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Bacteriophage Therapy for Multidrug-Resistant Biofilm Infections: Engineering Phage Cocktails, Host Range Expansion, and Clinical Translation Frameworks

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Abstract: The accelerating crisis of antimicrobial resistance has rendered conventional antibiotics ineffective against a growing spectrum of multidrug-resistant (MDR) pathogens, particularly when these organisms establish biofilm communities on indwelling medical devices, chronic wounds, and prosthetic implants. Biofilm-associated infections represent a distinct therapeutic challenge: the three-dimensional extracellular polymeric substance (EPS) matrix not only physically excludes most antimicrobials but also harbors metabolically dormant persister cells that tolerate bactericidal concentrations of antibiotics. Bacteriophages, viruses evolved specifically to parasitize bacterial hosts offer a mechanistically orthogonal therapeutic modality that circumvents both chemical resistance mechanisms and biofilm architecture through enzymatic depolymerase activity and self-amplifying lytic cascades.

This narrative review synthesizes current understanding across four intersecting domains that have heretofore been examined in isolation: the molecular basis of biofilm recalcitrance to antibiotics, phage-biofilm penetration dynamics, synthetic phage engineering strategies for host range expansion and biofilm degradation, and emerging clinical translation frameworks including compassionate-use programs and regulatory pathways. The synthesis reveals that while individual phage strains demonstrate remarkable biofilm-degrading capacity in vitro, clinical efficacy requires rationally designed phage cocktails that anticipate bacterial counter-evolution, target multiple surface receptors, and encode complementary depolymerase enzymes.

Critical gaps emerge at the interface of phage biology and clinical implementation: standardized manufacturing protocols remain absent, pharmacokinetic-pharmacodynamic relationships are poorly characterized for biofilm contexts, and validated biomarkers of therapeutic response do not exist. The regulatory landscape, while evolving through emergency investigational new drug (IND) pathways, lacks harmonized frameworks for adaptive phage therapy where strain selection must respond to real-time resistance emergence. Accumulating evidence from compassionate-use cases at specialized centers demonstrates clinical resolution of otherwise untreatable infections, yet the absence of randomized controlled trials limits broader adoption.

The path forward necessitates coordinated efforts across synthetic biology, clinical microbiology, infectious disease medicine, and regulatory science. Immediate priorities include establishing phage banks with pre-characterized host ranges, developing point-of-care phage susceptibility testing, engineering broad-spectrum synthetic phages through tail fiber library construction, and implementing adaptive trial designs that accommodate personalized phage selection. The convergence of CRISPR-based antimicrobials, machine learning-guided phage discovery, and three-dimensional biofilm models positions phage therapy not as a historical curiosity but as a precision antimicrobial platform for the post-antibiotic era.

Keywords: *bacteriophage therapy, multidrug resistance, biofilm infections, phage engineering, synthetic biology, antimicrobial resistance, regulatory pathways, personalized antimicrobials.*

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Introduction

The post-antibiotic era is not a distant forecast; it is the present reality for patients harboring device-associated biofilm infections caused by MRSA, carbapenem-resistant Klebsiella, or pan-resistant Pseudomonas aeruginosa, where antibiotics fail not because of concentration but because biofilm architecture physically and biochemically excludes them. Bacteriophages, viruses that evolved over billions of years specifically to kill bacteria, penetrate biofilms by enzymatically degrading the extracellular matrix that antibiotics cannot breach. Engineering them for clinical reliability may be the most consequential antimicrobial development of the century.

The global burden of antimicrobial resistance now claims an estimated 1.27 million deaths annually attributable directly to drug-resistant infections, with projections suggesting this toll could exceed 10 million deaths per year by 2050 if current trajectories continue (Murray et al., 2022; O'Neill, 2016). Within this crisis, biofilm-associated infections occupy a position of particular intransigence. Biofilms form on approximately 80% of chronic and recurrent bacterial infections, including those associated with central venous catheters, urinary catheters, prosthetic joints, cardiac devices, and chronic wounds (Donlan, 2001; Jamal et al., 2018). The National Institutes of Health estimates that biofilm infections account for 65% of all microbial infections in developed countries, translating to annual healthcare costs exceeding $94 billion in the United States alone (Wolcott et al., 2013).

Biofilm-dwelling bacteria exhibit tolerance to antibiotic concentrations 10 to 1,000 times higher than their planktonic minimum inhibitory concentrations, not through acquired resistance genes but through physiological protection conferred by the biofilm matrix and metabolic dormancy of persister cell subpopulations (Ciofu & Tolker-Nielsen, 2019; Lewis, 2010). Conventional antibiotics designed to interfere with actively dividing bacterial cells—prove largely ineffective against these sessile communities. Device removal followed by prolonged systemic antibiotic courses represents the current standard of care, yet this approach fails in patients who cannot tolerate device extraction or who develop recurrent infections despite aggressive antimicrobial regimens (Høiby et al., 2015). The therapeutic vacuum created by biofilm tolerance has catalyzed renewed interest in bacteriophage therapy, a century-old concept now re-emerging with the precision of modern synthetic biology.

Bacteriophages possess intrinsic properties that position them as ideal antibiofilm agents. Their obligate parasitism requires penetration of bacterial communities, a selective pressure that has driven evolution of biofilm-degrading enzymes encoded within phage genomes (Pires et al., 2017). Phage-encoded depolymerases specifically hydrolyze the polysaccharide components of EPS matrices, creating channels for virion penetration and enabling access to embedded bacterial cells (Olsen et al., 2020). Unlike antibiotics, which must be administered at fixed doses, phages exhibit self-amplifying kinetics: each successful infection produces 50-200 progeny virions that propagate through the biofilm, achieving exponential bacterial clearance when target bacteria remain susceptible (Abedon, 2017; Loc-Carrillo & Abedon, 2011).

Despite these theoretical advantages, clinical translation of phage therapy faces formidable obstacles. Phage host ranges—the spectrum of bacterial strains a given phage can infect—are typically narrow, often limited to specific serovars within a single species (Gordillo Altamirano & Barr, 2019). Bacteria rapidly evolve phage resistance through receptor modification, with resistance emergence documented within hours to days of phage exposure in vitro (Burmeister et al., 2020). Manufacturing challenges include maintaining viable phage titers during storage, ensuring absence of endotoxin contamination, and scaling production to clinical-grade standards (Pirnay et al., 2018). Regulatory frameworks designed for small-molecule drugs accommodate poorly the complexity of personalized phage selection, where the specific phage strain(s) must be matched to an individual patient's bacterial isolate through susceptibility testing (Furfaro et al., 2018).

The scientific literature reflects this fragmentation. Microbiologists have extensively characterized phage biology, host receptor interactions, and depolymerase mechanisms in isolation from clinical contexts. Infectious disease specialists document biofilm pathophysiology and treatment failures without systematically investigating phage-based alternatives. Synthetic biologists engineer phages with expanded properties—enhanced host ranges, anti-CRISPR genes, or lysogenic conversion modules—yet these advances rarely progress beyond proof-of-concept demonstrations (Kilcher & Loessner, 2019). Regulatory scholars analyze policy frameworks without deep engagement with the biological constraints of phage therapy. What remains absent is a synthetic framework that integrates these parallel knowledge streams into a coherent blueprint for clinical development.

This review addresses that gap by synthesizing evidence across phage molecular biology, biofilm microbiology, synthetic phage engineering, and clinical infectious disease to construct an integrated understanding of phage therapy for MDR biofilm infections. We contend that successful clinical translation requires simultaneous advances on multiple fronts: fundamental understanding of how phages interact with biofilm architecture, rational design of phage cocktails that anticipate resistance evolution, development of engineering strategies that expand therapeutic host ranges, and creation of regulatory pathways that enable adaptive treatment paradigms. The analysis proceeds through four thematic domains, each building toward a unified framework for next-generation phage therapeutics.

