Volume 3, Number 3, 2026
Pages 1481-1498
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

CRISPR-BASED ENGINEERING OF BACTERIOPHAGES AGAINST ANTIBIOTIC-RESISTANT BACTERIA

Muhammad Numan Ashraf[*1], Muhammad Awais Saleem[2], Sajjad Ahmad[3], Muhammad Nouman Nadeem[4], Syed Aoun Abbas Kazmi[5]

[*1,2,3,4]*Department of Biosciences and Technology, Emerson University Multan, Multan, Punjab, Pakistan*
[5]*Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Rawalpindi, Punjab, Pakistan*

[*1]numanashraf744@gmail.com, [2]awaissaleemjhn152@gmail.com, [3]sajjadmalik0147@gmail.com
[4]jamnoumannadeem444@gmail.com, [5]saounabbask@gmail.com[5]

https://doi.org/10.5281/zenodo.24009142

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 31, 2026

Keywords:
Bacteriophage; CRISPR-Cas; antimicrobial resistance; phage therapy; genome engineering; synthetic biology; Cas3; plasmid curing; XDR typhoid; Pakistan

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Muhammad Numan Ashraf

Abstract

Antimicrobial resistance is now a leading infectious cause of death worldwide, and the small-molecule pipeline is not replacing the drugs it is eroding. Bacteriophages offer a self-amplifying, narrow-spectrum alternative unaffected by the mechanisms that disable antibiotics, but wild phages are poorly matched to clinical requirements: host range is too narrow, bacteria escape within hours, temperate phages carry lysogeny and virulence cargo, and their genomes long resisted deliberate modification. CRISPR-Cas changed this twice over. It supplied a counterselection method efficient enough to make phage genomes routinely editable, turning phage therapy from a screening exercise into a design discipline; and it supplied a payload – a programmable nuclease that, delivered into a bacterium and aimed at its chromosome or a resistance plasmid, kills or disarms that cell by sequence rather than by biochemical class. Combining the two gives an agent whose host range, resistance profile and kill mechanism are all specified in DNA. This review covers the mechanistic basis of CRISPR self-targeting; the toolbox for editing phage genomes; design strategies including receptor-binding protein swapping, lysogeny deletion and anti-CRISPR counter-defence; the delivery problem that remains the field's binding constraint; the clinical pipeline, where engineered CRISPR-phages have now completed controlled human trials; and Pakistan as a regional case study in which XDR typhoid presents an unusually well-matched target. We conclude that the engineering is largely solved and that delivery, pharmacology and manufacturing now determine the pace.

Scope and sources

This is a narrative review. Literature was identified by targeted searching of PubMed and Google Scholar using combinations of *bacteriophage*, *CRISPR*, *Cas3*, *phage therapy*, *antimicrobial resistance* and *phage*

engineering, supplemented by citation chaining from the retrieved articles, covering 2007 — the year CRISPR's adaptive-immune function was demonstrated — to October 2026, with earlier work included where foundational to phage biology or therapy. Peer-reviewed reports and trial registrations were prioritised, and trial status was verified against ClinicalTrials.gov in November 2026. The review covers engineered phages and CRISPR-based antibacterials; phage therapy without a genetic-engineering component and CRISPR applications in eukaryotic cells fall outside its scope.

1. INTRODUCTION

Bacterial antimicrobial resistance (AMR) was associated with 4.71 million deaths in 2021 and directly attributable to 1.14 million, and the GBD 2021 reference forecast puts those figures at 8.22 million and 1.91 million by 2050 — a 69.6% rise in attributable deaths between 2022 and 2050, with 39.1 million cumulative attributable deaths over 2025–2050 (Naghavi et al., Lancet 2024). Resistance to carbapenems, the drugs of last resort for Gram-negative infections, rose faster than to any other class: from 619,000 associated deaths in 1990 to 1.03 million in 2021 [1,2].

The conventional answer — discover another small molecule — is failing on both scientific and commercial grounds. Most clinically useful scaffolds were found between 1940 and 1962; the chemical space accessible to whole-cell screening is heavily mined, and the Gram-negative outer membrane excludes most of what remains. Commercially, a new antibiotic is a drug prescribers are told to use sparingly, which is why several companies that reached approval in the last decade went bankrupt soon after. Small molecules also share a structural weakness: they act on targets conserved across a genus or phylum, so resistance arising anywhere spreads everywhere.

This suggests a different design principle — an agent defined by a nucleotide sequence rather than by its chemistry. Two technologies make that possible, and each solves the other's problem.

Bacteriophages are obligate bacterial parasites that adsorb to specific surface receptors, inject their genome, replicate and lyse the cell. They are self-amplifying at the site of infection, narrow-spectrum enough to spare commensals, and indifferent to β-lactamases, efflux pumps and ribosomal modifications. Their limitation is that natural phages are not designed for medicine: host range is often too narrow to cover a species, resistance can arise within one overnight culture, temperate phages integrate rather than kill, and some carry toxin or resistance genes that therapy would disseminate.

CRISPR-Cas systems use a short RNA guide to direct a nuclease to a complementary sequence. Their therapeutic relevance here is a quirk of context: in a bacterium an unrepaired double-strand break is lethal, so a Cas nuclease programmed against the cell's own genome is a sequence-specific suicide switch; aimed at a plasmid, it excises resistance determinants and restores susceptibility. The limitation is the mirror image of the phage's — a potent killing agent with no way into a Gram-negative bacterium in the gut, bladder or lung.

The convergence is therefore natural. Phages supply delivery; CRISPR supplies programmable killing, and also the counterselection that made phage genome engineering tractable in the first place, so the vector itself can be redesigned rather than merely selected from the environment.

This review is organised around that dual role. Sections 2 and 3 set out the biology of phages and CRISPR-Cas systems and the specific deficiencies that motivate engineering. Section 4 treats CRISPR as an antimicrobial payload. Section 5 surveys the genome-engineering toolbox, Sections 6 and 7 the design strategies and the delivery bottleneck, and Section 8 the preclinical and clinical evidence now available. Section 9 examines Pakistan as a regional case study, and Sections 10 to 12 assess what still blocks translation and where the field is heading.

2. Phage biology and the limits of classical phage therapy

2.1 The infection cycle

A phage infection begins with reversible then irreversible adsorption to a surface receptor — LPS core sugars, outer-membrane porins such as OmpC or LamB, pili, flagella, teichoic acids in Gram-positives, or capsular polysaccharide. Receptor identity is determined by the phage's receptor-binding proteins (RBPs), typically tail fibres or tail spikes, and it is the principal determinant of host range. The genome is then injected, and the phage follows one of two programmes.

