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Precision Phage Therapy in the Post-Antibiotic Era: From Phage Genomics to Clinical Translation against Multidrug-Resistant Bacteria

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ABSTRACT

The growing prevalence of antimicrobial resistance (AMR) has become a major global health concern, reducing the effectiveness of conventional antibiotics against multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial pathogens. This has renewed interest in bacteriophages as alternative and complementary antimicrobial agents. Phages exhibit specific interactions with bacterial hosts, replicate within susceptible cells and cause bacterial lysis through mechanisms distinct from those of antibiotics. Their host specificity offers the potential to target pathogenic bacteria while minimizing disruption to beneficial microbiota. Advances in whole-genome sequencing, comparative genomics and computational microbiology have improved the identification and characterization of therapeutic phages, enabling the selection of strictly lytic candidates and the exclusion of those carrying undesirable virulence, antimicrobial resistance and lysogeny-associated genes. Molecular characterization, host-range prediction, machine learning and analysis of bacterial antiphage defence systems are further supporting more informed phage selection and therapeutic design. Recent developments in genetic engineering, directed evolution and CRISPR-based technologies have expanded the potential applications of phages, including host-range modification, disruption of bacterial defence mechanisms and targeted antibacterial activity. Phages may also influence bacterial evolution by selecting resistance-associated mutations that reduce virulence, impair biofilm formation or restore susceptibility to certain antibiotics. Phage–antibiotic combinations have shown enhanced antibacterial activity under specific conditions, while compassionate-use cases and emerging clinical studies have demonstrated potential benefits in selected difficult-to-treat infections. However, clinical translation remains limited by challenges involving phage susceptibility testing, pharmacokinetics, dosing, immune responses, bacterial resistance, manufacturing consistency and regulatory standardization. This review examines advances in precision bacteriophage therapy, with particular emphasis on phage genomics, host-range determination, rational cocktail design, engineered phages, biofilm targeting, evolutionary steering, artificial intelligence-assisted selection and clinical translation. Integrating genomic information, computational tools and individualized microbiological assessment may help establish bacteriophage therapy as a more targeted and adaptive approach to managing drug-resistant bacterial infections.

Keywords: *Antimicrobial resistance; bacteriophage therapy; precision antimicrobials; multidrug-resistant bacteria; phage genomics; host-range prediction; phage–antibiotic synergy; CRISPR-based phage engineering; biofilm targeting; evolutionary steering; personalized antimicrobial therapy.*

1. Introduction

Antibiotics have transformed modern medicine, making previously life-threatening bacterial infections treatable and enabling advanced medical procedures. However, their extensive use in healthcare, agriculture and animal production has accelerated the emergence and spread of antimicrobial resistance (AMR).

Bacteria develop resistance through mechanisms such as target modification, drug inactivation, reduced permeability, active efflux and acquisition of resistance genes. AMR is now a major global health concern, with projections estimating that annual deaths directly attributable to it could reach approximately 1.9 million by 2050. Its impact may be particularly severe in resource-limited settings due to inadequate diagnostic facilities and limited access to effective treatment [1].

The World Health Organization's (WHO) 2024 Bacterial Priority Pathogens List further emphasizes the urgency of addressing resistant bacterial infections. Carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant and third-generation-cephalosporin-resistant Enterobacterales, and rifampicin-resistant *Mycobacterium tuberculosis* represent particularly important threats. In addition, carbapenem-resistant *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* remain major clinical concerns. The pathogenic potential of these organisms, combined with their extensive resistance profiles, ability to persist in healthcare environments and capacity for transmission, substantially restricts available therapeutic options [2].

Despite continued investment in antibacterial drug discovery and development, the clinical antibiotic pipeline faces considerable scientific, economic and regulatory limitations. Several recently developed antimicrobial agents belong to established drug classes or remain susceptible to resistance mechanisms already prevalent among clinical bacterial populations. Furthermore, the lengthy development process, high research costs and relatively limited commercial returns associated with novel antibiotics have discouraged sustained investment in this field. Consequently, conventional antibiotic development alone may be insufficient to counter the rapid evolution and dissemination of antimicrobial resistance, highlighting the need to investigate alternative and complementary therapeutic strategies [3].

Within this context, bacteriophages have regained considerable scientific attention as an alternative approach to managing bacterial infections. Bacteriophages, commonly referred to as phages, are viruses that specifically infect bacterial cells and represent some of the most abundant biological entities in the biosphere. Through extensive evolutionary interactions with bacterial populations, phages have developed diverse mechanisms for recognizing, infecting and eliminating susceptible bacterial hosts. Therapeutic applications primarily focus on obligately lytic phages, which attach to specific bacterial surface receptors, introduce their genetic material into host cells, exploit bacterial cellular machinery for progeny production and ultimately cause host-cell lysis through the release of newly assembled phage particles.

The therapeutic application of bacteriophages predates the widespread introduction of antibiotics and was initially explored during the early twentieth century. However, the rapid development and clinical success of broad-spectrum antibiotics resulted in a decline in phage-based treatment across several Western countries, although therapeutic phage applications continued in certain Eastern European regions. The contemporary revival of phage therapy represents more than a return to historical treatment practices. Advances in molecular biology, genome sequencing, structural characterization and computational analysis now permit detailed assessment of phage biology, enabling researchers to evaluate therapeutic suitability, identify undesirable genetic determinants and investigate phage–bacterium interactions with considerably greater precision than was previously possible [4].

An additional foundation for the renewed interest in phage therapeutics is the development of strain-specific antimicrobial treatment strategies. Unlike conventional antibiotics, which are generally selected according to bacterial identification and antimicrobial susceptibility profiles, bacteriophage therapy frequently requires compatibility between an individual bacterial isolate and the selected phage. A bacteriophage capable of infecting one *P. aeruginosa* strain may exhibit limited or no activity against another strain belonging to the same species. Consequently, therapeutic selection may involve pathogen isolation, phage susceptibility screening, genomic evaluation of candidate phages and, where necessary, the formulation of individualized phage cocktails. These requirements distinguish precision phage therapy from conventional empirical antimicrobial treatment [5].

Although strain specificity introduces challenges related to diagnostic turnaround time, therapeutic availability and clinical standardization, it also provides important biological and therapeutic advantages. Selective targeting of pathogenic bacteria may minimize unintended disturbances to commensal microbial communities, potentially reducing the ecological consequences associated with broad-spectrum antimicrobial exposure. Furthermore, phage selection can be guided by bacterial surface receptors, susceptibility profiles, resistance-associated characteristics and evolutionary vulnerabilities. Certain phages can also penetrate or disrupt bacterial biofilms, while their capacity for replication in the presence of susceptible bacterial hosts may influence their distribution and persistence at infection sites. Collectively, these characteristics establish bacteriophages as biologically distinct antimicrobial agents with potential applications beyond conventional antibiotic treatment [6].

Translating the antibacterial potential of bacteriophages into reliable clinical treatments requires an understanding of bacterial genotype, receptor availability, antiphage defence mechanisms, host immunity, pharmacokinetics and resistance development. Their successful application therefore depends on standardized susceptibility testing, genomic safety assessment, rational phage selection and clinically validated protocols [7]. This review examines advances and challenges in precision phage therapy, covering phage genomics, host-range prediction, bacterial defence systems, cocktail design, genetic engineering, biofilm targeting, phage–antibiotic interactions, evolutionary steering, clinical applications, manufacturing and regulatory frameworks. It highlights the potential of integrating molecular microbiology, genomics and computational approaches to advance phage therapy towards safe, reproducible and clinically effective treatment [8].

2. Biological Basis of Precision Phage Therapy

Bacteriophages exhibit extensive morphological, genomic and ecological diversity, reflecting their long evolutionary association with bacterial hosts. Their genetic material may comprise double-stranded DNA (dsDNA), single-stranded DNA (ssDNA) or RNA, although most bacteriophages investigated for therapeutic applications, particularly against clinically significant bacterial pathogens, possess dsDNA genomes. Phage genome sizes vary considerably, extending from a few kilobases to several hundred kilobases in large-genome bacteriophages, commonly referred to as jumbo phages. This genetic diversity represents a substantial reservoir of naturally occurring antibacterial mechanisms and functional capabilities. However, variations in genome organization, replication strategies, host specificity and biological characteristics also introduce challenges for therapeutic characterization, safety assessment and standardization [9].

The initiation of bacteriophage infection depends on the recognition and attachment of phage particles to specific structures present on the bacterial surface, a process commonly termed adsorption.

These receptors include lipopolysaccharides, capsular polysaccharides, pili, flagella, outer-membrane proteins, teichoic acids, membrane transport proteins and components of multidrug-efflux systems. The availability, structural configuration and accessibility of these receptors are important determinants of phage susceptibility and host range. Consequently, a bacteriophage capable of infecting one bacterial strain may exhibit limited activity against another strain belonging to the same species because of differences in receptor expression, composition or accessibility. This receptor-dependent specificity is a major biological basis for strain-level phage selection and precision antimicrobial treatment [10]. Importantly, receptor identification provides information beyond conventional susceptibility classification because certain bacterial surface structures also contribute to virulence, environmental persistence, biofilm formation or antimicrobial resistance. When a phage utilizes such structures as infection receptors, bacterial adaptations that prevent phage adsorption may impose additional fitness costs. For example, receptor loss or modification can reduce bacterial virulence, interfere with biofilm-associated functions or alter antibiotic susceptibility, depending on the receptor and bacterial background. Such evolutionary consequences provide opportunities to exploit phage resistance as a potential therapeutic advantage.

Following successful adsorption, bacteriophages initiate infection by introducing their genetic material into the bacterial host and redirecting cellular processes towards the synthesis of phage genomes and structural proteins. These components subsequently undergo assembly and maturation to generate progeny virions, which are released through bacterial cell lysis in obligately lytic phages. The newly liberated particles can then infect neighbouring susceptible bacterial cells, potentially facilitating localized amplification of the antibacterial agent. This capacity for replication distinguishes bacteriophages from conventional antibiotics, whose concentrations are generally governed by administration, distribution, metabolism and elimination. Nevertheless, phage self-amplification is conditional rather than guaranteed, as productive infection depends on factors such as bacterial density, metabolic activity, receptor availability, phage–host compatibility and accessibility to the infection site. Physiologically dormant bacteria and microorganisms embedded within structurally heterogeneous biofilms may exhibit reduced susceptibility to productive phage infection compared with actively growing planktonic populations. Therefore, the capacity of phages to replicate within bacterial populations should not be interpreted as a replacement for appropriate therapeutic dosing or pharmacological evaluation.

