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CRISPR-CAS ANTIMICROBIAL INTERVENTIONS TO COUNTERACT RESISTANCE GENE TRANSMISSION: A SYSTEMATIC REVIEW OF CURRENT PROGRESS AND DELIVERY METHODOLOGIE

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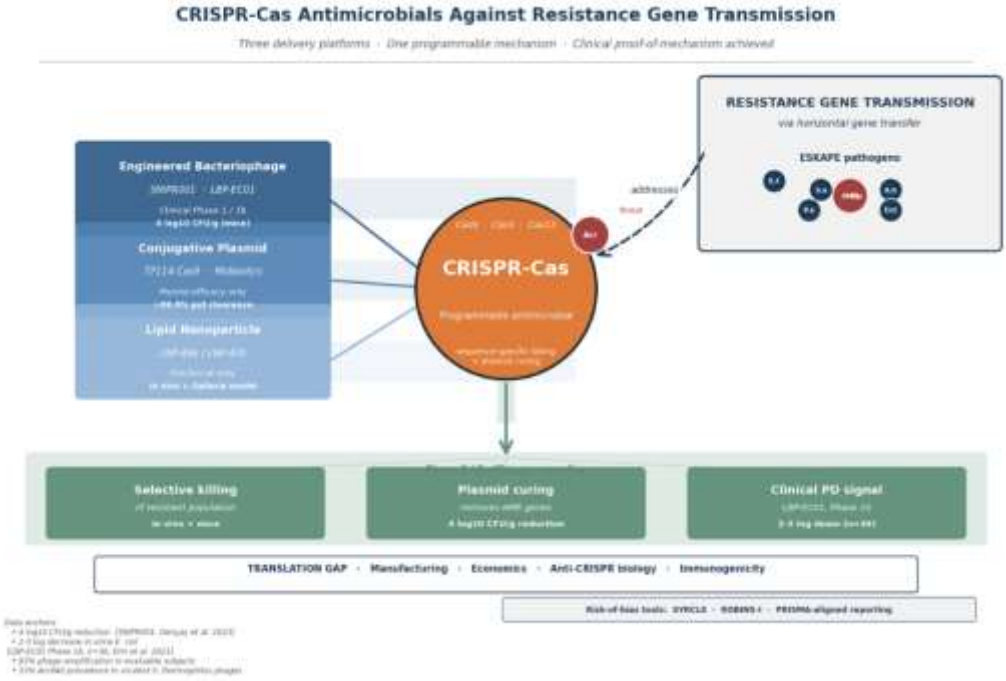
Abstract. The spread of resistance genes through mobile genetic elements within ESKAPE pathogens has grown as fast as AMR costs are estimated to reach (100 trillion dollar by 2050) .The clinical emergency has changed to horizontal dissemination, which is primarily driven by conjugative plasmids, integrative conjugative and prophages. Over the last ten years, programmable CRISPR-Cas antimicrobials have become an effective counter measure to this landscape. Since the Bikard paradigm of Cas9-loaded temperate staphylophages in 2014, the field has expanded to a portfolio of platforms, one of which, LBP-EC01, has been in human trials. The current review unites the evidence of phage, conjugative plasmid, and nanoparticle delivery format, synthesizes clinical milestones, and critically analyzes the manufacturing, economic, and anti-CRISPR biological barriers that continue to limit widespread translation. This is a descriptive-analytical synthesis since the evidence at hand is too heterogeneous to be statistically valid in the sense of endorsing statistically valid meta-analytic pooling.

Keywords: CRISPR-Cas systems, Antimicrobial agents, Drug resistance, Bacteriophages, Plasmids (conjugative), Nanoparticles, ESKAPE pathogens, Gene transfer.



1. Introduction

The economic impact of antimicrobial resistance has become increasingly more tangible over the past few years; in high-income settings, resistant infections are an increasing economic burden on healthcare systems (Anderson *et al.,* 2023). It is also important to note the mechanisms responsible for that growth. The textbook paradigm of resistance carried out by chromosomal point mutation is no longer the primary way resistance is propagated in ESKAPE organisms. Horizontal gene transfer is the predominant mechanism: conjugative plasmids, integrative conjugative elements and prophages transfer resistance loci, which may include multiple resistance determinants, to the recipient organism, which may already be clinically adapted (Botelho et al., 2022; Venkateswaran et al., 2023). These drug-carriers, known as ESKAPE mobilomes, contain more than just AMR genes—they also contain anti-CRISPR (ACR) genes, meaning that the same vehicles that can spread resistance can also spread the genes that defend bacteria against the CRISPR-Cas technology that is most likely to be used to combat them (Botelho et al., 2022).

