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CRISPR-Cas Systems: Transformative Precision Tools to Combat Antimicrobial Resistance in Multidrug-Resistant Gram-Negative Pathogens

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Abstract

The global rise of antimicrobial resistance (AMR) in Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, threatens the effectiveness of current antibiotics. This crisis, fueled by horizontal gene transfer and a slowing antibiotic pipeline, demands innovative solutions. The CRISPR-Cas system, looking forward, the integration of artificial intelligence, wearable biosensor technologies, and innovative enzyme systems promises to further enhance the diagnostic capabilities of enzyme-based biosensors, making them more accessible and effective in clinical diagnostics. This review explores the latest advancements, challenges, and future directions in the development and application of enzyme-based biosensors for rapid disease diagnosis. Originally a bacterialzadaptive immune mechanism, has been repurposed as a precise, programmable gene-editing tool with significant potential to combat AMR. It offers two primary strategies: targeted killing of resistant bacteria and genetic inactivation of resistance determinants. This review outlines the molecular mechanisms underlying CRISPR function and evaluates advanced delivery methods such as engineered bacteriophages, conjugative plasmids, outer membrane vesicles, and synthetic nanoparticles for their specificity and clinical potential.

Additionally, CRISPR-based diagnostics enable rapid, sensitive, and multiplexed detection of resistance genes, supporting personalized antimicrobial therapy. Despite promising preclinical data, challenges remain in optimizing delivery within complex microbial communities, reducing off-target effects, and addressing ethical considerations of genome editing in microbiomes. Continued technological progress and integration with existing therapies position CRISPR-based approaches as a transformative tool in managing antimicrobial resistance.

Keywords

Antimicrobial resistance; CRISPR-Cas; Gram-negative; bacteria; Gene editing; bacteriophage delivery.

Introduction

The rise of antimicrobial resistance (AMR) in Gram-negative bacteria has emerged as one of the most pressing threats to global health, undermining the efficacy of conventional antibiotics and increasing morbidity, mortality, and healthcare costs worldwide [1,2]. Gram-negative pathogens such as Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Acinetobacter baumannii have developed complex resistance mechanisms, including the production of extended-spectrum β-lactamases (ESBLs), carbapenemases, efflux pump overexpression, and reduced membrane permeability [3,4]. The convergence of high adaptability, horizontal gene transfer capacity, and environmental persistence in these bacteria poses an urgent challenge to infection control [5].

Historically, the treatment of bacterial infections entered a golden era following the discovery of penicillin in 1928 by Alexander Fleming, which paved the way for the antibiotic revolution of the mid-20th century [6]. However, bacterial adaptation was rapid—penicillin resistance was documented as early as the 1940s [7]. Subsequent decades witnessed the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, largely fueled by overuse and misuse of antibiotics in human medicine, agriculture, and veterinary practice [8,9]. This cycle of antibiotic development followed by the swift emergence of resistance highlighted the limitations of relying solely on conventional drug pipelines [10].

In parallel, the molecular biology revolution of the late 20th century brought forth tools for precise genetic manipulation. Among these, the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins) system emerged from fundamental microbiology studies of *Streptococcus thermophilus* in 1987, where peculiar repetitive DNA sequences were first reported [11]. Initially viewed as a genetic curiosity, CRISPR-Cas was later recognized in the early 2000s as an adaptive immune system in bacteria and archaea, defending against invading phages and plasmids [12]. A breakthrough came in 2012 when researchers demonstrated that the Cas9 protein, guided by synthetic RNA, could be reprogrammed to cut DNA at virtually any desired location [13]. This transformative advance converted CRISPR-Cas into a versatile genome-editing platform with applications ranging from agriculture to medicine. The importance of CRISPR-Cas in the context of AMR lies in its potential to target resistance determinants at their genetic source [14,15]. Unlike antibiotics, which exert broad-spectrum pressure and can inadvertently promote resistance, CRISPR-based antimicrobials can be engineered to selectively disable resistance genes without harming beneficial microbiota [16]. Furthermore, CRISPR constructs can be delivered via bacteriophages, conjugative plasmids, or synthetic nanoparticles, enabling

precise eradication of resistance plasmids or chromosomal loci [17]. For Gram-negative bacteria—where the impermeable outer membrane and robust efflux systems complicate treatment—CRISPR-Cas offers a precision-based alternative that aligns with the principles of next-generation antimicrobial stewardship [18].

This review synthesizes recent advances in CRISPR-Cas applications for combating AMR in Gram-negative bacteria, focusing on both mechanistic insights and translational potential. By combining historical lessons from antibiotic resistance evolution with cutting-edge genome editing technology, we aim to highlight how CRISPR-based strategies could reshape the future of infectious disease management, offering targeted, sustainable, and adaptable solutions in the fight against resistant pathogens [19,20].

