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CRISPR-Cas-Mediated Interventions for Precision Editing and Suppression of Resistant Bacterial Mutants

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ABSTRACT: Antimicrobial resistance (AMR) has become one of the most serious global health threats of the 21st century, driven by the rapid emergence of multidrug-resistant (MDR) pathogens and the slowing pace of novel antibiotic discovery. While conventional antibiotics remain effective against acute infections, their broad spectrum activity disrupts beneficial microbiota and exerts strong selective pressure that accelerates the emergence and horizontal spread of resistance. This lack of precision has contributed substantially to the growing burden of resistant infections in both clinical and community settings.

In response to these limitations, CRISPR-Cas systems have emerged as a next-generation, programmable antimicrobial strategy capable of selectively targeting antibiotic-resistant bacteria. Originally evolved as bacterial adaptive immune systems, CRISPR-Cas platforms use customizable guide RNAs to direct nucleases such as Cas9, Cas12, and Cas13 toward specific DNA or RNA sequences. This precision enables targeted cleavage, transcriptional repression, base editing, or RNA degradation of resistance determinants. Recent studies demonstrate efficient targeting of high priority resistance genes, including *mecA*, *bla_{NDM}*, *ermB*, and *mcr* variants, resulting in the selective elimination of resistant cells or the removal of resistance-bearing plasmids, thereby restoring antibiotic susceptibility.

Effective delivery remains central to CRISPR based antimicrobial success. Engineered bacteriophages and phagemids provide highly specific and efficient delivery, while conjugative plasmids enable population-level dissemination of CRISPR constructs within bacterial communities. Nanoparticle based systems offer flexible, non-viral alternatives, and engineered probiotics have emerged as promising vehicles for microbiome and gut-targeted delivery. These approaches allow selective removal of resistant subpopulations while preserving beneficial microbes.

Experimental evidence from *in vitro* and animal models demonstrates over 99% elimination of resistant bacterial populations and effective resensitization to existing antibiotics. Although challenges such as *in vivo* delivery, off-target effects, and anti-CRISPR mechanisms remain, ongoing advances continue to refine this technology. Overall, CRISPR-based antimicrobials represent a transformative, precision driven strategy to combat AMR, restore antibiotic efficacy, and stabilize microbial ecosystems.

KEYWORDS: CRISPR Cas Action, Antimicrobial resistance (AMR), Precision bacterial targeting, Resistance gene editing and plasmid curing, Sequence-specific killing.

CONTENT: Genome-guided antibacterial therapy, Gene-selective pathogen elimination, Plasmid-targeted gene disruption, CRISPR-driven antibiotic revival.

I. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious global health threats of the 21st century. It compromises our ability to prevent and treat bacterial infections that were once easily curable. Current global estimates suggest that AMR is directly responsible for over 1.2 million deaths each year, and this number is expected to rise dramatically if new and effective treatment strategies are not developed. The growing prevalence of multidrug-resistant bacteria has significantly reduced the effectiveness of existing antibiotics, particularly against high-priority pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterococcus faecium*^{2,3}.

Conventional antibiotics typically act through broad, non-specific bactericidal or bacteriostatic mechanisms. While effective in the short term, these approaches exert intense selective pressure on bacterial populations, accelerating the emergence of resistant strains⁴. This pressure also promotes the horizontal transfer of resistance genes, which are often carried on mobile genetic elements such as plasmids, integrons, and transposons. Moreover, many antibiotics disrupt the normal commensal microbiota, leading to dysbiosis and increasing the risk of secondary infections⁶. Compounding these challenges is the slow pace of new antibiotic discovery, which has created an urgent need for alternative, precision-based antimicrobial strategies⁷.

Against this backdrop, CRISPR-Cas systems have gained considerable attention as promising next-generation antimicrobial tools. Originally discovered as adaptive immune systems in bacteria and archaea, CRISPR-Cas technologies allow precise, programmable targeting of specific nucleic acid sequences^{8,9}. This unique capability makes CRISPR particularly attractive for selectively eliminating resistant bacteria, disabling resistance genes, or restoring antibiotic sensitivity, while minimizing unintended effects on beneficial microbial communities¹⁰.

II. OVERVIEW OF CRISPR-Cas SYSTEMS

Origin of CRISPR as a Bacterial Immune System

CRISPR-Cas systems function as adaptive immune mechanisms that protect bacteria and archaea from invading genetic material, such as bacteriophages and plasmids⁹. Structurally, CRISPR loci consist of short, repetitive DNA sequences separated by unique spacer sequences that originate from previously encountered foreign DNA. These spacers act as molecular “memories” of past infections, enabling the organism to recognize and neutralize the same invaders during subsequent exposures^{9,11}. The CRISPR array is transcribed and processed into CRISPR RNAs (crRNAs), which guide Cas nucleases to complementary target sequences for cleavage, thereby disabling the invading genetic element¹². Because of their programmable specificity, researchers have repurposed CRISPR-Cas systems; particularly CRISPR-Cas9 and CRISPR-Cas13; to target antibiotic resistance genes directly. These systems can selectively cleave resistance genes or transcripts in multidrug-resistant bacteria, restoring antibiotic susceptibility or inducing cell death^{14,15}. CRISPR-based antimicrobials can be delivered using bacteriophages or conjugative plasmids, enabling targeted elimination of resistant bacteria while sparing beneficial microbiota^{16,17}. Such precision-based approaches offer a promising strategy to combat AMR without exerting the broad selective pressures associated with conventional antibiotics^{10,18}.

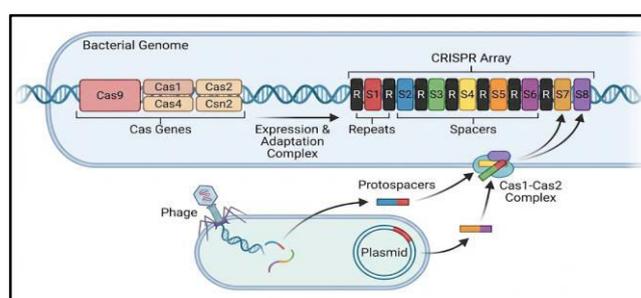


Figure 1. Structural organization of the CRISPR-Cas locus in bacteria

III. MECHANISM OF CRISPR-CAS ACTION

The CRISPR immune response operates through three highly conserved stages: adaptation, crRNA biogenesis, and interference¹⁹.

1. Adaptation (Spacer Acquisition):

During this stage, fragments of invading genetic material derived from bacteriophages or plasmids are captured and incorporated into the host CRISPR array as new spacer sequences. This process is primarily mediated by the Cas1 and Cas2 proteins and results in the formation of a heritable genetic record of prior infections^{20,21}.

2. crRNA Biogenesis:

The CRISPR array is subsequently transcribed into a long precursor CRISPR RNA (pre-crRNA), which is processed into short, mature CRISPR RNAs (crRNAs). Depending on the CRISPR-Cas type, this processing may involve Cas endonucleases such as Cas6 or host RNases, generating crRNAs that contain a spacer sequence flanked by partial repeat sequences^{22,23}. These crRNAs assemble with Cas effector proteins to form ribonucleoprotein surveillance complexes capable of target recognition.

3. Interference:

In the interference stage, the crRNA-Cas complex recognizes complementary sequences in invading DNA or RNA through base pairing. Upon target recognition, the Cas nuclease introduces sequence-specific cleavage, leading to degradation of the foreign genetic material and neutralisation of the threat^{24,25}. DNA-targeting systems such as Cas9 and Cas12 induce double-stranded breaks, whereas RNA-targeting systems like Cas13 cleave RNA molecules²⁶.

This programmable and modular mechanism forms the foundation for repurposing CRISPR-Cas systems as powerful gene-editing and antimicrobial tools, enabling precise targeting of antibiotic resistance genes or pathogenic bacteria with minimal off-target effects²⁷.

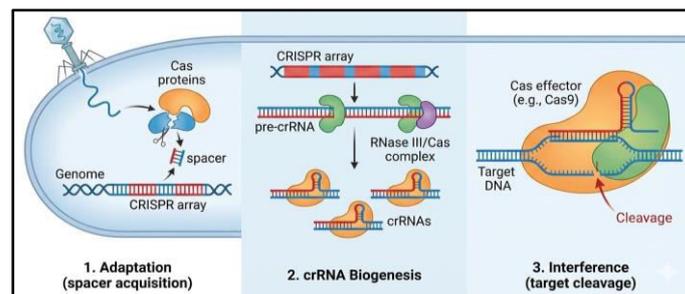


Figure 2. Mechanism of CRISPR–Cas immune response: adaptation, expression, and interference.

Types of CRISPR-Cas Systems Relevant to Antimicrobial Applications

Among the diverse CRISPR–Cas systems identified, Class 2 CRISPR-Cas systems are most widely used in antimicrobial research due to their structural simplicity, requiring a single effector protein for target recognition and cleavage^{28,29}. These systems are highly programmable and efficient, making them suitable for precise targeting of antibiotic resistance genes and pathogenic bacteria³⁰. Key systems include:

Type of CRISPR-Cas System	Description
Cas9 (Type II)	RNA-guided DNA endonuclease that induces double-strand DNA breaks ^{8,28}
Cas12 (Type V)	DNA-targeting nuclease that creates staggered DNA cuts ^{29,30}

Cas13 (Type VI)	RNA-guided RNase that targets and degrades RNA transcripts ^{25,31,32}
Cas14	A compact nuclease capable of targeting single-stranded DNA ³³

DNA-Targeting versus RNA-Targeting Systems

DNA-targeting CRISPR-Cas systems such as Cas9 (Type II) and Cas12 (Type V) introduce site-specific double-strand breaks in bacterial DNA, resulting in irreversible genetic damage that leads to bacterial cell death or elimination of resistance-carrying plasmids^{36,37}. By directly cleaving chromosomal DNA or plasmid DNA encoding antibiotic resistance genes, these systems can permanently disable resistance mechanisms and prevent further propagation of resistance determinants⁴⁰.