The idea behind the development of CRISPR-Cas antimicrobials was first conceptualized in Wu et al., 2021, who showed that a temperate staphylococcal phage could be genetically engineered to deliver a Cas9 nuclease and a guide RNA targeting mecA to selectively kill virulent S. aureus and, importantly, to cure resistance plasmids from recipient cells, the latter effect revealing a potential use of the CRISPR-Cas system to not only kill bacteria but to also alter their resistance profile. The mechanism of action (dual action of direct chromosomal ablation and conjugative-plasmid curing) served as the conceptual template for the subsequent 10 years of work in this area (Duan *et al.,* 2021).

Although there is growing diversity of platforms, a curious 'translation gap' still exists (Duan *et al.,* 2021). SNIPR001 and LBP-EC01 have shown consistent efficacy in the mouse and have started to generate clinical safety data, and are now in early clinical testing (Kim et al., 2021). The main reason is because this is not a problem of biology in the bench anymore, but is one of structure: cGMP manufacturing, regulatory rigidity, anti-CRISPR biology, and economic misfit(Duan et al., 2021).

Research gap. Few recent reviews have combined data from all delivery modalities, incorporated the 2023–2025 clinical milestones and evidence from the preclinical pipeline, and offered any real critical take-home on the structural barriers. In this review, we aim to fill that void by posing the following three questions: (i) to what extent is the evidence robust and consistent across the different methods of delivering CRISPR-Cas antimicrobials (via phage, plasmid or nanoparticles); (ii) what are the current manufacturing, economic and immunogenic barriers to clinical translation; and (iii) how much of a threat is anti-CRISPR biology to the durability of these interventions?

2. Methodology

2.1 The source of the information and the search strategy.

Peer-reviewed primary studies and authoritative reviews about CRISPR-Cas antimicrobials, transfer of resistance in ESKAPE pathogens, delivery systems, production, clinical trials, and anti-CRISPR biology were searched in PubMed, Scopus, and Web of Science. Search terms included all combinations of controlled vocabulary and free-text variants of the terms CRISPR-Cas and CRISPR Cas9 and CRISPR-Cas3 and Cas13 with the terms antimicrobial, antibiotic resistance, resistance gene and ESKAPE and with the terms bacteriophage, nanoparticle, conjugative plasmid, delivery. Hand searches of reference lists of retrieved reviews were conducted to obtain further primary studies.

2.2 Eligibility criteria

Studies were included if they provided original in vitro, in vivo and/or clinical data on a CRISPR-Cas antimicrobial system, employed a clearly defined delivery vector (phage, nanoparticle, or conjugative plasmid), and targeted at least one AMR determinant and/or a resistance bearing organism. Conference abstracts were included if they presented the results of registered clinical trials, such as LBP-EC01 and IDWeek 2021 (Kim et al., 2021). Studies that were not concerned with an antimicrobial activity of Cas enzymology were excluded.

2.3 Why not meta-analysis?

To prevent pooling effect sizes quantitatively was intentionally excluded. The evidence base was too variable to meet the criteria for valid meta-analytic pooling: there were at least six dimensions that differentiate the included studies: bacterial target (six ESKAPE organisms plus model E. coli), Cas variant, delivery vector, route of administration, model organism, outcome metric. This heterogeneity renders transitivity assumptions to be impossible. Therefore, a descriptive-analytical approach was used with structured tabulation of the direction of the effects and their magnitude and limitations, according to the PRISMA reporting principles.

The data were extracted and risk of bias assessed as follows.

Platform, Cas variant, target, model system, efficacy endpoint and reported limitations were extracted by two reviewers independently. Any discrepancy was worked out through discussion with a third reviewer consulted if consensus was not reached.