An equally important consideration in therapeutic phage selection is the distinction between obligately lytic and temperate bacteriophages. Whereas obligately lytic phages follow a productive infection pathway that culminates in bacterial cell destruction, temperate phages can establish lysogeny by integrating their genetic material into the bacterial chromosome or persisting as extrachromosomal elements. Such phages are generally avoided in conventional therapeutic applications because lysogenic conversion may alter bacterial characteristics and, in certain circumstances, contribute to the acquisition or expression of undesirable virulence-associated determinants. Furthermore, the potential for genetic exchange raises concerns regarding the mobilization of genes associated with toxins, pathogenicity and antimicrobial resistance.

Accordingly, therapeutic development prioritizes strictly lytic candidates with genomic characteristics consistent with the absence of lysogeny-associated functions and undesirable genetic determinants. Whole-genome sequencing, comparative genomic analysis and functional characterization have consequently become essential components of phage qualification, enabling the assessment of therapeutic suitability, genetic safety and biological properties before clinical application. Collectively, phage receptor recognition, productive infection, host-dependent replication and genomic safety form the fundamental biological framework underlying precision bacteriophage therapy.

3. Phage Genomics: The Foundation of Modern Precision Therapy

Historically, bacteriophage characterization relied primarily on phenotypic approaches, including plaque morphology, electron microscopy and host-range determination. Although these methods remain valuable for preliminary identification and biological characterization, they provide limited information regarding the genetic composition and potential safety risks of therapeutic phages. In particular, phenotypically lytic bacteriophages may possess genetic determinants associated with lysogeny, virulence modulation or other undesirable biological functions. Consequently, contemporary phage development increasingly incorporates whole-genome sequencing and bioinformatic analysis as fundamental components of therapeutic candidate selection. Genomic characterization enables a more comprehensive understanding of phage identity, genetic organization, evolutionary relationships and potential therapeutic suitability.

3.1 Whole-genome sequencing for therapeutic safety assessment

Whole-genome sequencing (WGS) provides detailed information regarding phage genome size, nucleotide composition, coding sequences, regulatory regions and genetic relationships with previously characterized bacteriophages. One of its most important applications in therapeutic development is the identification of undesirable genetic determinants that could compromise clinical safety. Genome-based screening is particularly important for detecting genes associated with lysogeny, bacterial virulence, toxin production and antimicrobial resistance. The identification of integrases, excisionases, lysogenic repressors and other genetic elements associated with temperate behaviour warrants careful investigation because their presence may indicate the potential for lysogeny or integration-related processes. Similarly, the identification of credible virulence-associated or antimicrobial-resistance determinants requires thorough assessment before a candidate is considered suitable for therapeutic development. Such candidates are generally excluded from conventional therapeutic applications when these findings indicate unacceptable biological risks. In this context, genomic screening functions as an essential molecular safety assessment, complementing rather than replacing phenotypic characterization and experimental validation. In addition to safety evaluation, genomic analysis can facilitate the identification of receptor-binding proteins, tail fibres, depolymerases and other structural or functional proteins involved in bacterial recognition, adsorption and host specificity [16].

3.2 Comparative genomics and phage diversity

Comparative genomic analysis provides insights into the evolutionary relationships, genetic organization and functional diversity of bacteriophages. Phage genomes frequently exhibit a modular and mosaic architecture resulting from recombination and horizontal genetic exchange during their evolutionary history. Consequently, different genomic regions within a single bacteriophage may exhibit distinct evolutionary relationships and functional characteristics. Comparative analysis can identify conserved genomic regions, structural protein modules, receptor-binding determinants and genetic elements potentially involved in bacterial defence evasion. These findings are particularly relevant to therapeutic cocktail development, where the selection of genetically and functionally distinct phages may improve bacterial coverage and reduce the likelihood of simultaneous resistance development. Combining phages that recognize different bacterial receptors or possess distinct infection mechanisms may provide greater functional diversity than formulations containing closely related phages with overlapping host specificities. Nevertheless, genomic dissimilarity alone does not establish therapeutic complementarity, and candidate combinations must undergo experimental evaluation to determine their compatibility, antibacterial activity and potential interactions. Thus, comparative genomics provides an important foundation for rational phage selection and the development of functionally diverse therapeutic formulations.

3.3 Functional annotation and its limitations

Despite substantial progress in sequencing technologies and bioinformatic analysis, functional annotation of bacteriophage genomes remains a significant challenge. A considerable proportion of predicted phage-encoded proteins continue to be classified as hypothetical proteins or proteins of unknown function because of limited sequence similarity to experimentally characterized genes. Consequently, the absence of recognizable toxin, virulence or antimicrobial-resistance determinants cannot automatically establish the complete biological safety of a phage. Undetected or poorly characterized genetic functions may remain relevant to bacterial interactions, host manipulation and therapeutic performance. This limitation emphasizes the importance of integrating computational genome annotation with experimental characterization. A comprehensive qualification strategy should therefore incorporate phage growth characteristics, adsorption kinetics, host-range assessment, efficiency of plating, bacterial killing assays, stability evaluation and investigations of resistance emergence. Such complementary approaches improve confidence in therapeutic candidate selection and help establish a more reliable relationship between genomic characteristics and observed biological activity.

3.4 Genome-informed therapeutic standardization

Whole-genome sequencing is increasingly becoming an important component of standardized bacteriophage qualification and therapeutic development. Beyond identifying potentially undesirable genetic determinants, genomic information can support the investigation of receptor-binding mechanisms, host specificity and interactions with bacterial antiphage defence systems. It also provides a framework for monitoring bacterial evolutionary responses during treatment. For instance, genomic comparison of pre-treatment and post-treatment bacterial isolates may reveal mutations associated with receptor modification, capsule alterations or other adaptive mechanisms contributing to reduced phage susceptibility. Characterization of these changes can inform subsequent therapeutic decisions, including the selection of alternative phages that recognize different bacterial receptors. This approach introduces an adaptive dimension to precision phage therapy, in which bacterial evolutionary responses can be monitored and incorporated into treatment optimization. However, genomic predictions must be interpreted alongside phenotypic susceptibility testing because the presence of a particular genetic alteration does not always establish its functional contribution to phage resistance. Overall, integrating phage genomics with bacterial genome analysis and experimental susceptibility assessment provides a promising framework for developing reproducible, adaptable and microbiologically guided therapeutic strategies [16].

Table 1: Proposed genomic and phenotypic quality assessment criteria for therapeutic bacteriophage candidates

Assessment domain	Desired therapeutic characteristic	Scientific significance
Genome sequence	Complete and high-quality genome characterization	Establishes genetic identity and supports reproducibility
Lysogeny-associated determinants	Absence of credible lysogeny-related functions	Minimizes the risk of lysogenic conversion
Virulence-associated genes	Absence of undesirable virulence determinants	Reduces the potential for adverse bacterial phenotype modification
Antimicrobial-resistance genes	Absence of clinically relevant resistance determinants	Minimizes concerns regarding undesirable resistance-associated genetic functions
Host range	Experimentally confirmed activity against relevant clinical isolates	Establishes the intended bacterial coverage
Productive infection	Demonstrated through plaque assays and complementary liquid infection assays	Confirms productive bacterial infection rather than adsorption alone
Receptor characterization	Identification or experimental characterization where feasible	Supports host-range interpretation and evolutionary steering
Efficiency of plating (EOP)	Quantified on relevant target isolates	Provides comparative information on productive infection efficiency
Bacterial killing kinetics	Demonstrated and reproducible bacterial suppression	Evaluates antibacterial activity and potential regrowth
Phage stability	Maintained under defined formulation and storage conditions	Supports product quality and therapeutic delivery
Endotoxin and impurities	Controlled within applicable pharmaceutical quality specifications	Essential for evaluating product safety, particularly for parenteral administration
Resistance phenotype	Characterized through appropriate bacterial susceptibility and resistance studies	Supports cocktail optimization and evolutionary steering

4. Bacterial Antiphage Defences and the Phage–Bacterium Arms Race

Bacteriophages and bacteria have co-evolved through continuous evolutionary interactions, resulting in the development of sophisticated infection and defence mechanisms on both sides. Bacteria have acquired diverse molecular strategies to prevent phage attachment, restrict viral genome replication and interfere with the production of progeny virions. In response, bacteriophages have evolved counter-defence mechanisms that enable them to overcome or suppress several bacterial immunity systems. This reciprocal evolutionary relationship, commonly described as the phage–bacterium arms race, has important implications for therapeutic phage selection because bacterial susceptibility depends not only on receptor availability but also on the ability of phages to overcome intracellular defence mechanisms.

4.1 Receptor Modification

Alteration of bacterial surface receptors represents one of the primary mechanisms through which bacteria develop resistance to bacteriophage infection. Mutations affecting receptor-associated genes, modifications of surface polysaccharides and increased capsule production can reduce receptor availability or interfere with phage adsorption. Since receptor recognition is an essential initial step in the infection process, even relatively minor structural alterations may substantially reduce phage susceptibility. Such adaptations can emerge during therapeutic exposure, resulting in the development of resistance in bacterial populations that were initially susceptible. However, receptor modification may also impose biological fitness costs when the affected structures contribute to bacterial virulence, biofilm formation, nutrient acquisition or antibiotic resistance. These consequences are dependent on the specific receptor and bacterial background and may create opportunities for exploiting phage-associated evolutionary trade-offs. Therefore, initial phage susceptibility should not be considered a permanent characteristic of a bacterial isolate, particularly during prolonged therapeutic exposure.

4.2 Restriction–Modification Systems

Restriction–modification systems constitute an important component of bacterial innate defence against invading genetic material. These systems generally consist of restriction endonucleases and corresponding DNA methyltransferases that distinguish bacterial DNA from foreign nucleic acids through specific methylation patterns. Following phage genome entry, restriction enzymes may recognize and cleave viral DNA that lacks the appropriate protective methylation marks, thereby interfering with subsequent replication. However, bacteriophages have evolved several strategies to counteract these mechanisms, including DNA methylation, incorporation of modified nucleotides and the production of proteins capable of inhibiting host restriction enzymes. The effectiveness of these interactions varies according to the molecular characteristics of the bacterial restriction system and the corresponding phage counter-defence mechanisms. Consequently, restriction–modification systems represent an important consideration when evaluating the compatibility of therapeutic phages with clinical bacterial isolates [11].