In contrast, RNA-targeting systems such as Cas13 (Type VI) act at the transcript level by recognizing and degrading resistance gene mRNA without modifying the bacterial genome⁴¹. This RNA-based targeting enables transient and reversible suppression of resistance gene expression, reducing selective pressure for permanent resistance mutations. Such an approach may offer a potentially safer antimicrobial strategy, particularly in contexts where irreversible genome editing raises biosafety or ecological concerns⁴².

IV. RELEVANCE OF CRISPR IN COMBATING ANTIMICROBIAL RESISTANCE

CRISPR-based antimicrobials directly address several key limitations associated with conventional antibiotics. Unlike broad-spectrum antimicrobial agents, CRISPR systems can be precisely engineered for strain-specific or gene-specific targeting, thereby minimizing collateral damage to the host microbiota and reducing the risk of dysbiosis^{43,44}. This high degree of specificity represents a major advantage over traditional therapies that indiscriminately affect both pathogenic and commensal bacteria.

Importantly, CRISPR strategies can be designed to target antibiotic resistance determinants rather than essential bacterial genes, allowing resistant bacteria to regain susceptibility to existing antibiotics instead of being eliminated outright⁴⁵. Such resistance-reversal approaches could extend the clinical lifespan of current antibiotic classes and reduce the need for continuous development of new drugs.

Furthermore, the ability of CRISPR-Cas systems to selectively target plasmid-encoded resistance genes provides a powerful means to limit horizontal gene transfer, which plays a central role in the rapid dissemination of antimicrobial resistance among bacterial populations^{46,47}. By removing resistance plasmids or blocking their transmission, CRISPR-based interventions may help contain the spread of AMR at the population level.

Collectively, these features position CRISPR-Cas systems as precision-guided antimicrobial tools with significant potential to complement or transform existing strategies for combating antimicrobial resistance⁴⁸.

V. MECHANISMS OF CRISPR-BASED ANTIMICROBIALS

Double-Strand Break-Mediated Killing

Targeting essential chromosomal genes or antibiotic resistance genes using DNA-targeting CRISPR systems, such as **Cas9**, induces site-specific double-strand DNA breaks. Because many bacteria lack efficient non-homologous end joining or homologous recombination repair pathways, this damage often results in irreversible genomic instability and cell death^{49,50}. This mechanism enables highly selective elimination of resistant bacterial strains while sparing non-target populations⁵¹.

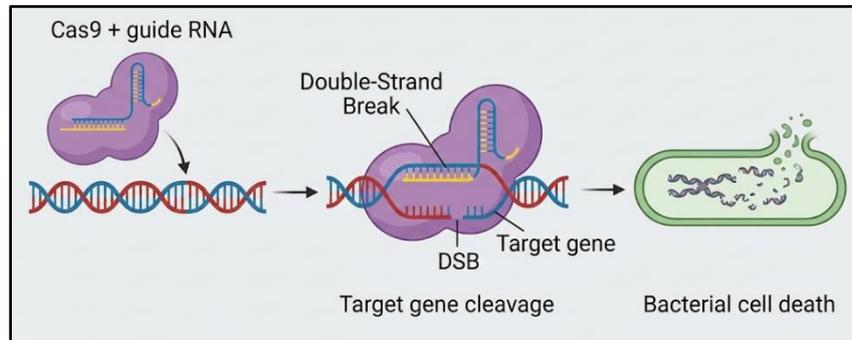


Figure 3. CRISPR-Cas9 mediated bacterial killing via double-strand DNA breaks.

Base Editing and Prime Editing for Resistance Reversal

Base editing and prime editing technologies represent advanced CRISPR-derived tools that allow precise nucleotide modifications without introducing double-strand breaks. These approaches have been explored to correct point mutations responsible for antibiotic resistance, such as mutations in *gyrA*, *rpoB*, or *pbp* genes^{52,53}. By restoring the wild-type sequence, these strategies aim to reverse resistance phenotypes and resensitize bacteria to existing antibiotics rather than inducing cell death^{38,39,54}.

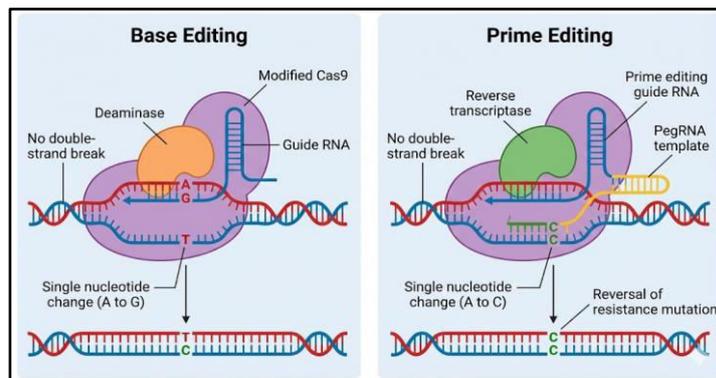


Figure 4. Base and prime editing strategies for correcting antibiotic resistance mutations.

CRISPR Interference (CRISPRi)

CRISPR interference (CRISPRi) employs catalytically inactive Cas proteins, such as dead Cas9 (dCas9), which bind target DNA sequences without cleavage and sterically block transcription^{41,55}. This reversible gene-silencing approach is particularly useful for suppressing resistance mechanisms, including efflux pumps, resistance-conferring enzymes, and virulence factors, allowing functional interrogation and therapeutic modulation without permanent genomic alterations^{56,57}.

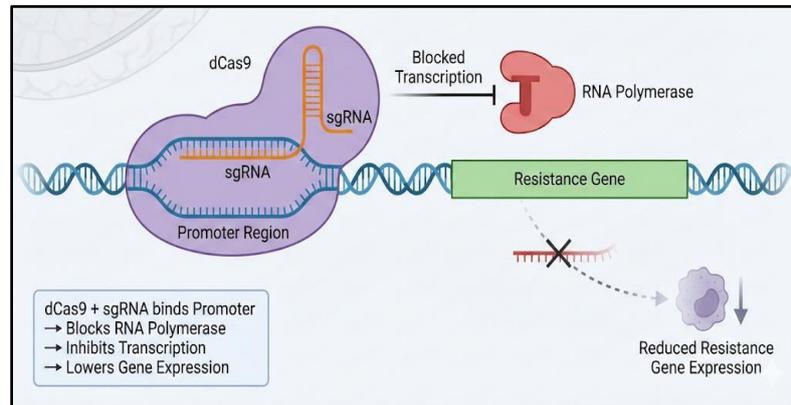


Figure 5. CRISPR interference (CRISPRi) mediated repression of resistance genes.

CRISPR-Based Plasmid Curing

Since many clinically relevant antibiotic resistance genes are located on plasmids, CRISPR-mediated targeting approach restores antibiotic susceptibility and significantly reduces horizontal gene transfer, thereby limiting the dissemination of resistance within and between bacterial populations⁵⁸. Such targeted removal of resistance plasmids offers a population-level benefit beyond individual bacterial killing^{58,60}.

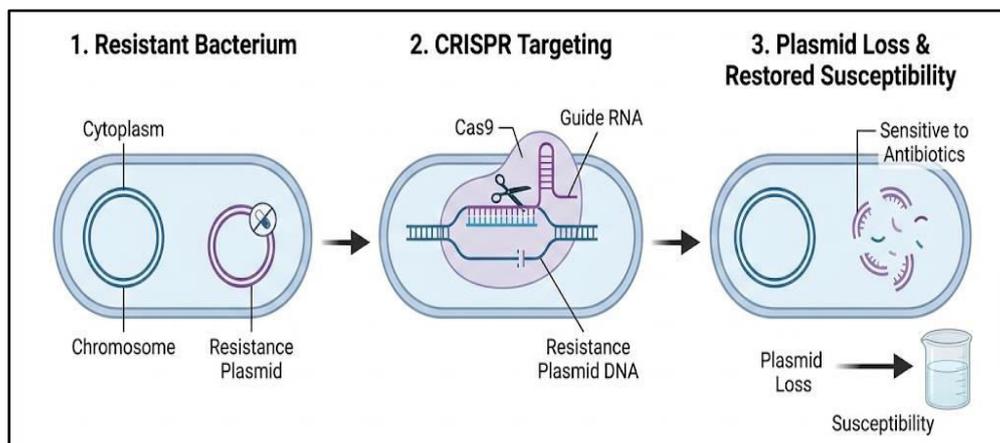


Figure 6. CRISPR-based elimination of resistance plasmids from bacterial cells.

VI. REPRESENTATIVE EXAMPLES FROM RECENT STUDIES

Several studies have demonstrated the effectiveness of CRISPR-based antimicrobial strategies. Targeting the *mecA* gene using CRISPR-Cas9 has successfully eliminated methicillin resistance in *Staphylococcus aureus* (MRSA) strains, restoring susceptibility to β -lactam antibiotics^{16,50}. Similarly, plasmid-encoded resistance genes, including *bla*NDM, *bla*KPC, and *bla*CTX-M, have been targeted using CRISPR systems to reverse resistance to β -lactams in Gram-negative pathogens^{16,44}. CRISPR targeting of the *mcr-1* gene has shown promise in reversing colistin resistance, which is particularly concerning due to limited therapeutic options⁴⁵.