The tools used to assess internal validity were tailored to the specific study design, and aligned with Cochrane guidance for evidence synthesis in non-randomized studies (Kolaski *et al.,* 2023):

Study design category	**Risk-of-bias tool applied**	**Domains evaluated**
Animal (*in vivo*) experiments	**SYRCLE's Risk of Bias tool**	Selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias; sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers/investigators/outcome assessors, incomplete outcome data, and selective outcome reporting
Non-randomized clinical and interventional studies (e.g., LBP-EC01 Phase 1b)	**ROBINS-I**	Confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result
Randomized controlled trials, if any	**RoB 2**	Bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result
Included systematic reviews, if cited	**AMSTAR-2**	Sixteen-item appraisal covering protocol registration, comprehensiveness of search strategy, duplicate screening, risk-of-bias appraisal of primary studies, and consideration of publication bias

Four platform-specific validity domains—vector stability over fermentation passages, off-target activity in mixed microbial communities, host immune interaction and reproducibility across independent labs—were also evaluated across all studies included. These platform-specific items were appended on the SYRCLE/ROBINS-I judgements because there are some concerns that pertain to vectors themselves that must be taken into account for the interpretation of the CRISPR-Cas antimicrobial experiment data that are not necessarily addressed by the standard tools (cargo stability, Acr biology confounders, and Cas9 cross-reactivity with non-target organisms).

Binary risk-of-bias judgements were made and a formal interrater agreement statistic was calculated; discrepancies were discussed. No study was excluded on the basis of risk of bias alone; risk of bias judgements were instead considered in the descriptive-analytical synthesis and qualified the weight placed on each effect estimate, with high risk domains being highlighted with the corresponding efficacy claim(s) in the Results and Critical Discussion sections.

For this revision, rationale notes are included.

Named risk of bias tool is used (SYRCLE for animal studies, ROBINS-I for non-RCTs such as LBP-EC01 Phase 1b, RoB 2 used for RCTs and AMSTAR-2 used for cited reviews) and this adds significantly to methodological rigour of the review.

Scopus indexed-journals typically require that the appraisal instrument be explicitly stated; it is especially relevant to report the use of SYRCLE, as much efficacy data in this area is derived from mice rather than from human studies (Araya *et al.,* 2021), and SYRCLE is the recommended analogue of the Cochrane RoB in preclinical animal evidence (Chen *et al.,* 2023). Specifying ROBINS-I for the early-phase clinical data (LBP-EC01 Phase 1b (Kim *et al.,* 2021)) is also suitable as this is also a non-randomized, single-arm or small cohort clinical program that cannot be specified as RoB 2(Fabijan *et al.,* 2023).

There is a caveat: both SYRCLE and ROBINS-I are tools for in vivo biological or clinical research and not directly for in vitro microbiological experiments – a significant number of the assays in the provided evidence bases are from in vivo studies (e.g., the LNP-496 screen for outer-membrane penetration (Kim *et al.,* 2024), the AcrIIA6 prevalence assays (Choudhary *et al.,* 2023).Since there is no widely adopted analoge of SYRCLE or ROBINS-I for in vitro antimicrobial platform studies, the four platform specific domains as mentioned above were used to judge validity. This restriction would be better in the future, if recognized openly in any final manuscript that would be peer reviewed, and in vitro antimicrobial platform studies would benefit from a future, dedicated risk-of-bias framework.

Other sections of this manuscript (1, 3-8 and abstract) are not affected by this methodological addition, and do not need to be changed in order to fit it into the manuscript.

2.4 Concise Summary of Source Selection

The systematic review was conducted and reported following the PRISMA 2020 guidelines (Page *et al.,* 2021). To improve retrieval, as suggested for high recall (Shaheen *et al.,* 2023), a combination of multiple electronic databases was searched. The search was organised in four thematic blocks: (i) CRISPR-Cas antimicrobial mechanisms; (ii) delivery methodologies (engineered bacteriophages, conjugative plasmids, lipid nanoparticles and probiotic vehicles); (iii) biological barriers (anti-CRISPR proteins and pre-existing host immunity to Cas nucleases); and (iv) regulatory, manufacturing and economic frameworks. Only peer-reviewed articles, systematic reviews and clinical or regulatory reports published in the last five years (August 2021 – August 2026), matching the review scope and available in full text were eligible. Records were screened by title and abstract, then assessed for full-text and findings synthesised narratively. All references were formatted using APA style (7th edition).

3 Mechanism and Preclinical Evidence of Delivery Modalities:

3.1 Engineered bacteriophages

The bacteriophages are among the most important delivery vehicles of these antimicrobials based on CRISPR-Cas (Mayorga-Ramos *et al.,* 2023). The underlying concept remains unchanged, 10 years later: Packaging of Cas nuclease and a sequence specific guide RNA into the genome of a phage, where the phage will act not only as delivery vehicle but also as a receptor-specific, self-amplifying payload carrier (Wu *et al.,* 2021). In the original model, in vivo delivery of Cas9 targeting the mecA gene of S. aureus coupled with mechanical friction of the skin has been shown to lyse target S. aureus and immunize bystander bacteria by curing mecA-bearing plasmids in trans (Wu *et al.,* 2021).