CRISPR-cas mechanisms and classification

CRISPR–Cas systems operate through a coordinated, three-stage immune process—adaptation, expression, and interference—which allows prokaryotes to recognize and defend against invading genetic elements such as bacteriophages and plasmids [8-10].

During the adaptation stage, short fragments of foreign DNA, known as spacers (approximately 20–40 base pairs), are excised from the invader's genome and inserted into the host's CRISPR array. This process is primarily mediated by the Cas1–Cas2 complex, which integrates the new spacer adjacent to the leader sequence of the CRISPR locus, ensuring its priority in subsequent transcription [8,9]. Over time, this growing array serves as a chronological record of past infections, enabling sequence-specific immunity.

In the expression stage, the CRISPR array is transcribed into a long precursor CRISPR RNA (pre-crRNA). In many systems, the pre-crRNA undergoes processing to produce mature CRISPR RNAs (crRNAs), each containing a single spacer sequence flanked by repeat-derived segments. In Type II CRISPR systems, processing requires the participation of a trans-activating crRNA (tracrRNA) and the bacterial enzyme RNase III, which together produce guide RNAs that can precisely direct Cas effectors [9,11].

The interference stage is the execution phase, where crRNA-loaded Cas effector complexes survey cellular nucleic acids for complementary target sequences. Target recognition often requires an adjacent protospacer adjacent motif (PAM) in DNA-targeting systems, ensuring that only foreign sequences—rather than the host's own CRISPR loci—are cleaved [12,13]. Upon successful recognition, the Cas protein induces a double-stranded break in DNA or cleaves RNA, depending on the type of CRISPR–Cas system.

CRISPR–Cas systems are broadly divided into two major classes based on the composition of their effector complexes [13,14]. Class 1 systems employ multi-protein complexes for target recognition and cleavage, encompassing Types I, III, and IV. Class 2 systems, in contrast, rely on a single, large, multi-domain effector protein, making them more straightforward to harness for genome engineering. Prominent examples of Class 2 effectors include Cas9 (Type II), Cas12a, formerly Cpf1 (Type V), and Cas13 (Type VI), which target DNA or RNA with high specificity and have become the preferred tools in biotechnological and therapeutic applications [14-16] (Table 1).

Class	Type	Signature effector(s)	Target nucleic acid	Effector complex structure	Notable features	Representative examples
Class 1	Type I	Cas3	DNA	Multi-protein (Cascade complex)	Requires PAM; Cas3 is a helicase–nuclease	*E. coli* Type I-E
	Type III	Cas10	DNA and RNA	Multi-protein (Csm/Cmr complexes)	Can target both DNA and RNA; transcription-dependent targeting	Thermus thermophilus Type III-B
	Type IV	Csf proteins	DNA	Multi-protein	Often plasmid-associated; poorly characterized	Pseudomonas aeruginosa
Class 2	Type II	Cas9	DNA	Single-protein	Requires tracrRNA; most widely used in genome editing	Streptococcus pyogenes Cas9
	Type V	Cas12a (Cpf1)	DNA	Single-protein	Produces staggered DNA cuts; no tracrRNA required	Francisella novicida Cas12a
	Type VI	Cas13	RNA	Single-protein	RNA-guided RNA cleavage; collateral cleavage activity	Leptotrichia wadei Cas13a

Table 1: Classification of CRISPR–Cas systems

Applications of CRISPR-Cas against antimicrobial resistance

The advent of CRISPR–Cas technology has opened a new frontier in the battle against antimicrobial resistance, offering unprecedented precision in selectively eliminating resistant bacterial strains while sparing susceptible or beneficial members of the microbiome. This specificity arises from the programmable nature of CRISPR guide RNAs, which can be tailored to recognize unique genetic sequences associated with resistance determinants, thereby avoiding the collateral damage often caused by conventional antibiotics [7,17].

One of the most promising approaches involves strain-specific killing by targeting essential genes or resistance-conferring loci. Conjugative plasmid-based delivery of CRISPR–Cas9, for example, has been shown to selectively eradicate resistant *Escherichia coli* populations in mixed microbial communities, effectively reducing resistance gene prevalence without disrupting the surrounding microbiota [7] (Table 2).

Bacteriophage-mediated delivery represents another powerful strategy; wherein engineered phages serve as precision vehicles to deliver CRISPR payloads into target bacteria. In animal infection models, phages carrying CRISPR–Cas systems have demonstrated significant pathogen clearance, with modular phage platforms enabling rapid reprogramming to address newly emerging resistance genes [17,18]. Such modularity enhances the feasibility of creating tailored therapies for diverse Gram-negative pathogens (Table 2).

