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CRISPR-Enhanced Phage Therapy for Multi-Drug Resistant Bacterial Infections: Mechanisms, Engineering Strategies, and Clinical Prospects

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Abstract. Antibiotic resistance represents one of the most urgent global health threats, rendering conventional antimicrobial therapies increasingly ineffective. Bacteriophage therapy has re-emerged as a promising alternative, offering microbial specificity and the capacity to target pathogens unresponsive to existing antibiotics. However, natural phages face limitations including narrow host range, unpredictable pharmacokinetics, and the rapid evolution of phage-resistant bacterial mutants. Advances in CRISPR-Cas genome editing have enabled the development of engineered phages that incorporate programmable DNA or RNA targeting systems, greatly enhancing their therapeutic precision and versatility. CRISPR-enhanced phages can selectively disrupt chromosomal genes essential for survival, eliminate antibiotic resistance plasmids, modulate virulence factor expression, and expand their natural host range. This review synthesises current knowledge on CRISPR–bacteria interactions, engineering strategies for constructing enhanced phages, and their demonstrated applications against multidrug-resistant pathogens, such as MRSA, Pseudomonas aeruginosa, CRE, and Acinetobacter baumannii. Challenges related to delivery, biosafety, immune responses, and regulatory oversight are critically evaluated. Overall, CRISPR-enhanced phage therapy represents a transformative approach with significant potential to address the rising burden of antibiotic resistance.

Keywords: Phage therapy, Genome engineering, Multidrug-resistant bacteria

1. Introduction

1.1. Global burden of antibiotic resistance

Antibiotic resistance has intensified into one of the most pressing global healthcare threats. Decades of antibiotic overuse and misuse, combined with the rapid evolutionary adaptability of bacterial populations, have accelerated the emergence of multidrug-resistant (MDR) pathogens. According to the World Health Organization, antimicrobial resistance is currently responsible for at least 700,000 deaths annually, a figure projected to rise to 10 million by 2050 if no effective interventions are implemented [1]. The impact of MDR infections is particularly significant in clinical settings, where treatment options are increasingly limited, and mortality rates remain high.

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1.2. Clinical significance of major MDR pathogens

Among the most concerning MDR organisms are methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacteriaceae (CRE), Pseudomonas aeruginosa, and Acinetobacter baumannii. These pathogens are responsible for persistent, recurrent, and often life-threatening infections, especially in hospitals and intensive care units [2]. Their intrinsic resistance mechanisms—combined with acquired genes such as mecA, blaNDM-1, and blaKPC—render many frontline antibiotics ineffective, underscoring the urgent need for alternative therapeutic strategies.

1.3. Limitations of conventional bacteriophage therapy

Bacteriophage therapy has re-emerged as a promising solution due to the specificity of phages and their ability to target bacteria resistant to conventional antibiotics. However, natural phages face several significant limitations. Their narrow host range restricts broad clinical applicability, and bacteria can rapidly evolve phage resistance through receptor mutations or anti-phage defense systems [3]. In addition, many naturally occurring phages are temperate and may integrate into bacterial genomes rather than undergoing lytic replication, potentially transferring undesirable genetic elements. The pharmacokinetics of phages in vivo are also highly unpredictable, complicating optimization, standardization, and regulatory approval.

1.4. Emergence of CRISPR-enhanced phage therapy

The integration of CRISPR-Cas systems into therapeutic phages represents a transformative innovation aimed at overcoming the limitations of natural phage therapy. Engineered CRISPR-phages can introduce programmable functionalities, including the disruption of essential chromosomal genes, elimination of antibiotic resistance plasmids, and inhibition of multiple bacterial pathways simultaneously [4]. These engineered functions not only improve the precision and lethality of phage therapy but also reduce the likelihood of resistance development. As a result, CRISPR-enhanced phage therapy has emerged as a next-generation antibacterial strategy with the potential to fundamentally reshape how multidrug-resistant infections are treated.