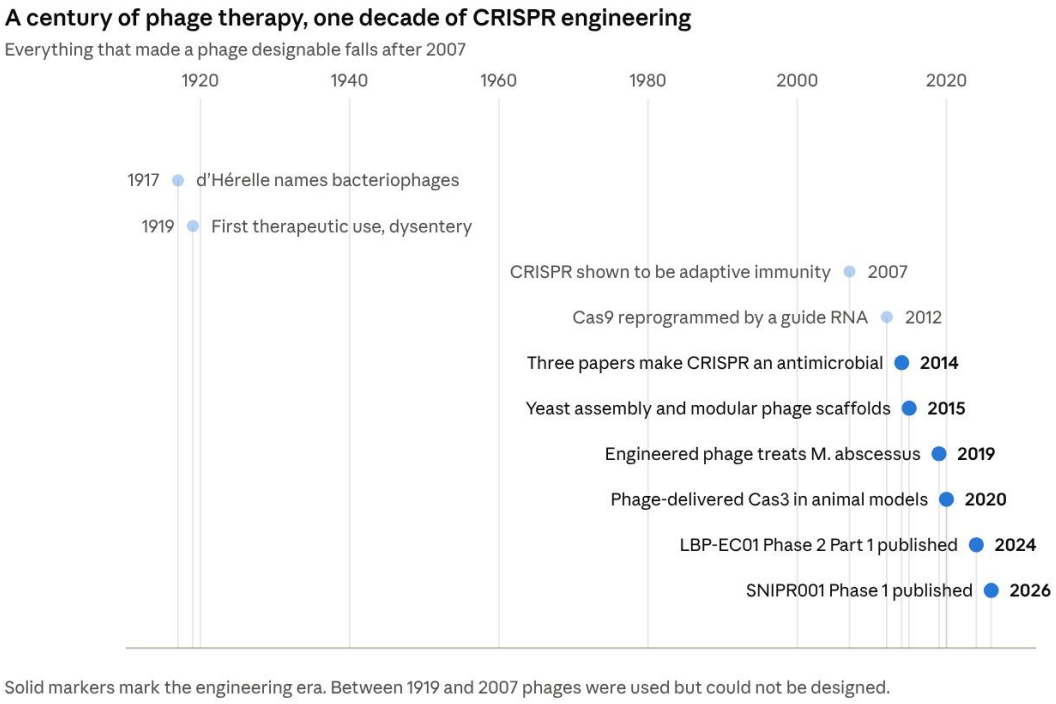
Lytic (virulent) phages redirect host transcription and translation, replicate, assemble progeny and lyse the cell within 20–60 minutes, releasing 10–200 virions. This is the cycle therapy exploits: the dose amplifies itself where the target is present and decays where it is absent, a pharmacokinetic profile no small molecule has.

Temperate (lysogenic) phages instead integrate as a prophage, replicate passively and confer superinfection immunity. Prophages are also major vehicles of horizontal gene transfer — Shiga toxin in *E. coli* O157:H7, cholera toxin in *Vibrio cholerae* and Panton–Valentine leukocidin in *S. aureus* are all prophage-encoded. Classical therapy therefore excludes temperate phages, which removes most phages in any environmental sample from consideration; engineering lifts that constraint by deleting the lysogeny module.

2.2 A century of uneven evidence

Phage therapy predates antibiotics. Félix d'Hérelle named bacteriophages in 1917 and treated dysentery with them in 1919, and clinical use continued at the Eliava Institute in Tbilisi and the Hirszfeld Institute in Wrocław through the antibiotic era. Western interest lapsed because early trials were uncontrolled, preparations poorly characterised, and antibiotics simply easier.

The modern revival has been driven by compassionate-use cases in patients with no remaining options: a disseminated multidrug-resistant *Acinetobacter baumannii* infection resolved with an intravenous phage cocktail at UC San Diego in 2016 [30], and a disseminated *Mycobacterium abscessus* infection in a cystic fibrosis patient treated in 2019 with a three-phage cocktail — one of which was an engineered, lysogeny-deleted derivative of phage Muddy, the first clinical use of a genetically modified phage [31]. Controlled trials have been more equivocal: several randomised studies, including a burn-wound trial (PhagoBurn) and a *Staphylococcus aureus* bacteraemia study, failed to show clear superiority [33], usually because of underdosing, poor host-range matching or manufacturing variability rather than failure of the principle.



Field milestones · 10 events, 1917–2026

The shape of this timeline is the argument of the review in miniature: phage therapy is a century old, but everything that made a phage designable arrived in the last twenty years, and the clinical readouts only in the last three.

2.3 Why wild phages are not drugs

Six properties of natural phages conflict with clinical requirements, and together they define the engineering agenda.

Limitation	Consequence for therapy	Engineering response
Narrow host range (often strain-level)	Needs diagnosis to the strain; cocktails or personalised matching	RBP swapping, directed evolution of tail fibres
Rapid receptor-loss resistance	Bacterial escape within hours; relapse	CRISPR payload kills escape mutants; receptor-loss as a fitness cost
Lysogeny	No killing; superinfection immunity; HGT	Delete integrase / repressor; obligately lytic derivatives
Virulence and AMR cargo	Therapy spreads toxin or resistance genes	Genome curation and removal
Bacterial anti-phage defences (R-M, CRISPR, Abi, retrons, CBASS)	Infection aborted before replication	Anti-restriction and anti-CRISPR proteins; methylation matching
Endotoxin release on lysis	Inflammatory burden in sepsis	Non-lytic or non-replicative delivery particles

The recurring theme is that the therapeutic properties of a phage are encoded, and therefore editable. The obstacle until recently was that phage genomes were extraordinarily difficult to edit — which is where CRISPR entered the field as a tool before it arrived as a drug.

3. CRISPR-Cas systems as programmable nucleases

3.1 Native function

CRISPR-Cas is prokaryotic adaptive immunity against phages and plasmids, present in roughly 40% of sequenced bacteria and 85% of archaea. It operates in three stages. In **adaptation**, Cas1–Cas2 excise a short fragment (a protospacer) from invading DNA and insert it as a new spacer into the CRISPR array, between repeats. In **expression**, the array is transcribed and processed into individual CRISPR RNAs (crRNAs). In **interference**, a crRNA loads into a Cas effector and directs it to cleave sequences complementary to the spacer [3,4].

Self-versus-non-self discrimination relies on the protospacer-adjacent motif (PAM), a short sequence flanking the target in the invader but absent beside the spacer in the host array. The PAM is both a safety feature for the bacterium and a design constraint for the engineer.

A detail that is a footnote in human genome editing is central here: because bacteria lack non-homologous end joining and usually cannot repair a Cas-induced double-strand break, self-targeting is lethal. In human cells a Cas9 cut starts an editing event; in a bacterium it usually ends the cell. That asymmetry is the whole mechanistic basis of CRISPR antimicrobials.