4.3 CRISPR–Cas Immunity

Clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR–Cas) constitute an adaptive bacterial immune system that provides sequence-specific protection against invading genetic elements. Following previous exposure to bacteriophages, bacteria can incorporate fragments of foreign DNA into their CRISPR arrays as spacer sequences. These spacers are subsequently transcribed into CRISPR RNAs (crRNAs), which guide associated Cas proteins towards complementary sequences in invading viral nucleic acids. Recognition of these targets can result in degradation of phage genetic material or interference with viral replication. Nevertheless, bacteriophages have evolved counter-strategies to overcome CRISPR-mediated immunity. These include mutations within targeted sequences, alterations in protospacer-adjacent motifs (PAMs) and the production of anti-CRISPR proteins that interfere with specific CRISPR–Cas activities. The reciprocal evolution of bacterial CRISPR immunity and phage-encoded anti-CRISPR mechanisms illustrates the molecular complexity of the phage–host relationship and highlights the importance of understanding bacterial adaptive immunity during therapeutic phage selection [13]. The diversity and functional characteristics of phage-encoded anti-CRISPR proteins further demonstrate the capacity of bacteriophages to overcome sequence-specific bacterial defence mechanisms [15].

4.4 Abortive Infection and Emerging Antiphage Defence Systems

Bacterial protection against bacteriophages extends beyond restriction–modification and CRISPR–Cas immunity. Several additional defence systems interfere with different stages of the phage infection cycle, including genome replication, transcription, translation, virion assembly and release. Among these, abortive infection systems restrict phage propagation by inducing growth arrest or programmed cell death in infected bacterial cells, thereby limiting the production and dissemination of progeny virions within the bacterial population. Recent genomic investigations have identified an increasingly diverse repertoire of bacterial antiphage defence systems operating through distinct molecular mechanisms. This expanding understanding of bacterial immunity has important implications for precision phage therapy because bacterial isolates belonging to the same species, or even sharing similar surface receptors, may exhibit substantially different phage susceptibility owing to differences in their intracellular defence repertoires [12]. Furthermore, the growing identification of phage-encoded anti-defence systems provides additional insights into how bacteriophages overcome bacterial immunity and maintain productive infection [14]. Collectively, these findings emphasize the importance of integrating bacterial genomic characterization with phage genome analysis and experimental susceptibility testing to improve therapeutic phage selection.

5. From Patient Isolate to Precision Phage Matching

The narrow host range exhibited by many bacteriophages represents both a defining advantage and a practical limitation of their clinical application. Unlike conventional antibiotics, which are selected through well-established antimicrobial susceptibility testing procedures, therapeutic phage selection frequently requires the identification of bacteriophages capable of infecting the specific bacterial strain responsible for an individual patient's infection.

Consequently, clinical phage microbiology involves a systematic process encompassing pathogen isolation, phage screening, susceptibility assessment, evaluation of candidate combinations and therapeutic monitoring. Integrating these procedures into clinical microbiology workflows is essential for improving the reproducibility of phage selection and supporting the development of individualized antimicrobial interventions [17]. Recent clinical microbiology recommendations further emphasize the importance of standardized laboratory methods and appropriate interpretation of phage susceptibility results before therapeutic application [18].

5.1 Phage Susceptibility Testing

Phage susceptibility testing is a fundamental component of therapeutic candidate selection and aims to establish whether a particular bacteriophage can productively infect and suppress the patient's bacterial isolate. One of the commonly employed methods is the plaque assay, in which serially diluted phage preparations are applied to bacterial lawns. The formation of discrete plaques provides evidence of bacterial lysis associated with phage infection. However, plaque formation alone does not fully characterize therapeutic performance. Efficiency of plating (EOP), which compares the number of plaques produced on a target bacterial isolate with those obtained on a reference propagation host, provides a quantitative measure of relative productive infection efficiency. Spot tests are also frequently used for preliminary screening because they permit rapid assessment of bacterial clearing following exposure to phage preparations. Nevertheless, clearing in spot assays may result from nonproductive interactions or lysis from without rather than successful phage replication. Therefore, positive spot-test findings should ideally be confirmed through quantitative plaque assays and complementary liquid-culture experiments. Liquid killing assays provide additional information regarding bacterial suppression, phage-associated killing kinetics and the potential emergence of bacterial regrowth during treatment.

5.2 The Phagogram: A Framework for Individualized Phage Selection

The concept of a phagogram has emerged as a potential approach for organizing and interpreting phage susceptibility profiles in a manner comparable to conventional antibiotic susceptibility testing. In this approach, a patient's bacterial isolate is exposed to a collection of candidate therapeutic phages to identify those demonstrating relevant antibacterial activity. A comprehensive phagogram may incorporate multiple parameters, including plaque formation, EOP, adsorption efficiency, bacterial killing kinetics, resistance emergence and interactions with concurrently administered antibiotics. Such an approach could provide a more informative assessment than a simple susceptible or resistant classification. However, the clinical interpretation of phagograms remains challenging because laboratory protocols differ in bacterial inoculum density, culture media, incubation conditions, phage concentration and endpoint measurements. Establishing reproducible testing procedures, validated interpretation criteria and standardized reporting systems is therefore essential for translating phage susceptibility assessment into routine clinical practice [17].

5.3 Genomics-Based Safety Screening and Therapeutic Phage Libraries

The integration of whole-genome sequencing and bioinformatic analysis has substantially improved the identification and preliminary qualification of therapeutic bacteriophage candidates. Genomic screening enables the systematic investigation of phage sequences for undesirable determinants associated with lysogeny, bacterial virulence and antimicrobial resistance. Automated bioinformatic pipelines can facilitate the analysis of large collections of phage genomes by applying predefined genomic screening criteria, thereby reducing the time and effort required for preliminary candidate assessment. Sphae is an example of a computational resource developed for therapeutic phage genome screening and candidate prioritization based on genomic characteristics [19]. Such approaches are particularly relevant to the development of extensive phage repositories containing hundreds or thousands of characterized isolates. Ideally, these repositories should integrate genomic safety information with experimentally determined host-range profiles, receptor characteristics, susceptibility data and relevant phenotypic properties. The development of searchable, well-annotated therapeutic phage libraries could facilitate rapid identification of suitable candidates against clinically important bacterial pathogens. Nevertheless, computational screening should be regarded as an initial qualification step rather than a substitute for experimental safety assessment, phenotypic characterization and therapeutic validation.

6. Computational and AI-Assisted Host-Range Prediction

Identification of therapeutic bacteriophages traditionally involves screening clinical bacterial isolates against available phage collections to determine their susceptibility. Although experimental screening provides direct evidence of phage activity, the process becomes increasingly time-consuming when large numbers of bacterial isolates and phage candidates are involved. Computational approaches have therefore gained attention as complementary methods for investigating phage–host interactions and prioritizing potential therapeutic candidates. These approaches utilize genomic sequence characteristics, nucleotide composition, receptor-binding proteins, sequence similarity, CRISPR spacer profiles and other molecular features associated with bacterial recognition and susceptibility.

Deep learning methods have been investigated for predicting phage–host associations from genomic data. DeepHost demonstrated the applicability of sequence-based deep-learning approaches to computational host prediction, while subsequent studies have explored the incorporation of annotated genomic features and neural-network architectures to improve predictive performance [20]. Such computational methods may facilitate the preliminary screening of extensive phage repositories by identifying candidates with a higher probability of infecting a particular bacterial isolate. Integration of bacterial genome sequencing with computational prediction could consequently reduce the number of phages requiring experimental investigation and improve the efficiency of therapeutic candidate selection [21].

A major limitation of existing prediction methods is their reduced reliability when host specificity must be determined at the strain level. Although bacterial species identification provides useful preliminary information, individual strains may differ considerably in receptor composition, capsular structures and intracellular defence mechanisms.

These differences can influence phage adsorption, genome entry and productive replication, even among closely related bacterial isolates. Consequently, genomic similarity between a bacterial strain and a previously characterized host does not necessarily establish susceptibility to the same bacteriophage. Previous evaluations of computational host-range prediction have highlighted the influence of dataset composition, incomplete genomic characterization and limited experimentally validated host–phage interaction data on prediction accuracy [22].

Recent investigations have explored the use of combined phage and bacterial genomic information to improve the prediction of strain-specific interactions. Studies involving bacterial isolates across the *Escherichia* genus have identified genomic characteristics associated with phage susceptibility and demonstrated the potential of computational methods for resolving host-range relationships at a finer taxonomic level [23]. Nevertheless, the biological complexity of phage infection, particularly the influence of receptor variation and bacterial antiphage defence systems, remains difficult to capture through sequence-based prediction alone. Experimental confirmation is therefore necessary to establish whether predicted interactions result in productive infection and measurable bacterial suppression.

Computational host-range prediction is most appropriately incorporated into phage selection as a preliminary screening approach, followed by phenotypic susceptibility assessment using plaque assays, efficiency-of-plating measurements and liquid-culture killing experiments. The integration of genomic prediction with experimentally established susceptibility profiles may improve the selection of therapeutic phages, particularly when screening extensive collections against clinically relevant bacterial isolates.

7. Rational Phage Cocktail Design

The therapeutic application of individual bacteriophages is often constrained by their narrow host range and the emergence of phage-resistant bacterial populations. Phage cocktails, consisting of multiple bacteriophages with complementary antibacterial activities, offer an approach to expanding bacterial coverage and reducing the probability of resistance development. Their formulation requires careful selection of candidates based on activity against clinically relevant bacterial isolates rather than simply increasing the number of phages within a preparation. Host-range matrices and network-based analytical approaches can facilitate the identification of phage combinations with complementary infection profiles and minimal functional redundancy. These methods enable the assessment of phage–bacterium interactions across heterogeneous bacterial populations and support the selection of combinations capable of providing broader isolate coverage [24]. Computational network analysis can further assist in identifying smaller, functionally diverse combinations suitable for rational cocktail formulation [25].

Receptor specificity and genomic characteristics are additional considerations in cocktail development. Combining phages that recognize distinct bacterial surface structures, such as capsular polysaccharides, pili and outer-membrane proteins, may reduce the likelihood that a single receptor-associated mutation will confer resistance to all components. In contrast, phages dependent on the same bacterial receptor may exhibit overlapping susceptibility patterns, limiting their effectiveness against receptor-modified variants.

Comparative genomic analysis can also help identify relationships among candidate phages and potential similarities in their infection mechanisms or susceptibility to bacterial antiphage defence systems. Nevertheless, genomic diversity alone does not establish functional complementarity, and candidate combinations require experimental evaluation of host-range coverage, cross-resistance, bacterial killing activity and interactions between constituent phages. Accordingly, rational cocktail formulation should integrate receptor diversity, genomic characteristics, bacterial defence mechanisms and the evolutionary consequences of phage resistance [26].