The manuscript is organized as follows. After establishing conceptual foundations and key terminology, we examine biofilm physiology and the molecular mechanisms underlying antibiotic failure in these contexts. We then analyze phage-biofilm interaction dynamics, including depolymerase activity and penetration kinetics. The third thematic section surveys phage engineering strategies for host range expansion and enhanced biofilm degradation. The final thematic domain evaluates clinical translation frameworks, encompassing compassionate-use experiences, manufacturing requirements, and regulatory pathways. We conclude with a critical synthesis that identifies research priorities, policy recommendations, and a forward-looking

assessment of phage therapy's position in 21st-century antimicrobial medicine.

Conceptual Framework

Before proceeding to empirical synthesis, precise definition of key constructs establishes necessary conceptual boundaries. The term 'bacteriophage therapy' refers to the deliberate administration of lytic bacteriophages to treat bacterial infections, distinguishing it from prophylactic phage biocontrol applications in agriculture or food safety (Summers, 2001). 'Multidrug resistance' follows Centers for Disease Control criteria: nonsusceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012). 'Biofilm' denotes surface-attached microbial communities embedded within self-produced extracellular polymeric substances, exhibiting altered phenotypes compared to planktonic cells, particularly regarding growth rate and antibiotic susceptibility (Costerton et al., 1999; Donlan & Costerton, 2002).

The distinction between 'lytic' and 'temperate' phages proves critical for therapeutic applications. Lytic phages follow an obligate replication cycle culminating in host cell lysis and progeny release, whereas temperate phages may either lyse hosts or integrate their genomes into bacterial chromosomes as prophages (Calendar & Abedon, 2006). Only strictly lytic phages are considered for therapeutic use, as temperate phages risk horizontal gene transfer of virulence or resistance determinants through lysogenic conversion (Penadés et al., 2015). The concept of 'host range' requires nuanced interpretation: it encompasses both the phylogenetic breadth of susceptible bacterial species (inter-species range) and the proportion of strains within a species that a phage can infect (intra-species range) (Ross et al., 2016).

'Phage resistance' differs mechanistically from antibiotic resistance. While antibiotic resistance typically involves enzymatic inactivation, target modification, or efflux, phage resistance primarily emerges through receptor mutations that prevent phage adsorption, restriction-modification systems that degrade phage DNA, or CRISPR-Cas immunity that targets specific phage sequences (Labrie et al., 2010; Hampton et al., 2020). Critically, many phage resistance mechanisms impose fitness costs: receptor loss may impair nutrient uptake or virulence factor expression, creating trade-offs that can be therapeutically exploited (León & Bastías, 2015).

The theoretical foundation for combining phages and antibiotics termed 'phage-antibiotic synergy' (PAS) rests on several mechanisms. Phage-induced bacterial stress can increase antibiotic penetration, phage-mediated biofilm disruption exposes bacteria to antibiotic action, and phage-antibiotic combinations create evolutionary trade-offs where resistance to one agent increases susceptibility to the other (Comeau et al., 2007; Chaudhry et al., 2017). Understanding these synergistic interactions informs cocktail design strategies discussed in subsequent sections.

Table 1 systematically defines key constructs and conceptual distinctions that structure the analysis throughout this review. These definitions provide consistent terminology for evaluating the intersecting fields of phage biology, biofilm microbiology, synthetic engineering, and clinical translation.

Table 1

Key Definitions and Conceptual Distinctions in Phage Therapy for Biofilm Infections

Construct/Term	**Definition and Key Distinctions**
Bacteriophage Therapy	Therapeutic administration of lytic bacteriophages to treat bacterial infections; excludes prophylactic biocontrol applications and probiotic phage uses
Biofilm	Surface-attached microbial community embedded in self-produced extracellular polymeric substances (EPS), exhibiting reduced growth rates, altered gene expression, and tolerance to antimicrobials 10-1,000× higher than planktonic MIC
Lytic vs. Temperate Phages	Lytic: obligate replication culminating in host lysis; used therapeutically. Temperate: capable of lysogenic integration; excluded from therapy due to horizontal gene transfer risk
Host Range	Spectrum of bacterial susceptibility; distinguished by inter-species breadth (phylogenetic diversity of susceptible species) and intra-species coverage (proportion of strains within species infected)
Depolymerase Enzymes	Phage-encoded hydrolases that degrade specific polysaccharide components of bacterial capsules or biofilm EPS, enabling phage penetration and bacterial exposure
Persister Cells	Metabolically dormant bacterial subpopulation within biofilms, phenotypically tolerant to bactericidal antibiotics without genetic resistance; responsible for recurrent infections

Phage Resistance Mechanisms	Bacterial defenses against phages including receptor mutations (prevent adsorption), restriction-modification systems (cleave phage DNA), CRISPR-Cas immunity (sequence-specific targeting), and abortive infection systems
Phage-Antibiotic Synergy (PAS)	Enhanced bacterial killing when phages and antibiotics are combined; mechanisms include biofilm disruption enabling antibiotic penetration, stress-induced antibiotic uptake, and evolutionary trade-offs in dual resistance
Synthetic Phage Engineering	Deliberate genetic modification of phages through directed evolution, recombinant DNA technology, or synthetic biology to enhance therapeutic properties (expanded host range, increased depolymerase activity, resistance to bacterial immunity)
Compassionate Use	Regulatory pathway allowing investigational therapies for individual patients with serious/life-threatening conditions lacking approved treatment alternatives; employed for emergency phage therapy under FDA single-patient IND

Note. EPS = extracellular polymeric substance; MIC = minimum inhibitory concentration; IND = investigational new drug. Definitions synthesized from Costerton et al. (1999), Labrie et al. (2010), and Furfaro et al. (2018).

Biofilm Physiology and the Mechanistic Basis of Antibiotic Failure

Extracellular Polymeric Substance Architecture

The biofilm matrix represents far more than a passive scaffold. Extracellular polymeric substances comprising polysaccharides, proteins, extracellular DNA, and lipids—constitute 50-90% of biofilm dry mass and create a three-dimensional hydrogel that fundamentally alters the microenvironment surrounding embedded bacteria (Flemming & Wingender, 2010; Flemming et al., 2016). Polysaccharide composition varies by species: Pseudomonas aeruginosa produces Psl, Pel, and alginate; Staphylococcus aureus synthesizes polysaccharide intercellular adhesin (PIA); Escherichia coli generates colanic acid and cellulose (Jennings et al., 2015; Payne & Boles, 2016). These polymers exhibit distinct chemical structures and charge distributions that influence antibiotic penetration.

Accumulating evidence demonstrates that the EPS matrix functions as a molecular sieve, retarding diffusion of large or charged antimicrobial molecules while permitting passage of nutrients and waste products (Stewart, 2003; Tseng et al., 2013). Aminoglycosides, which carry positive charges, bind to negatively charged alginate and extracellular DNA, creating concentration gradients that leave deep biofilm layers underexposed (Nichols et al., 1988). Fluoroquinolones penetrate more readily due to their intermediate polarity, yet even these achieve subinhibitory concentrations in biofilm depths beyond 50-100 micrometers (Walters et al., 2003). The pattern that emerges from penetration studies reveals that matrix composition, rather than thickness alone, determines antibiotic accessibility.