3.2 Effectors relevant to antibacterial design

Systems divide into Class 1 (multi-protein effector complexes; Types I, III, IV) and Class 2 (single-protein effectors; Types II, V, VI) [5]. Both are used therapeutically, and the choice of effector materially changes the outcome. Cas9 (Type II) is the best characterised and compact enough for phage packaging; Cas12a (Type V) processes its own crRNA array, which simplifies multiplexing, and its AT-rich PAM suits many genomes; Cas13 (Type VI) targets RNA; Cascade–Cas3 (Type I) degrades DNA processively; and catalytically dead dCas9 silences transcription without killing (CRISPRi).

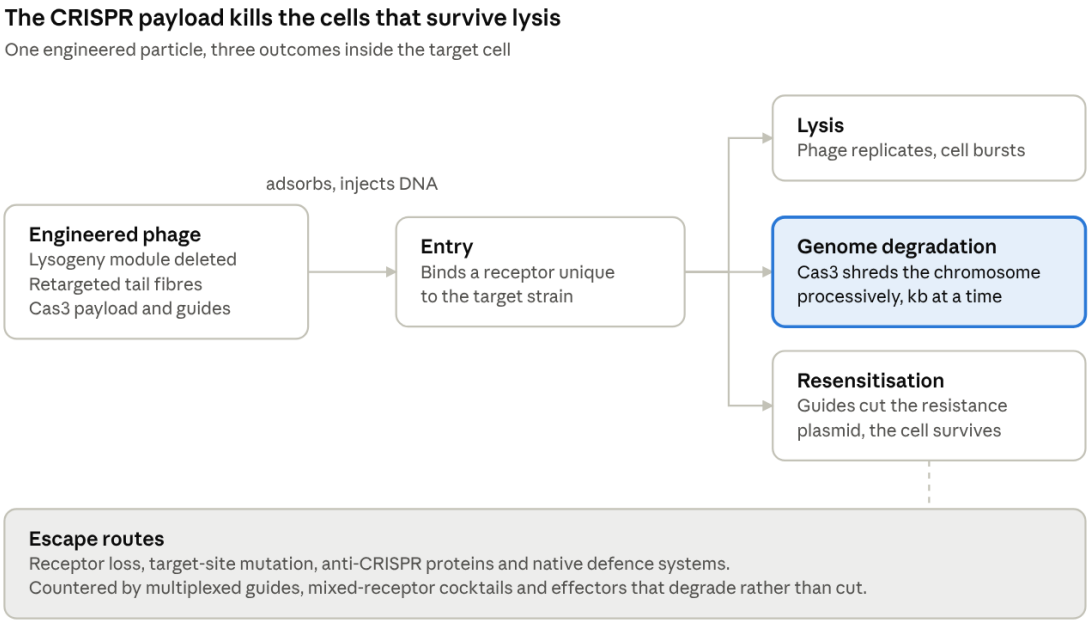
The Cas9-versus-Cas3 distinction deserves emphasis. A Cas9 cut is a single blunt lesion at a defined site, so a cell that mutates that site or acquires an anti-CRISPR can survive. Cascade–Cas3, once it recognises a target, recruits a helicase-nuclease that degrades DNA processively over kilobases. The result is genome destruction rather than a point lesion, and escape by point mutation is correspondingly less available — the reason Locus Biosciences built its clinical platform on a Type I-E system rather than Cas9. Cas13 offers a third logic: cleaving RNA with collateral trans-cleavage of bystander transcripts, it induces growth

arrest and death without creating a DNA lesion at all [6,13], sidestepping DNA repair and the commonest escape routes, at the cost of a less durable, non-heritable effect.

3.3 Anti-CRISPRs: the counter-move that cuts both ways

Phages encode anti-CRISPR (Acr) proteins that inhibit Cas effectors by blocking DNA binding, PAM recognition, nuclease activity or complex assembly; more than 100 families are now described across Types I, II, V and VI [7]. For therapy they are a double-edged consideration. A target strain carrying an Acr-encoding prophage can neutralise a CRISPR payload, which is a resistance mechanism that must be screened for. But Acrs are also a useful engineering reagent: supplying an Acr in *trans* protects a phage genome during editing in a Cas-expressing host, and Acr-based circuits can be used to confine nuclease activity in space or time.

4. CRISPR as a sequence-specific antimicrobial



Mechanism of an armed lytic phage · 3 outcomes, 1 escape band

The lytic cycle and the nuclease are complementary rather than redundant: lysis handles productive infections, and the payload reaches the cells where infection would otherwise abort.

4.1 Killing by self-targeting

The founding experiments were reported in 2014 by three groups working in parallel. Bikard and colleagues delivered Cas9 with guides against *mecA* and the *S. aureus* chromosome using a staphylococcal phagemid packaged in ϕNM1, killing methicillin-resistant *S. aureus* selectively within mixed populations and reducing colonisation in a mouse skin model [9]. Citorik, Mimee and Lu delivered Cas9 into *E. coli* on an M13 phagemid, achieving several orders of magnitude of sequence-specific killing against carbapenemase (*bla*NDM-1) and ESBL (*bla*SHV-18) carriers and extending survival in a *Galleria mellonella* infection model [10]. Yosef and colleagues built a lysogenisation system in which a temperate phage delivers Cas9 that destroys resistance plasmids and simultaneously confers sensitivity to lytic phage, so that surviving cells are selectively removed [11,12].

The design consequence is that a therapeutic guide RNA is chosen the way a PCR primer is: a sequence present in every target isolate and absent from everything else in the community — a species-specific housekeeping gene, a virulence determinant, or the resistance gene itself.

4.2 Curing plasmids and restoring antibiotic susceptibility

Targeting a plasmid rather than the chromosome produces a different and sometimes preferable outcome: the cell survives, minus its resistance. Because mobile genetic elements carry most clinically important transmissible resistance — *bla*CTX-M and *bla*SHV ESBLs, *bla*KPC, *bla*NDM and *bla*OXA-48 carbapenemases, *mcr-1* colistin resistance, *van*A in enterococci — a plasmid-curing agent converts a resistant population back into a treatable one and restores the existing antibiotic armamentarium rather than replacing it.

This has been demonstrated repeatedly: Cas9 delivered by conjugative plasmid re-sensitised *E. coli* and *Klebsiella* to β-lactams; CRISPR-mediated removal of *mcr-1* and *bla*NDM-5 restored colistin and carbapenem susceptibility; and conjugative delivery into *Enterococcus faecalis* cleared *ermB* and *tetM* in the mouse gut [15]. A subtlety worth noting is that plasmid curing has an important failure mode: if the plasmid encodes a toxin–antitoxin addiction system, losing it kills the cell anyway, so curing and killing are not always cleanly separable.

4.3 The three design levers

Taken together, the literature identifies three variables that determine whether a CRISPR antimicrobial works.

Guide choice sets the spectrum — species-wide, clone-specific or resistance-gene-specific — and therefore the collateral effect on the microbiota. **Effector choice** sets the escape rate: point-cutting Cas9 permits escape by target-site mutation, while processive Cas3 and RNA-targeting Cas13 raise that barrier. **Delivery efficiency** sets everything else, and it is the field's central problem. In all three 2014 studies, and essentially every study since, killing in culture reached 2–4 logs while in vivo effects were far smaller — not because the nuclease failed but because it reached a minority of cells. A nuclease that kills 99.99% of the bacteria it enters is useless if it enters 1% of them, which makes the vector the real object of study and the subject of the rest of this review.