2. CRISPR–bacteria interactions relevant to phage therapy

2.1. Overview of CRISPR-Cas systems and their functional diversity

The CRISPR-Cas system operates as an adaptive immune mechanism in bacteria and archaea, enabling the recognition and degradation of invading genetic elements such as phages and plasmids. Among the numerous CRISPR classes and types, three systems—Type II Cas9, Type I Cas3, and Type VI Cas13—have become central to engineered phage therapy due to their distinct and powerful nucleic acid–targeting mechanisms. Cas9 introduces precise double-stranded DNA breaks at target sequences, Cas3 performs extensive DNA degradation through a processive nuclease–helicase activity, and Cas13 uniquely targets RNA transcripts, providing a means to suppress bacterial gene expression without altering genomic DNA [4,5]. These functional differences expand the antimicrobial capabilities of engineered phages, allowing them to disrupt bacterial chromosomes, degrade plasmids, or silence essential transcripts.

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2.2. Bacterial anti-phage defense systems

Phage infection outcomes are heavily shaped by bacterial defense strategies, many of which hinder the efficacy of natural phage therapy. Restriction–modification (RM) systems degrade unmodified foreign DNA, while abortive infection pathways induce programmed cell death to prevent phage replication within the population. Newer defense systems such as BREX and DISARM also restrict phage success by recognizing and blocking phage replication processes [6]. Moreover, bacteria that carry endogenous CRISPR-Cas systems can directly target therapeutic phages, limiting their ability to establish productive infections. These defense layers contribute to the evolutionary arms race between bacteria and phages, emphasizing the need for engineered phages that can circumvent or disable host immunity.

2.3. CRISPR programmability and its implications for engineered phage therapy

The programmable nature of CRISPR enables phages to achieve highly selective and potent antibacterial activity. By incorporating customized guide RNAs, CRISPR-enhanced phages can target essential bacterial genes, disrupt virulence pathways, or eliminate antibiotic resistance plasmids such as blaNDM-1 or mecA. Multiplexed guide RNA strategies further reduce the likelihood of bacterial escape mutations by simultaneously targeting multiple genomic regions or plasmids [4,5]. This programmability also allows engineered phages to bypass bacterial defense systems by specifically inactivating genes required for phage restriction. Ultimately, integrating CRISPR into phage therapy enhances precision, decreases host resistance evolution, and broadens the therapeutic utility of phages against multidrug-resistant pathogens.

3. Engineering strategies for CRISPR-enhanced phages

The development of CRISPR-enhanced phages relies on the incorporation of CRISPR-Cas cassettes into lytic phages or phagemid vectors, allowing precise genetic interference once the phage infects a bacterial cell [5]. Through these systems, engineered phages can cleave chromosomal DNA, disrupt essential genes, or eliminate plasmids encoding antibiotic resistance [7,8], as demonstrated in studies targeting the mecA gene in MRSA or blaNDM-1 in carbapenem-resistant Enterobacteriaceae [9]. Expanding the host range of engineered phages remains a critical challenge, which researchers have approached by modifying tail fiber proteins, engineering receptor-binding domains, or using CRISPR to disable bacterial defense barriers that restrict phage entry [10]. CRISPR also facilitates the reprogramming of temperate phages into obligate lytic variants by removing integrases and lysogeny regulators, thereby enhancing therapeutic safety. Collectively, these engineering strategies significantly broaden the functional capacity, safety, and versatility of phage therapeutics.

4. Applications against major MDR pathogens

4.1. Methicillin-Resistant Staphylococcus Aureus (MRSA)

CRISPR-enhanced phages have shown strong potential in targeting MRSA, one of the most clinically significant multidrug-resistant pathogens. Engineered phages carrying Cas9 constructs designed to cleave the mecA gene—the key determinant of methicillin resistance—can effectively eliminate this resistance determinant and restore susceptibility to β-lactam antibiotics [11]. In addition to reversing resistance, these engineered phages significantly reduce bacterial load in both

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in vitro and in vivo infection models, demonstrating their capacity to act as precision antibacterial agents against resistant S. aureus strains.