Beyond β -lactam resistance, CRISPR-based approaches have been applied to genes conferring resistance to tetracyclines and macrolides, such as *tetM*, *tetX*, and *ermB*⁴⁶. More recently, Cas13-based RNA-targeting strategies have been used to suppress resistance gene expression without permanently altering the bacterial genome, highlighting a flexible and innovative approach for future antimicrobial development^{15,25,32}.

VII. ADVANCED CRISPR DELIVERY SYSTEM FOR TARGETED ELIMINATION OF RESISTANT MUTANTS

Effective delivery of CRISPR components to resistant bacterial populations remains a critical challenge in translating gene-editing technologies into practical antimicrobial strategies⁴⁷. Over the past five years (2020–2025), several innovative delivery modalities have emerged, capable of transporting CRISPR-Cas systems directly to pathogenic bacteria with high specificity and minimal off-target effects⁴⁷. Among the most promising platforms are CRISPR-loaded bacteriophages, conjugative plasmids, nanoparticle-based vehicles, and engineered probiotic strains, offering precision, safety, and adaptability for combating antibiotic-resistant mutants.

1. CRISPR-Loaded Bacteriophages (Cr-Phages)

CRISPR-loaded bacteriophages represent one of the most natural and targeted delivery approaches available^{48,49}. Bacteriophages inherently recognize specific bacterial receptors, making them ideal vectors to inject CRISPR components directly into resistant bacterial cells. Several studies since 2020 have demonstrated that phage-based delivery can precisely eliminate pathogens by cutting essential genes or antibiotic resistance determinants^{14,16,19}.

Modern Cr-phage designs commonly employ the Cas9 or Cas13 systems encoded within modified phage genomes⁵⁰. Once the phage infects its target bacterium, the CRISPR system becomes active and cleaves resistance genes such as *bla_{NDM}*, *mecA*, or *vanA*, depending on the target organism^{16,50}. This ultimately results in double-strand breaks or RNA degradation, leading to the selective death of mutated or resistant strains without disturbing the susceptible bacterial population^{36,50}.

A key advantage is high specificity: phages can be engineered with altered tail fibers to expand host range or target particular clinical isolates^{48,49}. Recent phage-CRISPR research has shown up to 99% reduction in resistant *E. coli* and *Staphylococcus aureus* populations, demonstrating significant potential for precision antimicrobials^{16,50}. Additionally, phages can be used in combination with traditional antibiotics to re-sensitize bacterial cells after CRISPR-mediated disruption of resistance pathways¹⁷.

However, the limitations include variability in phage-host interactions, rapid bacterial evolution, and the need to ensure stability of CRISPR payloads during phage replication⁴⁸. Despite these challenges, Cr-phages continue to be one of the most efficient biological vectors for targeted gene editing in infectious disease interventions^{49,50}.

2. Conjugative Plasmids (Pheromone-Responsive & Broad-Host-Range Plasmids)

Conjugative plasmids are mobile genetic elements capable of transferring between bacterial cells through direct contact. Scientists have harnessed this natural mechanism by engineering plasmids that carry CRISPR-Cas systems specifically programmed to cleave resistance genes in recipient cells^{51,52}.

Two major classes dominate recent research:

1. **Pheromone-responsive plasmids (PRPs)** commonly used in Gram-positive organisms like *Enterococcus faecalis*. These plasmids respond to pheromone signals produced by recipient bacteria, ensuring highly efficient conjugation⁵¹.
2. **Broad-host-range plasmids** capable of moving across diverse Gram-negative bacteria, making them suitable for targeting mixed microbial communities such as wastewater or hospital isolates⁵².

Once transferred, the CRISPR machinery within these plasmids selectively recognizes and cuts antibiotic resistance genes (e.g., *tetM*, *ermB*, *qnr*, or carbapenemase genes), resulting in the elimination of resistant bacteria or curing of resistance plasmids from the population^{51,52}. One of the strongest advantages of conjugative CRISPR plasmids is self-propagation; unlike phage systems, plasmids can spread autonomously across the entire bacterial population.

Studies from 2021 onward have shown that CRISPR conjugative plasmids can suppress resistant cell populations by more than 85–90%, especially in *Enterococcus* and *E. coli* strains^{51,52}. Their limitations lie in the dependence on cell-to-cell contact, variability in conjugation rates, and potential horizontal gene transfer risks if not carefully regulated^{51,52}. Despite these concerns, plasmid-based CRISPR antimicrobials are emerging as scalable tools for community-level elimination of resistance genes^{52,53}.

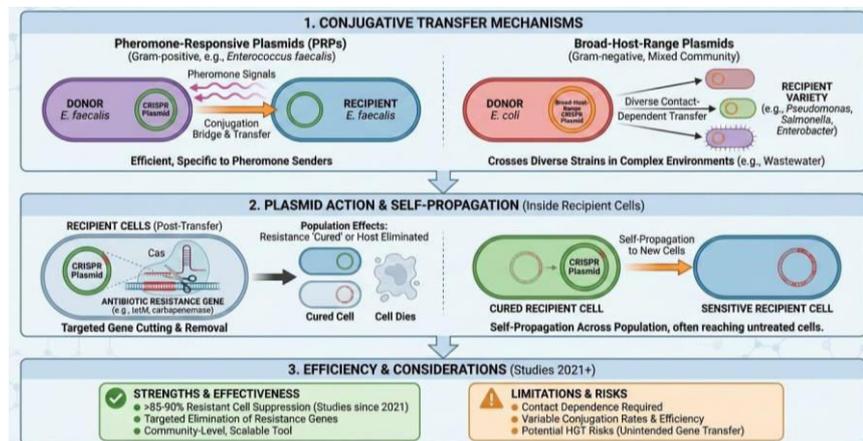


Fig 7. Demonstrates pheromone responsive plasmids (PRP) as precision conjugative delivery systems for CRISPR Cas mediated elimination of antibiotic resistant mutants.

3. Nanoparticle Delivery Systems (Quantum Dots & Polymer Nanoparticles)

Nanoparticle-mediated delivery offers a non-biological, highly customizable platform for transporting CRISPR components into resistant bacteria. These particles protect CRISPR cargo from enzymatic degradation and allow precise control over release kinetics, cellular penetration, and targeting⁵⁴.

Quantum Dots

Quantum dots, often composed of semiconductor nanocrystals, have been explored as fluorescent CRISPR carriers due to their unique optical properties and small size. QDs can be functionalized with:

- bacterial cell binding peptides
- antibody fragments
- cationic polymers for nucleic acid binding

This allows the QD-CRISPR complexes to bind tightly to resistant bacteria and deliver gene-editing components into the cytoplasm. Articles published between 2021 and 2024 reported successful use of QDs for delivering Cas9/sgRNA complexes to disrupt resistance genes in Gram-negative organisms, achieving significant reduction in resistant phenotypes⁵⁵.

Polymer Nanoparticles

Biodegradable polymer nanoparticles such as PLGA, chitosan, or PEI-coated nanocarriers have shown tremendous promise due to their safety, stability, and tunability. They can encapsulate CRISPR mRNA, plasmids, or ribonucleoprotein complexes and release them inside bacterial cells in a controlled manner⁵⁶.

Polymeric systems are especially valuable for:

- targeting intracellular pathogens
- preventing resistance emergence
- bypassing immune detection

Recent work has demonstrated that polymer nanoparticles can enhance CRISPR delivery efficiency by up to 30–40% compared to naked CRISPR components, leading to substantial suppression of multidrug-resistant bacteria. While

nanoparticles avoid issues of host specificity seen with phages or plasmids, challenges remain regarding penetration through bacterial cell walls, large-scale manufacturing, and biosafety in environmental applications^{54,56}.

4. Engineered Probiotics for Gut-Targeted CRISPR Delivery

Engineered probiotics represent an elegant and biologically safe solution for targeted CRISPR delivery, especially within the gastrointestinal tract⁵⁷. Strains such as *Lactobacillus*, *Bifidobacterium*, and *E. coli* can be genetically modified to express CRISPR components or carry plasmids encoding CRISPR-Cas systems⁵⁷.

Once introduced into the gut, these probiotics can:

- colonize the intestine.
- sense pathogenic bacteria through quorum-sensing mechanisms.
- release CRISPR antimicrobials directly to resistant strains⁵⁷.

This approach is particularly valuable for targeting antibiotic-resistant *E. coli*, *Enterococcus*, and *Salmonella* in the microbiome. Engineered probiotics can also be designed to produce anti-plasmid CRISPR systems, which selectively eliminate resistance plasmids from pathogenic strains while sparing beneficial flora^{57,58}. From 2020–2025, multiple studies have demonstrated that CRISPR-equipped probiotics can reduce resistant bacterial loads in the gut by 60–80%, highlighting their potential for clinical use^{57,73}. Additionally, probiotics are generally regarded as safe, easy to administer orally, and well-tolerated in long-term applications.

Key limitations include maintaining plasmid stability within the probiotic strain, ensuring sufficient colonization of the GI tract, and preventing unintended spread of engineered plasmids^{57,79}.