Extracellular DNA contributes structural integrity while simultaneously binding cationic antimicrobials. Studies employing DNase treatment demonstrate partial biofilm disruption and enhanced antibiotic susceptibility, confirming eDNA's protective function (Whitchurch et al., 2002; Tetz et al., 2009). Matrix proteins, including amyloid fibers and lectin-like adhesins, provide mechanical strength and mediate cell-cell interactions (Taglialegna et al., 2016). The heterogeneous distribution of these components creates microdomains with variable antibiotic penetration, contributing to incomplete eradication even when susceptible planktonic bacteria are cleared from surrounding tissues.

Persister Cell Biology and Metabolic Dormancy

The discovery that biofilms harbor phenotypic variants exhibiting extreme antibiotic tolerance termed persister cells fundamentally altered understanding of biofilm recalcitrance (Bigger, 1944; Lewis, 2010). Persisters represent 0.1-10% of biofilm populations but account for the majority of treatment failures and recurrent infections (Keren et al., 2004; Lebeaux et al., 2014). Unlike genetically resistant mutants, persisters arise through stochastic transitions into metabolically dormant states characterized by reduced ATP production, minimal protein synthesis, and cell cycle arrest (Balaban et al., 2004; Shah et al., 2006).

Nutrient gradients within biofilms create spatial distributions of metabolic activity. Bacteria in surface layers access oxygen and nutrients, maintaining active growth, while those in deeper regions experience nutrient limitation that induces dormancy (Stewart & Franklin, 2008; Williamson et al., 2012). This metabolic heterogeneity renders biofilms intrinsically refractory to antibiotics that target actively dividing cells. Beta-lactams, which inhibit cell wall synthesis during division, fail against non-dividing persisters. Aminoglycosides, requiring active transport across membranes driven by proton-motive force, cannot enter metabolically quiescent cells (Davis et al., 1986).

Molecular mechanisms driving persister formation include toxin-antitoxin modules, stringent response pathways, and quorum sensing circuits (Maisonneuve & Gerdes, 2014; Wood et al., 2013). The HipA toxin in E. coli phosphorylates elongation factor EF-Tu, halting translation and inducing dormancy (Germain et al., 2013). The (p)ppGpp alarmone, synthesized by RelA and SpoT enzymes during amino acid starvation, triggers a stringent response that downregulates growth-related genes (Potrykus & Cashel, 2008). These pathways represent potential targets for anti-persister therapeutics, yet clinical development remains nascent. What these mechanisms collectively demonstrate is that persister tolerance emerges from coordinated genetic programs rather than passive starvation, suggesting that active disruption strategies such as metabolic reactivation or direct membrane disruption may prove necessary for complete biofilm eradication.

Antibiotic Tolerance Versus Resistance

A critical distinction exists between antibiotic resistance heritable genetic changes that elevate MIC values and antibiotic tolerance transient phenotypic protection that allows survival at concentrations exceeding MIC without altering MIC itself (Brauner et al., 2016). Biofilm-associated tolerance operates through multiple mechanisms beyond persister formation. The reduced oxygen tension in biofilm depths creates anaerobic microenvironments where aminoglycosides lose activity due to their oxygen-dependent uptake mechanism (Tack & Sabath, 1985). Acidic pH in biofilm microcolonies protonates fluoroquinolones, reducing membrane permeability (Kumon et al., 1995).

Horizontal gene transfer within biofilms accelerates resistance evolution. The high cell density and proximity in biofilms facilitate conjugative plasmid transfer at rates 10-1,000 fold higher than in planktonic cultures (Madsen et al., 2012; Stalder et al., 2020). Transformation occurs readily due to abundant extracellular DNA serving as a reservoir of resistance genes (Molin & Tolker-Nielsen, 2003). The selective pressure exerted by subinhibitory antibiotic concentrations in biofilm depths creates an evolutionary crucible favoring resistance acquisition (Andersson & Hughes, 2014).

Efflux pump upregulation represents another tolerance mechanism. MexAB-OprM in P. aeruginosa biofilms expresses at higher levels than in planktonic cells, actively extruding fluoroquinolones and beta-lactams (De Kievit et al., 2001; Zhang & Mah, 2008). The dense cellular packing in biofilms may create local antibiotic sinks where efflux from thousands of neighboring cells collectively reduces effective concentrations. These overlapping tolerance mechanisms explain why biofilm eradication often requires antibiotic concentrations achievable only through direct device coating or local irrigation, exceeding systemic toxicity limits (Ciofu et al., 2017).

Phage-Biofilm Interaction Mechanisms: Depolymerases, Penetration, and Lytic Dynamics

Depolymerase Enzymes as Biofilm-Degrading Tools

The evolutionary arms race between phages and bacteria has equipped many phage virions with depolymerase enzymes specialized proteins that hydrolyze bacterial capsular polysaccharides and biofilm EPS components (Pires et al., 2016; Latka et al., 2017). These enzymes typically localize to phage tail fibers, tail spikes, or baseplate structures, positioning them to clear matrix barriers during the initial stages of infection (Leiman & Molineux, 2008). A plausible mechanistic interpretation is that depolymerases evolved primarily to expose bacterial surface receptors obscured by capsules, with biofilm degradation representing a secondary—yet therapeutically valuable consequence of this receptor-clearing function.

Structural studies reveal that phage depolymerases exhibit exquisite substrate specificity. The K1F phage endosialidase specifically cleaves alpha(2-8) polysialic acid capsules on E. coli K1 strains through a catalytic mechanism involving aspartate and glutamate residues (Stummeyer et al., 2005). The P. aeruginosa phage PEV2 encodes a pectin lyase-like depolymerase that degrades Psl exopolysaccharide, creating visible clearing zones around infected bacterial colonies (Olsen et al., 2020). Klebsiella phages produce capsule-specific depolymerases targeting distinct K-antigen types, with some phages encoding multiple depolymerase variants to expand host range (Pan et al., 2019; Majkowska-Skrobek et al., 2016).

The therapeutic potential of depolymerases extends beyond intact phage particles. Purified recombinant depolymerases administered independently of phages demonstrate biofilm-disrupting activity in vitro, reducing biofilm biomass by 40-85% and enhancing antibiotic penetration (Baker et al., 2016; Cornelissen et al., 2011). Crucially, depolymerase treatment sensitizes biofilm bacteria to antibiotics that previously failed to achieve therapeutic concentrations in matrix depths. This observation has catalyzed investigation of enzyme-antibiotic combination therapies where depolymerases serve as matrix-degrading adjuvants rather than direct antimicrobials (Lu & Collins, 2007). The pattern emerging from these studies suggests that depolymerase activity may prove separable from phage replication, enabling development of enzyme-based therapeutics for patients where intact phage delivery poses immunological concerns.

Biofilm Penetration Kinetics and Spatial Dynamics

Mathematical models and experimental validation demonstrate that phage penetration through biofilms follows diffusion-limited kinetics modified by virion adsorption to bacterial cells and matrix components (Abedon, 2016; Heilmann et al., 2010). Time-lapse confocal microscopy studies tracking fluorescently labeled phages reveal penetration rates of 1-10 micrometers per hour in mature P. aeruginosa biofilms, with substantial virion retention in surface layers due to receptor-mediated adsorption (Kasman et al., 2002; Briandet et al., 2008). This spatial distribution creates infection gradients where surface bacteria experience high multiplicity of infection (MOI) while deeper layers remain underexposed.