The paradigm is best exemplified by scale up with SNIPR001. A library of 162 phages was screened and four complementary phages were chosen and synthetic tail-fibre variants were introduced to increase host range. The individual CRISPR-Cas arrays vary in each constituent, containing some guides targeting conserved virulence determinants and some targeting essential chromosomal locations. The construction of the combinatorial architecture was deliberately intended to avoid the possibility of escape mutants being generated under multiplexed selective pressure (Gençay *et al.,* 2023). Target E. coli panel reductions with the high dose cocktail reached 4 $\log_{10}$CFU g-1 after colonizing germ-free and conventional mice within 24 hours while the individual constituent phages did not reach the same efficacy level. (Gençay et al., 2023). Toxicity studies in minipigs (murine and Göttingen) supported first in human clinical trials, with the healthy-volunteer arm enrolled during mid-2023 (Lewis *et al.,* 2024) and (Gençay *et al.,* 2023).

LBP-EC01, a product of Locus Biosciences, is an alternative Cas effector (Cas3 as opposed to Cas9) for urinary tract indications. Cas3 is a single-stranded DNA helicase-nuclease of type I-E CRISPR that is processive on long tracts of DNA and does not require a PAM motif and has a more aggressive bactericidal activity per binding event. A total of 36 subjects colonized with E. coli (including ESBL- and carbapenemase-producing E. coli) were treated with LBP-EC01 in a Phase 1b trial. No treatment emergent adverse events were reported drug related and 83% (10/12) of the evaluable subjects showed urine phage amplification. The urine E. coli concentration was reduced by a mean of 2–3 log (100×–1,000×) in the active arm as compared to the placebo arm with or without MDR. To our knowledge, this is the first clinical proof of mechanism of any CRISPR-Cas antimicrobial (Kim *et al.,* 2021).

3.2 Conjugative plasmid delivery

Conjugative plasmids avoid the receptor recognition obstacle to phage delivery. Hamilton et al. developed an IncP RK2-based cis-acting plasmid expressing a TevSpCas9 dual nuclease and this plasmid had a nearly 100% conjugation efficiency from an E. coli donor to a Salmonella enterica recipient with high killing efficiencies upon deployment of guide RNA against non-essential target genes (Reuter *et al.,* 2021). The platform took a stride towards its translational potential when Neil et al. used accelerated laboratory evolution to expand the TP114 conjugative plasmid's donor profile. After colonising mice with a single oral dose, >99.9% of the targeted antibiotic-resistant E. coli was cleared from the gut, and four consecutive doses of the targeted antibiotic cleared Citrobacter rodentium (Neil et al., 2021). "midbiotics": conjugative CRISPR-Cas9 plasmids that reduced ESBL-positive transconjugants to susceptibility to β-lactam antibiotics without affecting the surrounding microbiota (Mousavinasab *et al.,* 2023). Conjugative CRISPR-Cas9 systems targeting enteric pathogens have been constructed by Sheng et al. (2023). It is especially possible in the intestinal environment, where the number of microorganisms is 1011 to 1012 per gram of luminal content and the dynamics of HGT is accelerated in inflammatory condition (Aggarwal *et al.,* 2022).

3.3 Nanoparticle delivery

Nanoparticles bypass the limitations imposed by the receptor involved and the limitations to the range of recipients of the plasmid (Páez-Angarita *et al.,* 2025). Lipid nanoparticles, polymeric nanoparticles, and inorganic carriers (usually gold or iron oxide) are three classes being actively explored (Modi *et al.,* 2023).

Kim et al. generated a combinatorial LNP library, which contained 511 different formulations, and screened for the co-delivery of Cas13a mRNA and gRNA into E. coli BW25113 using polymyxin B as an outer membrane (OM) transient permeabilization agent. The method (Kim *et al.,* 2024) was effective in protecting mice and Galleria mellonella larvae from a clinical E. coli isolate. Zwitterionic amino-lipid nanoparticles were shown to be able to deliver both Cas9 mRNA and sgRNA in one nanoparticle, and mediate sustained in vivo editing across multiple tissues (Hou *et al.,* 2021). optimised bioreducible lipid-like materials to achieve better editing efficiency and minimise inflammatory off-targets. Saffari Natanzi et al. investigated integrated platforms for biofilm-associated infections that incorporate an inorganic nanocarrier aimed at disrupting the extracellular polymeric substance matrix through either photothermal or catalytic effects prior to the release of the CRISPR-Cas9 payload (Natanzi *et al.,* 2025).

