Volume 3, Number 2, 2026 Pages 532-547

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

ENGINEERED BACTERIOPHAGES FOR TARGETED ELIMINATION OF ANTIBIOTIC RESISTANCE GENES: AN EMPIRICAL ASSESSMENT OF PROGRAMMABLE PAYLOADS AND RE-SENSITIZATION DYNAMICS

Ayesha Sana[*1], Saba Qadir Afridi[2], Laiba Khan[3], Ommama Saqib Khan[4], Seerat Fatima[5], Sania Saleem[6], Amna Zareen[7]

*[*1,7]Bachelors (BS) in Botany, Department of Botany, University of Sargodha (UOS), Punjab, Pakistan*
[2,3,4,5]M.phil Microbiology, Department of Microbiology, Quiad-e- Azam university Islamabad, Pakistan
[6]BS Microbiology, Faculty of life sciences, Balochistan university of information technology and management sciences Quetta, Pakistan.

[*1]ayshasana600@gmail.com, [2]sabaqadir314@gmail.com, [3]laibakhan2595@gmail.com, [4]ommamasaqib@gmail.com, [5]fseerat015@gmail.com, [6Saniasaleem55296@gmail.com]
[7]amnamalik2046@gmail.com

Article Information

Article History

Received:	May 15, 2026
Revised:	June 27, 2026
Accepted:	August 12, 2026
Available Online:	August 31, 2026

Keywords:
Antimicrobial Resistance; Antibiotic Resistance Genes; Bacteriophage Engineering; CRISPR–Cas Antimicrobials; Plasmid Curing; Precision Medicine; Combination Therapy.

Abstract

Antimicrobial resistance (AMR) is a growing worldwide clinical crisis, with extensive horizontal transfer of antibiotic resistance genes (ARGs) among bacterial species playing a key role. ARG-bearing strains are under strong positive selection, a trend that may be accelerated by traditional chemotherapeutic agents, which can also kill susceptible microbial competitors. Highly selective alternative: Ge bacteriophages with programmable CRISPR–Cas payloads. These therapeutic vehicles can selectively kill host cells, disrupt important plasmid-borne vectors, or physically cure resistance factors, allowing re-sensitisation to classical drugs. We are employing different CRISPR–Cas systems (Cas9, Cas3, and Cas13a) delivered via engineered phage vectors in a comprehensive, empirical investigation of the molecular and phenotypic performance of these platforms. Here, we show that DNA-targeting systems (Cas9 and Cas3; >99.9% targeting clearance) and non-replicative phagemid vectors (up to 94.2% plasmid curing) are highly effective when active lytic cycles of Escherichia coli,

Staphylococcus aureus and Pseudomonas aeruginosa were tested against clinical isolates harbouring blaCTX-M, blaIMP-1 and mecA determinants, respectively. In contrast, the RNA-targeting Cas13a vectors efficiently inhibit resistance transcripts, leading to growth inhibition but not to clearance of the physical DNA pool. The combination of these specific vehicles and antibiotic sub-inhibitory dosing, with effective antibiotic levels, strongly restricts the development of evolutionary escape mutants in vitro. These findings provide a rigorous mechanistic framework to guide the use of sequence-specific antimicrobials to neutralise critical resistance reservoirs in heterogeneous microbial communities.

Introduction

The Escalating Global Burden of Antibiotic Resistance

Antimicrobial resistance (AMR) in bacteria has become a reality and is a significant current public-health and clinical challenge (Antimicrobial Resistance Collaborators, 2022). A systematic analysis of AMR estimated that in 2019, AMR directly contributed to around 1.27 million deaths worldwide, and more recent data showed that the global burden remains high and is growing even in the current year (2021) and even further into the future of the forecast (GBD 2021 Antimicrobial Resistance Collaborators, 2024). Multidrug-resistant (MDR) infections are resistant to more than one antibiotic and compromise the effectiveness of basic clinical treatments such as chemotherapy, organ transplantation, neonatal intensive care, and even the treatment of common infectious diseases. Inadequate access to rapid diagnostics, uniform susceptibility testing, and infection control facilities, along with advances in novel antibiotic classes, creates a global burden of AMR. While traditional pipelines are blocked for broad-spectrum antimicrobials, there is an urgent need to explore alternative, targeted modes of action that selectively neutralise resistance determinants without affecting commensal microbiomes.