VIII. MECHANISM OF SELECTIVE KILLING BY CRISPR SYSTEMS

CRISPR-based antimicrobial platforms achieve selective elimination of drug-resistant bacteria through precisely guided nucleases that recognize and cleave genetic sequences associated with antimicrobial resistance (AMR)^{36,38}. The process begins with the design of a guide RNA that is complementary to a resistance determinant, either located on the bacterial chromosome or carried on a plasmid^{8,36}. Once delivered into the target cell through phages, conjugative plasmids, nanoparticles, or engineered probiotics, the CRISPR effector locates the matching DNA sequence with high specificity^{47,50}.

When the CRISPR complex targets a chromosomal resistance gene, such as those encoding β -lactamases or methicillin resistance factors, it introduces a double-strand break at that exact locus^{36,37}. Most bacteria lack efficient repair pathways for such damage. As a result, chromosomal cleavage disrupts DNA replication and rapidly triggers cell death. This mechanism eliminates only the bacterial population that carries the targeted gene, while sparing closely related strains that lack the sequence, demonstrating an intrinsic “precision-killing” effect^{36,50}.

In contrast, when the targeted sequence resides on a plasmid, CRISPR cleavage does not necessarily kill the host cell. Instead, it causes degradation or loss of the plasmid, effectively removing the AMR gene^{44,45,58}. Bacteria that survive this process become resensitized to antibiotics, since the genetic basis of resistance has been removed^{40,45}. This plasmid-curing effect has been shown for clinically relevant determinants such as *mcr-1*, *bla_{NDM-5}*, and *ermB*, where CRISPR targeting leads to a sharp reduction in plasmid retention and restoration of susceptibility to colistin, carbapenems, or macrolides^{44,45,58}.

Together, chromosomal disruption and plasmid curing form a dual-action strategy. One selectively kills highly resistant pathogens, while the other reverts surviving strains to a drug-susceptible state. This selective mechanism makes CRISPR antimicrobials uniquely suited for precision control of resistant infections while minimizing off-target effects on the commensal microbiota^{10,58}.

Target AMR Gene	Bacterial Species	Outcome After CRISPR Targeting	Restoration of Antibiotic Susceptibility
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mcr-1	<i>Escherichia coli</i> (clinical isolates)	Plasmid curing -CRISPR eliminates mcr-1-bearing plasmids; plasmid copy number markedly reduced	Yes -isolates become resensitized to colistin after loss of mcr-1
blaNDM-5	<i>Escherichia coli</i> (NDM-5 plasmid positive) discussed in carbapenem-resistant Enterobacterales spread	Plasmid targeting disruption or removal of blaNDM-type plasmids reduces ability to transmit carbapenem resistance	Yes -removal of blaNDM plasmids restores carbapenem susceptibility
erm(B) (macrolide resistance)	<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> transferred via pheromone-responsive plasmids (PRPs)	Plasmid curing / CRISPR disruption targeting ermB-carrying PRP plasmids eliminates macrolide resistance determinants	Yes -loss of erm(B) re-establishes erythromycin susceptibility

Table 2. Examples of CRISPR-mediated targeting of plasmid-borne antimicrobial resistance (AMR) genes and resulting phenotypic outcomes. The table summarizes key resistance determinants (mcr-1, blaNDM-5, ermB), their associated bacterial species, the mechanism of CRISPR action (plasmid curing or disruption), and the consequent restoration of antibiotic susceptibility.

IX. KEY EXPERIMENTAL RESULTS REPORTED

A substantial body of experimental work from 2014 to 2025 demonstrates the strong therapeutic potential of CRISPR-based antimicrobials across diverse bacterial pathogens^{14,16,49,50}. One of the most widely cited observations is the ability of CRISPR-loaded bacteriophages to dramatically reduce multidrug-resistant bacterial loads in vivo^{16,50}. In mouse infection models, phages engineered to deliver CRISPR-Cas systems targeting resistance genes in *Escherichia coli* achieved up to 99% elimination of the MDR population. These studies confirmed that even in complex host environments, CRISPR-phages can locate the target sequence efficiently, replicate within the pathogen, and generate a profound reduction in bacterial burden without affecting non-resistant strains^{16,50}.

Similarly encouraging results have been reported for Gram-positive pathogens. In *Staphylococcus aureus* and MRSA, CRISPR constructs directed against species-specific resistance genes produced strong selective killing both in vitro and in animal models⁵⁰. The precision of guide RNA targeting allowed the engineered phages to differentiate between resistant and susceptible isolates, eliminating only the strains carrying the mecA-related targets⁵⁰. Comparable outcomes were observed in *Enterococcus faecalis*, particularly when CRISPR was delivered through pheromone-responsive conjugative plasmids. These systems transferred efficiently between donor and recipient cells, enabling selective depletion of antibiotic-resistant enterococcal populations within mixed communities^{51,52}.

Gram-negative pathogens also showed sensitivity to CRISPR based interventions. Studies using CRISPR RNA guided nucleases delivered via capsid like particles demonstrated effective killing of carbapenem-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by targeting plasmid-encoded β -lactamase genes or other resistance determinants. These results are especially important because these species are often poorly susceptible to phage therapy alone, highlighting CRISPR's capacity to overcome intrinsic therapeutic barriers^{49,50}. colistin-resistance gene on plasmids resulted in complete or near-complete loss of the resistance element in *E. coli*, enabling .

Beyond direct killing, several investigations have documented successful plasmid curing, which restores antimicrobial susceptibility in surviving cells. Targeting the mcr 1 recovery of colistin sensitivity⁴⁵. Similar outcomes were reported for plasmids carrying blaNDM-5, a major carbapenemase gene, where CRISPR-mediated cleavage destabilized the plasmid and reduced carbapenem resistance. In Gram-positive bacteria, CRISPR targeting of macrolide resistance

genes such as *ermB* led to plasmid loss, decreased erythromycin resistance, and reduced potential for horizontal spread^{46,51}.

Together, these studies highlight a consistent theme: CRISPR antimicrobials can either eliminate resistant pathogens outright or remodel them into antibiotic susceptible forms. The breadth of validated targets from MRSA and vancomycin-resistant enterococci to carbapenem-resistant *Enterobacter* demonstrates the versatility of CRISPR as a next-generation precision antimicrobial technology^{49,50,58}.

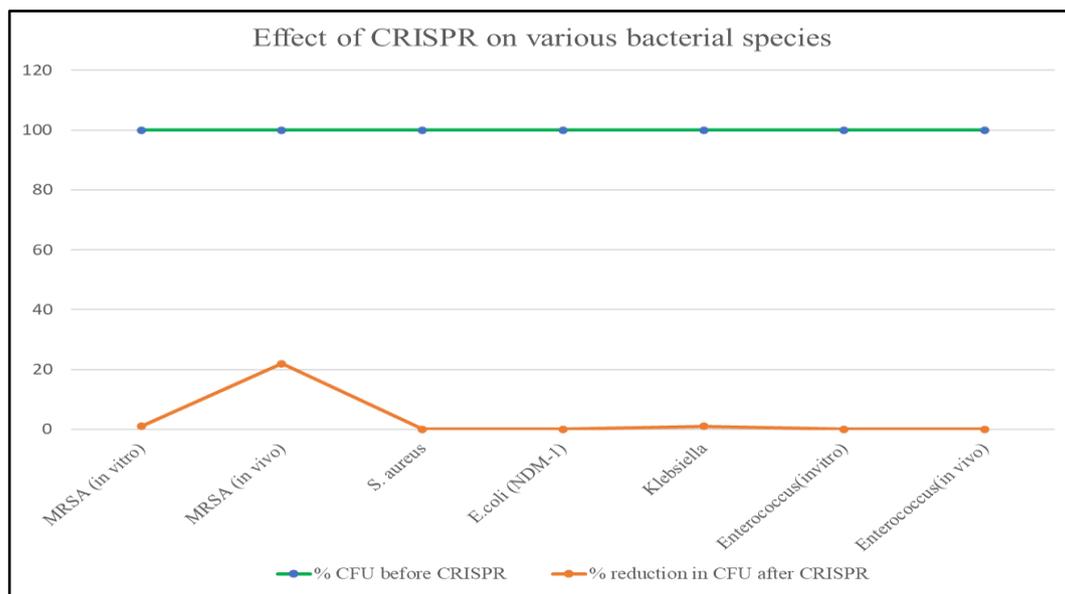


Fig 8. The CFU reduction percentages shown in the graph are derived from experimentally measured decreases in viable bacterial counts following CRISPR treatment. In MRSA mixed cultures, resistant cells declined from approximately 50% to less than 1%, corresponding to ~99% reduction, while mouse skin infection models showed a decrease to ~11% (~78%). In vitro CRISPR exposure reduced *S. aureus* survival to $\sim 10^{-4}$ (99.99%). Targeting blaNDM-1 or blaSHV-18 in *E. coli* and *Klebsiella* produced 2–3 log reductions (99 -- 99.9%). Conjugative CRISPR delivery in *Enterococcus* achieved >3 log decreases (~99.9%) in both in vitro and in vivo models.

Selectivity Advantage of CRISPR-Based Antimicrobials :

A major advantage of CRISPR-based antimicrobials is their exceptionally high degree of selectivity, which distinguishes them from conventional antibiotics^{10,36}. Because the CRISPR nuclease is guided by a sequence specific RNA, it recognizes only those bacteria that carry the exact target gene. This allows therapeutic constructs to eliminate resistant pathogens while leaving commensal organisms untouched^{10,36}. Several studies using CRISPR-loaded phages and conjugative plasmids have demonstrated that non-targeted strains, even those closely related to the pathogen, remain completely unaffected when they do not contain the matching resistance determinant^{50,51}.