4.4 Beyond the coliforms

Most of the literature above uses *E. coli*, for convenience rather than clinical priority, and the design problem changes outside that comfort zone. Gram-positive phages use wall teichoic acids or capsular polysaccharide as receptors rather than LPS and porins, so host-range engineering targets different proteins; and because transformation of large phage genomes into intact Gram-positive cells usually fails, the L-form route is in practice the only general method of synthetic assembly for *Listeria*, *Bacillus* and *Staphylococcus* [19]. Temperate phages dominate outside the coliforms — in *S. aureus*, where the earliest CRISPR antimicrobial work was done [9], and in *Clostridioides difficile*, where narrow spectrum is the entire point since the disease is caused by antibiotic disruption of the microbiota and phage-delivered Cas3 reduces burden in animal models [14] — so lysogeny deletion becomes a prerequisite rather than a refinement. Enterococci conjugate efficiently, making them natural targets for plasmid curing [15] though that same property is the biosafety concern, while mycobacteria, edited by BRED [16], combine a waxy envelope, an intracellular niche and documented neutralising-antibody responses [32] into the field's hardest delivery problem. Two generalisations hold: the vehicle, not the nuclease, must be re-engineered for each genus, and for anaerobes and intracellular pathogens the relevant reservoir — spores, macrophages, granulomas — may be inaccessible to any particle-based agent.

5. Engineering phage genomes: the toolbox

5.1 Why phage genomes were hard

Until roughly 2014, editing a phage genome meant homologous recombination between the infecting phage and a plasmid carrying homology arms, then screening for the rare recombinant. Recombination frequencies of 10^{-6} to 10^{-4} meant thousands of plaques screened per edit. The problem was not making the edit but finding it, because there is no selectable marker on a virion.

CRISPR solved this as a counterselection problem. Design a guide against the wild-type sequence being replaced; the edited genome, lacking the protospacer or PAM, is immune, while every unedited genome is destroyed. Enrichment of 10^{2}–10^{4}-fold turns an impractical screen into a routine one [17]. This is the

reason CRISPR matters twice in this field — the payload application is more publicised, but the counterselection application is what made engineered phages buildable at all.

5.2 The methods

CRISPR-Cas counterselection. A host expressing Cas9 or Cas12a with a guide against the parental sequence is infected with a mixed lysate after recombination with a donor template, so only recombinants plaque. The constraint is that the phage must not already be resistant — many encode Acrs or carry modified genomes (hydroxymethylated cytosine in T4, for instance) that Cas9 cannot cleave, in which case Type I-E or Cas12a systems serve as alternatives.

BRED. Developed for mycobacteriophages by the Hatfull laboratory, bacteriophage recombineering of electroporated DNA co-electroporates phage genomic DNA with a short double-stranded substrate into cells expressing Che9c recombineering functions. Recombinants arise at 10–15% without selection, high enough to screen by PCR; BRED produced the lysogeny-deleted Muddy derivative used in the 2019 *M. abscessus* case [16,31].

Yeast assembly and cell-free rebooting. The genome can be split into overlapping fragments, modified in vitro, assembled by homologous recombination in *Saccharomyces cerevisiae* and rebooted in a host; Ando and colleagues used this to build a modular T7 platform and retarget it by swapping tail-fibre genes, redirecting host range from *E. coli* to *Klebsiella* and *Yersinia* [18]. Arbitrary multi-site edits are made at once in a host the phage cannot kill, but rebooting efficiency is poor for large genomes and most Gram-positive hosts, where *Listeria* L-forms are used instead. Cell-free transcription–translation extracts synthesise infectious phages from purified DNA in hours with no living host at all, having rebooted T4, T7, ϕX174 and several *Bacillus* phages [20].

Retrons, base editing and variant libraries. Retron reverse transcriptases produce single-stranded donor DNA inside the cell, raising recombineering efficiency without separate template delivery, and base editors introduce point changes without a double-strand break [23]. The ORACLE approach builds libraries of thousands of RBP variants in one background and selects them against panels of clinical isolates [24], mapping host range rather than testing variants singly — the basis of most machine-learning work in the field.

5.3 Choosing a platform

Method	Typical host range	Edit types	Efficiency before selection	Main limitation
CRISPR counterselection	Broad; any Cas-tractable host	Point edits, deletions, insertions	10^{-6}–10^{-4}, enriched 10^{2}–10^{4}×	Phage Acrs; modified genomes resist cleavage
BRED	Mycobacteria, some actinobacteria	Deletions, substitutions, tags	10–15%	Host-specific recombineering functions needed
Yeast assembly + reboot	Gram-negative, mostly coliphages	Whole-genome redesign, multi-site	Assembly near-quantitative; reboot low	Rebooting efficiency; poor for Gram-positives
Cell-free TXTL	Genome-dependent, host-independent	Whole-genome synthesis and variants	Hours per cycle	Extract compatibility; scale
L-form rebooting	Gram-positive (*Listeria*, *Bacillus*, *Staphylococcus*)	Whole-genome	Moderate	Few validated L-form hosts
Retron / base editing	Broad	Point edits, gene inactivation	High, no DSB required	Limited edit types; PAM-constrained windows

Method	Typical host range	Edit types	Efficiency before selection	Main limitation
ORACLE libraries	Model phages (T7, T3)	RBP variant libraries	Thousands of variants per round	Needs a tractable modular backbone

In practice, programmes combine them: yeast or TXTL assembly to build the backbone, CRISPR counterselection to install payloads, and library screening to tune host range.

6. Design strategies for engineered therapeutic phages

An engineered therapeutic phage is a composition of independent design decisions. The seven below are the ones that recur across published programmes.

6.1 Arming the phage with a CRISPR payload

The payload is a Cas effector plus one or more guides, inserted into a non-essential region of the phage genome or supplied on a packaged phagemid. Three considerations dominate.

Three considerations dominate. *Cargo capacity*: *S. pyogenes* Cas9 is 4.1 kb before its promoter and guide cassette, a substantial fraction of a 40 kb genome and prohibitive for small phages, so compact orthologues (*S. aureus* Cas9, 3.2 kb; CasΦ, ~0.8 kb) or native Type I systems, where only the guide array needs delivering, are preferred. *Multiplexing*: guides against several loci raise the escape barrier multiplicatively, and Cas12a is convenient because it processes its own crRNA array. *Layered killing*: the key insight from the clinical programmes is that payload and lytic cycle should be complementary rather than redundant — a lytic phage already kills what it infects productively, so the nuclease adds value against cells where lysis fails, in abortive infections, biofilm and stationary phase, and in phage-resistant mutants.