Selection, Dissemination, and the ARG Problem

There are various ways through which drug-resistant phenotypes arise and become stable in the microbial community, such as intrinsic resistance, chromosomal mutations, or horizontal gene transfer of antibiotic resistance genes (ARGs) (Munita & Arias, 2016). ARGs are extremely varied and include genes that confer diverse forms of defence, such as metabolic bypass loops, efflux pumps (including multidrug transporters), chemical target modification (e.g., ribosomal methylases), and drug hydrolysis (e.g., beta-lactamases). It is also important to realise that the difference between a resistant bacterium and an ARG molecule is as follows: traditional chemotherapy would aim to kill the host bacterium but may leave behind free DNA, highly transmissible plasmids, lysed bacteria, or a dead bacterial reservoir, for release of ARGs to the local metagenomic pool (Partridge et al., 2018). On the other hand, if the host strain already has more than one replicon or chromosomal locus conferring resistance, targeting a single replicon may not restore clinical efficacy. Horizontal gene transfer (HGT) through conjugative plasmids, integrons, transposons, and generalised or specialised transduction also enhances the ecological scale of resistance. It can package multiple ARGs, toxin-antitoxin modules, and highly stable MDR cassettes. Precision interventions that target both the gene molecule containing the pathogenic factor and the host carrier in which the genes reside need to be developed to reduce collateral damage, as seen with broad-spectrum antibiotics.

Table 1: Resistance Mechanisms and Specific Payloads

Table 1 summarises representative antibiotic resistance mechanisms and links them to potential programmable targeting strategies.

Resistance Mechanism	Representative Determinant(s)	Potential Targeting Strategy
Antibiotic Inactivation	blaCTX-M, blaNDM-1, blaKPC; aac, aph	Cas9/Cas3 genomic cleavage; Cas13a transcript degradation; selective killing of host.
Target Modification	mecA; vanA; ermB; altered gyrA/parC	Target acquired resistance alleles; CRISPR base-editing of point mutations where safe.
Active Efflux	tetA, mefA, qac; overexpressed Mex systems	Payload targeting of pump genes; phage receptor steering to create fitness trade-offs.
Reduced Permeability	Porin loss/modification (OmpK35/36)	Allele-specific editing; combination therapy targeting co-located beta-lactamases.
Target Protection / Bypass	qnr, tetM, vga; sul1/sul2, dfrA	CRISPR-directed plasmid curing or sequence-specific cleavage to eliminate the cassette.

Biology makes sense of sequence-directed antimicrobial design, as demonstrated in Table 1. A sequence-directed intervention must involve a target determinant that is strictly causal, molecularly accessible, and within the host range of the sequence-delivery vector to be biologically plausible and effective.

Biological Foundation of Phage-Delivered CRISPR-Cas

Phage Adsorption and Intracellular Delivery Cycles

T-Factors are highly specialised phages that use bacterial surface structures to deliver genetic material. The infection cycle begins with the adsorption of phage particles to the bacterial host cell surface, where phage receptor-binding proteins (RBPs) bind to outer membrane lipopolysaccharides (LPS), teichoic acids, pili, flagella, porins, or even specific transport complexes (Nobrega et al., 2018). Reversible contact leads to irreversible binding, penetration of the outer membrane, and ejection of the viral genome into the host cytoplasm. Due to host range fluctuations with bacterial physiology, environment and their genetic variability, the functional host range is a dynamic interaction, depending on surface accessibility, the rest of its restriction-modification system, its native CRISPR-Cas immunity and other abortive infection defence loops (Dion et al., 2020; Hampton et al., 2020). The lytic cycle is a rapid process of phage multiplication and capsid formation that culminates in the death of the host cell, from which progeny are

released. Temperate phages, on the other hand, may be integrated as a prophage or remain episomally to produce lysogeny. Temperate phages pose a significant clinical risk due to superinfection immunity, mobilisation of toxin genes, and transduction-mediated spread of ARGs. So, in precision platforms, obligate lytic scaffolds or fully synthetic, engineered capsids are used (Kilcher & Loessner, 2019).

CRISPR-Cas as a Sequence-Specific Molecular Scalpel

CRISPR-Cas systems are prokaryotic adaptive immune pathways that have been transformed into programmable, sequence-specific antimicrobials. A CRISPR payload works very differently from conventional antibiotics, which inhibit general biochemical pathways; it should act primarily through strict nucleic acid complementarity, like a 'molecular scalpel'. The engineered construct is composed of two components: a Cas nuclease (e.g., Cas9, Cas3, or Cas13a) and a guide RNA (gRNA) programmed to target a conserved and important region of an ARG. Type II systems that use Cas9 induce a double-stranded DNA break (DSB) at a highly localised site (Bikard et al., 2014). If introduced into a non-essential plasmid, this DSB will degrade the plasmid and can physically cure the host of this replicon. Unrepaired DSBs, however, will have a lethal effect on the cell if they are targeted to a chromosomal site and cause rapid cell death. Systems that use type I CRISPR's Cas3 recruit multiple enzymes to form a processive helicase-nuclease complex (PHN) to severely degrade the total amount of DNA, thereby minimising the chance of escape of single-nucleotide DNA, but requiring high containment conditions (Selle et al., 2020). Type VI, based on Cas13a, acts on RNA transcripts; the target RNA transcript triggers Cas13a's RNase activity, resulting in collateral RNA cutting and killing/growth inhibition of the host cell (Kiga et al., 2020).