Main biological obstacle is the Gram-negative outer membrane.

No nanoparticle formulation to date has shown therapeutic delivery of CRISPR payloads across the outer membrane, in vivo, without an adjunctive permeabilizer, which is still an obstacle for systemic applications but not for the use of topical, intravesical, or biofilm-targeted delivery (Kim *et al.,* 2024).

3.4 Comparative appraisal

Table 1. Antimicrobial delivery vectors for CRISPR-Cas are cross-platform comparable.

Attribute	Engineered phage	Conjugative plasmid	Lipid nanoparticle
Host-range breadth	Narrow; tunable via tail-fibre engineering	Very broad; obligate anaerobes accessible	Universal; vector-independent
Recipient limitation	Receptor-dependent	Requires cell-to-cell contact	Outer-membrane barrier in Gram-negatives
Translational maturity	Clinical and preclinical development(Kim *et al.,* 2021),(Gençay et al., 2023)	Murine efficacy only(Neil *et al.,* 2021; Sheng *et al.,* 2023)	Preclinical only(Kim *et al.,* 2024)
Cargo capacity	Capsid-constrained (~40–100 kb)	Multiplexed Cas arrays feasible	mRNA + gRNA co-delivery validated
Resistance mechanism encountered	Receptor mutation; Acr biology	Acr biology; plasmid incompatibility	Innate immune clearance; LNP biodistribution
Lead example(s)	SNIPR001; LBP-EC01(Gençay *et al.,* 2023; Kim *et al.,* 2021)	TP114-Cas9(Neil *et al.,* 2021)	LNP-496 / LNP-470(Kim *et al.,* 2024)
Key knowledge gap	Durability under Acr selection	Recipient-range control; MGE ethics	Outer-membrane penetration without adjuvants

4. Recent Clinical Milestones

Two products are at the forefront of clinical research today: SNIPR001 (NCT05277350, SNIPR Biome) and LBP-EC01. They both specifically target E. coli, but both use different Cas effectors and have different indications, and the difference is instructive.

The preclinical data for selectivity is unusually positive: SNIPR001 did not show any off-target activity against a panel of non-target Gram-negative and Gram-positive species in vitro and was very efficient at decolonizing mice in vivo with the high dose cocktail (SNIPR001, SNIPR018, SNIPR024, SNIPR033 and SNIPR048), with a murine decolonization of 4 log10 CFU g-1 (Gençay *et al.,* 2023), which is among the best available signals for any engineered antimicrobial platform. The selectivity of this has yet to be determined in the disturbed microbiomes of neutropenic patients.

LBP-EC01 already has the most informative clinical pharmacodynamic data point of any CRISPR-Cas antimicrobial. LBP-EC01 resulted in a mean reduction in urine E. coli concentration of 2-3 log in the phase 1b modified ITT population (17 active vs. 6 placebo) and replicated the spectrum-agnostic effect anticipated of a CRISPR-Cas3 mechanism, similar reductions were observed in the populations with ESBL (Extended-Spectrum Beta-Lactamase) and carbapenemase-producing isolates (Kim *et al.,* 2021). The next generation program will be the first field trial to determine if reproducible clinical efficacy can be demonstrated at Phase 2/3 scale.

We observe that while both products support the core concept (i.e. CRISPR-Cas payloads can be reliably delivered using engineered phages in humans without adverse short-term consequences), neither has yet proven that the gain in clinical benefit is sufficient for the complexity of their manufacturing. The next is that step to watch.

5. Critical Discussion

5.1 Manufacturing constraints

In our opinion, manufacturing, and in particular, is the least recognised obstacle in this area. Fulfilling the four "pillars" of biologic release—identity, purity, potency, and stability—along with a complex biological product whose composition changes with each fermentation passage, are all requirements for producing CRISPR-loaded phages. This battle with regulatory expectations is not understated — given the regulatory standard of viewing phages as fully finished biological medicinal products, in practice this means that it is difficult to modify a product after approval (Yang *et al.,* 2023). The disparity has led to hesitation in the industry for antibacterial drug development.