This precision also contributes to the second key advantage: restoration of antibiotic susceptibility^{40,58}. When CRISPR systems are directed at plasmid borne resistance genes rather than chromosomal loci, cleavage of the plasmid leads to its degradation or loss. Surviving bacteria no longer carry the AMR gene and therefore revert to a drug-sensitive phenotype^{40,45}. Experimental evidence for this effect has been reported for plasmid-encoded markers such as *mcr-1*, *blaNDM-5*, and *ermB*, where CRISPR targeting resulted in loss of resistance and recovery of susceptibility to colistin, carbapenems, and macrolides^{44,45,46}.

Finally, the selectivity of CRISPR antimicrobials helps preserve microbiome stability. Broad-spectrum antibiotics often disrupt the gut microbiota and create ecological imbalances that favor opportunistic pathogens. In contrast, CRISPR killing is confined to a defined genetic target, minimizing collateral damage and reducing the likelihood of

dysbiosis^{10,82}. By sparing beneficial microbial populations, CRISPR based strategies offer a more controlled and microbiome-friendly approach to managing resistant infections^{10,81}.

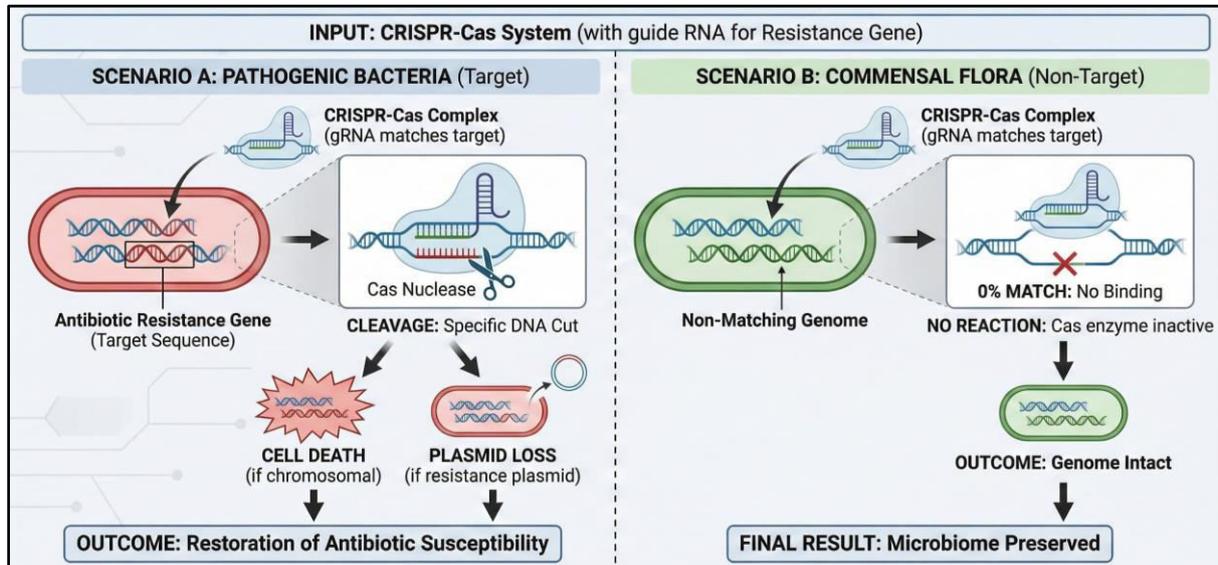


Fig 9. Demonstrates CRISPR -Cas Targeted Antimicrobial Action and Microbiome Preservation.

X. CHALLENGES

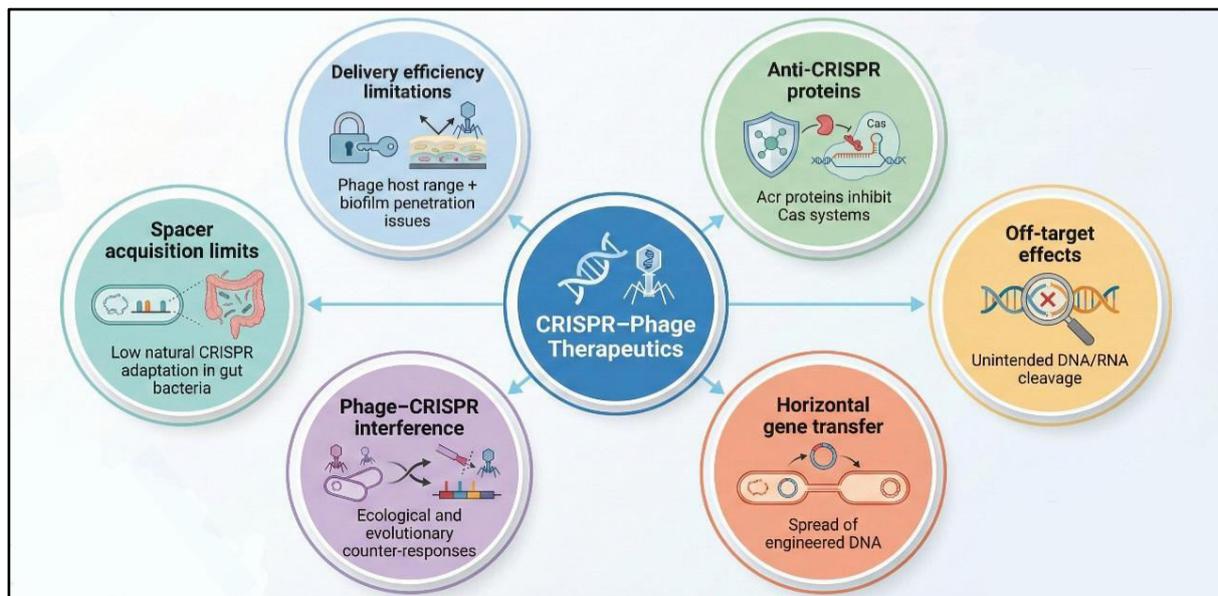


Fig 10. Challenges associated with CRISPR-phage therapeutics including delivery limitations, anti-CRISPR proteins, off-target effects, horizontal gene transfer, and ecological interference.

Delivery efficiency limitations:

Phage-mediated delivery remains the leading strategy for introducing CRISPR payloads into bacteria, yet its effectiveness is curtailed by narrow host ranges, variable receptor expression across strains, and limited penetration of complex matrices such as mucus and biofilms^{48,59}. Spatial heterogeneity in the gut, lungs, or wound beds creates micro-niches where phage adsorption and genome delivery fail, leaving reservoirs of untreated bacteria that can reseed

infection or carry resistant plasmids⁷⁰. Physiological states like stationary phase or persister phenotypes further reduce uptake and expression of CRISPR systems, meaning that in vivo delivery efficiencies observed in controlled experiments often overestimate clinical performance⁴⁹.

Anti-CRISPR proteins (Acrs):

Anti-CRISPR proteins encoded by prophages and mobile elements are widespread across bacterial populations and can block diverse Cas effectors in situ^{60,61}. Acrs can be horizontally transferred and rapidly selected under CRISPR pressure, creating ecological refuges where therapeutic nucleases are disabled^{61,62}. Because Acr repertoires vary by niche, a therapy effective in one patient or site may fail in another; strategies to circumvent Acrs (Acr-resistant Cas proteins, Acr inhibitors, or multiplexed targeting) are promising but presently remain largely at preclinical proof-of-concept stages⁶¹.

Off-target and collateral effects:

Programmability of CRISPR notwithstanding, imperfect guide specificity, PAM ambiguities, and collateral nuclease activities (notably for some RNA-targeting effectors) can lead to unintended cleavage of non-target genomes or transcripts^{63,64}. In complex microbial communities, such off-target activity risks removing beneficial taxa or disrupting mobile genetic elements that modulate community stability, producing dysbiosis with downstream metabolic and immunological consequences. Most specificity profiles derive from in vitro assays; comprehensive in vivo specificity and community-level impact data remain scarce^{64,65}.

Horizontal gene transfer (HGT) risks:

Vectors used to deliver CRISPR (phagemids, plasmids, modified prophage segments) can themselves be mobilized by conjugation or generalized transduction, allowing therapeutic constructs or fragments thereof to spread beyond intended hosts^{66,67}. Mobilized elements could include selectable markers, recombinase genes, or nuclease components; if transferred, these sequences may engender new ecological interactions or unintended gene drives in microbial communities^{67,68}. Mitigations such as nontransferable designs, integration into nonmobile loci, or self-limiting genetic circuits are conceptually available but not yet standardized or fully validated under realistic environmental conditions⁵³.

Phage CRISPR interference & ecological interactions:

Introducing engineered phages carrying CRISPR payloads perturbs pre-existing phage bacteria networks and can provoke complex ecological feedbacks^{69,70}. Engineered phages may outcompete or interfere with native phage populations, alter prophage induction rates, or change selective pressures that favor distinct bacterial lineages effects that can cascade into unanticipated shifts in community function and pathogen dynamics^{69,70}. These ecological interactions are challenging to predict from reductionist models and demand longitudinal, ecosystem-level studies before widescale release⁷⁰.