6.2 Host-range engineering

Host range is largely a property of the RBPs, making it the most tractable engineering target. Three approaches are in use:

Modular swapping replaces the tail-fibre or tail-spike gene of a characterised backbone (T7 and T3 are the standard chassis) with that of a phage having the desired specificity; directed evolution recovers host-range mutants by serial passage or RBP mutagenesis, often from single amino acid changes at the fibre tip [25]; and library screening tests thousands of designed variants against isolate panels in one experiment [24]. The therapeutic goal is a small cocktail whose combined coverage approaches species level — for uncomplicated UTI, enough *E. coli* strains that empirical dosing is possible without prior susceptibility testing.

6.3 Genome curation, biofilm penetration and resensitisation

Deleting the integrase and repressor converts a temperate phage into an obligately lytic one, opening up the large majority of characterised phages; this was the key modification in the *M. abscessus* case, where three phages were engineered to remove the repressor gene [31]. Curation also means removing any toxin, resistance or virulence gene, a requirement regulators treat seriously. For biofilm — where most chronic resistant infection lives, and whose matrix excludes antibiotics and phages alike — phages can be armed with depolymerases that degrade capsular polysaccharide and matrix EPS, or with a dispersal enzyme such as DspB, as in the classic T7-DspB construct that reduced biofilm counts by about 4.5 logs, two orders of magnitude better than the unmodified phage [26]. A third option is to deliver a payload that restores drug susceptibility rather than killing: guides against resistance genes, CRISPRi silencing of efflux pumps or β-lactamases, or sensitising constructs such as the T7 phage expressing *lexA3*, which suppressed the SOS response and potentiated quinolone killing [27]. This last category treats resistance itself as the target and keeps the existing formulary in play.

6.4 Counter-defence and non-replicative delivery

Bacteria carry an expanding catalogue of anti-phage systems — restriction–modification, native CRISPR-Cas, abortive infection, retrons, CBASS, Thoeris, Gabija [8] — and engineered phages can carry counter-measures: anti-CRISPR proteins, anti-restriction proteins such as Ocr of T7, and methylation matched to the target strain's R-M system. Where lysis itself is undesirable, as in sepsis where endotoxin release is dangerous, the phage can instead be used purely as a delivery particle. A **phagemid** is a plasmid carrying a packaging signal and the CRISPR cassette, packaged into capsids by a helper system; the resulting particle injects DNA but cannot replicate. This was the format of the original Bikard and Citorik studies [9,10] and is the basis of Eligo Bioscience's platform. The trade-off is severe and defines the delivery problem of Section 7: with no self-amplification, the full dose must be delivered at the start and every bacterium reached by at least one particle.

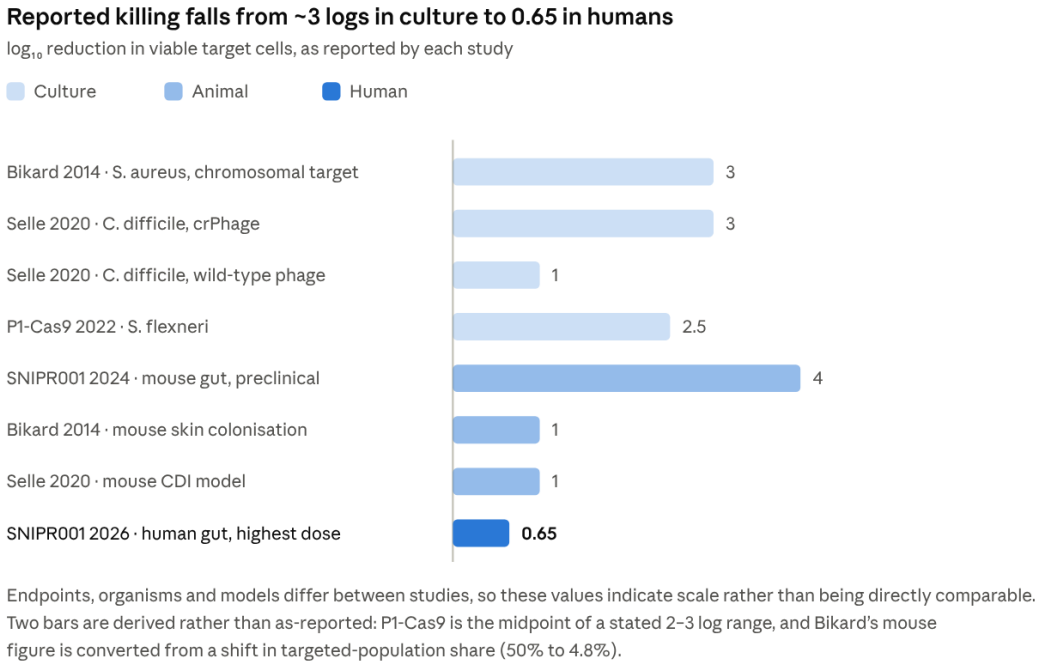
7. Delivery: the rate-limiting step

7.1 The arithmetic

CRISPR antimicrobials fail in vivo for an unglamorous reason. The fraction of cells infected depends on the multiplicity of infection (MOI) achieved at the site, not the dose administered. For a non-replicative particle, an average MOI of 10 — enough that Poisson statistics leave under 10^{-4} of cells uninfected — requires about 10^9 particles per mL of infected tissue, sustained against dilution, mucus binding, immune clearance and flow.

A replicating phage escapes this arithmetic because it amplifies where its host is present, provided bacterial density exceeds a critical value (typically 10^4–10^6 CFU/mL). Below that threshold replication does not sustain itself and the phage behaves like a conventional drug that is cleared. This is why residual low-density populations often survive treatment, and it is a strong argument for combining phages with antibiotics, which act efficiently at low density where phages do not.

7.2 The gap, quantified



Reported $\log_{10}$ reductions · 8 observations from 5 studies, 2014–2026

The most informative comparison is between the two SNIPR001 bars, because they are the same agent. In the murine gut it produced a 4-log reduction in *E. coli*; in the human gut, at the highest tolerated dose, it produced 0.65 logs [35,36]. Nothing about the nuclease, the guides or the capsid changed between

those two experiments. What changed was the host, the resident community and the scale and structure of the compartment the particles had to penetrate.

The broader pattern is consistent: agents that remove 99.9% of their target in culture remove far less in an animal, and less again in a person. The wild-type comparator in the Selle study is a useful control — arming the phage with Cas3 moved killing from 1 log to 3 in culture [14], so the payload works. The attrition happens between the flask and the body, which is why Sections 7.3 to 7.5 treat delivery, not nuclease engineering, as the binding constraint.