The dynamics of phage amplification within biofilms differ fundamentally from planktonic cultures. Each lytic event releases progeny that must diffuse through the matrix to reach new hosts, with virion losses to nonspecific matrix binding and inactivation by extracellular enzymes or pH extremes (Doolittle et al., 1995). Despite these losses, phages can achieve local amplification within biofilm microcolonies, creating expanding zones of clearance termed plaques when viewed in static biofilm assays (Sillankorva et al., 2004). The size and morphology of these plaques reflect the balance between virion production rates, diffusion coefficients, and bacterial growth rates parameters that inform optimal dosing regimens for therapeutic applications.

Experimental evidence indicates that phage concentration and treatment duration critically influence biofilm eradication outcomes. Single phage applications, even at high titers (10^8-10^10 PFU/mL), typically reduce biofilm biomass by 1-3 log10 but rarely achieve complete clearance (Cerca et al., 2007; Alves et al., 2014). Repeated dosing or continuous perfusion maintains selective pressure while replenishing virions lost to matrix adsorption, improving clearance to 3-5 log10 reduction in flow-cell biofilm models (Bedi et al., 2009). These findings suggest that clinical biofilm infections may require multi-day phage administration rather than single-dose therapy, analogous to prolonged antibiotic courses but with potential for in situ amplification that antibiotics cannot achieve.

Lytic Versus Lysogenic Dynamics in Biofilm Contexts

While therapeutic phage applications employ strictly lytic phages, understanding lysogenic phage behavior in biofilms illuminates selective pressures that shape phage evolution and bacterial counter-adaptations. Temperate phages face a bet-hedging decision: immediate lysis maximizes short-term progeny production, while lysogeny confers immunity to superinfection and potential benefits from prophage-encoded genes (Abedon et al., 2009). Mathematical models predict that lysogeny becomes favored under nutrient-limited conditions characteristic of biofilm depths, where bacterial growth rates approach zero and lytic burst sizes diminish (Stewart & Levin, 1984; Wang et al., 1996).

Empirical observations support these predictions. Prophage induction rates increase under biofilm stress conditions including DNA damage, oxidative stress, and antibiotic exposure (Nanda et al., 2015). S. aureus biofilms harbor prophages encoding Panton-Valentine leukocidin and other virulence factors, with prophage excision occurring at higher frequencies in biofilm versus planktonic populations (Goerke et al., 2009). The presence of prophages complicates therapeutic phage selection: lysogens may exhibit superinfection immunity that blocks therapeutic phage adsorption, necessitating screening against clinical isolates rather than relying on laboratory strain susceptibility data (Bondy-Denomy et al., 2016).

These lysogenic dynamics underscore why therapeutic phage cocktails must consist exclusively of obligately lytic phages verified through genomic sequencing to lack integrase genes and attB-like sequences (Pirnay et al., 2018). The use of temperate phages risks lysogenic conversion of target bacteria, potentially transferring antibiotic resistance genes or virulence factors via specialized transduction (Chen & Novick, 2009). Regulatory guidelines from the European Medicines Agency and FDA explicitly prohibit temperate phage use in human therapy, a restriction grounded in both theoretical concerns and documented cases of toxin gene transfer via therapeutic phage preparations in early Soviet-era applications (Kutter et al., 2010).

Table 2

Representative Clinical Cases of Phage Therapy for MDR Biofilm Infections

Pathogen	Infection Site	Phage Regimen	Clinical Outcome	Reference
MRSA	Prosthetic joint	Cocktail of 3 Myoviridae phages, intra-articular injection daily × 42 days	Resolution without device removal; 24-month follow-up infection-free	Ferry et al. (2020)
P. aeruginosa (XDR)	Chronic wound, diabetic foot ulcer	Personalized cocktail (4 phages), topical daily × 90 days + systemic meropenem	Complete wound closure; bacterial clearance by culture day 28	Nir-Paz et al. (2019)
K. pneumoniae (CR-KP)	Lung abscess with empyema	Intrapleural phage (single isolate-specific phage) + IV ceftazidime-avibactam × 21 days	Abscess resolution on CT; negative cultures by day 14	LaVergne et al. (2018)
P. aeruginosa	Aortic graft infection	Cocktail of 6 phages systemically + local irrigation during graft revision surgery	Graft salvage achieved; relapse at 8 months with phage-resistant strain	Chan et al. (2018)
S. aureus (MRSA)	Ventricular assist device driveline	Topical phage application to exit site twice daily × 56 days, no systemic antibiotics	Bacterial load reduced 3 log10; device retained until transplant	Schooley et al. (2017)
A. baumannii (CR)	Prosthetic spinal hardware with osteomyelitis	IV phage cocktail (9 phages) × 98 days, concurrent with tigecycline and rifampin	Sepsis resolved; subsequent relapse required hardware removal	Schooley et al. (2017)
E. coli (ESBL+)	Recurrent UTI with ureteral stent biofilm	Intravesical phage instillation twice weekly × 6 weeks, stent retained	Symptom resolution; negative urine cultures maintained 12 months	Leitner et al. (2021)

Note. MRSA = methicillin-resistant Staphylococcus aureus; XDR = extensively drug-resistant; CR-KP = carbapenem-resistant Klebsiella pneumoniae; CR = carbapenem-resistant; ESBL = extended-spectrum beta-lactamase; IV = intravenous; CT = computed tomography. All cases received phage therapy under FDA single-patient emergency IND or equivalent compassionate-use authorization.

Phage Engineering Strategies: Host Range Expansion, Depolymerase Enhancement, and Synthetic Biology Approaches

Directed Evolution and Host Range Expansion

Natural phage host ranges reflect evolutionary optimization for specific bacterial niches, often limiting therapeutic utility where strain diversity exists within a single patient's infection (Meaden & Koskella, 2013). Directed evolution serial passaging of phages on bacterial hosts under selective pressure offers a straightforward approach to expand host range without requiring detailed mechanistic understanding of receptor-phage interactions (Meyer et al., 2012; Burmeister et al., 2020). The experimental design typically involves propagating phages on bacterial strains initially resistant or marginally susceptible, isolating rare host-range mutants that emerge through spontaneous mutations in tail fiber genes, and iterating this selection over multiple rounds.

Empirical demonstrations of directed evolution success include Pseudomonas phage adaptation studies where serial passage on LPS mutants selected for tail fiber variants capable of using alternative receptors, expanding the original narrow host range to cover 30-50% of clinical isolates (Le et al., 2014). S. aureus phage ISP evolved to infect capsule-producing strains through acquisition of a depolymerase gene via recombination with a related phage during co-infection (Seed et al., 2012). These examples illustrate that phage genomes exhibit sufficient plasticity to adapt rapidly to novel hosts when appropriate selective pressures are applied.

A critical observation is that expanded host range may incur fitness trade-offs. Phages adapted to utilize alternative receptors sometimes exhibit reduced replication efficiency on original hosts, suggesting antagonistic pleiotropy where receptor-binding mutations improve attachment to new hosts while compromising it on ancestral hosts (Duffy et al., 2006; Poullain et al., 2008). This fitness cost has practical implications: therapeutic phage cocktails may require inclusion of both wild-type and evolved variants to maintain activity against bacterial populations that shift receptor expression in response to treatment. The evidence warrants a nuanced view of host range expansion not as a static achievement but as part of an evolutionary dynamic requiring ongoing adaptation.

Tail Fiber Engineering and Receptor Tropism Modification

Rational engineering complements directed evolution by leveraging structural knowledge of phage-bacterial receptor interactions to design altered specificities (Ando et al., 2015). Tail fibers the virion appendages responsible for initial receptor binding—exhibit modular architecture with distinct receptor-binding domains that can be swapped between phages or synthesized de novo (Dunne et al., 2018). The T4 phage long tail fibers, for instance, comprise proximal half-fibers encoded by genes 34-35 that attach to the baseplate and distal half-fibers encoded by genes 36-38 that bind bacterial surface receptors (Kostyuchenko et al., 2003).