Application Strategy	Delivery System	Target(s)	Mechanism	Outcome	References
Strain-specific	Conjugative plasmids	β-lactamase genes, essential	Cas9-mediated double-	Selective elimination of resistant *E. coli*;	[7]

killing		chromosomal genes	strand breaks	preservation of commensals	
Therapeutic phage delivery	Engineered lytic or temperate phages	Plasmid-borne or chromosomal resistance genes	Cas9 or Cas12a cutting	Clearance of target pathogens in animal models	[17,18]
Modular phage systems	Reprogrammable phage scaffolds	Multiple AMR genes	Swappable guide RNAs with Cas effectors	Rapid adaptation to new resistance profiles	[18]
Base editing via non-replicative phages	Phagemid particles	β-lactamase genes	Cytidine/adenine base editors	Gene inactivation without double-strand breaks; restored antibiotic sensitivity	[19]
CRISPR interference (CRISPRi)	Plasmids or phages	Integrons, transposons, conjugative plasmids	dCas9-mediated transcriptional repression	Inhibition of resistance gene expression and transfer	[20–22]
CRISPR-based diagnostics (SHERLOCK, DETECTR)	Cas12/Cas13 with reporter probes	AMR genes in clinical samples	Collateral cleavage of reporter molecules	Rapid, point-of-care AMR detection	[23,24]
Multiplex detection (FLASH)	Hybridization + CRISPR	Multiple AMR genes	Cas13 collateral cleavage with guide multiplexing	Comprehensive resistance profiling	[25]

Table 2: Applications of CRISPR–Cas Systems Against Antimicrobial Resistance.

Recent innovations also include non-replicative phage particles equipped with base editors. These systems, rather than introducing double-stranded DNA breaks, induce precise nucleotide substitutions that inactivate resistance genes, such as β-lactamases, in gut-colonizing *E. coli*. This results in restored antibiotic susceptibility while minimizing the risk of bacterial cell death-induced toxin release or fitness costs [19] (Table 2).

Beyond direct bacterial killing, CRISPR interference (CRISPRi) and plasmid-targeting strategies have been developed to disarm mobile genetic elements—including integrons, transposons, and conjugative plasmids—that serve as vehicles for multidrug resistance gene dissemination [20–22]. By disabling these mobile resistance platforms, CRISPR systems can slow the horizontal spread of AMR within microbial populations (Table 2).

In parallel, CRISPR-based molecular diagnostics are revolutionizing AMR detection and surveillance. Platforms such as SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) leverage the collateral cleavage activity of Cas12 and Cas13 enzymes to detect AMR genes at the point of care [23,24]. Enhanced multiplexing platforms like FLASH (Finding Low Abundance Sequences by Hybridization) further enable simultaneous detection of multiple resistance genes, facilitating rapid and comprehensive resistance profiling in clinical settings [25].

Clinical progress and challenges

Although CRISPR-based antimicrobials are still in their infancy compared to other therapeutic domains, the field has witnessed notable progress in recent years. Historically, the use of CRISPR-Cas systems as antimicrobials was first demonstrated in the early 2010s when researchers engineered CRISPR-Cas9 constructs to selectively target antibiotic resistance genes in pathogenic bacteria. This proof-of-concept established the feasibility of reprogramming bacterial adaptive immunity for therapeutic purposes, paving the way for translational efforts.

As of early 2025, no CRISPR-based antibacterial therapy has reached late-stage (Phase 3) clinical trials, but Locus Biosciences has made a significant milestone by advancing its CRISPR-Cas3 bacteriophage therapy to Phase 2 clinical trials for treating recurrent urinary tract infections (UTIs) caused by *Escherichia coli*. In early human studies, this therapy demonstrated promising efficacy and safety, with some patients achieving complete bacterial clearance without recurrence over several months (Table 3).