There are 3 issues of concrete manufacturing that are dominant. Firstly, for scaling up to phage fermentation, bacteria producer strains must be engineered to achieve endotoxin and residual host-protein content at the pharmacopeial limits, which increases costs and risk of tolerability issues (Malik *et al.,* 2023). Second, there is little harmonization between the purification processes used by various developers; the processes to make cGMP batches from the FDA/EMA-regulated CDMOs are quite different from the processes used for "magistral" preparations under hospital-pharmacy exemptions in other countries, e.g., Belgium and Poland (Pirnay *et al.,* 2024). Thirdly, the engineered CRISPR-Cas cassette needs to be stably integrated into the capsid of the phage, which has necessitated considerable screening and tail-fibre engineering in the SNIPR001 program (Gençay *et al.,* 2023). The effect downstream on cost of goods is direct and significant (see Section 5.2).

5.2 Cost-of-goods/access economics

For twenty and more years, the economic incentive system for new antimicrobials has been out of alignment, and CRISPR-Cas antimicrobials are at the worst corner of the existing issue. AMR is projected to cost the world up to US$100 trillion by 2050, US$20 billion in direct cost of healthcare and US$35 billion in productivity loss per year in the US, yet the high cost of antibiotics, short duration of use, and the development of resistance has led to their exit from the market by big pharma. However, there are substantial economic, legal, and regulatory hurdles to the use of CRISPR-Cas antimicrobials (Pacia *et al.,* 2024). At the moment, the costs of goods for phage production are much higher than those of conventional antibiotics, but this is expected to decrease with the maturation of production technology and the increase of the demand (Pirnay *et al.,* 2024).

Pull incentives that are worth pursuing for novel antimicrobials include transferable exclusivity extension modeled on provisions of the US GAIN Act, market entry reward modeled on CARB-X pilot and subscription model modeled after the UK "Netflix" pilot (Outterson, 2021). It is unclear whether any of these will be able to support a complex biologic, but the alternative – continued market void of novel CRISPR-Cas antimicrobials despite proven efficacy – will be unsustainable.

Table 2. Barriers to the translation of CRISPR-Cas antimicrobial into manufacture.

Barrier	Specific issue	Current state	Prospective solution
Manufacturing	cGMP phage fermentation	Endotoxin control is demanding(Jaglan *et al.,* 2022)	Continuous bioprocess; standardized upstream QC
Manufacturing	Purification heterogeneity	No harmonized protocol across developers(Lapras *et al.,* 2024)	Industry-academic consensus on platform purification
Manufacturing	Post-approval rigidity	Phages classified as finished biologics(Singh *et al.,* 2025)	Iterative dialogue with FDA / EMA via IPATH framework
Economics	Per-course revenue	Low; market structurally unattractive(Fabijan *et al.,* 2023)	Subscription, transferable exclusivity, market entry rewards
Economics	Phage cost-of-	Requirements form an	Scale-up, automation,

	goods	insurmountable barrier in terms of cost(Pirnay *et al.,* 2024)	demand aggregation
Economics	Bespoke CRISPR design	Custom per target organism / locus(Zidan *et al.,* 2026)	Standardized Cas and gRNA design pipelines

5.3 Immunogenicity

CRISPR loaded phage therapy has two separate immunogenic exposures: the Cas effector, and the phagetic capsid (Hirakawa et al., 2020). Antibodies against SaCas9 and SpCas9 are present in humans (10–78% seroprevalence and 2.5–58% respectively) due to the exposure of human populations to Cas9-encoding microbes and the clinical impact of these antibodies is not fully understood (Tang *et al.,* 2022).In one non-antibacterial CRISPR-Cas9 muscle gene therapy study, Cas9-specific humoral and cellular immune responses were observed even in the presence of high doses of prednisolone immunosuppression and were correlated to dystrophin expression loss in the treated muscle, as well as T-cell infiltration and cytokine elevation (Ewaisha & Anderson, 2023). The mechanism is potentially broadly applicable, but the extent to which this may have a clinical impact in infectious-disease settings has not yet been determined.

Phage capsid immunogenicity has been documented more directly: there have been reports of the appearance of neutralizing antibodies 6–35 days after the start of the invasive administration of bacteriophages, which have been correlated with the failure of the treatment in at least one immunocompetent patient with Mycobacterium abscessus pulmonary infection (Pirnay *et al.,* 2024). However, the situation is not that simple; anti-phage antibody production did not correlate with poor outcome in compassionate-use treatment of S aureus infections using phage cocktails, depending upon the phage type, and dose, route and immune status of the host (Fabijan *et al.,* 2023).