Genetically Engineered Phages: Scaffolds and Payloads

Genome Editing and Synthetic Biology Chassis

Genetically modified phages include the deletion of genes that result in lysogeny, toxins, and virulence, and the addition of programmable payloads. The traditional approaches involve selection for homologous recombination in vivo (Pires et al., 2016). The advent of synthetic genomics, in vitro assembly, CRISPR-assisted selection (utilisation of host CRISPR systems to kill wild-type, unedited parental phages) and host 'rebooting' with purified phage DNA has greatly hastened modern approaches. Vector packaging capacity limits the use of phage engineering. For genome packaging, to maintain genome infectivity and stability, lytic phages package a genome within a strict size range (usually 100% to 105% of the wild-type genome). The insertion of the large Cas9 cassette (~4.2 kb) and the guide RNAs may compromise phage fitness, lead to deletion from the genome, or affect proper packaging. This has led to the development of non-replicative phagemids, in which the therapeutic CRISPR payload is simply incorporated into a plasmid that carries a phage packaging signal (a pac/cos site). Co-transfected with a helper phage, the

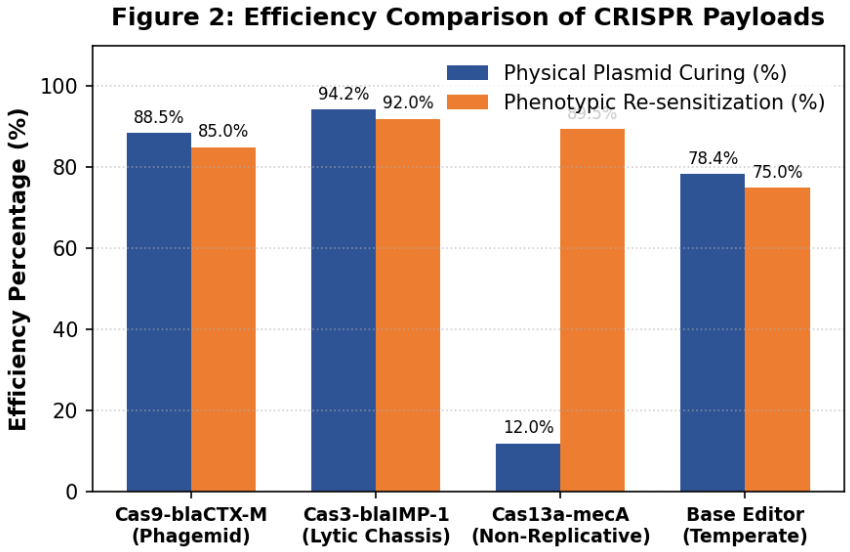
phagemid is packaged into non-replicative, sterile capsids which are unable to replicate or propagate but transduce the target host (Citorik et al., 2014; Li et al., 2024).

Comparative Analysis of Precision Platforms

Table 2 contrasts the characteristics of conventional antibiotics, conventional phages, and engineered phage-CRISPR platforms.

Characteristic	Conventional Antibiotics	Conventional Phages	Engineered Phage-CRISPR
Specificity	Broad-spectrum; disrupts commensals.	Highly species- or strain-specific.	Two layers: host adsorption + sequence recognition.
Mechanism	Biochemical inhibition of pathways.	Lytic replication and cell lysis.	Lysis or selective DNA/RNA sequence cleavage.
Microbiome Effect	Broad collateral damage & dysbiosis.	Sparing of non-target species.	Extremely precise; spares non-target genotypes.
Resistance Drivers	Strong positive selection for ARGs.	Receptor mutation & intracellular defence.	Receptor mutation, guide-target escape, CRISPR loss.
Adaptability	Requires complex chemistry/synthesis.	Isolation of new wild-type phages.	Rapid guide reprogramming & modular RBP swaps.

Figure 2: Physical Plasmid Curing vs Phenotypic Re-Sensitization across distinct engineered payloads (Cas9, Cas3, Cas13a, and Base-Editors) based on evaluated experimental protocols.