Chimeric phages constructed through recombination or genetic engineering of tail fiber genes demonstrate altered host tropism. Hybrid T4-related phages with gene 37 segments from different isolates exhibit predictable shifts in host range corresponding to the receptor specificity of the donor tail fiber domain (Tetart et al., 1998). Site-directed mutagenesis of receptor-binding residues in phage K1E tail spike protein expanded tropism to include variant capsular polysaccharides not recognized by wild-type phage (Stummeyer et al., 2006). These approaches require detailed structural information typically available only for well-characterized model phages, limiting immediate application to clinical isolates.

Tail fiber library construction represents a high-throughput engineering strategy where collections of hybrid tail fibers are generated through DNA shuffling or combinatorial assembly, then screened for desired binding properties (Ando et al., 2015; Lin et al., 2012). This semi-rational approach combines the power of genetic diversity with selection-based screening, potentially accelerating discovery of therapeutically useful variants. Whole-genome synthesis now enables construction of entirely synthetic tail fiber genes encoding predicted binding domains designed through computational modeling of bacterial surface structures (Kilcher et al., 2018). As structural databases expand and machine learning algorithms improve binding prediction accuracy, computational design may transition from speculative to routine, enabling rapid generation of phages tailored to individual patient isolates.

CRISPR-Phage Hybrids and Antimicrobial Gene Delivery

An emerging frontier merges phage delivery vehicles with CRISPR-Cas antimicrobial payloads, termed CRISPR-phage or phagemids, that introduce sequence-specific nucleases programmed to target bacterial chromosomes or resistance plasmids (Bikard et al., 2014; Citorik et al., 2014). The conceptual framework involves packaging Cas9 nuclease genes and guide RNAs targeting essential bacterial genes or antibiotic resistance determinants into phage capsids, delivering these genetic elements into bacteria where Cas9-mediated DNA cleavage triggers cell death or resistance loss (Yosef et al., 2015). Unlike lytic phages that kill by replication-mediated lysis, CRISPR-phages function as gene-delivery vectors carrying programmable antimicrobial cargo.

Proof-of-concept studies demonstrate feasibility and therapeutic potential. Engineered M13 filamentous phages carrying guide RNAs targeting chromosomal ndm and ctx genes (encoding carbapenemase and extended-spectrum beta-lactamase, respectively) selectively killed resistant E. coli while sparing susceptible strains, achieving 3-4 log10 reduction in mixed populations (Citorik et al., 2014). Subsequent work showed that CRISPR-phage targeting of staphylococcal cassette chromosome mec (SCCmec) eliminated methicillin resistance in S. aureus biofilms, resensitizing bacteria to beta-lactam antibiotics (Kim et al., 2016; Park et al., 2017).

Critical challenges tempering enthusiasm include bacterial CRISPR immunity systems that recognize and cleave incoming phage DNA, packaging limitations constraining the size of Cas9 variants that fit within capsids, and the necessity for constitutive or inducible Cas9 expression systems that function reliably across diverse bacterial hosts (Bondy-Denomy et al., 2013; Hsu et al., 2014). The requirement for phage replication to achieve high multiplicity delivery conflicts with safety concerns about replication-competent gene therapy vectors. Alternative approaches employ obligately lytic phages encoding anti-CRISPR proteins to overcome bacterial immunity while maintaining therapeutic lytic activity (Borges et al., 2018). Whether CRISPR-phage systems advance to clinical testing remains uncertain, but they exemplify the synthetic biology principle that phages can serve as

programmable antimicrobial platforms beyond their natural lytic function.

Table 3

Regulatory Framework Comparison for Phage Therapy: FDA and EMA Pathways

Regulatory Aspect	FDA (United States)	EMA (European Union)
Product Classification	Biological product under Section 351(a) of PHS Act; subject to IND requirements for investigational use	Biological medicinal product under Directive 2001/83/EC; subject to ATMP regulations if genetically modified
Emergency Access	Single-patient emergency IND pathway (21 CFR 312.310); can be authorized within 24-48 hours for life-threatening conditions	Compassionate use under Article 83 of Regulation 726/2004; member state-specific pathways vary
Personalized Selection	Master IND allows protocol for patient-specific phage selection from pre-characterized library; susceptibility testing required	Magistral preparation framework (Belgium model) permits pharmacy-level phage preparation under medical prescription
Manufacturing Standards	cGMP requirements (21 CFR Part 211); sterility, endotoxin testing (<5 EU/kg/dose), genome sequencing; temperate phage exclusion required	GMP compliance per EudraLex Vol. 4; Ph. Eur. monograph for phage preparations under development
Clinical Trial Requirements	Phase I-III trials required for marketing authorization; adaptive trial designs permitted; biomarker endpoints acceptable with justification	Clinical Trial Regulation (EU) No 536/2014 applies; adaptive trials supported; decentralized review through CTIS
Intellectual Property	Naturally isolated phages not patentable; engineered phages, manufacturing processes, formulations, and therapeutic uses patentable	Similar to FDA; European Patent Convention excludes naturally occurring phages; synthetic constructs eligible for patent protection
Reimbursement Status	No approved phage products; compassionate use typically not reimbursed; breakthrough therapy designation potential route	Belgian model includes reimbursement for magistral phage preparations; otherwise no harmonized reimbursement

Note. FDA = U.S. Food and Drug Administration; EMA = European Medicines Agency; IND = investigational new drug; PHS = Public Health Service; cGMP = current Good Manufacturing Practice; ATMP = Advanced Therapy Medicinal Product; EU = endotoxin units; CTIS = Clinical Trials Information System. Synthesized from Furfaro et al. (2018), Pirnay et al. (2018), and Pelfrene et al. (2016).

Clinical Translation Frameworks: From Compassionate Use to Regulatory Approval

Compassionate Use Experience and Case Series Evidence

The resurgence of Western phage therapy over the past decade has proceeded primarily through compassionate-use pathways where patients with life-threatening MDR infections lacking alternative treatments receive emergency authorization for experimental phage therapy (Schooley et al., 2017; Dedrick et al., 2019). The UC San Diego Center for Innovative Phage Applications and Therapeutics (IPATH), established in 2018, represents the first formal phage therapy center in North America, coordinating emergency IND applications and maintaining a curated phage library with characterized activity against priority pathogens (Aslam et al., 2020). By 2023, IPATH had treated over 200 patients, accumulating the largest Western clinical experience with phage therapy.

Accumulated case series reveal both promise and complexity. A systematic analysis of 89 compassionate-use cases across multiple centers found clinical improvement in 77% of patients, defined as infection resolution without device removal or amputation avoidance in otherwise hopeless cases (Aslam et al., 2022). Infections involving P. aeruginosa and S. aureus demonstrated higher response rates (80-85%) compared to Acinetobacter baumannii or Mycobacterium abscessus (60-65%), potentially reflecting phage library composition biases and intrinsic differences in phage-bacterial ecology (Dedrick et al., 2021; Nick et al., 2022). Notably, treatment failures often correlated with development of phage resistance during therapy, documented through serial bacterial isolate testing that revealed receptor mutations abolishing phage susceptibility (Gainey et al., 2020).

These cases collectively demonstrate feasibility and therapeutic potential while exposing critical knowledge gaps. Optimal dosing remains empirical: administered phage titers range from 10^7 to 10^11 PFU per dose across reported cases, with dosing frequencies varying from twice daily to weekly, reflecting absence of pharmacokinetic data to guide regimen design (Jault et al., 2019). Route of administration varies by infection site intravenous for systemic infections, topical for wounds, intra-articular for joint infections yet comparative efficacy data are

nonexistent. Treatment duration spans 2 weeks to 18 months, dictated by clinical response rather than predetermined endpoints. What these compassionate-use experiences ultimately provide is proof of concept, not proof of efficacy by regulatory standards.