7.3 Delivery formats and their trade-offs

Format	Self-amplifies	Host range	Lysis / endotoxin	Biosafety profile	Typical use
Engineered lytic phage + CRISPR cargo	Yes	Phage-determined	Yes	Replicating GMO; environmental shedding questions	UTI, wound, respiratory infection
Phagemid in packaged particle	No	Capsid-determined	No	Non-replicative; self-limiting	Skin, gut, sepsis-adjacent settings
Temperate phage delivering lysogenic cargo	Partially	Phage-determined	No	Integration into host genome	Microbiome editing
Conjugative plasmid	Spreads by conjugation	Very broad, promiscuous	No	Hardest to contain; HGT risk	Gut decolonisation (preclinical)
Nanoparticle / LNP	No	Formulation-determined	No	Conventional drug profile	Early research; poor bacterial uptake

The tension is visible in the table: formats that spread well are the ones regulators find hardest to accept, and formats with clean biosafety profiles cannot reach enough cells. Conjugation is the most efficient means of moving DNA between bacteria in a dense community such as the gut, but an engineered conjugative element that spreads autonomously is close to the definition of what containment rules exist to prevent.

7.4 Site-specific obstacles

Delivery problems are not uniform. In the **gut**, phages must survive gastric acid and bile and find targets in a dense, spatially structured community, where mucus-adherent phages outperform planktonic ones [28]. In the **bloodstream**, the mononuclear phagocyte system clears particles within minutes to hours, and endotoxin from synchronous lysis is itself dangerous. In the **lung**, cystic fibrosis mucus is near-impenetrable at phage dimensions. In **biofilm**, diffusion and matrix binding limit penetration to the outer layers unless depolymerases are supplied. Each has an engineering answer — mucin-binding Ig-like domains, capsid engineering to reduce clearance, depolymerase arming — and none is solved.

7.5 Mitigating immune clearance

Phages are proteinaceous and immunogenic. Neutralising antibodies appear within one to two weeks of exposure and can reduce efficacy on re-treatment, which matters for chronic infection requiring repeated courses. Serial-passage selection of phages that resist clearance in vivo, capsid surface engineering, and rotating distinct phages between courses are all under investigation [32]. Encouragingly, the clinical experience to date suggests immune responses reduce exposure rather than cause harm, and single courses of intravenous phage have been generally well tolerated.

8. From bench to bedside

Engineered CRISPR-phages are no longer a preclinical concept. Two programmes have completed randomised human trials and published them in peer-reviewed journals, which makes this one of the few areas of synthetic biology where translational evidence can be assessed rather than anticipated.

Programme	Agent	Target / indication	Format	Status (Nov 2026)
Locus Biosciences	LBP-EC01 (crPhage)	*E. coli* uncomplicated UTI	Six-phage lytic cocktail armed with Cas3	Phase 2/3 ELIMINATE (NCT05488340); Part 1 published, Part 2 dosing
SNIPR Biome	SNIPR001	*E. coli* gut decolonisation in haematological cancer	Four CRISPR-Cas3-armed phages, oral	Phase 1 published Mar 2026; Phase 1b/2a recruitment complete
Eligo Bioscience	EB005	*C. acnes* in acne vulgaris	Non-replicative phage-derived particle (Eligobiotic)	Preclinical / IND-enabling; FDA Fast Track
Eligo Bioscience	EB003	Shiga-toxin-producing *E. coli*	Eligobiotic targeting *stx*	Preclinical

8.1 LBP-EC01: the furthest-advanced programme

LBP-EC01 is a six-phage cocktail of lytic *E. coli* phages engineered to express a Type I-E CRISPR-Cas3 system targeting the *E. coli* chromosome, with a reported in vitro host range covering 96% of a 356-strain panel of uropathogenic isolates [39]. The design logic is the layered killing described in Section 6.1: the phages lyse what they can infect productively, and Cas3 degrades the genome of cells where the lytic cycle would otherwise abort.

In the Phase 1b study no drug-related treatment-emergent adverse events occurred, and among subjects whose isolates were sensitive, 83% showed phage amplification — in vivo confirmation that the agent replicates at the infection site rather than merely being excreted.

Part 1 of the Phase 2 ELIMINATE trial was a randomised, open-label study in 39 adult female patients at six US centres, defining a dosing regimen for LBP-EC01 with trimethoprim–sulfamethoxazole. Primary and secondary outcomes were met and a well-tolerated regimen achieving high exposure at the infection site was identified [34]. The regimen combines intraurethral instillation with intravenous dosing, which is itself informative: reaching the bladder epithelium required local delivery as well as systemic administration. Subsequent review notes tolerability limits at higher systemic exposure [51], which constrains how far dose can simply be raised to close the gap in Section 7.2. Part 2 is randomised, double-blind and placebo-controlled, enrolling up to 288 women and following recurrence over six months — the more interesting endpoint, because it tests whether depleting the reservoir changes the natural history of the disease rather than only clearing an episode.

8.2 SNIPR001: oral decolonisation, and an instructive effect size

SNIPR001 is a cocktail of four phages, each armed with CRISPR-Cas3 and tail-fibre engineered [36] for coverage of fluoroquinolone-resistant *E. coli*. The indication is prophylactic — preventing *E. coli* bloodstream infection in neutropenic haematology patients by reducing the gut reservoir — a setting where broad-spectrum antibiotic prophylaxis causes significant collateral damage.

The Phase 1 trial (NCT05277350) randomised 36 healthy participants carrying more than 10^7 *E. coli* CFU per gram of stool to SNIPR001 or placebo across three dose cohorts (10^8, 10^{10} and 10^{12} PFU per dose, twice daily for seven days). No grade 3–4 and no serious adverse events occurred in the SNIPR001 groups, no phage was measurable in plasma, and phage recovered from faeces remained functional in amounts corresponding to the dose given [35].

The efficacy signal was more modest than the safety profile. The largest reduction in stool *E. coli* relative to placebo came at day 14 in the highest-dose group: 78%, or -0.65 $\log_{10}$ with a standard error of 0.64 — an estimate whose uncertainty exceeds its magnitude. This deserves stating plainly, because a well-

engineered, well-tolerated agent that reached its target compartment and demonstrably engaged its target still produced a sub-log reduction in a dense, spatially structured community. The limiting factor was delivery and ecology, not nuclease activity.

A randomised Phase 1b/2a trial in 24 haematological cancer patients has completed recruitment, and the programme holds FDA Fast Track designation. An expanded-access case in a kidney transplant recipient with progressive multidrug-resistant *E. coli* disease reported a marked clinical response with no phage-related safety signals — though a single case cannot establish that the CRISPR component was decisive.

8.3 Eligo and the non-replicative approach

Eligo Bioscience's "Eligobiotics" are phage-derived capsids carrying a CRISPR cassette but no phage genome, so they inject and edit without replicating. EB005 targets the subpopulation of *Cutibacterium acnes* carrying pro-inflammatory determinants, formulated as a topical gel — a well-chosen first indication, since the skin surface removes most of the delivery barriers that defeat systemic agents and the goal is to edit a community rather than sterilise a site. Recent pipeline updates still list EB005 as preclinical while vector bioproduction is scaled, a reminder that manufacturing rather than biology is often the gating step. EB003 applies the platform to excising the Shiga-toxin gene from STEC, an elegant case where disarming beats killing, since lysing a Shiga-toxin producer releases the toxin.