4.2. Carbapenem-Resistant Enterobacteriaceae (CRE)

In Enterobacteriaceae, CRISPR-engineered phages have been successfully applied to disrupt carbapenem resistance mediated by genes such as blaNDM-1 and blaKPC. Studies have shown that CRISPR-Cas constructs delivered via lytic phages or phagemid systems can selectively cleave plasmids encoding these carbapenemases, leading to the eradication of resistance plasmids from bacterial populations. In some cases, targeting chromosomal copies of resistance genes induces double-stranded DNA breaks that result in rapid bacterial cell death, highlighting the lethal potential of CRISPR-phages beyond plasmid curing alone [7].

4.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa remains one of the most difficult MDR pathogens to treat due to its intrinsic resistance mechanisms, including efflux pumps, adaptive virulence regulation, and robust biofilm formation. Cas13-based CRISPR phages represent a promising strategy by degrading essential RNA transcripts required for virulence and metabolic functions [12]. Through RNA-level targeting, Cas13 systems bypass some of the DNA repair-mediated escape mechanisms commonly seen in this species. Studies also show the ability of CRISPR-phages to attenuate quorum-sensing pathways and weaken biofilm integrity, both of which are critical to the pathogenicity and persistence of P. aeruginosa infections.

4.4. Acinetobacter baumannii

Acinetobacter baumannii, a frequent cause of ventilator-associated pneumonia and intensive-care–related infections, has demonstrated vulnerability to Cas3-equipped CRISPR phages. Cas3 induces extensive chromosomal degradation through its processive nuclease-helicase activity, resulting in efficient bactericidal effects even in extensively drug-resistant strains [13]. These engineered phages not only achieve high killing efficiency but also markedly reduce the probability of resistance development due to the broad genomic degradation triggered by Cas3 activity [14]. This makes Cas3-phages particularly promising for clinical scenarios involving highly recalcitrant A. baumannii infections.

4.5. Summary of therapeutic versatility

Collectively, these applications highlight the broad therapeutic versatility of CRISPR-enhanced phages across multiple high-risk MDR species. By enabling targeted DNA or RNA disruption, removing resistance determinants, impairing virulence pathways, and triggering lethal genomic degradation, CRISPR-engineered phages offer a multifaceted strategy for addressing infections that remain refractory to traditional antibiotics. Their success across diverse bacterial taxa reinforces CRISPR-phage therapy as a compelling next-generation approach for combating the growing threat of multidrug resistance.

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5. Advantages, limitations, and biosafety considerations

5.1. Advantages of CRISPR-enhanced phage therapy

CRISPR-enhanced phage therapy provides several clear advantages over conventional phage therapy and antibiotic-based treatments. By integrating programmable CRISPR-Cas systems, engineered phages can selectively cleave essential bacterial genes or eliminate antibiotic resistance determinants, enabling highly precise antibacterial activity that minimizes disruption to commensal microbiota [4,5]. This targeted approach lowers the likelihood of non-specific killing and reduces selective pressures that drive broad-spectrum resistance. In addition, the use of multiplexed guide RNAs allows simultaneous targeting of multiple loci within bacterial chromosomes or plasmids, substantially decreasing the probability of bacterial escape mutations and making resistance evolution far more difficult [8]. These combined features highlight the unique capacity of CRISPR-phages to achieve both specificity and robustness in combating multidrug-resistant bacteria.