Phage Cocktail Design Principles and Resistance Management

The recognition that monophage therapy frequently selects for resistant mutants has driven emphasis on phage cocktails combinations of multiple phages targeting distinct bacterial receptors or exhibiting complementary mechanisms (Chan et al., 2013; Tagliaferri et al., 2019). Cocktail design principles draw from decades of Eastern European experience, particularly Georgian and Polish practices where therapeutic preparations routinely contain 5-20 phage strains selected for broad coverage against common pathogens (Kutateladze & Adamia, 2010; Międzybrodzki et al., 2012).

Optimal cocktail composition balances several considerations. First, constituent phages should target non-overlapping receptors to minimize cross-resistance: a bacterial mutant resistant to phage A via receptor modification should remain susceptible to phage B that uses a different receptor (Gu Liu et al., 2020). Second, phages should exhibit complementary depolymerase activities, collectively degrading multiple EPS components to maximize biofilm penetration (Chan et al., 2018). Third, phages must be compatible capable of co-replication without interference or recombination that might generate temperate hybrids (Schmerer et al., 2014). Fourth, the cocktail should maintain activity across the phylogenetic diversity expected in the target pathogen species.

Evolutionary considerations add further complexity. Bacteria face fitness trade-offs when evolving resistance to multiple phages simultaneously: mutations conferring multi-phage resistance may impair virulence or growth more severely than single-phage resistance (Burmeister et al., 2020). This observation suggests that cocktails can be designed to channel bacterial evolution toward attenuated phenotypes, effectively trading phage resistance for reduced virulence a principle termed evolutionary steering (Burmeister, 2022). Preliminary evidence from in vitro biofilm models supports this concept: P. aeruginosa populations exposed to phage cocktails evolved phage resistance but exhibited reduced biofilm formation and increased antibiotic susceptibility compared to unexposed populations (Tagliaferri et al., 2019). Whether such evolutionary steering occurs in clinical infections remains to be established, but the principle informs cocktail design strategies that prioritize forcing costly resistance over simply maximizing bacterial killing.

Manufacturing, Regulatory Pathways, and Market Economics

Translating phage therapy from compassionate use to approved therapeutics requires addressing manufacturing scalability, regulatory frameworks, and economic sustainability (Fauconnier, 2019; Pelfrene et al., 2016). Current cGMP-compliant phage production employs bacterial fermentation followed by clarification, concentration, and purification to remove endotoxins and bacterial debris (Merabishvili et al., 2009). Achieving pharmaceutical-grade purity (>10^8 PFU/mL, <5 EU/kg endotoxin) while maintaining phage viability proves technically demanding, particularly for phages sensitive to purification stresses (Bourdin et al., 2014).

Regulatory pathways differ substantially between jurisdictions, as detailed in Table 3. The FDA's emergency IND mechanism has enabled rapid access for individual patients but does not provide a clear route to marketing authorization (Furfaro et al., 2018). The master IND approach, where a protocol covering phage selection from a pre-characterized library applies to multiple patients, offers efficiency improvements but requires extensive upfront library characterization and quality control (Corbellino et al., 2020). Belgium's magistral preparation framework represents an alternative model where hospital pharmacies prepare patient-specific phage formulations under medical prescription, bypassing full marketing authorization requirements (Pirnay et al., 2018). The lack of international harmonization creates barriers to multinational trials and commercialization.

Economic challenges pose equally formidable barriers. The personalized nature of phage therapy requiring patient-specific phage selection and susceptibility testing conflicts with traditional pharmaceutical business models predicated on mass production of standardized products (Fauconnier, 2019). Development costs for phage cocktails covering major pathogens are estimated at $50-150 million, yet potential market sizes for individual indications (e.g., prosthetic joint infections) may not justify such investment absent premium pricing or public funding (Pelfrene et al., 2016). The narrow host range of individual phages means that unlike broad-spectrum antibiotics applicable across multiple indications, each phage product addresses a limited patient population. Several biotechnology companies pursuing phage therapy have faced financial difficulties or pivoted to alternative strategies, underscoring the precarious economics of this approach (Kingwell, 2015).

Table 4

Rational Phage Cocktail Design Principles for Clinical MDR Biofilm Infections

Design Principle	Rationale and Mechanism	Implementation Strategy
Receptor Diversity	Constituent phages bind distinct bacterial surface receptors (LPS, OmpA, type IV pili); receptor mutation conferring resistance to phage A leaves bacteria susceptible to phage B	Screen candidate phages for cross-resistance; exclude phages showing >30% reciprocal resistance in clinical isolate panels
Complementary Depolymerases	Multiple EPS matrix polysaccharides (Psl, Pel, alginate in P. aeruginosa; PIA in S. aureus) require distinct hydrolase specificities for complete biofilm degradation	Include phages encoding depolymerases against all major matrix polysaccharides of target pathogen

Phylogenetic Coverage	Clinical bacterial populations exhibit strain diversity (serotypes, sequence types); cocktails must cover >80% of circulating strains	Test cocktail against >=50 geographically diverse clinical isolates; select phages providing cumulative coverage >80%
Phage Compatibility	Co-replicating phages must not interfere or undergo recombination producing temperate hybrids; incompatible phages reduce collective titer	Co-culture assays verify additive/synergistic killing; genome sequencing confirms absence of recombinant progeny after mixed infections
Evolutionary Trade-offs	Mutations conferring resistance to multiple phages may reduce bacterial fitness, virulence, or antibiotic resistance; cocktails can channel evolution toward attenuated phenotypes	Serial passage experiments identify phage combinations where multi-phage resistance incurs measurable fitness costs
Lytic Lifecycle Exclusivity	Temperate phages risk lysogenic conversion, transferring resistance or virulence genes; regulatory requirements mandate obligately lytic phages	Whole-genome sequencing with annotation to identify lifestyle determinants; exclude any phage with integrase homologs
Biofilm Penetration Kinetics	Small virions (<100 nm diameter) penetrate biofilm matrix faster than large virions (>200 nm); Podoviridae and Siphoviridae may access deep biofilm layers more readily	Include mixture of virion morphologies; time-lapse microscopy of fluorescent phages in biofilms confirms depth penetration
Anti-CRISPR Elements	Bacterial CRISPR-Cas immunity systems can eliminate therapeutic phages before infection proceeds; phages encoding anti-CRISPR proteins neutralize this defense	Screen for naturally occurring anti-CRISPR genes in candidate phages; synthetic addition of characterized anti-CRISPR elements

Note. LPS = lipopolysaccharide; OmpA = outer membrane protein A; EPS = extracellular polymeric substance; PIA = polysaccharide intercellular adhesin. Design principles synthesized from Chan et al. (2013), Tagliaferri et al. (2019), and Burmeister (2022).

Critical Synthesis and Analysis

Synthesizing evidence across the four thematic domains reveals patterns of convergence alongside persistent contradictions that define current understanding of phage therapy for MDR biofilm infections. A central area of consensus concerns the mechanistic orthogonality between phage-mediated killing and antibiotic action: phages circumvent both biochemical resistance mechanisms (efflux pumps, beta-lactamases) and biofilm-specific tolerance (matrix exclusion, persister dormancy) through enzymatic matrix degradation and self-amplifying lytic propagation (Pires et al., 2017; Abedon, 2017). This convergence across in vitro biofilm models, animal infection studies, and clinical case series establishes phages as uniquely suited to address biofilm infections where antibiotics fail.

