-bacteria interactions within the gastrointestinal tract, particularly under lytic and lysogenic cycles, and (iii) experimental and clinical applications of phage therapy targeting multidrug-resistant bacterial strains. Non-peer-reviewed sources, publications lacking primary research data, and studies unavailable in English were excluded to maintain scientific rigor and relevance.

A narrative approach was applied to synthesize the selected literature, with particular focus on phages' capabilities to modulate microbial populations, selectively target pathogenic bacteria, and promote bactericidal effects through the lytic cycle. In addition, clinical studies were analyzed for insights into the efficacy, safety, and potential limitations of phage therapy, providing a balanced assessment of phages as adjuncts to traditional antibiotics for gastrointestinal and systemic infections involving resistant pathogens. Additionally, recent studies were reviewed to explore the interactions between phages and the host immune system, emphasizing their potential in reducing

inflammation and reestablishing microbial balance during infections.

3. Results

Phages and the Gut Microbiome

The human gut harbors a vast and diverse microbial population, including bacteria and viruses, where phages play a fundamental role in shaping bacterial communities (19). The antibiotic resistance crisis has spurred a renewed interest in leveraging phages to address microbial imbalances and infections (20). The gut contains approximately 10^{14} bacterial cells, making it an intricate ecosystem where phages interact closely with bacteria, affecting microbiome composition and human health (21). Phages primarily operate in two cycles: the lytic cycle, where they destroy bacteria, and the lysogenic cycle, where they integrate into the bacterial genome and may lie dormant. Lytic phages are particularly valuable for therapeutic applications due to their ability to kill bacteria rapidly, while lysogenic phages can confer new traits to bacteria, including drug resistance (22). The discovery of the abundant crAss-like phages and other core phage groups has provided new insights into the phageome's role in human health (23).

Phages' Role in Regulating Gut Bacteria

Phages selectively infect bacteria by recognizing specific receptors on bacterial cells, leading to infection (24). In lytic phages, this binding results in bacterial lysis, where phage-encoded enzymes break down the cell wall, releasing new phages (25). These enzymes also assist in breaching bacterial biofilms, aiding phage access to otherwise protected bacteria (8). Phage-driven interactions influence bacterial resistance: bacteria that evolve antibiotic resistance may become more susceptible to phages and vice versa. This dynamic, often termed the "seesaw effect," exemplifies the evolutionary balance between bacterial resistance and susceptibility (26). Moreover, phages can integrate with bacterial DNA in a process called lysogenic conversion, which sometimes enhances bacterial resilience against environmental stresses, promotes colonization, or adds antibiotic resistance (24).