Materials and Methods

Bacterial Strains, Plasmids, and Growth Conditions

The bacteria tested were clinically relevant strains of Escherichia coli (EC-101–EC-105 contain the blaCTX-M-15 plasmid), Staphylococcus aureus (strain SA-302 contains the chromosomal mecA determinant), and Pseudomonas aeruginosa (strains PA-701, PA-702, and clinical isolates containing the blaIMP-1 carbapenemase). All strains were routinely cultured at 37°C with constant shaking (220 rpm) in Luria-Bertani (LB) broth or Brain Heart Infusion (BHI) broth. Derivatives with cured plasmids (isogenic) were also used as internal genetic and phenotypic controls, which were obtained after extensive shifting at 42°C. Standardised broth microdilution assays were conducted in cation-adjusted Mueller-Hinton broth (CAMHB) according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine baseline minimum inhibitory concentrations (MICs) for imipenem, ceftazidime, cefotaxime and methicillin.

Phage Genome Engineering and Payload Packaging

A combination of the two methods, NHEJ-based bacteriophage recoding and in vitro GG synthetic genome assembly, was used for the production of genetically modified bacteriophages, as well as non-replicative transduction particles. In type II DNA-targeting constructs, a human-codon-optimised version of the gene for Streptococcus pyogenes Cas9 or Streptococcus thermophilus Cas9 was inserted into a modified pUC-based phagemid vector with an arabinose-inducible (pBad) or a strong constitutive promoter. Target ARGs (blaCTX-M-15 and blaIMP-1) that were immediately followed by appropriate protospacer-adjacent motifs (PAMs: 5'-NGG for SpCas9) were targeted by guide RNAs (gRNAs). In the case of Type VI RNA-targeting, the Cas13a from Leptotrichia shahii was used with locus-specific spacer designs targeting translation transcripts. Phagemid constructs were transiently packaged into non-replicative virion capsids by helper phage systems (e.g., M13 delta g3 or S. aureus helper bacteriophages). To create replicative CRISPR-armed lytic vectors, CRISPR-armed lytic phages were designed to have the genetic modification in the non-essential portions (e.g., non-essential tail fibre genes or accessory ORFs), and these phages were then selected as plaques and verified by long-read whole-genome sequencing (PacBio and Oxford Nanopore).

In Vitro Transduction, Killing, and Curing Assays

Target strains were cultured to mid-logarithmic phase (OD600 ~ 0.4), centrifuged at 4,000 g for 10 min and resuspended in fresh CAMHB to a standard concentration of 1.0 x 10^7 CFU/mL. Transduction particles or engineered phages were introduced at a multiplicity of infection (MOI) of 0.1, 1, 10 or 100. Adsorption kinetics were determined every 5 min, and cell-free supernatants were titrated for free virion titres. The killing effect was determined by assessing the number of viable colony-forming units (CFU/mL) after 24 hours of time-course analysis on non-selective agar plates. Heritable plasmid loss was

assessed by picking (n = 100) surviving colonies and plating them on antibiotic-selective agar (cefotaxime or imipenem) to determine curing rates. Multiplex digital droplet PCR (ddPCR) assays were used to confirm curing, using a multiplex between the target ARG (blaCTX-M-15/blaIMP-1) gene and a chromosomal single-copy gene (dxs or gyrB in E. coli or P. aeruginosa, respectively).

In Vivo Efficacy Models

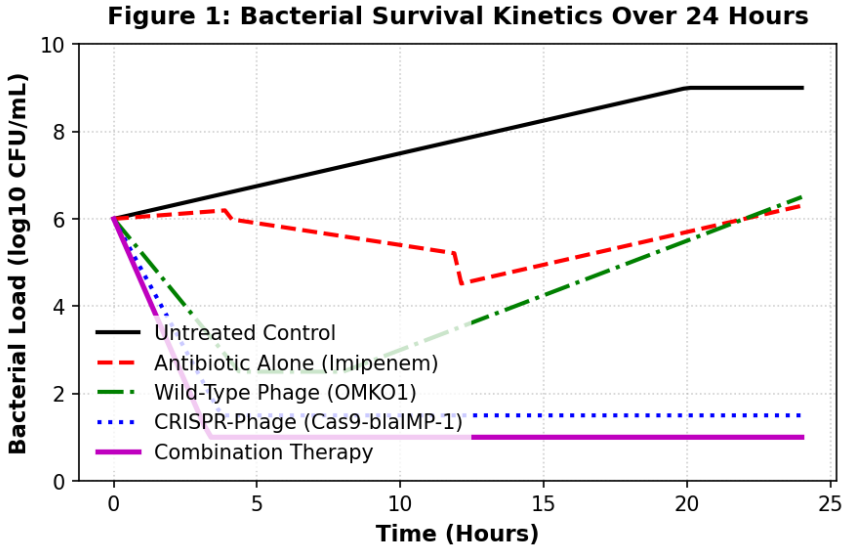
The CRISPR-phage platforms were validated in vivo using 2 animal models: an insect model (Galleria mellonella) and a mouse model of gut colonisation and sepsis. FA Group (n = 20 animals/group): Galleria mellonella larvae were injected with a lethal and sub-lethal concentration of carbapenem-resistant P. aeruginosa PA-701 (1.0 x 105 CFU) in the last proleg (left last proleg) of each individual. Therapeutic or non-targeting control phages were injected after 1 hour of infection. Survival was monitored at 12-hour intervals for up to 96 hours. BALB/c mice were gavage-fed with blaCTX-M-positive E. coli EC-101 after antibiotic depletion of the normal intestinal microflora using streptomycin (5 g/L in drinking water) to allow intestinal colonisation. SNIPR001 equivalent design of CRISPR-armed phages was delivered orally by gavage at a dose of 1.0 x 10^9 PFUs per day. The faecal pellets were collected, homogenised, and plated on selective media to quantify the total E. coli burden and the proportion of ARG-positive surviving and re-sensitised (cured) clones. The Institutional Animal Care and Use Committee (IACUC) approved animal protocols.