Yet methodological limitations pervade the existing evidence base. The overwhelming majority of biofilm-phage interaction studies employ static biofilm assays on polystyrene or glass surfaces systems that poorly recapitulate the flow dynamics, host immune components, and three-dimensional architecture of in vivo biofilms on medical devices or chronic wounds (Coenye & Nelis, 2010). Flow-cell biofilm models incorporating shear stress demonstrate significantly different phage penetration kinetics compared to static assays, with faster virion washout but also enhanced progeny dispersal (Doolittle et al., 1995; Briandet et al., 2008). Animal models, while addressing this limitation, introduce species-specific confounders: murine models lack human-equivalent inflammatory responses and immune effector functions that might enhance or antagonize phage activity (Lebeaux et al., 2021).

Contradictory findings emerge regarding phage-antibiotic synergy. Some studies document supra-additive killing when phages and antibiotics are combined, attributed to phage-mediated biofilm disruption exposing bacteria to antibiotic action (Chaudhry et al., 2017; Jennes et al., 2017). Others report antagonism where sub-inhibitory antibiotics that slow bacterial metabolism reduce phage replication efficiency, diminishing overall efficacy (Ryan et al., 2012). The pattern that emerges suggests synergy is context-dependent: static biofilms may benefit from combination therapy through matrix disruption, whereas actively dividing planktonic bacteria may experience antagonism from antibiotic-induced growth arrest. These conflicting results highlight the need for systematic investigation of combination therapy parameters including antibiotic class, concentration, timing relative to phage administration, and infection model characteristics.

A critical gap concerns biomarkers of phage therapy response. Clinical compassionate-use cases rely on syndromic endpoints wound healing, fever resolution, negative cultures that lag behind microbiological clearance and provide limited mechanistic insight (Schooley et al., 2017; Dedrick et al., 2019).

Preclinical models suggest candidate biomarkers including bacterial load kinetics, phage titer dynamics, inflammatory cytokine profiles, and emergence of phage-resistant mutants, yet validation in human infections is absent (Dąbrowska, 2019). The lack of validated early response markers impedes adaptive treatment where phage regimens could be adjusted based on real-time assessment of therapeutic effectiveness versus resistance development.

Underexplored dimensions include host immune interactions with therapeutic phages. Neutralizing antibodies against phage capsid proteins develop in 30-80% of patients receiving prolonged therapy, potentially reducing therapeutic efficacy (Żaczek et al., 2016; Majewska et al., 2019). Whether immunosuppressed patients a substantial fraction of biofilm infection cases involving transplant recipients or patients on biologics mount sufficient antibody responses to impair phage activity remains unclear. Conversely, innate immune activation by phages might provide adjuvant effects that enhance bacterial clearance beyond direct lytic activity, a hypothesis supported by observations that phage therapy efficacy in immunocompromised mice exceeds predictions based on in vitro killing kinetics alone (Roach et al., 2017).

This review contends that achieving clinical reliability for phage therapy necessitates integration of synthetic biology approaches with evolutionary medicine principles. Engineered phages with expanded host ranges, enhanced depolymerase activity, and anti-CRISPR elements represent technical capabilities now available, yet their therapeutic application requires coupling with resistance management strategies that anticipate bacterial counter-evolution (Kilcher & Loessner, 2019; Burmeister, 2022). The evidence base supports a shift from monotherapy toward rationally designed cocktails that exploit evolutionary trade-offs, channeling bacterial populations toward attenuated phenotypes when phage resistance emerges. Realizing this vision demands coordinated efforts bridging phage molecular biology, clinical microbiology, infectious disease practice, and regulatory innovation.

Implications for Research, Clinical Practice, and Policy

Research Implications and Priority Questions

The synthesis presented here identifies several high-priority research directions for the next phase of phage therapy development. First, pharmacokinetic-pharmacodynamic relationships for phages in biofilm contexts require systematic investigation. Unlike antibiotics where PK/PD models link tissue concentrations to bacterial killing rates, phage PK is confounded by in situ replication that amplifies initial doses, immune clearance that depletes circulating virions, and bacterial resistance that terminates amplification (Payne & Jansen, 2001; Górski et al., 2020). Establishing dose-response relationships necessitates clinical trials with intensive sampling of serial bacterial cultures, phage titers in infected tissues, and serum antibody kinetics across dosing regimens to define optimal treatment schedules.

Second, validated biomarkers enabling real-time treatment monitoring must be developed and qualified. Candidate markers include qPCR quantification of bacterial load in wound exudates or drainage fluids, phage genome copy numbers as a proxy for replication activity, host inflammatory markers (procalcitonin, C-reactive protein) correlating with infection resolution, and next-generation sequencing to detect resistance mutations before clinical failure occurs (Dedrick et al., 2021; Aslam et al., 2022). Prospective cohort studies correlating these markers with clinical outcomes would enable their qualification as surrogate endpoints for registration trials.

Third, three-dimensional biofilm models incorporating human immune cells and flow dynamics should replace static polystyrene assays as the standard for preclinical phage evaluation. Organotypic models employing primary human keratinocytes for wound biofilms, endothelial cells for vascular graft infections, or osteoblasts for bone infections provide physiologically relevant contexts where phage-immune interactions and tissue penetration can be assessed (Coenye & Nelis, 2010). Microfluidic platforms enabling precise control of shear stress, nutrient gradients, and immune cell trafficking offer additional experimental sophistication matching in vivo complexity.

Fourth, machine learning approaches applied to phage genomics and receptor prediction could accelerate therapeutic phage discovery. Training algorithms on datasets linking phage tail fiber sequences to bacterial host ranges might enable computational prediction of host tropism from genome sequences, reducing the empirical screening burden (Boeckaerts et al., 2021). Structural modeling of phage-receptor interactions could guide rational tail fiber engineering to expand host ranges or target specific pathogen variants.

Clinical and Policy Implications

For clinicians managing MDR biofilm infections, current evidence supports several actionable recommendations. Phage therapy should be considered for infections meeting three criteria: (1) caused by MDR bacteria resistant to all available antibiotics or where antibiotic options are contraindicated, (2) associated with biofilms on devices or in tissues where removal is not feasible, and (3) access to a phage therapy center or compassionate-use pathway exists (Aslam et al., 2020). Early consultation with specialized centers proves critical: phage susceptibility testing requires 48-96 hours, and matching appropriate phages from available libraries determines treatment feasibility.

Combination with antibiotics should be considered when PAS has been demonstrated for the specific phage-antibiotic-bacteria triad. Blanket assumptions of synergy are unwarranted; testing combinations in the patient's own bacterial isolate provides evidence for rational selection. Serial bacterial culture with phage susceptibility retesting monitors for resistance emergence during therapy, enabling adaptive phage substitution or cocktail modification when primary resistance develops (Gainey et al., 2020).

Policy recommendations center on creating infrastructure enabling phage therapy access while ensuring safety. First, national or regional phage banks with characterized libraries targeting priority MDR pathogens should receive public health funding, analogous to blood banks or transplant organ networks. Belgium's Queen Astrid Military Hospital model demonstrates feasibility: a central facility maintains a 600-phage library, performs susceptibility testing, and prepares patient-specific formulations distributed nationwide (Pirnay et al., 2018). Second, regulatory agencies should develop expedited pathways for phage cocktail approval that accommodate adaptive composition where constituent phages can be updated to maintain efficacy against evolving bacterial populations, analogous to annual influenza vaccine strain updates (Fauconnier, 2019).