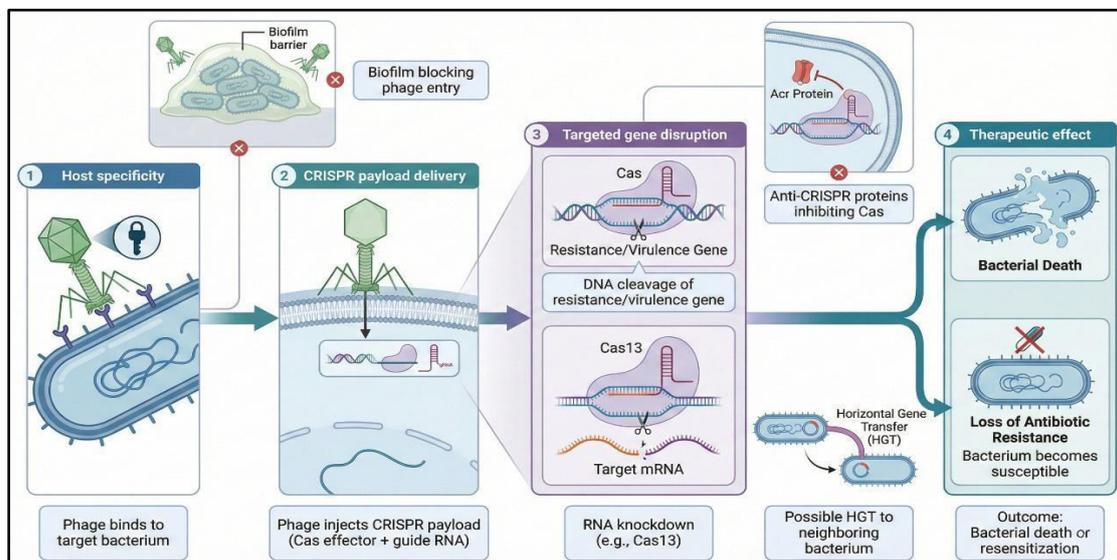
Spacer acquisition limitations in gut bacteria: The reliance on endogenous CRISPR adaptation (spacer acquisition) to maintain long-term immunity or adapt to phage escape is limited by intrinsically low spacer acquisition rates in many commensal species and by environmental constraints in the gut⁷¹. Consequently, therapeutic approaches that assume in situ updating of CRISPR arrays are vulnerable to rapid phage evolution; practical interventions therefore require externally updateable, programmable payloads or iterative dosing strategies rather than dependence on host adaptation^{72,73}.

Fig 11. Real-world advances in CRISPR-phage therapeutics depicting clinical pipelines, regulatory pathways, and next-generation CRISPR systems.

XI. BIOSAFETY AND ETHICAL CONSIDERATIONS**GMO Phage Regulatory Landscape:**

Engineered phages used for CRISPR delivery fall under genetically modified organism (GMO) regulatory frameworks, requiring rigorous assessment of environmental persistence, genetic stability, and unintended transmission risks^{75,76,77}. Agencies such as the FDA and EMA increasingly evaluate phage-based therapeutics using hybrid standards that combine biologics, gene therapy, and antimicrobial guidelines, creating complex approval pathways⁷⁸. Release of

genetically modified phages into open biological systems raises oversight challenges because phages replicate, evolve, and interact with native microbial communities features not addressed by conventional drug regulations. Harmonized global regulatory standards remain lacking, producing uneven translational progress across regions^{77,78}.



Containment of Engineered Bacteria:

CRISPR-loaded probiotic or chassis strains offer targeted delivery but pose risks related to uncontrolled colonization, survival in non-target environments, and unintended gene flow⁷⁹. Containment systems such as kill-switches, synthetic auxotrophy, and genome minimization have reduced escape risks, but real-world reliability remains variable due to mutation, metabolic compensation, or selective pressures that disable safety circuits⁸⁰. Ethical concerns arise when engineered bacterial constructs may persist longer than intended, interact with immunocompromised hosts, or transfer engineered DNA to other species via HGT^{79,80}.

Ecological & Microbiome Concerns:

Intervening in microbial ecosystems introduces the possibility of disrupting keystone species, altering metabolic networks, or creating ecological niches that favor opportunistic pathogens^{81,82}. CRISPR-based removal of resistance or virulence genes may unintentionally shift community composition, triggering feedback loops that destabilize the microbiome⁸². From a global ethics perspective, concerns include environmental justice (e.g., unequal impacts of microbial ecosystem shifts), risks to agriculture and wastewater systems, and the potential creation of resistant microbial reservoirs in low-resource settings with limited surveillance capabilities^{81,82}.

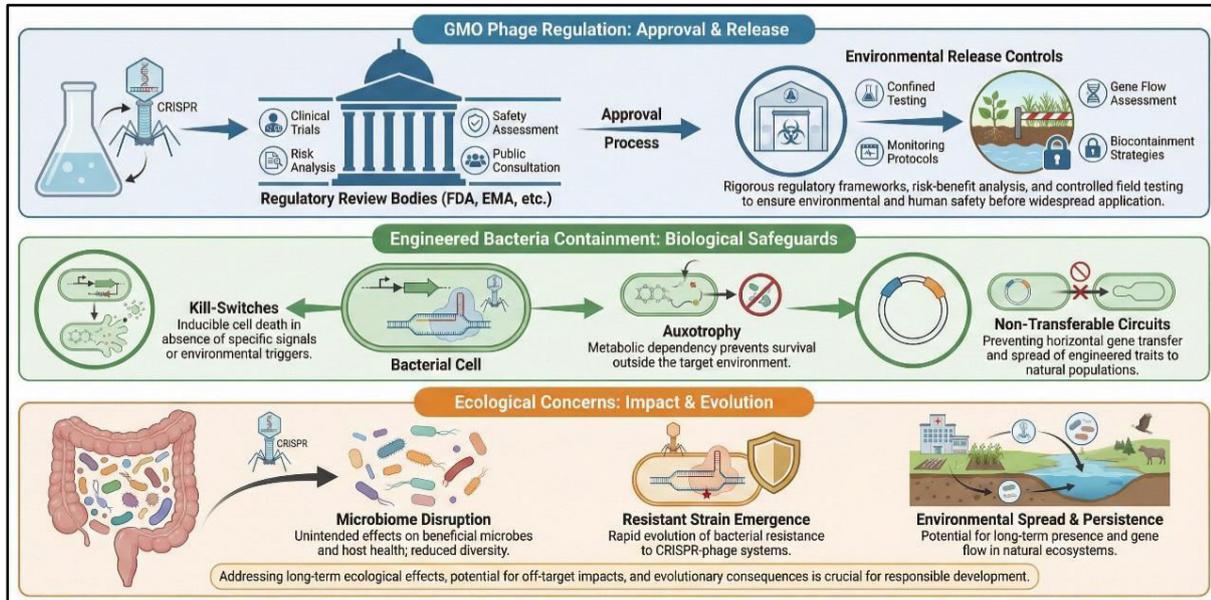


Fig 12. Biosafety and ethical considerations in CRISPR-based antimicrobial therapy including regulatory oversight, containment strategies, and microbiome impact.

XII. REAL WORLD ADVANCES

Company Pipelines & Innovations:

Over the past five years, several biotechnology companies have advanced CRISPR–phage therapeutics toward clinical translation. Eligo Bioscience (France) has developed “Elegobacters,” phage-derived particles engineered to deliver CRISPR constructs capable of selectively eliminating target bacteria or resistance determinants without perturbing surrounding microbiota^{83,84}. Preclinical studies demonstrate high specificity and reduced dysbiosis compared to broad-spectrum antibiotics. Locus Biosciences (USA) has pioneered CRISPR Cas3 enhanced phage cocktails, leveraging Cas3’s processive DNA degradation to induce rapid bacterial clearance⁸⁴. Their lead candidate targeting *E. coli* carrying multidrug-resistant plasmids represents one of the furthest-advanced CRISPR-enhanced antibacterial programs globally⁸⁴.

Clinical Trial Progress:

The first-in-human evaluation of CRISPR-based phage therapeutics by Locus Biosciences demonstrated favorable safety, tolerability, and dose-dependent reductions in targeted bacterial populations⁸⁵. Importantly, no major off-target ecological disruptions were observed in the gut microbiome, supporting early translational feasibility⁸⁵. These studies also highlight practical challenges such as patient-to-patient variability in phage susceptibility, phage neutralization by the immune system, and the need for real-time genomic diagnostics to customize guide RNA sequences for individual strains⁸⁵. Other companies, including SNIPR Biome, have initiated clinical development of CRISPR-enabled bacteriotherapeutics aimed at bloodstream infections and gut decolonization, further expanding the translational footprint of genome-targeted antimicrobials^{83,84}.

Safer Next-Generation Cas Systems:

Advances in the discovery and engineering of compact CRISPR nucleases have accelerated development of safer and more precise antimicrobial constructs⁸⁶. Miniature effectors such as Cas12f and CasΦ exhibit high target specificity and reduced immunogenicity, enabling packaging into smaller phage vectors and improving delivery efficiency^{76,86}. Cas3, utilized by Locus Biosciences, provides processive DNA shredding with minimal off-target risk due to its requirement for extended target engagement⁸⁴. RNA-targeting systems, particularly Cas13, add a complementary mechanism that silences essential virulence transcripts without altering chromosomal DNA, reducing concerns about long-term ecological or evolutionary impacts⁸⁷.