8.4 What the clinical record establishes

Three conclusions are supportable. Safety is reasonably well established: across the published exposures no serious drug-related adverse events have been reported for either programme, by oral, intravenous or intraurethral routes. Target engagement occurs in humans, shown by phage amplification at the infection site and dose-corresponding recovery of functional phage from faeces. And effect sizes are so far modest, sub-log in vivo against the 2–4 logs routine in culture. What is *not* established is clinical superiority: no engineered CRISPR-phage has beaten standard of care on a clinical endpoint in a controlled trial. ELIMINATE Part 2 is the first study positioned to answer that, and until it reports, claims about efficacy should be read as claims about mechanism.

9. A regional case study: Pakistan

Pakistan is an unusually informative test case for this technology, for three reasons: it carries one of the world's heaviest AMR burdens, it produced the clearest modern example of a resistance determinant that a CRISPR antimicrobial is well suited to remove, and it has an active phage-isolation literature but no engineered-phage programme. The gap between the second and third of those is the point of this section.

9.1 XDR typhoid: a purpose-built target for plasmid curing

In November 2016 the Aga Khan University laboratory detected an outbreak of ceftriaxone-resistant *Salmonella* Typhi in Hyderabad, Sindh, where such resistance had previously been found in under 1% of strains. The investigation identified 101 culture-confirmed cases between November 2016 and March 2017, concentrated in two subdistricts [41].

Whole-genome sequencing established what had happened, and the genetic detail is what makes this relevant. The organism was an endemic H58 (genotype 4.3.1) clone that had acquired a single promiscuous IncY plasmid carrying *bla*CTX-M-15 and *qnr*S, conferring ceftriaxone and fluoroquinolone resistance on top of a chromosomal MDR cassette already present. One plasmid acquisition therefore converted an MDR organism into an extensively drug-resistant one in a single step, leaving only azithromycin and carbapenems. By September 2017, 339 isolates with the same profile had been recovered from Sindh [40]; the clone reached Lahore, where sequenced isolates shared the Sindh genotype [42], and travel-associated cases were reported from five countries. Roughly 20,000 cumulative cases in Hyderabad and Karachi alone were documented by August 2021 [43].

Against the criteria in Section 4.2 this is close to an ideal target for plasmid curing. The resistance is plasmid-borne and the cell is viable without it, so removing the IncY plasmid should restore ceftriaxone and fluoroquinolone susceptibility rather than killing the host. The population is clonal, so a single guide set covers essentially the whole outbreak, removing the strain-matching problem that makes personalised

phage therapy slow. The targets are sequenced and public, and guides against *bla*CTX-M-15 would also act on the many other Enterobacteriaceae in the same settings that carry it. And with only azithromycin and carbapenems remaining, and azithromycin resistance already emerging, restoring a first-line drug has disproportionate value.

The obvious caveat is delivery, and it is a serious one. *S.* Typhi is an intracellular pathogen that resides within macrophages during systemic infection, which is the hardest compartment for any particle-based agent to reach, as Section 7.4 notes. A realistic near-term application would target gut carriage and the chronic gallbladder carrier state — where the organism is extracellular and the reservoir is accessible — rather than acute bacteraemia.

9.2 National burden

Country-level GBD analyses put Pakistan among the most heavily affected countries anywhere. The WHO Eastern Mediterranean Region analysis of GBD 2021 identifies Pakistan as the country with the highest absolute AMR burden in the region, with 31,000 deaths (95% UI 24,000–37,500) attributable to and 149,000 (121,000–177,000) associated with bacterial AMR in 2021 [49]; *Klebsiella pneumoniae* accounted for the largest attributable mortality, roughly 7,000 deaths. A South Asia analysis of the same cycle places Pakistan second only to India, with five South Asian countries exceeding 50 AMR-associated deaths per 100,000 [50]. GBD 2021 projects 11.8 million cumulative AMR-attributable deaths in South Asia between 2025 and 2050 [1] — roughly 30% of the global total from about a quarter of the world's population. Carbapenem resistance, where Pakistan's burden is particularly concentrated [45], is precisely where no small-molecule option remains.

9.3 Published Pakistani phage research

There is a substantial and growing domestic literature on phage isolation against MDR clinical isolates. The studies below are representative and all report quantitative antibacterial data.

Study	Institution	Target	Key reported data
Nawaz et al., Front Microbiol 2024 — phage ES10	Quaid-i-Azam University, Islamabad	MDR uropathogenic *E. coli*	[46] 99.34% reduction of host bacteria in human blood; 99.84% on contaminated fomites; genome free of resistance, virulence and lysogeny genes
Rasool et al., Jundishapur J Microbiol 2016;9(10):e36135	Government College University, Faisalabad	MRSA	[47] Lytic activity peaking 3–6 h post-infection; bacterial count reduction demonstrated in a rabbit model
Characterisation of phages PAA and PAM, 2025	TEM at NIBGE, Faisalabad	MDR uropathogenic *P. aeruginosa* (biofilm-forming)	[48] Synergy with sub-inhibitory meropenem and cefepime
Phage against MDR *P. aeruginosa* biofilm, 2019	Atta-ur-Rahman School of Applied Biosciences, Islamabad	MDR *P. aeruginosa*	Isolation from wastewater; activity against planktonic cells and biofilm

Two features deserve comment. The ES10 study [46] explicitly screened its phage genome for resistance, virulence and lysogeny genes and reported none — the genome-curation requirement of Section 6.3, performed by inspection rather than engineering. And hospital wastewater is the standard isolation source across all these studies, a genuine local advantage, since environments under high antibiotic selection are rich in phages adapted to resistant hosts. These values are not plotted in the Section 7.2 chart, whose inclusion rule is restricted to CRISPR-armed agents; converted for comparison, ES10's reductions correspond to roughly 2.2 and 2.8 $\log_{10}$.

9.4 The capability gap

Pakistan has demonstrated capacity for phage isolation, host-range testing, electron microscopy, genome sequencing and small-animal efficacy work across QAU, GCU Faisalabad, NIBGE and NUST. What is absent from the published record is any engineered phage: no lysogeny deletion, no RBP swapping, no CRISPR arming, no synthetic genome assembly.

That gap is narrower than it appears. CRISPR counterselection requires a Cas-expressing host strain and standard molecular biology rather than specialised equipment, and the sequencing capability needed to design guides already exists locally. The binding constraints are more likely regulatory — Pakistan has no approval pathway for a genetically modified live biological therapeutic — and the GMP standard discussed in Section 10.3, which no academic laboratory anywhere meets without dedicated investment.