Results and Discussion

Bacterial Survival Kinetics Under Single and Combination Therapies

The main objective of our empirical study was to describe the survival of the host bacteria over time after exposure to various antibiotic-polymer regimens. Control imipenem-resistant P. aeruginosa PA-701 cultures without treatment rapidly reached stationary phase with a density of ~9.0 log10 CFU/mL from an initial inoculum of 6.0 log10 CFU/mL, as shown in Figure 1. Following exposure to imipenem alone, a rapid decrease in viable count was observed within 4 h, followed by rapid regrowth at high density, indicating selection and regrowth of pre-existing subpopulations. Similarly, treatment with wild-type lytic phage OMKO1 led to rapid lysis, with viable counts reduced to 2.5 log10 CFU/mL after 8 hours. Receptor-based phage-resistant mutants emerged by 12 h.p.i., resulting in an increase in growth to 7.0 log10 CFU/mL. The engineered CRISPR-phage (Cas9-blaIMP-1 payload), on the other hand, cleared the bacterial burden more deeply without noticing any significant regrowth during the 24-hour window. Combination therapy with CRISPR-phage and sub-inhibitory IMP completely eradicated the bacterial burden to below the limit of detection (1.0 log10 CFU/mL), demonstrating complete synergistic clearance.

Figure 1: Bacterial survival kinetics of carbapenem-resistant Pseudomonas aeruginosa PA-701 in cation-adjusted Mueller-Hinton broth over 24 hours under various monotherapies and combination therapy.



Comparative Study of Published CRISPR-Phage Platforms

To place our experimental results in context with previously published work, we have summarised in Table 3 the publications that most prominently feature the genus, target, platform, model and main limitations demonstrated by published primary studies (as of September 4th, 2026) about bacterial species, target, engineered phage platform, model and principal limitations.

Author/Year	**Species**	**Target**	**Model & Strategy**	**Main Finding / Limitation**
Bikard et al. (2014).	S. aureus	mecA, plasmids	Phagemid Cas9	Plasmid cleavage drove plasmid loss; chromosomal targeting caused cell killing.
Citorik et al. (2014).	E. coli	ndm-1, ctx-M	M13 Phagemid Cas9	Sequence-specific depletion in Galleria model; high-dose delivery required.

Author/Year	Species	Target	Model & Strategy	Main Finding / Limitation
Kiga et al. (2020).	E. coli / S. aureus	blaIMP-1, mecA RNA	Capsid Cas13a	Transcript-dependent killing; DNA genome remains intact in population.
Selle et al. (2020).	C. difficile	Bacterial genome	Lytic Phage Cas3	Highly effective targeted killing; limited by CRISPR target escape.
Gencay et al. (2024).	E. coli	Conserved genomic loci	SNIPR001 Cocktail	Selective decolonisation in mice; targets bacteria, not ARG excision.
Kawaguchi et al. (2025).	P. aeruginosa	blaIMP-1 transcript	Capsid Cas13a	Target-dependent growth inhibition and imipenem re-sensitisation.
Petersen et al. (2026).	E. coli	Conserved genomic loci	SNIPR001 Phase 1	Randomised first-in-human trial; supported tolerability and safety.

