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CRISPR-Cas Approaches For Reversing Antibiotic Resistance Through Genetic Reprogramming : A Review

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ABSTRACT

Antimicrobial resistance claims over 1.27 million lives annually, driven by horizontal gene transfer of plasmids encoding beta-lactamases (blaNDM), efflux pumps (acrAB-tolC), and aminoglycoside modifiers. CRISPR-Cas9, Cas12a, Cas13, and interference variants (CRISPRi/a) offer programmable nucleases that restore antibiotic susceptibility by cleaving resistance cassettes, silencing transcription, or inducing collateral RNA degradation. This review synthesizes recent advances, including phagemid-delivered Cas9 curing >94% of carbapenem-resistant Enterobacteriaceae (CRE) plasmids, conjugative CRISPR drives converting vancomycin-resistant Enterococcus populations, and AI-optimized gRNAs minimizing off-target effects in GC-rich genomes[9].Fundamental processes involve DSBs capitalizing on error-prone bacterial NHEJ/HR for lethal outcomes, Cas13's HEPHN-driven mRNA shredding, and dCas9 conjugates enabling epigenetic silencing. Advanced vectors—phage fusions, nanocarriers, mobile plasmids—achieve biofilm invasion and herd-level propagation, demonstrated in rodent UTI/pneumonia against ESKAPE threats. Lab successes hit 99.9% resistance ablation, yet clinical obstacles linger: Cas immunogenicity, plasmid instability amid diverse flora, and adaptive AcrIF1-3 blockers. Pairing actinomycete BGC discovery with CRISPR-AMR holds promise for hybrid antibiotic innovations.

Figure : 00 References : 21 Table : 00

KEY WORDS : Actinomyc, Antibiotic susceptibility, Antimicrobial resistance, BGCs, Cas9/Cas12a/Cas13, CRISPRi, CRISPR-Cas, DNA repair pathways, ESKAPE pathogens, Horizontal gene transfer, NHEJ/HR, Phage delivery, Plasmid curing, Therapeutic reprogramming.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges, threatening to undermine the effectiveness of modern medicine and making even common bacterial infections increasingly difficult to treat[6]. The primary causes of AMR includes inappropriate antibiotic prescriptions, over use in agricultural and livestock for example feeding of antibiotics to chickens in the poultry farm where inadequate dosing and insufficient infection control systems are neglected resulting strong selective pressures that facilitate the emergence and spread of resistant strains[7]. These problems are further escalated by the distribution of substandard medicines, the release of antibiotic residues into the environment or the ecosystem, and the horizontal transfer of resistance genes. The discovery of new classes of low-molecular-weight antimicrobial[4] agents has been put into hold. While, the new age antibiotics are merely structural derivatives of existing scaffolds which lack any novel

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mechanism of action. This triggers the widening of gap between the rapid evolution of bacterial resistance and the saturation of antibiotic innovation which calls for the urgent need for alternative antimicrobial strategies[4].

Several approaches have been explored and pitched as a potential remediation for AMR including Bacteriophage therapy, antimicrobial peptides, vaccines and microbiome-based interventions. However, these approaches face limitations such as narrow host range, instability issues, immunogenicity[4] and inconsistent efficacy in clinical trials. Against these hindrances, Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR and CRISPR-associated[7] Cas proteins have emerged as flexible programmable molecular tools. CRISPR-Cas systems were discovered as adaptive immune systems in bacteria and archaea, CRISPR-Cas systems have been remodelled as targeted nucleic acid remodeling platforms capable of precise DNA or RNA cleavage and editing .

CRISPR-Cas Systems

The CRISPR-Cas system[8] is an adaptive immune defense mechanism in prokaryotes that protects cells by recognising and cleaving foreign nucleic acids. The CRISPR-Cas defence system operates in three different sequential steps which are as follows :

1. Adaptation (Spacer Acquisition) : In the initial adaptation phase, the Cas1 and Cas 2 proteins (the signature effectors for most CRISPR types) recognize and capture short fragments (typically 30-40 nucleotides) of foreign double-stranded DNA, such as from bacteriophages or conjugative plasmids. These fragments, termed protospacers, are precisely excised adjacent to a protospacer adjacent motif (PAM) sequence—usually 2-6 base pairs like NGG for Cas9 or TTTV for Cas12a—that serves as a recognition signal to avoid self-targeting the host CRISPR array. The protospacer is then integrated as a new spacer at the leader-proximal end of the CRISPR locus, expanding the array's memory repertoire and antibiotic resistance plasmids carrying genes like blaNDM[10].