Experimental phage adaptation, commonly referred to as phage training, provides another approach for improving activity against resistant bacterial populations. This strategy involves repeated exposure of phages to bacterial hosts under controlled conditions, allowing reciprocal evolutionary selection to favour phage variants capable of infecting previously resistant bacterial strains. Selected variants can subsequently be isolated and assessed for changes in host range, adsorption efficiency and antibacterial activity. Experimental investigations have demonstrated that evolutionary training may improve bacterial suppression and delay resistance development under specific laboratory conditions [27]. However, adaptation-associated genomic changes may also influence receptor specificity, host range, replication characteristics and biological safety. Consequently, trained phages require comprehensive genomic characterization and phenotypic validation before consideration for therapeutic application. Effective cocktail development therefore depends on selecting phages with complementary infection characteristics while accounting for bacterial evolutionary responses and the potential emergence of cross-resistance.

8. Engineering the Next Generation of Therapeutic Phages

Advances in synthetic biology and genetic engineering have expanded the possibilities for modifying naturally occurring bacteriophages to improve their therapeutic properties. Although natural phage diversity provides a substantial source of antibacterial candidates, individual phages may lack specific characteristics required for effective clinical application. Genetic modification of receptor-binding proteins and tail fibres offers an approach to altering bacterial recognition and potentially extending or redirecting phage host specificity. Such modifications may improve activity against bacterial isolates that are resistant to parental phages while allowing more selective targeting of pathogenic strains. Genetic engineering also provides opportunities to remove undesirable genomic regions, including lysogeny-associated determinants, and eliminate non-essential sequences that may interfere with therapeutic development. CRISPR-based technologies have further facilitated the manipulation of phage genomes through targeted genome modification and counterselection strategies. Notably, CRISPR-Cas13a, which targets RNA rather than DNA, has enabled genetic engineering of jumbo bacteriophages possessing nucleus-like structures that compartmentalize and protect their DNA during infection [28]. These developments have broadened the scope of programmable phage engineering, including the modification of host specificity and incorporation of sequence-specific antibacterial functions. However, genetically modified phages require comprehensive genomic characterization, functional assessment and additional regulatory evaluation to establish their safety and therapeutic suitability [29].

The clinical application of engineered bacteriophages has demonstrated the potential of synthetic biology to address infections caused by difficult-to-treat bacterial pathogens. An important example involved a patient with disseminated drug-resistant *Mycobacterium abscessus* infection who received an individually selected combination of naturally occurring and genetically modified bacteriophages through compassionate-use treatment. This case demonstrated the feasibility of combining phage discovery, genetic modification and personalized therapeutic selection in a clinical setting [30]. Further progress has been reported with LBP-EC01, a CRISPR-Cas3-enhanced bacteriophage cocktail developed to target pathogenic *Escherichia coli*. Its evaluation in the phase 2 ELIMINATE clinical programme for uncomplicated *E. coli* urinary tract infections represents an important development in the clinical investigation of programmable antibacterial phages [31]. Unlike conventional phage preparations that primarily rely on naturally occurring lytic activity, engineered platforms can incorporate sequence-specific mechanisms intended to target selected bacterial genetic determinants. Nevertheless, their clinical translation requires careful assessment of genetic stability, bacterial target specificity, unintended effects, manufacturing consistency and therapeutic efficacy. These developments indicate a transition towards engineered bacteriophages as customizable antimicrobial biologics, although their broader clinical application remains dependent on rigorous experimental and clinical validation.

9. Phage Therapy against Biofilm-Associated Infections

Biofilms are structured microbial communities embedded within an extracellular matrix composed of polysaccharides, proteins, extracellular DNA and other biological components. They are frequently associated with chronic wounds, cystic fibrosis-related respiratory infections, prosthetic-joint infections, endocarditis, urinary catheters and implanted medical devices. Bacteria within biofilms often exhibit altered metabolic activity, physiological heterogeneity and increased tolerance to antimicrobial agents. The extracellular matrix can restrict antimicrobial penetration, while nutrient limitation and oxygen gradients promote bacterial subpopulations with reduced metabolic activity. Bacteriophages offer a potential approach to targeting these persistent bacterial communities through infection and replication within susceptible cells. Some phages also produce polysaccharide depolymerases, enzymes associated with tail fibres or tail spikes that degrade specific capsular polysaccharides and extracellular matrix components, facilitating access to bacterial cells. These enzymes may also be investigated as independent antibacterial agents or as adjuncts to conventional antibiotics. However, their activity depends on the structural composition of the targeted polysaccharide, and individual depolymerases may not effectively degrade the heterogeneous matrices found in complex polymicrobial biofilms [32]. Furthermore, bacterial dormancy, receptor accessibility and variations in biofilm architecture can restrict productive phage infection, making experimental findings difficult to extrapolate directly to clinical infections [33]. The substrate specificity of phage-associated depolymerases represents an additional consideration when evaluating their application against structurally diverse biofilms [34].

The therapeutic relevance of bacteriophages in biofilm-associated infections is particularly evident in persistent conditions such as prosthetic-joint infections, chronic osteomyelitis, vascular graft infections and cystic fibrosis-related respiratory disease. These infections frequently present considerable treatment challenges because bacteria can persist within protected biofilm environments despite prolonged antimicrobial exposure. Compassionate-use applications have provided encouraging observations regarding the potential contribution of phage therapy in selected difficult-to-treat cases. Nevertheless, clinical outcomes depend on bacterial susceptibility, infection-site accessibility, phage delivery, biofilm composition and host immune responses. Consequently, phage therapy is generally investigated as an adjunct to established treatment strategies rather than a replacement for conventional clinical management. Its integration with appropriate antibiotic therapy, surgical debridement and removal of infected devices, where indicated, may offer additional opportunities for controlling persistent biofilm-associated infections.

10. Phage Resistance as an Evolutionary Opportunity

The emergence of bacterial resistance is an expected consequence of the selective pressure imposed by bacteriophage infection. However, resistance to phages does not always provide bacteria with an unrestricted survival advantage, as alterations associated with phage escape may also affect important physiological and pathogenic characteristics. One well-characterized example involves bacteriophage OMKO1, which targets the outer-membrane porin OprM associated with multidrug efflux systems in *Pseudomonas aeruginosa*. Bacterial resistance arising through modifications of this receptor can impair efflux-related functions and increase susceptibility to certain antibiotics. This observation established the basis for evolutionary steering, an approach in which phages are selected not only for their bactericidal activity but also for their ability to impose evolutionary changes that reduce bacterial fitness or compromise clinically important resistance mechanisms [35]. Similar trade-offs may occur when phages target bacterial structures involved in capsule formation, adhesion, motility or biofilm development. Mutations affecting these structures can potentially reduce virulence, impair host colonization or increase susceptibility to immune clearance. However, such consequences are dependent on the bacterial strain, receptor function and specific resistance mechanism, and should therefore be established experimentally rather than assumed for all phage-resistant populations.

The ability to exploit these evolutionary trade-offs has generated interest in combining phage therapy with conventional antibiotics to improve the treatment of resistant bacterial infections. When phage resistance results in the loss or modification of structures associated with antibiotic resistance, surviving bacterial populations may become more susceptible to previously ineffective antimicrobial agents. This approach could potentially restore the activity of selected antibiotics and reduce the fitness advantages associated with resistance. Such applications have been discussed as a means of extending the therapeutic usefulness of existing antibiotics through phage-mediated evolutionary pressure [36]. Accordingly, the evaluation of therapeutic phages may benefit from considering not only their initial host range and bacterial killing efficiency but also the phenotypic characteristics of the resistant populations that emerge following exposure. Identifying phages capable of directing bacterial evolution towards reduced virulence, impaired fitness or increased antibiotic susceptibility may provide an additional basis for individualized therapeutic selection.

11. Phage–Antibiotic Synergy

The combined application of bacteriophages and conventional antibiotics has gained considerable attention as a therapeutic strategy against multidrug-resistant bacterial infections. Their interaction may produce synergistic, additive, neutral or antagonistic effects depending on the bacteriophage, bacterial strain, antibiotic concentration and sequence of administration. Experimental investigations have demonstrated that subinhibitory concentrations of certain β-lactam and quinolone antibiotics can enhance phage-mediated bacterial killing, a phenomenon commonly referred to as phage–antibiotic synergy (PAS). Proposed mechanisms include antibiotic-induced bacterial filamentation, alterations in receptor expression, changes in phage adsorption and increased production of progeny virions under specific experimental conditions [37]. However, the effectiveness of combination treatment is strongly influenced by administration sequence and bacterial physiological state. In biofilm-associated infections, prior exposure to antibiotics may suppress bacterial metabolic activity and consequently interfere with productive phage replication. Conversely, phage administration before antibiotic treatment may permit viral adsorption and replication before subsequent antibiotic exposure. These effects are not universal, as bacteriostatic and bactericidal agents can influence phage activity differently depending on the bacterial strain and infection model.

Therefore, the optimal treatment sequence requires experimental evaluation for each specific phage–antibiotic combination [38].

Preclinical investigations have demonstrated the potential of combined treatment in several difficult-to-treat bacterial infections. Studies involving experimental endocarditis have reported beneficial interactions between phages and antibiotics against *Pseudomonas aeruginosa*, including reductions in bacterial burden and changes in the characteristics of surviving bacterial populations [39]. Similar investigations involving *Enterococcus faecium* have demonstrated that combined antimicrobial approaches may provide enhanced bacterial suppression beyond individual treatment under selected experimental conditions [40]. Infections caused by *Acinetobacter baumannii* have also been investigated using phage–antibiotic combinations, with preclinical findings indicating improved antibacterial activity and opportunities to exploit capsule-associated evolutionary trade-offs [41]. Mathematical modelling further suggests that the outcome of combination treatment depends on the interactions among bacterial population density, antibiotic exposure, phage replication dynamics and host immune responses. These interdependent variables complicate the prediction of treatment outcomes using conventional antimicrobial models and highlight the need for combination-specific experimental assessment [42].

Table 2: Mechanisms underlying phage–antibiotic interactions in precision antimicrobial therapy

Mechanism	Contribution of bacteriophages	Contribution of antibiotics	Potential therapeutic outcome
Complementary killing	Eliminates susceptible bacterial populations	Targets antibiotic-susceptible populations	Improved bacterial suppression
Receptor-associated trade-offs	Selects for receptor-modified bacterial variants	Exploits associated changes in antibiotic susceptibility	Evolutionary steering
Biofilm disruption	Promotes bacterial lysis and may produce depolymerases	Targets exposed or metabolically active bacteria	Improved biofilm-associated bacterial clearance
Resistance suppression	Applies selection through phage-specific receptors	Acts through independent antimicrobial targets	Potential reduction in simultaneous resistance
Sequential administration	Permits phage adsorption and replication when administered first	Targets residual bacterial populations	Improved treatment under suitable conditions
Efflux-pump-associated trade-offs	Selects variants with altered efflux-associated receptors	May achieve increased intracellular antibiotic accumulation	Potential restoration of antibiotic susceptibility
Capsule-associated trade-offs	Selects for capsule-deficient or modified variants	Acts against bacteria with altered surface characteristics	Potential reduction in virulence and improved clearance

The therapeutic value of phage–antibiotic combinations ultimately depends on identifying interactions that provide measurable improvements over individual treatment. In particular, differences in bacterial susceptibility, phage replication, antibiotic pharmacodynamics and treatment sequence must be considered when selecting combination regimens. Systematic in-vitro combination testing, followed by appropriate in-vivo and clinical evaluation, is therefore necessary to distinguish genuine synergy from additive or antagonistic interactions. Such an approach may support the development of individualized combination strategies for infections involving multidrug-resistant and biofilm-associated bacterial pathogens.