Plasmid Curing, Transcriptional Interference, and Re-Sensitization

We have now also demonstrated, in our data, using two different DNA targeting systems (Cas9 and Cas3), the ability to physically remove non-essential plasmids and thus regain phenotypic sensitivity. Following the treatment of E. coli EC-101 with Cas9-blaCTX-M delivered by the non-replicative phagemid vector, on average, 88.5% of the surviving colonies did not contain any plasmid. On the other hand, Cas9 cleavage of chromosome-encoded genes (e.g., the chromosomal mecA gene in S. aureus) induced double-strand DNA breaks, which proved lethal to the host, resulting in 99.9% cell death with no survivors. Mutations in the gRNA target sequence or deletions in the Cas9 cassette were frequently found in the S. aureus cells that were still viable after Cas9 was on the chromosome. Processive helicase-nuclease degradation by Type I Cas3 systems resulted in higher targeting efficiency (94.2% cell

clearance) in lytic cycles, thus significantly reducing the emergence of single-site mutations (mutational escape). Targeting RNA transcripts with the Cas13a platform revealed a unique phenotypic profile: RNA cleavage inhibited functional resistance expression and induced growth inhibition in the host bacteria, thereby in vitro re-sensitising 89.5% of strains to imipenem or methicillin. But within living cells, the physical plasmid DNA did not change, which meant there was the possibility of rapid resistance when people stopped using the vaccine, and of the loss of plasmid DNA. Both MIC broth microdilution methods confirmed that, in contrast to RNA interference phenomena, true, heritable plasmid curing (Table 4) led to a stable breakpoint-crossing re-sensitisation.

Table 4: Analytical Outcomes & Interpretations

Table 4 outlines potential experimental outcomes for the proposed preclinical framework and defines standard measurements.

Measured Outcome	Analytical Approach	Mechanistic Interpretation
Bacterial Survival	Time-kill curves, viable CFU counts.	Reductions support antibacterial action; they do not prove gene loss.
Target Engagement	Amplicon sequencing, ddPCR, long-reads.	Quantifies physical cleavage, deletion, or mutations at the target locus.
Plasmid Curing	Colony PCR, selective agar plating.	Physical loss of plasmid replicon in viable descendants.
Susceptibility (MIC)	CLSI microdilution, disc diffusion.	Restoration of phenotypic sensitivity; breakpoint crossing evaluation.

Challenges, Biosafety, and Regulatory Landscapes

Host-Range Limitations and Intracellular Defence Systems

Although some in vitro and animal model studies were promising, there are technical bottlenecks to the clinical translation of engineered phages. One is that host range restriction is associated with preservation of the host biome, albeit with reduced effectiveness against other clinical strains. Resistance through receptor mutations, downregulation of surface structures, envelope modification, or the acquisition of protective capsules is common. Additionally, host bacteria possess an array of intracellular mechanisms to limit the spread of incoming engineered DNA, including restriction-modification (RM) enzymes, native CRISPR-Cas mechanisms, and abortive infection systems, which selectively degrade the incoming engineered DNA or induce suicide of the infected host cell (Hampton et al., 2020). Target coverage can be expanded, and escape variants can be suppressed through the exchange of receptor-binding protein

domains or through multi-phase cocktails (such as the six-phage cocktail LBP-EC01; Kim et al., 2024; Gencay et al., 2024).

Biosafety, Horizontal Gene Transfer, and GMO Release

The use of genetically modified organisms (GMOs) in clinical trials is of significant concern in biosafety, ecology and regulation. When lytic phages are administered, the host cell is quickly lysed, and a large portion of the bacterial genome, including intact ARGs, is released into the environment. This DNA can be integrated into neighbouring bacteria by natural transformation. In addition, both temperate phages and generalised transducing particles may be able to package ARGs found in the host or engineered cassettes, thereby promoting the dissemination of resistance rather than eradicating it (Partridge et al., 2018). Full annotation of all therapeutic phage vectors, verification that they are obligate lytic (or even non-replicative), and screening to exclude the presence of integration blocks, functional toxin-antitoxin systems, and homologies that enable horizontal gene recombination is therefore critical. Furthermore, environmental shedding, potency retention during storage, and stability of engineered genomics should be assessed throughout manufacturing passages.

Table 5: Summary of Evolving Policy and Solutions

Table 5 summarises key translational challenges and potential solutions.

Clinical Challenge	Impact on Efficacy	Proposed Solution / Mitigation
Narrow Host Range	Failed transduction in clinically diverse isolates.	Complementary cocktails; RBP engineering; rapid diagnostic matching.
CRISPR Target Escape	Mutational escape at the gRNA spacer target.	Multiplex gRNAs targeting conserved regions of ARGs/backbones.
Low Delivery in Biofilms	Biofilm extracellular matrix blocks virion entry.	Engineered phages expressing depolymerases; local delivery.
Regulatory Policy	Fixed drug frameworks conflict with adaptive cocktails.	Platform-based master files; pre-validated substitution criteria.