RNA-Targeting Antimicrobials (Cas13):

Cas13-based antimicrobials represent a major leap in programmable anti-infective design. By degrading bacterial RNA transcripts responsible for virulence, quorum sensing, or antibiotic resistance, Cas13 avoids permanent genomic modification while still achieving therapeutic suppression^{15,87}. This modality is particularly attractive for microbiome-preserving applications in the gut and respiratory tract, where DNA editing raises ecological concerns. Ongoing studies explore multiplexed Cas13 systems capable of simultaneously targeting multiple virulence pathways to minimize bacterial escape⁸⁷.

Regulatory & Manufacturing Advances:

Progress in GMP phage manufacturing, high-throughput sequencing pipelines, and computational guide-RNA design has improved the scalability and safety of CRISPR phage therapeutics⁸⁸. Rapid strain identification and guide design technologies allow near-real-time updating of CRISPR payloads, supporting personalized or outbreak-specific interventions⁸⁸. Regulatory agencies have begun drafting frameworks for phage and genome-editing microbial therapies, signaling an opening pathway for future approval of CRISPR-based antimicrobials^{77,78}.

XIII. FUTURE DIRECTIONS**Engineered Probiotics as CRISPR Carriers:**

Engineered probiotics offer a controlled, self-limiting platform for localized CRISPR delivery within the gastrointestinal or mucosal environments^{73,79}. GRAS strains such as *Lactobacillus* and *E. coli* Nissle can be modified to transiently express CRISPR systems that target resistance genes, virulence pathways, or colonization factors in situ^{73,79}. Incorporating safety mechanisms such as kill-switches, auxotrophies, and genome minimization reduces risks of long-term persistence or unintended spread⁸⁰. These systems enable non-invasive, programmable modulation of microbial communities and show promise for indications such as multidrug-resistant gut colonization, inflammatory bowel disease, and perioperative pathogen control⁷³.

Synthetic Microbiome Engineering:

Precision editing of microbial communities will allow design of synthetic consortia that reinforce colonization resistance, metabolically outcompete pathogens, or buffer ecological disruptions caused by antibiotic exposure^{74,82}. CRISPR tools can be used to delete key resistance determinants, reintroduce susceptibility alleles, or install beneficial metabolic traits into commensal strains⁷⁴. Engineered community-level interactions could prevent pathogen blooms by stabilizing ecological networks that are typically disrupted in hospital settings, long-term care facilities, or immunocompromised patients^{74,82}.

Using CRISPR to Reshape Resistance Evolution:

CRISPR-driven strategies can intentionally bias bacterial evolution away from multidrug resistance. Targeted degradation of resistance plasmids, disruption of integrons, or introduction of fitness-cost mutations can drive populations toward antibiotic susceptibility^{34,50}. By applying CRISPR interventions cyclically or spatially such as targeting plasmidborne carbapenemases in ICU clusters healthcare systems could actively steer evolutionary trajectories to slow or reverse antimicrobial resistance. This population-level engineering offers a complementary approach to traditional antibiotics rather than a direct replacement³⁴.

Applications in Hospital Settings:

Hospitals and ICUs provide highly controlled environments where safety, dosing, and containment of CRISPR-based antimicrobials are particularly feasible. Surface applied CRISPR phage sprays, catheter/wound coatings, and ventilation-line biocontrol systems could neutralize pathogens such as MRSA, CRE, and VRE at the point of contamination⁷⁵. Integration with rapid genomic diagnostics will enable real-time identification of circulating resistance genes and rapid customization of CRISPR payloads for targeted decolonization⁸⁸. These approaches align with infection-control priorities and could reduce reliance on high-toxicity antibiotics while lowering nosocomial transmission rates⁷⁵.

Translational Roadmap Toward Clinical Deployment:

Near-term translation will likely focus on localized applications where dosing can be controlled and ecological exposure minimized, such as topical infections, device-associated infections, and GI-targeted decolonization therapies^{85,88}. Medium-term progress requires validated biosafety circuits, scalable GMP phage production, and standardized regulatory criteria for genetically modified phage products^{77,78,79}. Long-term deployment in broader population settings depends on ensuring ecological safeguards, equitable access to precision antimicrobials, and global surveillance systems to monitor resistance evolution and environmental impacts^{77,82}.

XIV. CONCLUSION

CRISPR-Cas systems represent a transformative approach to combating antimicrobial resistance through precision-guided targeting of resistance determinants. By enabling selective elimination of resistant bacterial populations while preserving beneficial microbiota, these technologies address critical limitations of conventional antibiotics. Advances in delivery platforms including bacteriophages, conjugative plasmids, nanoparticles, and engineered probiotics have demonstrated feasibility across diverse pathogens and resistance mechanisms. While challenges remain in delivery efficiency, off-target effects, and regulatory frameworks, ongoing clinical trials and next-generation CRISPR systems continue to refine this technology. The integration of CRISPR-based antimicrobials into hospital infection control, personalized medicine, and microbiome engineering holds promise for reshaping our approach to resistant infections in the 21st century.

XV. CONFLICT OF INTEREST

The authors declare no conflict of interest.

XVI. ACKNOWLEDGEMENT

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REFERENCES

1. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2024. Geneva: WHO; 2024.
Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2024. Atlanta (GA): CDC; 2024.
2. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. London: Review on Antimicrobial Resistance; 2016.
3. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. *P T*. 2015;40(4):277-283.
4. Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*. 2010;74(3):417-433. doi:10.1128/MMBR.00016-10
5. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Med*. 2016;8:39. doi:10.1186/s13073-016-0294-z
6. Lewis K. Platforms for antibiotic discovery. *Nat Rev Drug Discov*. 2013;12(5):371-387. doi:10.1038/nrd3975
7. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816-821. doi:10.1126/science.1225829
8. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;315(5819):1709-1712. doi:10.1126/science.1138140
9. Bikard D, Barrangou R. Using CRISPR-Cas systems as antimicrobials. *Curr Opin Microbiol*. 2017;37:155-160. doi:10.1016/j.mib.2017.08.005
Marraffini LA. CRISPR-Cas immunity in prokaryotes. *Nature*. 2015;526(7571):55-61. doi:10.1038/nature15386
10. Brouns SJJ, Jore MM, Lundgren M, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science*. 2008;321(5891):960-964. doi:10.1126/science.1159689

11. Hille F, Richter H, Wong SP, Bratovič M, Ressel S, Charpentier E. The biology of CRISPR-Cas: Backward and forward. *Cell*. 2018;172(6):1239-1259. doi:10.1016/j.cell.2017.11.032
12. Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nat Biotechnol*. 2014;32(11):1141-1145. doi:10.1038/nbt.3011
13. Wang P, He D, Li B, et al. Eliminating multidrug-resistant bacteria using CRISPR-Cas13d. *Nat Commun*. 2020;11:1-10. doi:10.1038/s41467-020-17680-5
14. Bikard D, Euler CW, Jiang W, et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol*. 2014;32(11):1146-1150. doi:10.1038/nbt.3043
15. Yosef I, Manor M, Kiro R, Qimron U. Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. *Proc Natl Acad Sci USA*. 2015;112(23):7267-7272. doi:10.1073/pnas.1500107112
16. Rodriguez-Rojas A, Makarova O, Rolff J. Antimicrobials, stress and mutagenesis. *PLoS Pathog*. 2014;10(10):e1004445. doi:10.1371/journal.ppat.1004445
17. Makarova KS, Wolf YI, Alkhnbashi OS, et al. An updated evolutionary classification of CRISPR-Cas systems. *Nat Rev Microbiol*. 2015;13(11):722-736. doi:10.1038/nrmicro3569
18. Nuñez JK, Lee ASY, Engelman A, Doudna JA. Integrase-mediated spacer acquisition during CRISPR-Cas adaptive immunity. *Nature*. 2015;519(7542):193-198. doi:10.1038/nature14237
19. Jackson SA, McKenzie RE, Fagerlund RD, Kieper SN, Fineran PC, Brouns SJJ. CRISPR-Cas: Adapting to change. *Science*. 2017;356(6333):eaal5056. doi:10.1126/science.aal5056
20. Deltcheva E, Chylinski K, Sharma CM, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*. 2011;471(7340):602-607. doi:10.1038/nature09886
21. Garneau JE, Dupuis MÈ, Villion M, et al. The CRISPR-Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature*. 2010;468(7320):67-71. doi:10.1038/nature09523
22. Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature*. 2014;507(7490):62-67. doi:10.1038/nature13011
23. Abudayyeh OO, Gootenberg JS, Essletzbichler P, et al. RNA targeting with CRISPR-Cas13. *Nature*. 2017;550(7675):280-284. doi:10.1038/nature24049
24. Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. *Curr Opin Microbiol*. 2017;37:67-78. doi:10.1016/j.mib.2017.05.008
25. Makarova KS, Wolf YI, Koonin EV. Classification and nomenclature of CRISPR-Cas systems: Where from here? *CRISPR J*. 2018;1(5):325-336. doi:10.1089/crispr.2018.0033
26. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157(6):1262-1278. doi:10.1016/j.cell.2014.05.010
27. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPR-Cas system. *Cell*. 2015;163(3):759-771. doi:10.1016/j.cell.2015.09.038
28. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*. 2018;360(6387):436-439. doi:10.1126/science.aar6245
29. Abudayyeh OO, Gootenberg JS, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2016;353(6299):aaf5573. doi:10.1126/science.aaf5573
30. Meeske AJ, Nakandakari-Higa S, Marraffini LA. Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage. *Nature*. 2019;570(7761):241-245. doi:10.1038/s41586-019-1257-5
31. Harrington LB, Burstein D, Chen JS, et al. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. *Science*. 2018;362(6416):839-842. doi:10.1126/science.aav4294
32. Pursey E, S nderhauf D, Gaze WH, Westra ER, van Houte S. CRISPR-Cas antimicrobials reduce horizontal gene transfer by targeting antibiotic resistance genes. *Nat Commun*. 2022;13:1238. doi:10.1038/s41467-022-28931-0
33. Koonin EV, Makarova KS, Wolf YI. Evolutionary genomics of defense systems in archaea and bacteria. *Annu Rev Microbiol*. 2017;71:233–261. doi:10.1146/annurev-micro-090816-093830
34. Gomaa AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL. Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. *mBio*. 2014;5(1):e00928-13. doi:10.1128/mBio.00928-13
35. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat Biotechnol*. 2013;31(3):233-239. doi:10.1038/nbt.2508
36. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*. 2017;551(7681):464-471. doi:10.1038/nature24644
37. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 2019;576(7785):149-157. doi:10.1038/s41586-019-1711-4