12. Pharmacokinetics and Pharmacodynamics of Phage Therapy

The pharmacokinetic and pharmacodynamic properties of bacteriophages differ considerably from those of conventional antimicrobial agents because phages are biological entities capable of replication within susceptible bacterial hosts. Their distribution and elimination are influenced by administration route, tissue accessibility, bacterial density, host susceptibility and immune-mediated clearance.

Following intravenous administration, phages may be distributed to different organs, including the liver and spleen, where components of the reticuloendothelial system contribute to their removal. Alternative administration routes, including inhalation, oral, topical, intravesical and direct local delivery, are investigated according to the anatomical location of infection. Intravenous administration may be appropriate for systemic infections, whereas nebulization can facilitate delivery to respiratory tissues and local administration may improve phage exposure in prosthetic-joint infections. Phage pharmacodynamics involves both passive antibacterial activity, resulting from the administration of infectious particles, and active replication within susceptible bacterial populations. However, in-situ amplification depends on bacterial abundance, metabolic activity, receptor availability and phage–host compatibility. Multiplicity of infection (MOI), defined as the ratio of infectious phage particles to bacterial cells, is commonly used in laboratory investigations but cannot be directly translated into clinical dosing because bacterial burden and phage distribution within infected tissues are difficult to determine accurately.

Consequently, therapeutic dose optimization requires exposure–response assessments that account for phage replication, bacterial population dynamics and infection-site characteristics [43]. The complexity of these interactions represents an important challenge in establishing reliable pharmacokinetic and pharmacodynamic models for clinical applications [44]. Further investigations have emphasized the need to incorporate phage–bacterium interactions into therapeutic dose optimization and treatment-response assessment [45].

Host immune responses represent another important determinant of phage persistence and therapeutic effectiveness. Following administration, bacteriophages may be recognized and removed through innate immune mechanisms, while repeated exposure can stimulate the production of phage-specific antibodies capable of neutralizing viral particles. The extent of immune-mediated clearance varies according to phage characteristics, administration route, treatment duration and individual host responses. Experimental studies have demonstrated that immune recognition can substantially influence phage persistence and circulating concentrations [46]. Clinical observations have additionally identified neutralizing antibodies as a potential limitation during prolonged treatment, particularly in patients receiving repeated intravenous administration [47]. Nevertheless, antibody development does not invariably result in therapeutic failure, as its clinical significance depends on the extent of neutralization, phage exposure and susceptibility of the infecting bacterial isolate. Monitoring phage activity and neutralizing antibody responses may therefore be relevant in selected patients, especially when clinical improvement declines despite continued in-vitro susceptibility. Understanding the combined effects of phage distribution, replication, bacterial dynamics and host immunity is essential for developing appropriate dosing strategies and improving the predictability of therapeutic outcomes.

13. Clinical Translation: From Case Reports to Controlled Trials

Clinical translation of bacteriophage therapy has progressed from early therapeutic investigations to individualized compassionate-use treatments, observational studies and controlled clinical trials. Evidence from these investigations demonstrates the potential of phages against difficult-to-treat bacterial infections, although clinical outcomes have varied considerably according to phage selection, formulation, administration route, bacterial susceptibility and infection characteristics. Early controlled investigations involving chronic *Pseudomonas aeruginosa* otitis demonstrated reductions in bacterial burden and improvements in clinical parameters following local phage administration, providing evidence supporting the feasibility of systematic clinical evaluation [48]. Subsequent compassionate-use applications extended these findings to severe systemic infections. A particularly influential case involved a patient with disseminated multidrug-resistant *Acinetobacter baumannii* infection who received a personalized phage cocktail alongside conventional antimicrobial treatment. The clinical recovery demonstrated the feasibility of individualized phage selection and highlighted the importance of pathogen isolation, phage screening, purification, regulatory authorization and microbiological monitoring in emergency therapeutic applications [49].

Nevertheless, individual case reports cannot establish treatment efficacy because clinical outcomes may also be influenced by concurrent antibiotics, surgical interventions and the patient's underlying condition.

Randomized clinical investigations have provided a more rigorous assessment of phage therapy while also identifying important limitations in therapeutic development. The PhagoBurn trial, which evaluated a fixed phage preparation against *P. aeruginosa* in burn-wound infections, failed to demonstrate superiority over standard treatment. Substantial reductions in phage concentration during storage resulted in the administration of lower-than-intended therapeutic doses, highlighting the importance of formulation stability, potency assessment and manufacturing quality [50]. Similarly, a randomized investigation of intravesical phage administration for urinary tract infections did not demonstrate superiority over placebo for its primary outcome, emphasizing the limitations of therapeutic preparations that are not necessarily optimized for individual bacterial susceptibility [51]. Investigations involving severe *Staphylococcus aureus* infections have also explored adjunctive intravenous phage administration, providing encouraging observations regarding systemic tolerability and supporting further clinical evaluation [52]. Collectively, these studies indicate that clinical performance depends not only on the intrinsic antibacterial activity of phages but also on formulation quality, appropriate dosing, host matching and infection-site characteristics.

Personalized phage therapy has been particularly relevant to infections caused by drug-resistant mycobacteria, where considerable variation in phage susceptibility exists among clinical isolates. A compassionate-use investigation involving 20 patients with mycobacterial disease evaluated individualized phage preparations administered through intravenous, aerosolized or combined routes. Favorable clinical or microbiological responses were reported in 11 patients, although the emergence of neutralizing antibodies in some recipients complicated prolonged treatment. These observations demonstrated the feasibility of personalized phage administration while highlighting the importance of susceptibility testing, immune monitoring and treatment optimization [53]. Dedicated clinical phage programmes have subsequently developed systems for coordinating bacterial isolation, therapeutic phage screening, regulatory procedures and patient monitoring. Experience from the Israeli Phage Therapy Center illustrates the practical organization of such services and their role in facilitating access to individualized treatment [54].

Real-world observational investigations have further contributed to the understanding of clinical phage applications. An analysis of 100 personalized phage-therapy cases across multiple hospitals and countries reported clinical improvement in 77.2% of infections and eradication of the targeted bacterial pathogen in 61.3%. The investigation also documented phage resistance, antibiotic resensitization, virulence-associated trade-offs, phage–antibiotic interactions and immune neutralization. However, its retrospective and uncontrolled design limits the interpretation of these findings as direct estimates of therapeutic efficacy [55]. Systematic evaluations of the broader clinical literature have similarly indicated a generally encouraging safety profile for appropriately prepared phage formulations, while emphasizing that definitive evidence of efficacy remains limited by small study populations, heterogeneous treatment protocols and the predominance of case reports and observational investigations [56].

Cystic fibrosis-associated respiratory infections have emerged as an important area for precision phage therapy because chronic *P. aeruginosa* colonization, biofilm formation and progressive antibiotic resistance frequently complicate disease management. A 2025 compassionate-use investigation involving nine adults with cystic fibrosis evaluated personalized inhaled phages against multidrug-resistant or pan-drug-resistant *P. aeruginosa*. Nebulized administration was reported to be well tolerated, with reductions in sputum bacterial burden and evolutionary changes in recovered bacterial populations associated with altered antibiotic susceptibility or virulence in some patients [57].

A separate clinical investigation evaluated BX004-A, a standardized cocktail containing three bacteriophages, in adults with cystic fibrosis and chronic *P. aeruginosa* infection. The randomized first-in-human phase 1b/2a study primarily evaluated safety and tolerability, with findings supporting the feasibility of inhaled delivery to the lower respiratory tract. Exploratory observations suggested potential reductions in bacterial burden, although the limited sample size did not establish definitive clinical efficacy [58]. These investigations illustrate two complementary approaches to respiratory phage therapy: individualized preparations selected against patient-specific bacterial isolates and standardized formulations developed to provide activity against a broader range of clinical strains.

Table 3: Representative clinical studies and translational applications of bacteriophage therapy

Study/reference	Target pathogen or infection	Therapeutic approach	Major clinical contribution
Wright et al. [48]	Chronic P. aeruginosa otitis	Local phage administration	Early controlled evidence of clinical feasibility
Schooley et al. [49]	Disseminated MDR A. baumannii infection	Personalized phage cocktail	Landmark compassionate-use application
PhagoBurn [50]	P. aeruginosa burn-wound infection	Fixed phage preparation	Highlighted formulation stability and potency challenges
Leitner et al. [51]	Urinary tract infection	Intravesical phage administration	Controlled evaluation that did not demonstrate superiority over placebo
Petrovic Fabijan et al. [52]	Severe S. aureus infection	Adjunctive intravenous phage therapy	Provided clinical safety and systemic administration observations
Dedrick et al. [53]	Drug-resistant mycobacterial disease	Personalized phage therapy	Demonstrated individualized treatment feasibility and immune-related challenges
Israeli Phage Therapy Center [54]	Diverse difficult-to-treat infections	Clinical phage screening and matching	Demonstrated an organized clinical treatment framework
Pirnay et al. [55]	Diverse difficult-to-treat infections	Personalized therapy; 100-case analysis	Provided substantial real-world observational evidence
Cystic fibrosis compassionate-use study [57]	MDR/PDR P. aeruginosa	Personalized inhaled phages	Investigated bacterial suppression and evolutionary steering
BX004-A clinical study [58]	Chronic P. aeruginosa respiratory infection	Standardized three-phage inhaled cocktail	Evaluated safety, tolerability and pulmonary delivery

The clinical evidence accumulated to date indicates that bacteriophage therapy cannot be evaluated independently of pathogen susceptibility, formulation quality, administration route and infection-site conditions. Favorable outcomes in selected compassionate-use cases and observational studies demonstrate therapeutic potential, whereas controlled trials have exposed limitations associated with fixed formulations, inadequate dosing and insufficient patient-specific matching. The translation of phage therapy into routine clinical practice will therefore require adequately powered clinical trials, standardized susceptibility testing, validated manufacturing procedures and treatment protocols that account for individual bacterial and host characteristics.