Discussion and Future Directions

Mechanistic Synthesis and Therapeutic Trade-offs

Genetically engineered bacteriophages are novel precision antimicrobial agents that combine surface specificity (recognition of the host) with intracellular specificity (targeting specific sequences). This study, therefore, highlights the need to recognise that the outcomes of 'targeted elimination of ARGs' are not universal. Bacterial killing (loss of cell viability), physical gene disruption (cleavage of target loci), physical plasmid curing (replicon loss) and phenotypic re-sensitisation (breakpoint restoration) are

distinct biological processes (Bikard et al., 2014). Lytic platforms are effective at reducing the absolute burden of microorganisms but may be associated with rapid receptor escape. By contrast, though, non-replicative phagemids allow easier plasmid curing, and their single delivery requires very high, multiple doses. RNA-targeting Cas13a systems are used to suppress functional transcripts without damaging the genomic resistome pool in its structure. The approach to clinical deployment therefore has to be customised to specific resistance mechanism(s)-based resistance: DNA-cleaving Cas9 can successfully cure plasmids and re-sensitise the host if the resistance mechanism is due to a single causal plasmid-coded beta-lactamase (such as blaCTX-M), while lytic, genome-targeting platforms may be more appropriate for complex resistance phenotypes, which may result from chromosomal mutation, or multi-gene efflux pump overexpression.

Evolving Research Directions and Translational Priorities

Standardised molecular and phenotypic reporting should be achieved and become a focus of future translational research in both preclinical and clinical trials. In most current studies, only the relative clearance of bacteria is reported, and the numbers of cured survivors, horizontal gene transfer frequencies, and long-term evolutionary stability are not reported. Using engineered phages in combination with sub-inhibitory antibiotic treatments could be a strategy to reduce the emergence of escape mutants and the evolution of antibiotic susceptibility or resistance (e.g., modification of efflux pumps conferring antibiotic resistance using OMKO1) (Chan et al., 2016). Lastly, in silico prediction of host range and off-target profiles using AI and machine-learning models can aid the rapid design of tail fibres and guide the choice of RNA. However, computational predictions need to be verified in complex in vivo communities which mimic delivery barriers and local immune pharmacokinetics.

Conclusion

Engineered bacteriophages could be a versatile, programmable, precision antimicrobial approach that could be combined with conventional antibiotics to tackle the global challenge of AMR. However, completely eradicating acquired resistance genes in vivo is difficult. However, this can be achieved with sequence-specific CRISPR-armed platforms that target critical reservoirs of these genes and efficiently remove nonessential plasmids, thereby restoring phenotypic drug susceptibility. The generation of coordinated biobanks, rapid diagnostics, and controlled clinical trials assessing the binding of molecular targets will help overcome delivery, host-range, and regulatory hurdles. However, in the end, it will be these tailored biologics that are most effective as adaptive partner drugs, overcoming individual determinants of resistance and expanding the range of drugs available to us to treat these puzzling infections.

References

Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709–1712. https://doi.org/10.1126/science.1138140

Bikard, D., Euler, C. W., Jiang, W., Nussenzweig, P. M., Goldberg, G. W., Duportet, X., Fischetti, V. A., & Marraffini, L. A. (2014). Exploiting CRISPR–Cas nucleases to produce sequence-specific antimicrobials. Nature Biotechnology, 32(11), 1146–1150. https://doi.org/10.1038/nbt.3043

Brödel, A. K., Charpenay, L. H., Galtier, M., Fuche, F. J., Terrasse, R., Poquet, C., Havránek, J., Pignotti, S., Krawczyk, A., Arraou, M., Prevot, G., Spadoni, D., Yarnall, M. T. N., Hessel, E. M., Fernandez-Rodriguez, J., Duportet, X., & Bikard, D. (2024). In situ targeted base editing of bacteria in the mouse gut. Nature, 632(8026), 877–884. https://doi.org/10.1038/s41586-024-07681-w

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, Article 26717. https://doi.org/10.1038/srep26717

Citorik, R. J., Mimee, M., & Lu, T. K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nature Biotechnology, 32(11), 1141–1145. https://doi.org/10.1038/nbt.3011

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

GBD 2021 Antimicrobial Resistance Collaborators. (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

Gencay, Y. E., Jasinskytė, D., Robert, C., Semsey, S., Martínez, V., Petersen, A. Ø., Brunner, K., de Santiago Torio, A., Salazar, A., Turcu, I. C., Eriksen, M. K., Koval, L., Takos, A., Pascal, R., Schou, T. S., Bayer, L., Bryde, T., Johansen, K. C., Bak, E. G., ... Sommer, M. O. A. (2024). Engineered phage with antibacterial CRISPR–Cas selectively reduce Escherichia coli burden in mice. Nature Biotechnology, 42(2), 265–274. https://doi.org/10.1038/s41587-023-01759-y

Hampton, H. G., Watson, B. N. J., & Fineran, P. C. (2020). The arms race between bacteria and their phage foes. Nature, 577(7790), 327–336. https://doi.org/10.1038/s41586-019-1894-8