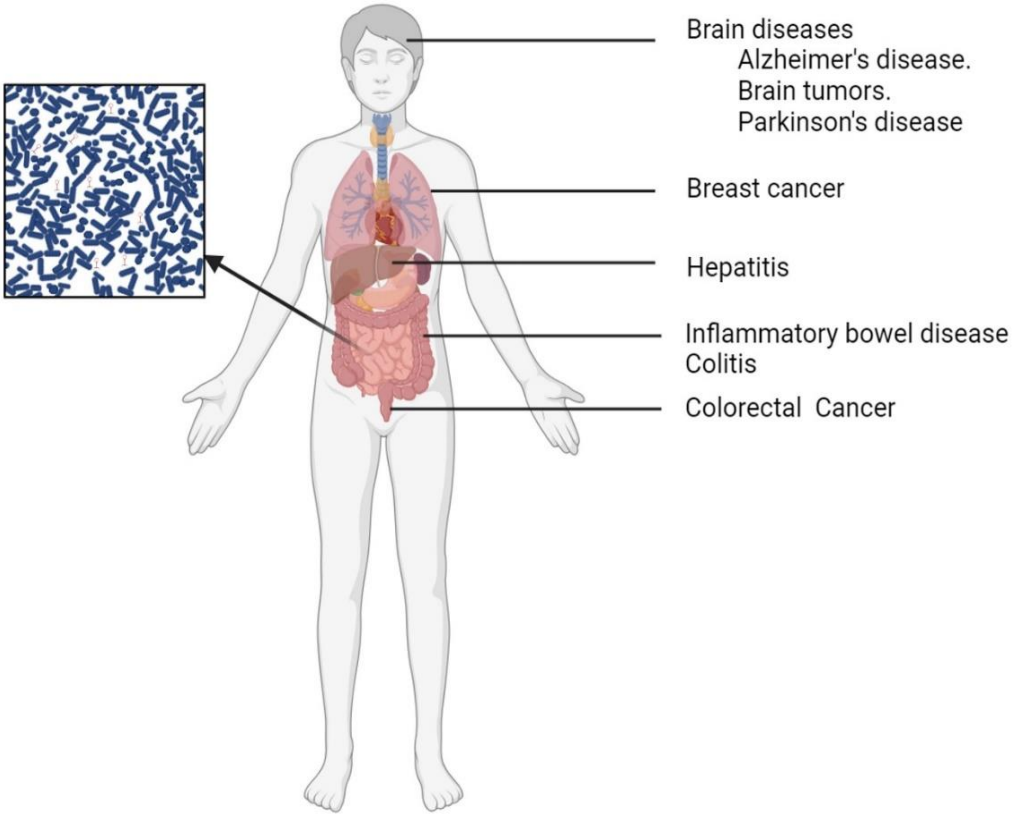

Figure 1. Therapeutic Applications of Phages in Infectious Disease Treatment

Phages and Infectious Disease Control

Historically, phages were used to treat infections like dysentery and cholera before antibiotics became mainstream (27). More recently, phage therapy has re-emerged as an effective intervention against multidrug-resistant pathogens (28). Studies have shown positive outcomes in treating infections caused by resistant bacteria like *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* and *Staphylococcus aureus* (29). In one notable case, a phage cocktail significantly improved the condition of a cystic fibrosis patient with a resistant Pseudomonas infection, while another patient with multidrug-resistant *Acinetobacter baumannii* benefited from a combined phage and antibiotic therapy. These examples underscore the potential of phages as effective treatments against persistent infections (30). Figure 1 illustrates the mechanisms by which bacteriophages can be employed as a treatment for infectious diseases, particularly those caused by antibiotic-resistant bacteria.

Phages for Gastrointestinal Infections

Phages have also shown potential in combating gastrointestinal infections caused by pathogens like *Escherichia coli* and *Salmonella* (31). Phage filtrates from healthy human feces have effectively treated chronic *Clostridium difficile* infections. Additionally, studies using phages against drug-resistant strains of *E. coli* and *Vibrio cholerae* in animal models have demonstrated phages' effectiveness in reducing bacterial load and supporting recovery, particularly when administered early in infection (32). Compared to antibiotics, phages have a minimal impact on the surrounding gut microbiota, offering a more targeted approach to infection control (33). Table 1 provides an overview of numerous studies focused on developing phage therapy targeting various drug-resistant bacterial strains.

4. Discussion

In conclusion, the human gut microbiota is a complex and dynamic ecosystem, with bacteriophages playing a pivotal role in regulating the microbial balance. As the global threat of antibiotic resistance continues to grow, phages offer a promising alternative to conventional antibiotics for treating multidrug-resistant bacterial infections (28). Their ability to specifically target pathogenic bacteria while preserving the beneficial components of the gut microbiota gives phages a distinct advantage in infection control. Recent studies have revealed the immense diversity and functional roles of phages in the gut, especially the widespread presence of crass-like phages (23). The interaction between phages and bacteria through lytic and lysogenic cycles influences both microbial populations and the development of antibiotic resistance. Lytic phages, with their ability to destroy harmful bacteria, have significant therapeutic potential, while lysogenic phages contribute to bacterial evolution and gene transfer (31). The resurgence of phage therapy, particularly in treating gastrointestinal and antibiotic-resistant infections, highlights the growing potential of phages as a viable

Table 1: Phage Therapy Development for Drug resistant Bacterial Strains

Target Bacteria	Phage	Phage Preparation	Model	Outcome	Reference
E. coli	ZCEC14	10^{10} PFU/ml	/	Strong and steady antibacterial action	(34)
E. coli	Uz-1	10^{13} PFU/ml	/	Restrict *E. coli* U1007 in vivo was proven.	(35)
K.pneumoniae	GWPB35	10^{9} PFU/ml	Mouse and zebrafish	Reduce the number of bacteria	
K.pneumoniae	P2	10^{8} PFU/ml	Mice	Significant reduced in a mouse model.	(36)
S. aureus	4086-1	10^{9} PFU/ml	Mice	Effectively reduced	(37)
S. aureus	SAML-4	10^{8} PFU/ml	mice, mastitis	Significantly reduced	(38)
A. salmonicida	AsM_ZHF	8×10^{4} PFU/ml	/	Significantly reduced	(39)

alternative to antibiotics. Despite promising results, challenges such as host specificity, immune system responses, and regulatory approval still remain and warrant further investigation. To fully unlock the therapeutic potential of phages, more comprehensive studies are needed to understand the diversity of the gut phageome, its interaction with the host, and its impact on microbial ecology.

5. Conclusion

As research progresses, phages could become a cornerstone in the fight against antibiotic-resistant infections, offering a natural and effective solution to a growing global health crisis. Future research should focus on optimizing phage delivery methods, overcoming bacterial resistance to phages, and developing strategies to harness phage therapy in a clinical setting. Additionally, exploring the combined use of phages and antibiotics may offer synergistic effects for managing drug-resistant infections.

Conflict Of Interest

The authors declare no conflict of interest

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