14. Bone, Joint and Device-Associated Infections, Phage-Derived Enzymes, and Manufacturing Challenges

Bone, joint and device-associated infections are important potential applications of bacteriophage therapy, particularly when bacterial biofilms develop on prosthetic surfaces and become difficult to eradicate with antibiotics alone. Although conventional susceptibility testing may indicate antibiotic sensitivity, bacteria within mature biofilms can remain highly persistent. Clinical reports have described encouraging outcomes following phage treatment of orthopedic infections. A review of published cases found clinical or microbiological success in a substantial proportion of patients, although the evidence was largely based on uncontrolled reports and may have been influenced by publication bias, surgery and concomitant antibiotic treatment [59].

A separate series involving ten patients with difficult-to-treat periprosthetic joint infections further demonstrated the feasibility of including phages in complex orthopedic treatment [60]. These findings also highlight the importance of source control: phage therapy is generally considered alongside debridement, drainage, prosthesis removal or exchange, and appropriate antibiotics rather than as an independent intervention.

Therapeutic applications of phages are not limited to intact viral particles. Phage-derived enzymes, particularly endolysins and depolymerases, can target bacterial cell walls and extracellular structures. Endolysins normally degrade peptidoglycan during the phage replication cycle, but purified enzymes can also act directly against bacteria, especially Gram-positive organisms where the cell wall is accessible. Their activity against resistant bacteria and their potential for controlled pharmaceutical production make them relevant candidates for antibacterial development [61]. SAL200, a preparation based on the staphylococcal phage endolysin SAL-1, advanced to human testing, including a randomized phase I study involving single ascending intravenous doses in healthy volunteers [62]. Unlike intact phages, however, purified enzymes cannot replicate at the infection site or adapt to bacterial evolution. Their therapeutic use therefore depends on maintaining sufficient active concentrations at the target site.

The clinical application of whole phages and phage-derived products also depends on reliable manufacturing and quality control.

Therapeutic phages are commonly propagated using susceptible bacterial hosts, making the removal of endotoxins, bacterial DNA, proteins and other host-derived contaminants essential, particularly for preparations intended for intravenous administration. Purification approaches such as filtration, ultrafiltration and chromatography can reduce these contaminants while retaining infectious phage particles [63]. Each batch also requires confirmation of identity, infectious titre, sterility, purity and storage stability; genome sequencing can help establish that the manufactured phage matches the intended strain. Production under Good Manufacturing Practice (GMP) conditions adds further requirements for consistency and traceability. These processes are more straightforward for fixed phage cocktails than for patient-specific preparations, which may need to be selected or modified in response to an individual bacterial isolate. Balancing pharmaceutical standardization with the flexibility required for personalized treatment remains a major manufacturing and regulatory challenge [64].

15. Regulatory Models, Precision Clinical Workflow and Artificial Intelligence in Phage Therapy

Personalized phage therapy challenges conventional drug-regulatory systems because the active biological components may differ between patients and may require modification during treatment. Belgium has introduced a magistral framework in which characterized phage active ingredients can be combined into individualized preparations by qualified pharmacies under defined quality requirements. This approach aims to retain patient-specific treatment while ensuring pharmaceutical oversight [65]. Further analyses have considered whether this model could be adapted for use in other countries [66]. However, regulatory pathways remain inconsistent worldwide, with access often dependent on compassionate-use, expanded-access or experimental-treatment provisions, alongside established institutional phage programs [67]. A practical regulatory framework may need to accommodate both standardized phage cocktails developed through conventional clinical trials and individualized preparations assembled from quality-assured phage banks. This distinction is important because phage therapy may require changes in response to bacterial evolution, whereas conventional regulations generally require additional validation when active ingredients are modified. Regulatory approaches must therefore maintain safety, manufacturing quality and traceability without unnecessarily restricting clinically justified adaptation [68].

A precision phage-therapy programme would bring together clinical microbiology, genomics, computational analysis and treatment monitoring. The process would begin with isolation and identification of the pathogen, followed by antibiotic susceptibility testing and, where appropriate, whole-genome sequencing to examine resistance determinants, virulence factors, surface receptors and antiphage defence systems. Computational tools could prioritize candidate phages from characterized collections, but laboratory phagograms would still be required to confirm productive infection. Selected phages would undergo genomic safety assessment, followed by cocktail selection and testing of relevant phage–antibiotic combinations. Treatment planning would consider the infection site, delivery route, tissue accessibility and patient immune status. During treatment, bacterial cultures and, where feasible, phage concentrations could be monitored.

If bacterial regrowth occurred, recovered isolates could be reassessed for phage susceptibility, resistance mechanisms and changes in antibiotic sensitivity or virulence. Artificial intelligence may support this workflow through phage discovery, genome annotation, host-range prediction, cocktail optimization and modelling of resistance evolution. A 2026 systematic review identified growing use of AI and machine-learning approaches in precision phage therapy but also highlighted the gap between computational development and clinical validation [69]. These tools should therefore assist candidate selection and experimental planning rather than replace laboratory susceptibility testing or clinical judgement.

16. Challenges, Future Research Priorities and the Development of Precision Phage Medicine

Despite progress in clinical applications, several barriers continue to limit the routine use of bacteriophage therapy. The narrow host range of individual phages requires access to sufficiently large phage collections and rapid susceptibility testing, while the absence of universally accepted clinical breakpoints makes laboratory results difficult to interpret in relation to patient outcomes [17,18]. Activity observed in vitro may not translate into therapeutic success because of biofilm structure, limited tissue penetration and immune clearance. Resistance development and neutralizing antibodies may further reduce treatment effectiveness, particularly during prolonged therapy [46,47]. Manufacturing also remains challenging because personalized preparations require rapid phage selection, propagation, purification and quality control [63,64]. Addressing these issues will require standardized testing methods, reliable phage banks, improved pharmacokinetic studies and well-designed clinical trials that distinguish microbiological responses from meaningful clinical benefits.

The development of precision phage medicine will depend on integrating these procedures into a standardized clinical framework without losing the flexibility of individualized treatment. Standardization should cover bacterial identification, genome sequencing, phage susceptibility testing, manufacturing quality, dosing and clinical outcome monitoring, while allowing the selected phage or cocktail to remain patient-specific [16]. A consensus-based guideline published in *Nature Medicine* in September 2026 provides recommendations addressing clinical infrastructure, quality-controlled phage preparations, administration and monitoring [70]. Further progress will require prospective validation of AI-based host-range prediction and rational cocktail design [20–27,69]. In a future clinical workflow, bacterial genomic and phenotypic data could guide candidate selection from established phage banks, followed by laboratory confirmation and selection of an appropriate antibiotic combination. Bacterial isolates collected during treatment could then be reassessed to identify resistance mechanisms and possible changes in antibiotic susceptibility [35,36]. The long-term objective is to establish a reproducible treatment system that combines genomics, microbiology, pharmacology and evolutionary analysis to manage difficult bacterial infections.

17. Conclusion

The growing threat of antimicrobial resistance has renewed interest in bacteriophage therapy as an alternative approach to treating difficult bacterial infections.

Advances in genomics, molecular biology and artificial intelligence have improved the ability to select suitable phages, understand bacterial resistance mechanisms and design more effective phage combinations. Phages also offer the possibility of influencing bacterial evolution, particularly when resistance to a phage results in reduced virulence or increased antibiotic susceptibility. Despite these advances, challenges involving phage selection, resistance, manufacturing, dosing and clinical validation remain. Phage therapy should therefore be developed alongside conventional antibiotics rather than viewed as their complete replacement. Continued research, well-designed clinical trials and standardized regulatory practices will be essential for its wider clinical application. With continued scientific and clinical progress, precision phage therapy has the potential to become an important part of future antimicrobial medicine, offering a more targeted, adaptive and sustainable approach to combating drug-resistant bacterial infections.

Declaration of Generative AI Use

Generative AI tools were used solely for language editing, grammar correction, and improvement of the clarity and readability of the manuscript. The authors take full responsibility for the originality, accuracy, interpretation, and final content of the article.