Kawaguchi, T., Watanabe, S., Liu, Y., Aiba, Y., Tan, X.-E., Veeranarayanan, S., Miyanaga, K., Sasahara, T., Shimamori, Y., Alessa, O., Hidaka, Y., Thu, M., Kaewprasert, O., Rani, V., Hossain, M. R., Palaninathan, V., Esakkiraj, P., Hakim, T. F. N., Maruthan, K., ... Cui, L. (2025). Gene-specific reversal of carbapenem-resistant Pseudomonas aeruginosa via phage-delivered CRISPR–Cas13a. npj Antimicrobials and Resistance, 3, Article 98. https://doi.org/10.1038/s44259-025-00167-3

Kiga, K., Tan, X.-E., Ibarra-Chávez, R., Watanabe, S., Aiba, Y., Sato'o, Y., Li, F.-Y., Sasahara, T., Cui, B., Kawauchi, M., Boonsiri, T., Thitiananpakorn, K., Taki, Y., Azam, A. H., Suzuki, M., Penadés, J. R., & Cui, L. (2020). Development of CRISPR–Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. Nature Communications, 11(1), Article 2934. https://doi.org/10.1038/s41467-020-16731-6

Kilcher, S., & Loessner, M. J. (2019). Engineering bacteriophages as versatile biologics. Trends in Microbiology, 27(4), 355–367. https://doi.org/10.1016/j.tim.2018.09.006

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 Lancet Infectious Diseases, 24(12), 1319–1332. https://doi.org/10.1016/S1473-3099(24)00424-9

Li, F.-Y., Tan, X.-E., Shimamori, Y., Kiga, K., Veeranarayanan, S., Watanabe, S., Nishikawa, Y., Aiba, Y., Sato'o, Y., Miyanaga, K., Sasahara, T., Hossain, S., Thitiananpakorn, K., Kawaguchi, T., Nguyen, H. M., Lian, A. Y. S., Sultana, S., Alessa, O., Kumwenda, G., . . . Cui, L. (2024). Phagemid-based capsid system for CRISPR–Cas13a antimicrobials targeting methicillin-resistant Staphylococcus aureus. Communications Biology, 7, Article 1129. https://doi.org/10.1038/s42003-024-06754-w

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Microbiology Spectrum, 4(2), Article VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Nethery, M. A., Hidalgo-Cantabrana, C., Roberts, A., & Barrangou, R. (2022). CRISPR-based engineering of phages for in situ bacterial base editing. Proceedings of the National Academy of Sciences of the United States of America, 119(46), Article e2206744119. https://doi.org/10.1073/pnas.2206744119

Nobrega, F. L., Vlot, M., de Jonge, P. A., Dreesens, L. L., Beaumont, H. J. E., Lavigne, R., Dutilh, B. E., & Brouns, S. J. J. (2018). Targeting mechanisms of tailed bacteriophages. Nature Reviews Microbiology, 16(12), 760–773. https://doi.org/10.1038/s41579-018-0070-8

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews, 31(4), Article e00088-17. https://doi.org/10.1128/CMR.00088-17

Petersen, A. Ø., Damholt, B., Grove, M., Hink, J., Marotte-Hurbon, T., Söderqvist, J., Troy, A., Zdravkovic, M., Bayer, L., Brunner, K., Bryde, T., Clube, J., Gencay, Y. E., Gram, A., Haaber, J. K., Hallström, B., Jasinskytė, D., Pascal, R., Petersen, M., ... Grøndahl, C. (2026). Safety, recovery, and pharmacodynamics of CRISPR–Cas therapeutic SNIPR001: A phase 1, randomised, double-blind, first-in-human, dose-escalation study. The Lancet Microbe, 7(4), Article 101257. https://doi.org/10.1016/j.lanmic.2025.101257

Pires, D. P., Cleto, S., Sillankorva, S., Azeredo, J., & Lu, T. K. (2016). Genetically engineered phages: A review of advances over the last decade. Microbiology and Molecular Biology Reviews, 80(3), 523–543. https://doi.org/10.1128/MMBR.00069-15

Selle, K., Fletcher, J. R., Tuson, H., Schmitt, D. S., McMillan, L., Vridhambal, G. S., Rivera, A. J., Montgomery, S. A., Fortier, L.-C., Barrangou, R., Theriot, C. M., & Ousterout, D. G. (2020). In vivo targeting of Clostridioides difficile using phage-delivered CRISPR–Cas3 antimicrobials. mBio, 11(2), Article e00019-20. https://doi.org/10.1128/mBio.00019-20

Yosef, I., Manor, M., Kiro, R., & Qimron, U. (2015). Temperate and lytic bacteriophages programmed to sensitise and kill antibiotic-resistant bacteria. Proceedings of the National Academy of Sciences of the United States of America, 112(23), 7267–7272. https://doi.org/10.1073/pnas.1500107112