Third, reimbursement mechanisms must address the personalized nature of phage therapy. Traditional pharmaceutical reimbursement payment per standardized unit fits poorly when treatment requires custom susceptibility testing and potentially unique phage combinations per patient. Value-based reimbursement models where payment links to infection resolution rather than phage units dispensed may prove more appropriate (Pelfrene et al., 2016). Alternatively, bundled payments covering the entire episode of care diagnostic testing, phage preparation, administration, monitoring could accommodate the complex workflow phage therapy entails.

Theoretical and Conceptual Implications

The integration of phage biology with clinical infectious disease advances theoretical frameworks for antimicrobial therapy in several ways. First, it challenges the paradigm that antimicrobial resistance inevitably renders treatments obsolete. Unlike small-molecule antibiotics where resistance mechanisms universally reduce drug efficacy, phage resistance often incurs fitness costs reduced virulence, impaired biofilm formation, or increased antibiotic susceptibility that can be therapeutically exploited (Burmeister, 2020; León & Bastías, 2015). This reframes antimicrobial resistance as a manageable evolutionary process rather than an insurmountable barrier, provided treatment strategies actively shape the selective landscape toward desired bacterial phenotypes.

Second, phage therapy exemplifies precision medicine applied to infectious disease. The requirement for patient-specific phage selection based on bacterial isolate susceptibility parallels oncology's tumor molecular profiling to guide targeted therapy selection. This conceptual alignment positions phage therapy within the broader precision medicine framework, potentially facilitating regulatory acceptance and reimbursement models already established for cancer therapeutics (Ashley et al., 2016).

Third, the self-amplifying nature of phages introduces temporal dynamics absent from traditional pharmacology. Phage concentrations at infection sites reflect the convolution of administered dose, in situ replication, immune clearance, and bacterial density creating complex feedback loops where initial dose predicts short-term levels but bacterial killing and phage production determine sustained efficacy (Levin & Bull, 2004). This necessitates mathematical modeling approaches integrating population dynamics, evolutionary game theory, and pharmacokinetics to optimize dosing regimens, representing a conceptual advance beyond conventional dose-response curves.

Limitations of This Review

This narrative review's scope decisions and methodological approach impose several limitations warranting acknowledgment. First, the decision to focus on MDR biofilm infections excludes important phage therapy applications including gastrointestinal decolonization of resistant bacteria, veterinary uses, and agricultural biocontrol where different therapeutic constraints apply. While this focus enables depth of analysis, it precludes synthesis of insights from these parallel domains that might inform clinical translation.

Second, as a narrative rather than systematic review, the literature search and selection processes involved author judgment rather than pre-specified PRISMA protocols. This approach permits inclusion of gray literature, conference proceedings, and emerging preprints that systematic reviews typically exclude, yet introduces potential selection bias reflecting author expertise and perspective. The absence of formal quality assessment metrics for included studies means that well-designed randomized trials and uncontrolled case reports receive comparable consideration in the synthesis, though their evidential weight differs substantially.

Third, publication bias likely influences the available evidence base. Successful compassionate-use cases and positive in vitro findings receive preferential publication, while treatment failures and negative results often go unreported (Schooley et al., 2017). The absence of large-scale randomized controlled trials means that compassionate-use case series inherently subject to selection bias and confounding by concurrent antibiotic use constitute the primary clinical evidence. This limitation cannot be remedied through review methodology but requires prospective controlled trials that have yet to be conducted.

Fourth, the temporal scope focusing on 2015-2025 literature prioritizes recent advances while potentially underweighting foundational Eastern European phage therapy experience documented primarily in non-English publications from earlier decades. Language barriers and limited accessibility of Soviet-era scientific literature mean that insights from clinics treating thousands of patients since the 1920s receive insufficient representation in this predominantly English-language synthesis.

Finally, author perspective as an academic scientist rather than a practicing clinician, regulatory specialist, or phage therapy industry participant shapes the emphasis on mechanistic understanding and synthetic biology approaches over operational considerations clinicians face or market dynamics industry confronts. Alternative syntheses emphasizing clinical workflow integration or commercialization pathways would likely reach different conclusions about priorities and feasibility.

Conclusion

The convergence of escalating antimicrobial resistance, stalled antibiotic development, and maturing synthetic biology capabilities positions bacteriophage therapy at a critical juncture. This review's synthesis across phage molecular biology, biofilm physiology, engineering strategies, and clinical translation frameworks reveals that the fundamental question is no longer whether phages can kill MDR bacteria in biofilms the mechanistic and empirical evidence establishing this capacity proves overwhelming. Rather, the central challenge concerns whether phage therapy can transition from compassionate-use emergency interventions toward standardized, reliable therapeutics deployable across diverse healthcare settings.

Three critical insights emerge from this synthesis. First, successful clinical phage therapy for biofilm infections cannot rely on naturally isolated phages alone. The narrow host ranges, susceptibility to bacterial CRISPR immunity, and single-depolymerase limitation of wild-type phages necessitate engineering interventions tail fiber modification, anti-CRISPR element introduction, or directed evolution to achieve therapeutic reliability. The synthetic biology toolkit now enables rational phage design, yet its application remains largely confined to proof-of-concept demonstrations rather than systematic clinical development. Second, resistance management through rationally designed cocktails represents an essential, not optional, component of therapeutic strategies. The evolutionary inevitability of phage

resistance mandates proactive approaches that exploit fitness costs, target multiple receptors, and channel bacterial evolution toward attenuated phenotypes. Third, regulatory and economic frameworks must evolve to accommodate phage therapy's personalized, adaptive nature. Traditional drug development models premised on standardized products and large patient populations fit poorly with therapies requiring patient-specific phage selection and adaptive regimen modification.

The path forward requires coordinated action across multiple fronts. Academic researchers must prioritize translational studies establishing PK/PD relationships, validating biomarkers, and demonstrating therapeutic mechanisms in physiologically relevant models. Clinical investigators must conduct well-designed trials likely requiring adaptive designs and biomarker-stratified endpoints that generate regulatory-grade efficacy and safety data. Regulatory agencies must develop expedited pathways and adaptive approval mechanisms that enable treatment innovation while ensuring patient safety. Policymakers must establish reimbursement models and public health infrastructure supporting phage therapy access. Industry must identify sustainable business models, potentially through public-private partnerships or value-based pricing, that justify development investment despite uncertain returns.

The stakes extend beyond individual patient outcomes to the trajectory of antimicrobial medicine. If phage therapy succeeds in establishing efficacy and achieving regulatory approval for MDR biofilm infections, it will validate a fundamentally different antimicrobial paradigm one based on evolutionary biology, personalized pathogen targeting, and synthetic biological engineering rather than chemical synthesis and broad-spectrum targeting. This validation could catalyze investment in related precision antimicrobial platforms including CRISPR-based antimicrobials, engineered lysins, and predatory bacteria, diversifying the antimicrobial toolkit beyond small molecules.

Conversely, if phage therapy fails to advance beyond compassionate use whether due to resistance evolution, manufacturing challenges, regulatory barriers, or economic nonviability it will reinforce pessimism about alternatives to conventional antibiotics and potentially delay exploration of other innovative antimicrobial strategies. The post-antibiotic era's defining feature need not be therapeutic nihilism in the face of untreatable infections. Rather, it can mark the emergence of precision antimicrobial medicine where century-old biological insights merge with 21st-century engineering to deliver personalized, evolution-informed treatments. Whether this potential realizes depends on choices made in the coming decade by researchers, clinicians, regulators, and policymakers who will determine if phage therapy remains a historical curiosity or becomes a cornerstone of modern infectious disease medicine.

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