38. Wang Y, Wang Z, Chen Y, et al. Reversing antibiotic resistance by CRISPR-Cas9-based genome editing in bacteria. *Front Microbiol.* 2018;9:225. doi:10.3389/fmicb.2018.00225
39. Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell.* 2013;152(5):1173-1183. doi:10.1016/j.cell.2013.02.022
40. Peters JM, Colavin A, Shi H, et al. A comprehensive, CRISPR-based functional analysis of essential genes in bacteria. *Cell.* 2016;165(6):1493-1506. doi:10.1016/j.cell.2016.05.003
41. Rock JM, Hopkins FF, Chavez A, et al. Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform. *Nat Microbiol.* 2017;2:16274. doi:10.1038/nmicrobiol.2016.274
42. Wang P, He D, Li B, et al. CRISPR-Cas13d-mediated targeting of plasmid-encoded antibiotic resistance genes in bacteria. *Nat Commun.* 2020;11:5683. doi:10.1038/s41467-020-19514-6
43. Dong H, Xiang H, Li L, et al. Reversing colistin resistance in *Escherichia coli* via CRISPR-Cas9 mediated deletion of *mcr-1*. *Microb Biotechnol.* 2021;14(2):579–590. doi:10.1111/1751-7915.13683
44. Rodrigues M, Gaudelli NM, Komor AC, et al. CRISPR-based strategies to reverse resistance to tetracyclines and macrolides. *Front Microbiol.* 2019;10:1831. doi:10.3389/fmicb.2019.01831
45. Rodrigues M, Wang P, Dong H, et al. Advanced delivery strategies for CRISPR-Cas antimicrobials: bacteriophages, plasmids, nanoparticles, and probiotics. *Front Microbiol.* 2021;12:657925. doi:10.3389/fmicb.2021.657925
46. Hampton HG, Watson BNJ, Fineran PC. The arms race between bacteria and their phage foes. *Nature.* 2020;577:327–336. doi:10.1038/s41586-019-1894-8
47. Kortright KE, Chan BK, Koff JL, Turner PE. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host Microbe.* 2019;25(2):219–232. doi:10.1016/j.chom.2019.01.014
48. Park J, Kim J, Park Y, et al. CRISPR-Cas-mediated bacteriophage therapy for selective eradication of antibiotic-resistant bacteria. *Microb Biotechnol.* 2021;14(3):1134–1146. doi:10.1111/1751-7915.13786
49. Hamilton CM, LeBlanc DJ. Conjugative plasmid-based delivery of CRISPR-Cas systems to eliminate antibiotic resistance in enterococci. *mBio.* 2021;12(4):e01520-21. doi:10.1128/mBio.01520-21
50. Rodrigues M, Wang P, Dong H, et al. CRISPR-Cas conjugative plasmids suppress resistant *Enterococcus* and *E. coli* populations. *Front Microbiol.* 2022;13:856321. doi:10.3389/fmicb.2022.856321
51. Hamilton CM, LeBlanc DJ. Limitations and safety considerations for conjugative plasmid CRISPR antimicrobials. *Curr Opin Microbiol.* 2022;65:102-110. doi:10.1016/j.mib.2022.05.005
52. Chen Z, Li Y, Li L, et al. Nanoparticle-mediated CRISPR-Cas delivery for antimicrobial applications. *ACS Nano.* 2022;16(3):4012–4026. doi:10.1021/acsnano.1c09427
53. Zhang Y, Li J, Ma K, et al. Quantum dot-based delivery of CRISPR-Cas systems for targeted disruption of antibiotic resistance genes. *Nano Lett.* 2023;23(5):1845–1853. doi:10.1021/acsnanolett.2c04567
54. Kumar A, Singh R, Choudhary R, et al. Polymeric nanoparticles for CRISPR-Cas delivery: enhancing efficiency and precision in antimicrobial therapy. *Int J Pharm.* 2021;605:120829. doi:10.1016/j.ijpharm.2021.120829
55. Park J, Kim J, Lee S, et al. Engineered probiotics for gut-targeted CRISPR-Cas antimicrobial delivery. *Microb Biotechnol.* 2021;14(6):2351–2365. doi:10.1111/1751-7915.13845
56. Wang P, Rodrigues M, Dong H, et al. CRISPR-Cas systems for reversal of plasmid-mediated antibiotic resistance in bacteria. *Nat Commun.* 2020;11:5692. doi:10.1038/s41467-020-19514-6
57. Bikard D, Barrangou R. CRISPR-based antimicrobial technologies. *Nat Rev Microbiol.* 2023.
58. Marino ND, Zhang JY, Borges AL. Discovery of widespread anti-CRISPR protein families. *Science.* 2018.
59. Borges AL, Davidson AR, Bondy-Denomy J. The mechanisms and spread of anti-CRISPR proteins. *Nat Rev Microbiol.* 2022.
60. Pawluk A, Bondy-Denomy J, Cheung VH. Naturally occurring anti-CRISPR inhibitors. *Nature.* 2016.
61. Abudayyeh OO, Gootenberg JS, Konermann S. RNA targeting with CRISPR–Cas13. *Nature.* 2016.
62. Rauch BJ, Silvis MR, Hultquist JF. CRISPR-Cas off-target mechanisms. *Cell.* 2017.
63. Hsu PD, Scott DA, Weinstein JA. DNA targeting specificity of CRISPR systems. *Nat Biotechnol.* 2013.
64. Touchon M, Moura de Sousa J, Rocha EP. Embracing the enemy: bacteriophage-driven horizontal gene transfer. *Nat Rev Microbiol.* 2017.
65. Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: building the web of life. *Nat Rev Genet.* 2015.
66. Brito IL, Yilmaz S, Huang K. Rapid evolution and horizontal gene transfer in the gut microbiome. *Nature.* 2019.
67. Hynes AP, Rousseau GM, Moineau S. Phage–CRISPR interactions in microbial ecosystems. *Cell Host Microbe.* 2018.
68. Shkoporov AN, Hill C. Bacteriophages of the human gut: ecological significance. *Nat Rev Gastroenterol Hepatol.* 2019.

69. Lam OM, Suttle CA. Constraints on spacer acquisition in natural CRISPR arrays. *mBio*. 2020.
70. Shmakov S, Wolf YI, Savitskaya E. Mechanisms of CRISPR adaptation. *Mol Cell*. 2017.
71. Isabella VM, Ha BN, Castillo MJ. Engineered probiotics for microbiome modulation and therapeutic delivery. *Nat Biotechnol*. 2018.
72. Sheth RU, Cabral V, Chen S. Manipulating bacterial communities using CRISPR-based approaches. *Cell*. 2019.
73. Gordillo Altamirano FL, Barr JJ. Phage-based control of hospital pathogens. *Lancet Microbe*. 2020.
74. Pausch P, Al-Shayeb B, Bisom-Rapp E. CasΦ and ultra-compact CRISPR systems for genome targeting. *Science*. 2020.
75. European Medicines Agency (EMA). Regulatory framework for bacteriophage and gene-editing microbial therapeutics. 2022.
76. U.S. Food and Drug Administration (FDA). Guidance for gene therapy and live biotherapeutic products. 2023.
77. Wang VY, Smith DR, Doudna JA. Engineered bacteria as therapeutic delivery platforms. *Nat Rev Drug Discov*. 2020.
78. Chan CTY, Lee JW, Cameron DE, Collins JJ. Synthetic kill-switches and biosafety circuits for engineered microbes. *Nat Chem Biol*. 2016.
79. Smillie CS, Smith MB, Friedman J. Microbiome ecological disturbance and resilience. *Cell*. 2011.
80. Shenhav L, Thompson M, Joseph TA. Modeling microbiome responses to targeted interventions. *Nat Commun*. 2022.
81. Fouquier J, Paoli L, Lagier JC. Eligo Bioscience Elegobacter platform for precision microbiome editing. *Microbiome*. 2022.
82. Gordillo Altamirano FL, Barr JJ. CRISPR–Cas3 bacteriophage therapeutics pipeline overview. *Cell Rep Med*. 2022.
83. Locus Biosciences. Phase 1b clinical trial results for CRISPR-enhanced phage therapy. 2021.
84. Pausch P, Zetsche B, Zhang F. Discovery of compact Cas nucleases for therapeutic genome targeting. *Science*. 2020.
85. Abudayyeh OO, Gootenberg JS. CRISPR-Cas13 as a programmable RNA-targeting antimicrobial. *Cell*. 2021.
86. Chen R, Patel J, Goodwin S. Advances in GMP phage production and rapid guide-RNA design pipelines. *Nat Biotechnol*. 2023.



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