Beyond bacterial applications, the bulk of clinical CRISPR trials are focused on genetic disorders, cancers, and viral infections (Table 3). For example:

Category	Example / Status	Details	Reference(s)
Bacterial CRISPR Therapy	Locus Biosciences – CRISPR-Cas3 phage therapy	Phase 2 trials for recurrent *E. coli* UTIs; promising bacterial clearance in early human studies	[WIRED, Wikipedia, 26]
Viral Applications	HIV & HBV genome disruption	Preclinical; CRISPR-mediated viral genome excision in cell and animal models	[2, 16]
Genetic Disorders	Exa-cel for sickle cell & β-thalassemia	Received first approval in the UK; regulatory review ongoing in US/EU	[26]
Ophthalmology	EDIT-101 for Leber congenital amaurosis	In vivo CRISPR gene editing in retinal cells	[3]
Challenges – Delivery	Targeting biofilms and polymicrobial infections	Limited penetration and heterogeneity hinder delivery efficiency	[24, 26]
Challenges – Off-target	Risk to commensals	Requires highly specific guide RNA design to avoid microbiome disruption	[16]
Challenges – Resistance	Anti-CRISPR proteins in bacteria	Naturally occurring inhibitors can block Cas activity	[2, 24]
Challenges – Regulation	Ethical, ecological, safety concerns	Stringent oversight delays trial progression	[26]

Table 3: Current Clinical Progress and Key Challenges of CRISPR-Based Antimicrobials.

- **Sickle cell disease and β-thalassemia: –** Vertex Pharmaceuticals' exa-cel (CRISPR-Cas9 gene editing) recently received regulatory approval in the UK and is under review in the US and EU.

- **Leber congenital amaurosis: –** Editas Medicine's EDIT-101 targets retinal cells to restore partial vision.
- **HIV and hepatitis B: –** Preclinical studies have shown CRISPR-mediated disruption of viral genomes.

According to recent reports, over 250 active CRISPR-related clinical trials are ongoing worldwide as of early 2025. Despite these advances, translation into antimicrobials faces unique and formidable challenges:

- **Target delivery: -** Efficiently reaching bacterial cells within polymicrobial communities and biofilms remains difficult, especially in chronic infections.
- **Off-target activity:** Even slight mismatches in guide RNAs could damage commensal or beneficial microbiota.
- **Anti-CRISPR proteins:-** Many bacteria encode proteins that inhibit CRISPR-Cas systems, which can reduce therapeutic efficacy.
- **Microbiome balance:-**Narrow-spectrum targeting must avoid unwanted disruption of the host microbiome.
- **Ethical and regulatory complexity:-** Safety, reversibility, and ecological impact assessments are still evolving under stringent regulatory frameworks.

Conclusion and future perspectives

CRISPR–Cas systems represent a transformative leap in antimicrobial therapeutics, redefining the approach to combating bacterial infections in an era of escalating antimicrobial resistance (AMR). Unlike conventional broad-spectrum antibiotics—which indiscriminately target both pathogenic and beneficial microbes—CRISPR-based interventions enable precision eradication of specific bacterial strains or the direct neutralization of their resistance determinants. This dual-action capability—simultaneously removing pathogens and dismantling the genetic basis of resistance—offers an unprecedented level of specificity that is difficult for conventional drugs to match.

The promise of CRISPR-based antimicrobials is reinforced by rapid advances in delivery technologies. Engineered bacteriophages, conjugative plasmids, liposomal nanoparticles, and synthetic biology–driven vectors are expanding the range of possible clinical applications, including in challenging environments such as biofilms, intracellular infections, and polymicrobial communities. Simultaneously, the integration of CRISPR-based antimicrobials with cutting-edge diagnostics—such as SHERLOCK, DETECTR, and FLASH—offers the possibility of real-time detection of resistance genes, enabling targeted therapy deployment within hours of diagnosis.

Looking forward, next-generation CRISPR therapeutics will likely focus on several key innovations:

- **Expanding Target Spectrum: -** Designing systems that target highly conserved resistance genes, mobile genetic elements, or integrons, enabling broad coverage across multiple species without harming the host microbiota.

- **AI-Assisted Guide RNA Optimization: -** Harnessing machine learning to improve guide RNA design, thereby increasing editing efficiency while minimizing off-target interactions.
- **Safe In Vivo Microbiome Editing:** - Developing delivery systems capable of selectively editing bacterial populations within the human microbiome, potentially reducing colonization by multidrug-resistant organisms without collateral ecological damage.
- **Therapeutic Synergy: -** Combining CRISPR-based antimicrobials with existing antibiotics or phage therapy to enhance bacterial clearance, delay resistance emergence, and restore efficacy to drugs compromised by widespread resistance.

As these technologies progress from preclinical experimentation to rigorously designed clinical trials, CRISPR antimicrobials are poised to become an integral part of the infectious disease treatment landscape. In the long term, their ability to precisely, adaptively, and sustainably control resistant pathogens could mark a turning point in the fight against AMR, restoring a level of control that was once thought to be slipping beyond our grasp.

Conflicts of interest

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Mohammad Fayyad-Kazan: Writing – review & editing, Writing – original draft, Supervision, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization**.**

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