2. Processing (crRNA biogenesis): During the processing stage, the entire CRISPR array is transcribed by host RNA polymerase III into a long precursor CRISPR RNA[10] (pre-crRNA) under the control of an upstream A/T-rich leader sequence and promoter. This pre-crRNA is subsequently cleaved into individual mature CRISPR RNAs (crRNAs)—each containing a single spacer flanked by repeat fragments—through two pathways: host RNase III-dependent processing (in type II systems like Cas9, requiring tracrRNA[17] hybridization for double-stranded stem-loop formation) or Cas protein-mediated cleavage (in type I/III systems *via* Cascade complex or Csm/Cmr effectors). This maturation yields functional crRNA guides (typically 39-42 nt) that retain the full spacer sequence for target recognition while the repeat ensures proper Cas protein binding.

3. Interference (Target Cleavage) : In the final interference stage, mature crRNA assembles with the relevant Cas effector complex (*e.g.,* Cas9-crRNA-tracrRNA ternary complex, Cas12a-crRNA R-loop, or Cascade-Cas3 helicase-nuclease) to scan complementary target DNA or RNA. Base-pairing between the crRNA[17] spacer and invader protospacer (requiring PAM for most type II/V systems) triggers conformational activation, leading to target strand nicking or double-strand breaks *via* conserved nuclease domains (RuvC/HNH in Cas9; HEPN in Cas13 for RNA). This cleavage generates lethal double-strand breaks (DSBs) or collateral RNA shredding, rapidly neutralizing the invader and preventing replication/integration. Critically, this process erects a formidable barrier against reinfection by identical phages (>99% protection efficiency) and blocks horizontal gene transfer (HGT) of resistance plasmids, as demonstrated by CRISPR arrays naturally rejecting blaKPC/mcr-1 acquisition in clinical E. coli isolates.

Nevertheless, CRISPR-Cas technologies face substantial obstacles for clinical deployment, such as PAM requirements restricting accessible targets, inadvertent off-target editing, immune system responses to Cas proteins, and inefficient delivery methods. Compact Cas variants like SaCas9, CjCas9, and NmCas9 better fit AAV vector size limits, improving transduction capabilities. Miniaturizing enzymes to d"1,000 amino acids—exemplified by CasÖ, Cas12f (CasX), and Cas14—broadens compatibility with AAVs, LNPs, and polymer-based carriers. Downsizing-related activity drops are offset by structure-guided modifications (domain removal, module replacement) alongside directed evolution strategies.

Protein engineering has also yielded PAM-flexible Cas versions (xCas9, SpCas9-NG, SpRY) for wider targeting and precision-enhanced variants (eSpCas9, SpCas9-HF1, HypaCas9) that curb off-target effects. Furthermore, continuous evolution systems like PACE have concurrently optimized editing accuracy, protein robustness, and performance, accelerating viable AMR interventions.

CRISPR-Cas Genome Modification In Bacteria : Role of DNA Repair Pathways

A successful gene editing in bacteria using the CRISPR-Cas system depends not only on the cleavage of Cas protein but also on the cellular by default DNA repair pathways that process resulting double stranded

breaks. Bacteria generally repair *via* two main mechanisms-

1. Homologous Recombination (HR)[18]
2. Non-homologous Recombination end joining (NHEJ) which is analogous to those in mammalian cells[11].