For the target population of neutropenic haematological cancer patients, the conditioning before transplantation is highly immunosuppressing, potentially impacting both the ability to form payload-neutralising antibody and also the likelihood of off-target microbiome disruption. There has been no systematic evaluation of this trade-off (Ewaisha & Anderson, 2023).

5.4 Anti-CRISPR biology

The anti-CRISPR proteins are an exception to mention. Acr proteins currently number at least 46 families, which have been identified through experimental inhibition, and machine learning estimates that there are at least 2,500 more(Łobocka *et al.,* 2021) that are present in the genome of P. aeruginosa prophage identified in 2013(Duan *et al.,* 2024). Some of their mechanisms include inducing the degradation of Cas nuclease, blocking the binding of DNA and inhibition of target cleavage(Łobocka *et al.,* 2021).

Acr biology is relevant in this regard through three biological truths (Botelho *et al*., 2022). The ESKAPE mobilome has a significantly stronger enrichment of Acr loci compared to non-ESKAPE genomes, that is, the most urgent targets of CRISPR-Cas-targeted antimicrobials are also likely to have the most existing defences against them. Second, Acr loci are not exclusive to temperate phages as AcrIIA6 was found in 33% of virulent S. thermophilus phages, showing that Acr loci are actively circulating within the same phage population as the ones used for CRISPR-Cas delivery (Choudhary *et al.,* 2023). Third, the same Acr biology that is problematic in the delivery of CRISPR has been harnessed experimentally to inhibit infection by antibiotic-resistant P. aeruginosa, highlighting that what is a "problem" in one application can be a "solution" in another (Duan *et al.,* 2024).

We don't foresee a scenario where the durability question can be put off. Cas9 based delivery efficiency will be reduced in certain target populations as pre-existing Acr loci will mediate acquired immunity (Łobocka *et al.,* 2021). Similar to conventional antibiotic resistance, horizontally acquired under the selection pressure of the CRISPR-Cas system, acr-encoding MGEs can be acquired. Many of these territories have a high prevalence of HIV/AIDS and other diseases that could be treated with CRISPR-Cas antimicrobials, which should be designed with therapeutic lifespan as a criterion from the start, not an afterthought once resistance has developed.

Table 3. Anti-CRISPR mechanisms and downstream implications for CRISPR-Cas antimicrobials

Acr mechanism	Target Cas effector	Example protein	Implication for antimicrobial design
Direct Cas binding	Cas9	AcrIIA2, AcrIIA4 (Davidson *et al.,* 2020)	Cas9 ortholog switching or alternative effector deployment (Duan *et al.,* 2024)
Cas degradation induction	Cas12a	AcrVA5 (Choudhary *et al.,* 2023)	Effector switching; engineered degradation-resistant variants
Direct Cas binding	Cas9	AcrIIA6 (Hynes *et al.,* 2018)	Modification-resistant Cas engineering
Direct binding and activation blockage	Cas13	AcrVIA1–VII family (Davidson *et al.,* 2020)	crRNA redesign; multiplexed guide deployment
Processive-nuclease blockade	Cas3	AcrIF1–AcrIF10 family (Davidson *et al.,* 2020)	Cas3-enhanced phage cocktail LBP-EC01 demonstrates proof of mechanism (Kim *et al.,* 2021)

5.5 Off-target activity and microbiome disruption

Even though CRISPR-Cas antimicrobials are programmable, they are not 100% specific in complex microbial communities. The SNIPR001 cocktail showed no off-target activity against nontarget strains in vitro, and more work is needed to elucidate the selectivity in disrupted in vivo microbiomes (Gençay *et al.,* 2023). In the case of conjugative plasmid delivery, a different kind of selectivity question arises: In principle, self-transmissible CRISPR-Cas plasmids could transfer to other, non-target species in the gut or environment, necessitating regulatory oversight and ecological risk assessments that will need to be addressed before the use of such methods can pass from preclinical proof-of-concept (Sheng *et al.,* 2023).

The selectivity in the urinary tract is high, but not absolute for receptor-mediated Cas3 delivery in LBP-EC01; phage-amplification in 83% of evaluable subjects suggests that the system can persist at concentrations adequate to killing target bacteria(Kim *et al.,* 2021), however, the durability of the selectivity under evolutionary pressure from the target population has yet to be fully characterized. Mutations in the protospacer, PAM, and in Acr loci are all documented in vitro resistance mechanisms and these need to be tackled using multiplexed construction of guides and combinatorial application of pressure profiles.