The practical implication is that the highest-value domestic contribution is probably not to replicate Western engineered-phage programmes but to supply what those programmes cannot generate: well-characterised panels of local clinical isolates with sequenced resistance plasmids, matched wild-type phages, and the host-range and defence-system data needed to design agents for the strains that actually circulate here. Host range is the parameter that fails first when a product designed against one region's strains is deployed in another.

10. Obstacles and open questions

10.1 Resistance, which does not disappear

The claim that CRISPR-phages are immune to resistance is marketing, not biology. Two of the escape routes are properties of the phage vector and are set out in the limitations table in Section 2.3: receptor loss or modification, and the host's native defence systems. Arming the vector with a nuclease adds three further routes that are specific to the payload.

The most useful framing is evolutionary rather than absolute. Receptor loss is a real escape route, but when the receptor is a virulence factor — an efflux component, LPS core, or a capsule — escape carries a fitness cost, and a phage can be chosen deliberately so that resistance to it entails attenuation. This "evolutionary steering" logic, demonstrated for *Pseudomonas aeruginosa* phages that bind the OprM component of a multidrug efflux pump [29], is arguably more valuable than any single-nuclease improvement: it makes the escape mutant easier to treat with antibiotics than the parent.

10.2 Pharmacology and immunogenicity

Standard pharmacokinetics assumes a dose that decays; a replicating phage instead grows where its substrate is abundant, so concentration–time curves are non-monotonic and depend on bacterial density. AUC, Cmax and half-life are poorly defined, and dose–response may be flat above a threshold rather than graded. What dose-finding means here is still unsettled — one reason ELIMINATE Part 1 was devoted entirely to defining a regimen.

Immunogenicity compounds this. The antibody response and its countermeasures are described in Section 7.5; what remains open is durability over repeated courses, which matters most for exactly the chronic and recurrent infections where a narrow-spectrum agent is otherwise most attractive. No engineered product has been studied over the multiple courses chronic use would require, so whether efficacy is maintained, degrades gradually, or fails after the second exposure is simply unknown.

10.3 Biosafety, manufacturing and regulation

An engineered, replicating, self-disseminating antibacterial raises containment questions a small molecule does not: horizontal transfer of the CRISPR cassette into non-target bacteria, environmental persistence and shedding from treated patients, and the possibility that a guide acquires new specificity. Mitigations — non-replicative particles, deletion of transduction functions, inducer-dependent kill switches, narrow host-range capsids — each trade efficacy against containment, the same trade-off that runs through Section 7.

Manufacturing is a separate obstacle. Phages are produced by infecting a host strain, so production requires culturing the pathogen, a GMP challenge when that pathogen is a multidrug-resistant clinical isolate; downstream processing must remove host endotoxin to parenteral standards, and genetic stability

over production passages must be demonstrated, since phage populations evolve during propagation. Plaque-forming-unit potency assays are variable and do not measure payload activity, so release testing needs orthogonal assays. Regulation compounds both. Engineered phages sit at the intersection of biological products, genetically modified organisms and, in some formulations, gene therapies; in the EU, GMO legislation applies alongside medicinal product law and clinical use requires environmental risk assessment. The deeper problem is that regulation is built around fixed products tested in large populations, whereas phage therapy's natural form is personalised matching to a patient's isolate. Accommodations are emerging — the Belgian magistral framework, FDA expanded access, platform designations where a validated backbone accepts new guides — but none yet constitutes a routine route to approval.

10.4 Microbiome effects

Precision is the central claim, and it is partly validated: SNIPR001 Phase 1 data showed target engagement without substantially disturbing overall microbiome composition. But targeting a conserved gene in *E. coli* also hits commensal *E. coli*; guides against resistance genes hit whichever commensals carry them; and removing a resident population opens a niche something else will occupy. Precision at the level of a sequence is not precision at the level of an ecosystem, and long-term community effects remain unmeasured.

11. Future directions

Computational design and synthetic genomes. Library methods such as ORACLE are mapping the sequence determinants of receptor binding at scale, producing the structured sequence–function data that protein language models consume; the near-term goal is to predict which RBP variants will bind a given isolate and synthesise a matched phage rather than screening for one, converting personalised therapy from a weeks-long search into a design task. Cell-free rebooting and whole-genome assembly together make it feasible to design a minimal, modular genome free of unknown ORFs, with defined payload insertion sites — which also answers a regulatory question natural phages cannot.

Phage–antibiotic synergy as the default. Phages amplify at high bacterial density, antibiotics act efficiently at low density, and both lead clinical programmes dose in combination rather than as monotherapy. The realistic near-term role is as an adjunct that restores the usefulness of existing drugs.

In situ microbiome editing and diagnostics-coupled deployment. The broader application is removing a gene from a community without removing the community — excising Shiga-toxin or resistance genes from gut *Enterobacteriaceae*, or inflammatory determinants from skin commensals — reframing the goal from sterilisation to genetic sanitation, which is probably where the technology's distinctive value lies. This requires precision diagnosis: rapid sequencing-based identification of the infecting strain, its receptor profile, its defence systems and its Acr content. The 2025–26 literature has begun to formalise this as a closed-loop model pairing molecular resistance profiling with matched engineered phage selection [52,53].

Access. AMR burden falls disproportionately on low- and middle-income countries, which is also where a locally manufacturable, strain-matched antibacterial would be most valuable and where cold-chain, GMP and regulatory infrastructure is least available. Phage production is technically simpler and cheaper than small-molecule synthesis at small scale, so distributed manufacturing is plausible in a way it is not for antibiotics — but only if regulatory frameworks and funding models are built for it deliberately.

12. Conclusion

The engineering problem is largely solved. A decade ago, modifying a phage genome was a screening ordeal and arming one with a programmable nuclease was a proof of concept in culture. Today phage genomes can be assembled in yeast, rebooted in cell-free extracts, edited by CRISPR counterselection or base editing, and screened as libraries of thousands of variants; host range, lysogeny, cargo and defence evasion are all design parameters. Two engineered CRISPR-phage products have completed randomised human trials with clean safety profiles and demonstrated target engagement.

What remains unsolved is pharmacology. The recurring finding is a gap of two to three orders of magnitude between killing in culture and killing in a patient, and it is not caused by the nuclease failing. It is caused by particles failing to reach bacteria — in biofilm, in mucus, in the structured interior of the gut, past immune clearance and dilution. Every major design decision in the field, from replicative versus non-replicative formats to combination dosing with antibiotics, responds to that single constraint.

The honest near-term expectation is not that CRISPR-phages replace antibiotics but that they occupy niches where their properties are decisive: decolonising reservoirs before high-risk procedures, treating recurrent infections where a narrow agent spares the microbiome, resensitising resistant populations so existing drugs work again, and removing virulence or resistance genes from communities that should not be destroyed. Pakistan's XDR typhoid clone is a concrete instance of the third. These are roles no small molecule can fill. Whether the field reaches them depends less on further advances in genome editing than on delivery, manufacturing and a regulatory framework built for programmable, self-replicating medicines.

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