5.2. Limitations and technical barriers

Despite these advantages, several important limitations currently hinder the widespread application of CRISPR-enhanced phages. Efficient delivery of CRISPR components into bacterial cells remains a major challenge, particularly in Gram-negative pathogens with impermeable outer membranes [15]. Additionally, many Cas proteins—such as Cas9 and Cas13—are large enzymes that may exceed the packaging capacity of certain bacteriophage capsids, restricting the range of compatible phage vectors [3]. There are also concerns regarding off-target editing, unintended interactions with non-pathogenic microbes, and unpredictable impacts on microbial ecology. These constraints underscore the need for improved delivery platforms, optimized Cas variants, and rigorous off-target assessment strategies.

5.3. Biosafety, gene transfer risks, and ecological considerations

Because CRISPR-enhanced phages incorporate engineered genetic material, they fall under gene therapy classifications in many regulatory jurisdictions. This designation requires strict oversight regarding biosafety, environmental risks, and ethical concerns related to genetically modified organisms [16]. Horizontal gene transfer is a specific area of caution, as phages can unintentionally mobilize bacterial DNA or spread engineered constructs if not properly designed. Environmental release of genetically modified phages raises additional concerns regarding ecological impact, viral evolution, and long-term persistence in microbial communities. Public acceptance of genetically engineered viruses also remains uncertain. Addressing these biosafety and regulatory challenges will be essential to ensuring the safe translation of CRISPR-phage therapies from laboratory research to clinical practice.

6. Future directions

Future innovations in engineered phage therapy are likely to center on improved specificity, broader host range, and enhanced delivery systems. Multi-guide RNA phages capable of simultaneously targeting several essential genes or resistance mechanisms will reduce escape mutations and provide broader antibacterial coverage. Combining CRISPR-phages with antibiotics may further enhance therapeutic outcomes through phage–antibiotic synergy. Rapid advances in machine learning are also expected to accelerate phage engineering, guiding the design of optimized tail fiber proteins,

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CRISPR target sites, and infection dynamics. RNA-targeting CRISPR systems, such as Cas13, represent a particularly promising avenue for treating Gram-negative pathogens that resist DNA-targeting strategies. Additionally, personalized phage therapy pipelines—supported by clinical phage banks, rapid genome sequencing, and automated gRNA design—may enable tailored treatment approaches for individual patients. These directions collectively highlight the transformative potential of CRISPR-enhanced phage therapy in the coming decade.

7. Conclusion

The accelerating spread of antibiotic resistance poses a severe threat to global public health, as the effectiveness of traditional antimicrobial strategies continues to diminish. In this context, bacteriophage therapy enhanced with CRISPR-Cas technology offers a revolutionary pathway for addressing drug-resistant infections. By integrating programmable gene-editing systems, CRISPR-engineered phages can precisely disrupt essential bacterial genes, eliminate antibiotic resistance plasmids, and modulate multiple pathogenic pathways. These capabilities fundamentally overcome the inherent limitations of natural phages, such as narrow host range, vulnerability to resistance development, and limited controllability. Accumulating research demonstrates that engineered CRISPR phages exhibit strong antibacterial activity against several clinically significant multidrug-resistant pathogens—including MRSA, CRE, Pseudomonas aeruginosa, and Acinetobacter baumannii—providing promising solutions for infections that currently lack effective treatment options.

Nevertheless, several challenges remain before CRISPR-enhanced phage therapy can achieve clinical translation. Key limitations include inefficient delivery, size constraints for large Cas proteins, potential immunogenicity, and biosafety concerns such as off-target effects, unintended gene transfer, and long-term impacts on microbial communities. As CRISPR phages function as gene-editing agents, their application will require stringent regulatory oversight. Future research should prioritise improving delivery efficiency, safety, and specificity, as well as optimising engineering and evaluation workflows.

Looking ahead, advances such as multi-target CRISPR strategies, phage–antibiotic combination therapies, and AI-assisted phage design are expected to further enhance the efficacy and scalability of engineered phage therapeutics. With responsible development supported by collaboration between researchers, regulators, and the public, CRISPR phages may become an important component of future antibacterial treatment strategies.

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