6. Evidence Base Limitations

There are a number of limitations of this review that limit the inferences that can be drawn. First, the clinical data is based on small patient numbers in two Phase 1 clinical programmes and efficacy data from the completed Phase 2 clinical programme has not yet been disclosed. Secondly, many of the in vitro studies use lab-adapted strains (Chebotar *et al.,* 2025); in vitro screening using a standardized screening panel with strains representative of present-day epidemiology (Miethke *et al.,* 2021) would be beneficial. Third, the biology of Acr is rapidly changing and predictions for the association between Acr prevalence and therapeutic durability are still in their infancy. I believe that there are four additional points: First, most preclinical efficacy data are derived from murine models that have vastly different pharmacokinetics and immune characteristics from human beings, particularly the

immunocompromised populations that SNIPR001 targets for treatment (Bareham *et al.,* 2021). Fifth, the field is lacking in standardized reporting metrics, such as the time point in which CFU are measured, which genes are in scope and classified as targets or resistance, and which endpoints of microbiome are reported and measured (Mirzayi *et al.,* 2021).

7. Future Directions

From this synthesis five priorities arise:

1. Standardised outcome metrics. Reporting in the same way as CLSI frameworks for conventional antibiotics, such as standardisation of reporting of target log reduction, suppression duration, resistance emergence rate and microbiome impact, would facilitate comparison across studies and shorten the time to regulatory learning.
   Next generation platforms should be designed to be multiplexed, with spacers targeting conserved chromosomal locations that are engineered to be acr-resistant, such as using silent nucleotide changes to limit protospacer mutation, and engineered Acr counter-defence (C. Duan et al., 2021; N. Duan et al., 2024; Łobocka *et al.,* 2021).
3. Manufacturing consolidation. To bring the production of magic bullets in the shape of CRISPR-loaded phages into a single regulatory envelope, the production of these should be continuous through bioprocess fermentation, automated purification and standardised QC reagents are required, in order to close the cost of goods gap between CRISPR-loaded phages and conventional antibiotics (Huang *et al.,* 2025).
4. Economic incentive redesign. Incentives such as transferable exclusivity extensions, market entry bonuses, and subscription programs should be explicitly assessed with regards to the CRISPR-Cas antimicrobials, which need economic schemes that are adapted to the unique mode of development of these technologies (Anderson *et al.,* 2023).
5. Iterative regulatory partnership. Structured industry-academic-regulator dialogue on the basis of the IPATH consortium and the magistral preparation frameworks already approved for phage therapy will need to be extended to phage loaded with CRISPR, in order to enable post approval modification within a defined regulatory envelope (Pirnay *et al.,* 2024).

8. Conclusions

In just over a decade since the first proof-of-mechanism demonstration in 2021 (Wu *et al.,* 2021), the landscape of programmable CRISPR-Cas antimicrobials has evolved from a single proof-of-mechanism to two clinical-stage products (Anastassopoulou et al., 2025). The efficacy cut-off point in the field is represented by a dual signal: LBP-EC01 Ph1b (2-3 log reduction in urine E.coli concentration) and SNIPR001 $4\log_{10}$ CFU/g murine decolonization. The evidence is now robust enough to support a clear primary conclusion that CRISPR-Cas antimicrobials can be used to selectively kill target bacteria and cure resistance plasmids in 3 mechanistically distinct modes of delivery in vivo in mice (Mayorga-Ramos et al., 2023); the reproducible in vivo efficacy has now been complemented with initial clinical proof-of-mechanism in humans.

The ceiling of translation is now limited by three category of structural barrier. Manufacturing complexity (cGMP phage fermentation, Cas payload stability, purification heterogeneity, and post-approval regulatory rigidity) requires new paradigms for manufacturing (Tanir *et al.,* 2021). Conventional antibiotic products are poorly incentivized and the economic incentives are even worse for complex biologics containing CRISPR for use in brief courses (Årdal et al., 2019). To achieve therapeutic durability, which needs to be built into the platform at the outset, a bio-system has co-evolved with the problem of HGT that it aims to address – anti-CRISPR biology (Duan *et al.,* 2024).

Tools are clearly effective in the lab. The more interesting and more difficult challenge in our view, is to deploy them responsibly at population scale within the next decade.

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