Bacteria mainly rely on homologous recombination (HR) driven by RecA, working with either the RecBCD complex (or AddAB/AdnAB systems in mycobacteria) and the RecFOR pathway. Species such as *Escherichia coli*, *Helicobacter pylori*, *Haemophilus influenzae* and *Lactococcus lactis* carry out accurate DNA repair via HR, often integrating recombination with external donor DNA for precise sequence replacements or insertions. *Deinococcus radiodurans*[12] demonstrates superior HR fidelity under radiation stress through RecA-PprA collaboration. Importantly, E. coli lacks a classical non-homologous end-joining (cNHEJ) mechanism, resulting in a clear preference for HR-mediated repair.

Sophisticated CRISPR-Cas based bacterial gene editing requires strategies that are tailored to not only to Cas nuclease[5] efficiency but also to strain-specific DNA repair pathways. When HR dominates, it is more effective to coordinate›-Red or RecET recombinase expression with Cas-induced cleavage via inducible expression systems. Although Cas9 is known for its generation of blunt cuts that can be cytotoxic[4], while Cas12a produces staggered 5'-overhang double stranded breaks that may produce Homologous Direct Repair. Most, interestingly targeting essential genes in bacteria with deficient repair capacity may lead to lethal DNA damage accumulation and these vulnerabilities can be used to design targeted antimicrobial strategies against key genes in repair-deficient bacteria.

Mechanism Of Antibiotic Resistance-Horizontal Gene Transfer

The tenacious global expansion of antimicrobial resistance[20] stems from sophisticated, evolutionarily refined bacterial defense mechanisms. Rather than arising from isolated genetic mutations, this crisis reflects coordinated transformations across cellular architecture, metabolic networks, and adaptive genomics. This resistance is not the result of a single mutation but a systemic phenomenon driven by multilayered alterations in cellular structure, metabolism, and genetic adaptation. These sophisticated resistance mechanisms are broadly classified into intrinsic resistance (naturally encoded) and acquired resistance (evolutionarily gained)[13].

[A] Intrinsic resistance : Intrinsic resistance originates from bacteria's fundamental structural and physiological properties. Gram-negative bacteria[1], for example, utilize lipopolysaccharide (LPS)-rich outer membranes to impede hydrophobic antibiotic entry, while RND- and MFS-family efflux pumps actively export accumulated drugs, maintaining non-lethal intracellular concentrations.

[B]Adaptive resistance : Acquired resistance, which directly contributes to clinical treatment failures, develops through mutations altering drug targets or acquisition of resistance-conferring genes. Point mutations in the quinolone resistance-determining region (QRDR) of gyrA or parC decrease fluoroquinolone binding, and horizontally transferred carbapenemase[19,20]genes (blaKPC, blaNDM, blaOXA-48)[19]render â-lactams completely ineffective. Selective pressure from antibiotics rapidly amplifies these adaptations, producing dramatic phenotypic differences between drug-sensitive and resistant strains of the same lineage.

Antibiotic-resistant bacteria and their resistance genes traverse linked ecological compartments connecting soil microbiomes, aquatic systems, animal agriculture, and human communities. Through the One Health lens, AMR ignores species and ecosystem boundaries, continuously reshuffled by horizontal gene transfer, phage transduction, and plasmid re-assortment that propel its worldwide expansion.

Effective counter measures require cross-disciplinary integration of microbiology, clinical therapeutics, environmental science, and public health initiatives to confront both resistance biology and dissemination routes. Precision therapeutic strategies hold particular promise for breaking transmission chains, with CRISPR-mediated gene targeting increasingly recognized for its capacity to selectively neutralize resistance determinants.

How CRISPR-Cas System can overcome antimicrobial resistance

CRISPR-Cas systems herald a precision revolution against antimicrobial resistance, deploying programmable gRNAs to orchestrate targeted interventions at DNA, RNA, and transcriptional tiers—contrasting sharply with conventional antibiotics' indiscriminate selective pressure. Cas9 and Cas12a nucleases execute surgical double-strand breaks at chromosomal resistance loci (blaNDM-1, mecA, vanA, tetM) or excise multi-drug plasmids via pCasCure platforms, achieving 94% elimination rates in carbapenem[19,21]-resistant Enterobacteriaceae (CRE) and restoring â-lactam efficacy; Cas13 variants, packaged in CapsidCas13a phagemids, recognize ermB or aph(3')-IIIa transcripts in MRSA, unleashing HEPN-mediated collateral RNA cleavage that

indiscriminately shreds essential bacterial transcripts for rapid, sequence-specific killing without genomic alteration.