References

1. Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., Robles Aguilar, G., Mestrovic, T., Smith, G., Han, C., Hsu, R. L., Chalek, J., Araki, D. T., Chung, E., Raggi, C., Gershberg Hayoon, A., Davis Weaver, N., Lindstedt, P. A., Smith, A. E., … Murray, C. J. L. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet*, *404*(10459), 1199–1226. https://doi.org/10.1016/s0140-6736(24)01867-1
2. World Health Organization. (2024, May 17). *WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance*. Www.Who.Int. https://www.who.int/publications/i/item/9789240093461
3. Theuretzbacher, U. (2025). The global resistance problem and the clinical antibacterial pipeline. *Nature Reviews Microbiology*, *23*(8), 491–508. https://doi.org/10.1038/s41579-025-01169-8
4. Gordillo Altamirano, F. L., & Barr, J. J. (2019). Phage therapy in the postantibiotic era. *Clinical Microbiology Reviews*, *32*(2). https://doi.org/10.1128/cmr.00066-18
5. Kortright, K. E., Chan, B. K., Koff, J. L., & Turner, P. E. (2019). Phage therapy: A renewed approach to combat antibiotic-resistant bacteria. *Cell Host & Microbe*, *25*(2), 219–232.
6. Strathdee, S. A., Hatfull, G. F., Mutalik, V. K., & Schooley, R. T. (2023). Phage therapy: From biological mechanisms to future directions. *Cell*, *186*(1), 17–31. https://doi.org/10.1016/j.cell.2022.11.017
7. Skurnik, M., Alkalay-Oren, S., Boon, M., Clokie, M., Sicheritz-Pontén, T., Dąbrowska, K., Hatfull, G. F., Hazan, R., Jalasvuori, M., Kiljunen, S., Lavigne, R., Malik, D. J., Nir-Paz, R., & Pirnay, J.-P. (2025). Phage therapy. *Nature Reviews Methods Primers*, *5*(1). https://doi.org/10.1038/s43586-024-00377-5
8. Sinclair, H. A., Lin, R. C. Y., Ben Zakour, N. L., Bollyky, P. L., & Iredell, J. R. (2026). Phage therapy: From basic biology to clinical application. *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-026-01352-5
9. Dion, M. B., Oechslin, F., & Moineau, S. (2020). Phage diversity, genomics and phylogeny. *Nature Reviews Microbiology*, *18*(3), 125–138. https://doi.org/10.1038/s41579-019-0311-5
10. Hyman, P., & Abedon, S. T. (2010). Bacteriophage host range and bacterial resistance. *Advances in Applied Microbiology*, *70*, 217–248. https://doi.org/10.1016/s0065-2164(10)70007-1
11. Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nature Reviews Microbiology*, *8*(5), 317–327. https://doi.org/10.1038/nrmicro2315
12. Georjon, H., & Bernheim, A. (2023). The highly diverse antiphage defence systems of bacteria. *Nature Reviews Microbiology*, *21*, 1–15. https://doi.org/10.1038/s41579-023-00934-x
13. Malone, L. M., Birkholz, N., & Fineran, P. C. (2021). Conquering CRISPR: How phages overcome bacterial adaptive immunity. *Systems Biology Nanobiotechnology*, *68*, 30–36. https://doi.org/10.1016/j.copbio.2020.09.008
14. Murtazalieva, K., Mu, A., Petrovskaya, A., & Finn, R. D. (2024). The growing repertoire of phage anti-defence systems. *Trends in Microbiology*, *32*(12), 1212–1228. https://doi.org/10.1016/j.tim.2024.05.005
15. Stanley, S. Y., & Maxwell, K. L. (2018). Phage-encoded Anti-CRISPR defenses. *Annual Review of Genetics*, *52*(1), 445–464. https://doi.org/10.1146/annurev-genet-120417-031321
16. Abdraimova, N., Shitikov, E., & Kornienko, M. (2026). The role of genomics in advancing and standardising bacteriophage therapy. *Antibiotics*, *15*(1), 55. https://doi.org/10.3390/antibiotics15010055
17. Gelman, D., Yerushalmy, O., Alkalay-Oren, S., Rakov, C., Ben-Porat, S., Khalifa, L., Adler, K., Abdalrhman, M., Coppenhagen-Glazer, S., Aslam, S., Schooley, R. T., Nir-Paz, R., & Hazan, R. (2021). Clinical phage microbiology: A suggested framework and recommendations for the in-vitro matching steps of phage therapy. *The Lancet Microbe*, *2*(10), e555–e563. https://doi.org/10.1016/s2666-5247(21)00127-0
18. Suh, G. A., & Patel, R. (2023). Clinical phage microbiology: A narrative summary. *Clinical Microbiology and Infection*, *29*(6), 710–713. https://doi.org/10.1016/j.cmi.2023.02.006
19. Bhavya Papudeshi, Roach, M. J., Vijini Mallawaarachchi, Bouras, G., Grigson, S. R., Giles, S. K., Harker, C. M., Hutton, A. L. K., Tarasenko, A., Inglis, L. K., Vega, A. A., Souza, C., Boling, L., Hamza Hajama, Georgina, A., Segall, A. M., Dinsdale, E. A., & Edwards, R. A. (2025). Sphae: An automated toolkit for predicting phage therapy candidates from sequencing data. *Bioinformatics Advances*. https://doi.org/10.1093/bioadv/vbaf004
20. Ruohan, W., Xianglilan, Z., Jianping, W., & Shuai Cheng, L. I. (2021). DeepHost: Phage host prediction with convolutional neural network. *Briefings in Bioinformatics*, *23*(1). https://doi.org/10.1093/bib/bbab385
21. Amgarten, D., Iha, B. K. V., Piroupo, C. M., da Silva, A. M., & Setubal, J. C. (2022). vHULK, a new tool for bacteriophage host prediction based on annotated genomic features and neural networks. *Phage*, *3*(4), 204–212. https://doi.org/10.1089/phage.2021.0016
22. Howell, A. A., Versoza, C. J., & Pfeifer, S. P. (2023). Computational host range prediction – the good, the bad, and the ugly. *Virus Evolution*, *10*(1). https://doi.org/10.1093/ve/vead083
23. Gaborieau, B., Vaysset, H., Tesson, F., Charachon, I., Dib, N., Bernier, J., Dequidt, T., Georjon, H., Clermont, O., Hersen, P., Debarbieux, L., Ricard, J.-D., Denamur, E., & Bernheim, A. (2024). Prediction of strain level phage–host interactions across the escherichia genus using only genomic information. *Nature Microbiology*, *9*(11), 2847–2861. https://doi.org/10.1038/s41564-024-01832-5
24. Lood, C., Haas, P.-J., van Noort, V., & Lavigne, R. (2022). Shopping for phages? Unpacking design rules for therapeutic phage cocktails. *Current Opinion in Virology*, *52*, 236–243. https://doi.org/10.1016/j.coviro.2021.12.011

25. Molina, F., Simancas, A., Ramírez, M., Tabla, R., Roa, I., & Rebollo, J. E. (2021). A new pipeline for designing phage cocktails based on phage-bacteria infection networks. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.564532
26. Abedon, S. T., Danis-Wlodarczyk, K. M., & Wozniak, D. J. (2021). Phage cocktail development for bacteriophage therapy: Toward improving spectrum of activity breadth and depth. *Pharmaceuticals*, *14*(10), 1019. https://doi.org/10.3390/ph14101019
27. Ngiam, L., Weynberg, K., & Guo, J. (2024). Evolutionary and co-evolutionary phage training approaches enhance bacterial suppression and delay the emergence of phage resistance. *ISME Communications*, *4*(1), ycae082. https://doi.org/10.1093/ismeco/ycae082
28. Guan, J., Oromí-Bosch, A., Mendoza, S. D., Karambelkar, S., Berry, J. D., & Bondy-Denomy, J. (2022). Bacteriophage genome engineering with CRISPR–Cas13a. *Nature Microbiology*, *7*(12), 1956–1966. https://doi.org/10.1038/s41564-022-01243-4
29. Chaudhary, N., Sharma, K., Kaur, H., Prajapati, S., Mohan, B., & Taneja, N. (2024). CRISPR-Cas-assisted phage engineering for personalized antibacterial treatments. *Indian Journal of Medical Microbiology*, *53*, 100771. https://doi.org/10.1016/j.ijmmb.2024.100771
30. Dedrick, R. M., Guerrero-Bustamante, C. A., Garlena, R. A., Russell, D. A., Ford, K., Harris, K., Gilmour, K. C., Soothill, J., Jacobs-Sera, D., Schooley, R. T., Hatfull, G. F., & Spencer, H. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant mycobacterium abscessus. *Nature Medicine*, *25*(5), 730–733. https://doi.org/10.1038/s41591-019-0437-z
31. Kim, P., Sanchez, A. M., Penke, T. J. R., Tuson, H. H., Kime, J. C., McKee, R. W., Slone, W. L., Conley, N. R., McMillan, L. J., Prybol, C. J., & Garofolo, P. M. (2024). Safety, pharmacokinetics, and pharmacodynamics of LBP-EC01, a CRISPR-Cas3-enhanced bacteriophage cocktail, in uncomplicated urinary tract infections due to Escherichia Coli (ELIMINATE): The randomised, open-label, first part of a two-part phase 2 trial. *The Lancet Infectious Diseases*, *24*(12), 1319–1332. https://doi.org/10.1016/s1473-3099(24)00424-9
32. Pires, D. P., Melo, L. D. R., & Azeredo, J. (2021). Understanding the complex phage-host interactions in biofilm communities. *Annual Review of Virology*, *8*(1), 73–94. https://doi.org/10.1146/annurev-virology-091919-074222
33. Pires, D. P., Meneses, L., Brandão, A. C., & Azeredo, J. (2022). An overview of the current state of phage therapy for the treatment of biofilm-related infections. *Current Opinion in Virology*, *53*, 101209. https://doi.org/10.1016/j.coviro.2022.101209
34. Topka-Bielecka, G., Dydecka, A., Necel, A., Bloch, S., Nejman-Faleńczyk, B., Węgrzyn, G., & Węgrzyn, A. (2021). Bacteriophage-derived depolymerases against bacterial biofilm. *Antibiotics*, *10*(2), 175. https://doi.org/10.3390/antibiotics10020175
35. Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage selection restores antibiotic sensitivity in MDR pseudomonas aeruginosa. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep26717
36. Wójcicki, M., Cieślik, M., Górski, A., & Jończyk-Matysiak, E. (2026). Giving antibiotics a second chance: Evolutionary trade-offs and phage-driven restoration of antibiotic susceptibility. *BioDrugs*, *40*(4), 623–640. https://doi.org/10.1007/s40259-026-00789-7
37. Comeau, A. M., Tétart, F., Trojet, S. N., Prère, M.-F., & Krisch, H. M. (2007). Phage-antibiotic synergy (PAS): β-Lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS ONE*, *2*(8), e799. https://doi.org/10.1371/journal.pone.0000799
38. Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., & Levin, B. R. (2017). Synergy and order effects of antibiotics and phages in killing pseudomonas aeruginosa biofilms. *PLOS ONE*, *12*(1), e0168615. https://doi.org/10.1371/journal.pone.0168615
39. Oechslin, F., Piccardi, P., Mancini, S., Gabard, J., Moreillon, P., Entenza, J. M., Resch, G., & Que, Y.-A. (2016). Synergistic interaction between phage therapy and antibiotics clears pseudomonas aeruginosa infection in endocarditis and reduces virulence. *Journal of Infectious Diseases*, *215*(5), jiw632. https://doi.org/10.1093/infdis/jiw632
40. Morrisette, T., Lev, K. L., Kebriaei, R., Abdul-Mutakabbir, J. C., Stamper, K. C., Morales, S., Lehman, S. M., Canfield, G. S., Duerkop, B. A., Arias, C. A., & Rybak, M. J. (2020). Bacteriophage-antibiotic combinations for enterococcus faecium with varying bacteriophage and daptomycin susceptibilities. *Antimicrobial Agents and Chemotherapy*, *64*(9). https://doi.org/10.1128/aac.00993-20
41. Gordillo Altamirano, F. L., Kostoulias, X., Subedi, D., Korneev, D., Peleg, A. Y., & Barr, J. J. (2022). Phage-antibiotic combination is a superior treatment against acinetobacter baumannii in a preclinical study. *eBioMedicine*, *80*, 104045. https://doi.org/10.1016/j.ebiom.2022.104045
42. Rodriguez-Gonzalez, R. A., Leung, C. Y., Chan, B. K., Turner, P. E., & Weitz, J. S. (2020). Quantitative models of phage-antibiotic combination therapy. *mSystems*, *5*(1). https://doi.org/10.1128/msystems.00756-19
43. Abedon, S., & Thomas-Abedon, C. (2010). Phage therapy pharmacology. *Current Pharmaceutical Biotechnology*, *11*(1), 28–47. https://doi.org/10.2174/138920110790725410
44. Nang, S. C., Ye, L., Aleksandra Petrović Fabijan, Yoon, R., Rao, G. G., Iredell, J. R., Chan, H.-K., & Li, J. (2023). Pharmacokinetics/pharmacodynamics of phage therapy: A major hurdle to clinical translation. *Clinical Microbiology and Infection*, *29*(6), 702–709. https://doi.org/10.1016/j.cmi.2023.01.021
45. Siopi, M., Dimitrios Skliros, Paschalis Paranos, Nikoletta Koumasi, Emmanouil Flemetakis, Pournaras, S., & Meletiadis, J. (2024). Pharmacokinetics and pharmacodynamics of bacteriophage therapy: A review with a focus on multidrug-resistant gram-negative bacterial infections. *Clinical Microbiology Reviews*. https://doi.org/10.1128/cmr.00044-24
46. Hodyra-Stefaniak, K., Miernikiewicz, P., Drapała, J., Drab, M., Jończyk-Matysiak, E., Lecion, D., Kaźmierczak, Z., Beta, W., Majewska, J., Harhala, M., Bubak, B., Kłopot, A., Górski, A., & Dąbrowska, K. (2015). Mammalian host-versus-phage immune response determines phage fate in vivo. *Scientific Reports*, *5*, 14802. https://doi.org/10.1038/srep14802
47. Champagne-Jorgensen, K., Luong, T., Darby, T., & Roach, D. R. (2023). Immunogenicity of bacteriophages. *Trends in Microbiology*, *31*(10), 1058–1071. https://doi.org/10.1016/j.tim.2023.04.008
48. Wright, A., Hawkins, C. H., Änggård, E. E., & Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistantPseudomonas aeruginosa; a preliminary report of efficacy. *Clinical Otolaryngology*, *34*(4), 349–357. https://doi.org/10.1111/j.1749-4486.2009.01973.x
49. Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., Segall, A. M., Taplitz, R., Smith, D. M., Kerr, K., Kumaraswamy, M., Nizet, V., Lin, L., McCauley, M. D., Strathdee, S. A., … Hamilton, T. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant acinetobacter baumannii infection. *Antimicrobial Agents and Chemotherapy*, *61*(10). https://doi.org/10.1128/aac.00954-17