CRISPR interference (CRISPRi)[2] harnesses catalytically dead dCas9 to sterically block transcription initiation at efflux operons (acrAB-tolC in E. coli, mexAB-oprM in P. aeruginosa), slashing MICs 4-64-fold for rifampicin, tetracycline, and aminoglycosides while repressing biofilm architects (icaA in S. epidermidis, csgD in E. coli, mrkA in K. pneumoniae) to dismantle persistence sanctuaries. Beyond core resistance genes, expanded arsenals target virulence determinants (fliC, motAB, pilT motility clusters), quorum sensing hubs (agrAC, luxS), and gelatinase (gelE), synergistically amplifying co-administered antibiotics by fracturing tolerance networks in ESKAPE pathogens[16].

Advanced delivery vectors—bacteriophage[3] chimeras for tropism-specific infection, conjugative "gene drive" plasmids propagating population-wide conversion (99.9% VRE resensitization in murine models), and biofilm-penetrating lipid nanoparticles—enable therapeutic scale-up. Theranostic dual-use emerges as Cas13 hybrids fuse diagnostics with treatment, while CRISPR activation (CRISPRa) dissects regulatory cascades (SoxS, MarA) for network rewiring. This specificity neutralizes HGT reservoirs without collateral microbiota disruption, cementing CRISPR-Cas as the vanguard for programmable AMR reversal.

CRISPR Regulation Of Antibiotic Susceptibility : Opportunities and Challenges in Therapy

CRISPR-based methods for controlling antibiotic sensitivity establish a ground breaking treatment model, offering nucleotide-level precision against resistance and virulence genes to overcome the non-selective drawbacks of current antibiotics.

Robust preclinical data validate multilayered CRISPR tactics—Cas9 DNA cleavage, Cas13 RNA targeting, and dCas9 CRISPRa/i regulation—successfully reinstating susceptibility, clearing plasmids, and disrupting biofilms in MDR/XDR pathogens including CRE (E. coli, K. pneumoniae), VRE, and Salmonella enterica across cellular and animal studies.

Key translation obstacles involve delivery challenges (restriction-modification systems, efflux pumps, biofilms) and specificity concerns (off-target editing, Acr proteins, PAM escape mutations). Phagemids, conjugative plasmids, and nanoparticles contend with tropism/stability issues, while Cas RNP complexes struggle with broad tissue penetration in polymicrobial/biofilm contexts.

Advanced engineering counter measures encompass high-fidelity Cas variants, PAM-independent nucleases, multiplexed gRNA arrays, and spatiotemporal controls (photo/ligand-inducible split-Cas). AI-optimized gRNA design alongside automated lab pipelines achieve 80-90% first-attempt precision.

Synergistic combination regimens pairing CRISPR with antibiotics or AMPs boost efficacy through enhanced permeability and pathway blockade, requiring meticulous timing/dosage calibration. One Health deployment leverages CRISPR-armed probiotics to deplete gut resistance reservoirs and interdict HGT (tra/int loci), curbing trans-ecosystem superbug cycling via integrated surveillance—tempered by GMO biosafety monitoring.

Conclusion

This review has comprehensively examined the pivotal contribution of CRISPR-Cas system approaches for reversing antibiotic resistance through genetic programming as CRISPR-Cas systems provide precise tools to fight antimicrobial resistance[15] by targeting resistance genes, plasmids, and biofilms more effectively than traditional antibiotics. Preclinical studies show they restore drug susceptibility in dangerous pathogens like CRE and MRSA while blocking resistance spread through horizontal gene transfer. Challenges like delivery barriers and off-target effects must be solved through better engineering and AI optimization. One Health strategies using CRISPR probiotics can disrupt resistance across humans, animals, and environments. Ultimately, CRISPR offers hope for sustainable antibiotic use and ending the AMR crisis.

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