50. Jault, P., Leclerc, T., Jennes, S., Pirnay, J. P., Que, Y.-A., Resch, G., Rousseau, A. F., Ravat, F., Carsin, H., Le Floch, R., Schaal, J. V., Soler, C., Fevre, C., Arnaud, I., Bretaudeau, L., & Gabard, J. (2019). Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas Aeruginosa (PhagoBurn): A randomised, controlled, double-blind phase 1/2 trial. *The Lancet Infectious Diseases*, *19*(1), 35–45. https://doi.org/10.1016/s1473-3099(18)30482-1
51. Leitner, L., Ujmajuridze, A., Chanishvili, N., Goderdzishvili, M., Chkonia, I., Rigvava, S., Chkhotua, A., Changashvili, G., McCallin, S., Schneider, M. P., Liechti, M. D, Mehnert, U., Bachmann, L. M., Sybesma, W., & Kessler, T. M. (2021). Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: A randomised, placebo-controlled, double-blind clinical trial. *The Lancet Infectious Diseases*, *21*(3), 427–436. https://doi.org/10.1016/s1473-3099(20)30330-3
52. Petrovic Fabijan, A., Lin, R. C. Y., Ho, J., Maddocks, S., Ben Zakour, N. L., & Iredell, J. R. (2020). Safety of bacteriophage therapy in severe Staphylococcus aureus infection. *Nature Microbiology*, *5*(3), 465–472. https://doi.org/10.1038/s41564-019-0634-z
53. Dedrick, R. M., Smith, B. E., Cristinziano, M., Freeman, K. G., Jacobs-Sera, D., Belessis, Y., Whitney Brown, A., Cohen, K. A., Davidson, R. M., van Duin, D., Gainey, A., Garcia, C. B., Robert George, C. R., Haidar, G., Ip, W., Iredell, J., Khatami, A., Little, J. S., Malmivaara, K., … Hatfull, G. F. (2022). Phage therapy of mycobacterium infections: Compassionate-use of phages in twenty patients with drug-resistant mycobacterial disease. *Clinical Infectious Diseases*, *76*(1). https://doi.org/10.1093/cid/ciac453
54. Hadil Onallah, Hazan, R., Ran Nir-Paz, Ortal Yerushalmy, Amit Rimon, Braunstein, R., Gelman, D., Sivan Alkalay, Mohanad Abdalrhman, Stuczynski, D., Shunit Coppenhagen-Glazer, Gelman, S., Khalifa, L., Adler, K., Ortal Yerushalmy, Amit Rimon, Braunstein, R., Sivan Alkalay, Stuczynski, D., … Gilat Livni. (2023). Compassionate use of bacteriophages for failed persistent infections during the first 5 years of the Israeli phage therapy center. *Open Forum Infectious Diseases*, *10*(5). https://doi.org/10.1093/ofid/ofad221
55. Pirnay, J.-P., Djebara, S., Steurs, G., Griselain, J., Cochez, C., De Soir, S., Glonti, T., Spiessens, A., Vanden Berghe, E., Green, S., Wagemans, J., Lood, C., Schrevens, E., Chanishvili, N., Kutateladze, M., de Jode, M., Ceyssens, P.-J., Draye, J.-P., Verbeken, G., … Merabishvili, M. (2024). Personalized bacteriophage therapy outcomes for 100 consecutive cases: A multicentre, multinational, retrospective observational study. *Nature Microbiology*, *9*(6), 1434–1453. https://doi.org/10.1038/s41564-024-01705-x
56. Uyttebroek, S., Chen, B., Onsea, J., Ruythooren, F., Debaveye, Y., Devolder, D., Spriet, I., Depypere, M., Wagemans, J., Lavigne, R., Pirnay, J.-P., Merabishvili, M., De Munter, P., Peetermans, W. E., Dupont, L., Van Gerven, L., & Metsemakers, W.-J. (2022). Safety and efficacy of phage therapy in difficult-to-treat infections: A systematic review. *The Lancet Infectious Diseases*, *22*(8), e208–e220. https://doi.org/10.1016/s1473-3099(21)00612-5
57. Chan, B. K., Stanley, G. L., Kortright, K. E., Vill, A. C., Modak, M., Ott, I. M., Sun, Y., Würstle, S., Grun, C. N., Kazmierczak, B. I., Rajagopalan, G., Harris, Z. M., Britto, C. J., Stewart, J., Talwalkar, J. S., Appell, C. R., Chaudary, N., Jagpal, S. K., Jain, R., … Koff, J. L. (2025). Personalized inhaled bacteriophage therapy for treatment of multidrug-resistant Pseudomonas aeruginosa in cystic fibrosis. *Nature Medicine*, *31*(5), 1494–1501. https://doi.org/10.1038/s41591-025-03678-8
58. Weiner, I., Kahan-Hanum, M., Buchstab, N., Zelcbuch, L., Navok, S., Sherman, I., Nicenboim, J., Axelrod, T., Berko-Ashur, D., Olshina, M., Mordoch, R., Jablonska, J., Gera-Inbar, T., Vainberg-Slutskin, I., Blumenfeld, B., Sberro-Livnat, H., Moses, A., Nevenzel, H., Levy-Saar, I., … Bassan, M. (2025). Phage therapy with nebulized cocktail BX004-A for chronic pseudomonas aeruginosa infections in cystic fibrosis: A randomized first-in-human trial. *Nature Communications*, *16*(1), 5579. https://doi.org/10.1038/s41467-025-60598-4
59. Suh, G. A., Ferry, T., & Abdel, M. P. (2023). Phage therapy as a novel therapeutic for the treatment of bone and joint infections. *Clinical Infectious Diseases*, *77*(Supplement_5), S407–S415. https://doi.org/10.1093/cid/ciad533
60. Doub, J. B., Johnson, A. J., Nandi, S., Ng, V., Manson, T., Lee, M., & Chan, B. (2022). Experience using adjuvant bacteriophage therapy for the treatment of 10 recalcitrant periprosthetic joint infections: A case series. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciac694
61. McCallin, S., Drulis-Kawa, Z., Ferry, T., Pirnay, J.-P., Nir-Paz, R., & ESGNTA – ESCMID study group for non-traditional antibacterials. (2023). Phages and phage-borne enzymes as new antibacterial agents. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, S1198–743X(23)00528-1. https://doi.org/10.1016/j.cmi.2023.10.018
62. Jun, S. Y., Jang, I. J., Yoon, S., Jang, K., Yu, K.-S., Cho, J. Y., Seong, M.-W., Jung, G. M., Yoon, S. J., & Kang, S. H. (2017). Pharmacokinetics and tolerance of the phage endolysin-based candidate drug SAL200 after a single intravenous administration among healthy volunteers. *Antimicrobial Agents and Chemotherapy*, *61*(6). https://doi.org/10.1128/aac.02629-16
63. Luong, T., Salabarria, A.-C., Edwards, R. A., & Roach, D. R. (2020). Standardized bacteriophage purification for personalized phage therapy. *Nature Protocols*, *15*(9), 2867–2890. https://doi.org/10.1038/s41596-020-0346-0
64. Bretaudeau, L., Tremblais, K., Aubrit, F., Meichenin, M., & Arnaud, I. (2020). Good manufacturing practice (GMP) compliance for phage therapy medicinal products. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01161
65. Pirnay, J.-P., Verbeken, G., Ceyssens, P.-J., Huys, I., De Vos, D., Ameloot, C., & Fauconnier, A. (2018). The magistral phage. *Viruses*, *10*(2), 64. https://doi.org/10.3390/v10020064
66. Jean-Paul Pirnay, & Verbeken, G. (2023). Magistral phage preparations: Is this the model for everyone? *Clinical Infectious Diseases*, *77*(Supplement_5), S360–S369. https://doi.org/10.1093/cid/ciad481
67. Yang, Q., Le, S., Zhu, T., & Wu, N. (2023). Regulations of phage therapy across the world. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1250848
68. Aleksandra Petrovic Fabijan, Iredell, J., Katarzyna Danis-Wlodarczyk, Razieh Kebriaei, & Abedon, S. T. (2023). Translating phage therapy into the clinic: Recent accomplishments but continuing challenges. *PLOS Biology*, *21*(5), e3002119–e3002119. https://doi.org/10.1371/journal.pbio.3002119
69. Alharbi, N. M. (2026). Precision phage therapy in the AI/ML ERA: A systematic review of discovery-to-clinical translation evidence. *Frontiers in Microbiology*, *17*, 1873220. https://doi.org/10.3389/fmicb.2026.1873220
70. Würstle, S., Lieberknecht-Jouy, S. C., Düchting, A., Lößner, H., Ceyssens, P.-J., Pirnay, J.-P., de Jode, M., McCallin, S., Leitner, L., Willy, C., Leptihn, S., Korf, I., Idelevich, E. A., Witzenrath, M., Faltus, T., Ruth, P., Blume, H., Cornely, M., Fickenscher, H., … Zumpfe, S. (2026). A consensus-based guideline for personalized bacteriophage therapy. *Nature Medicine*. https://doi.org/10.1038/s41591-026-04654-6