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Next-Generation Nano-CRISPR Therapeutics for Multidrug-Resistant Infectious Diseases

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Abstract

Antimicrobial resistance (AMR) has become a major global health challenge, threatening the effectiveness of antibiotics that have long been the foundation of modern medicine. The increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria has resulted in rising morbidity, mortality, and healthcare costs worldwide, highlighting the urgent need for innovative antimicrobial strategies. Conventional antibiotics often exert broad-spectrum effects, disrupting beneficial microbial communities and accelerating the emergence of resistance. In response, CRISPR-Cas systems have emerged as promising precision antimicrobial tools capable of selectively targeting bacterial genomes, resistance genes, virulence determinants, and mobile genetic elements with high specificity.

This review explores the molecular basis of AMR, including enzymatic drug inactivation, target modification, efflux-mediated resistance, biofilm formation, and horizontal gene transfer. It further examines the diversity of CRISPR-Cas systems and their application in bacterial killing, plasmid curing, gene silencing, and microbiome modulation. Particular attention is given to the delivery challenges associated with CRISPR therapeutics and the role of nanotechnology in overcoming these barriers. Nanocarrier platforms, including lipid nanoparticles, polymeric nanoparticles, biomimetic systems, and phage-derived carriers, provide enhanced protection, targeted delivery, improved biofilm penetration, and controlled release of CRISPR cargo. The review also highlights the growing contribution of computational approaches, such as molecular docking, molecular dynamics simulations, and artificial intelligence-assisted design, in optimizing guide RNAs and nanocarrier performance. Current experimental evidence demonstrates that Nano-CRISPR systems can effectively combat resistant pathogens, disrupt biofilms, and restore antibiotic susceptibility. Collectively, these advances position Nano-CRISPR therapeutics as a promising next-generation strategy for precision antimicrobial intervention in the fight against AMR.

Keywords: AMR, CRISPR, Nanocarrier, Precision Antimicrobials, MDR bacteria

Introduction

The need for antibiotics caused a revolution in medicine in the middle of the 20th century. The unseen backbone of modern clinical practice is antibiotics. Antimicrobial resistance (AMR) is a global crisis that has been brought on by the success of these medications. Bacteria started to build defences against the treatments shortly after penicillin introduction. Resistant strains had spread throughout towns, hospitals, and agriculture by the early 2000s. AMR is growing so quickly these days that many scientists view it as an existential danger to modern medicine (Antimicrobial Resistance Collaborators, 2022; WHO, 2023).

1.1 The Global Scale of AMR

According to estimates, bacterial AMR caused about 4.95 million fatalities in 2019, of which 1.27 million were directly related to resistant infections (Antimicrobial Resistance Collaborators, 2022; Murray et al., 2022). Approximately 929,000 of these deaths were caused by six pathogens: *E. coli, S. aureus, K. pneumoniae, S. pneumoniae, A. baumannii,* and *P. aeruginosa*. AMR would become the third most common cause of death worldwide if susceptible infections took the place of resistant ones, with especially dire repercussions in low- and middle-income nations (WHO, 2023; Laxminarayan et al., 2023).

1.2 Collapse of the Antibiotic Pipeline

The discovery of new antibiotics has stalled despite the increasing demand due to scientific complexity, the quick development of resistance, and financial disincentives (Laxminarayan et al., 2023; Wright, 2016; Spatz et al., 2023). Because of this, relying on older drugs makes the therapeutic gap bigger and shows how important it is to find new ways to treat people.

1.3 Limitations of Conventional Antimicrobials

Conventional broad-spectrum antibiotics, while life-saving, have several drawbacks:

- Disruption of beneficial microbiota, potentially causing dysbiosis, opportunistic infections, and immunological or metabolic abnormalities (Dethlefsen et al., 2008; Becattini et al., 2016).
- The development of resistance is increased by indiscriminate selective pressure (Davies & Davies, 2010; Fredriksen et al., 2023).
- Bacteria within biofilms can tolerate antibiotics up to 1,000 folds higher than free-living cells due to restricted penetration, delayed metabolism, and enhanced gene exchange (Hall & Mah, 2017; Flemming et al., 2016; Li et al., 2024a, 2024b).
- Resistance genes carried on mobile genetic elements, such as plasmids and transposons, spread fast (Partridge et al., 2018; Mortreux et al., 2025; Martínez Quintela et al., 2024).

These problems underscore the necessity for a paradigm change in favor of precision-targeted antimicrobials (O'Neill, 2016).

1.4 Precision Antimicrobials: Resulting the Emergence of CRISPR-Based Strategies

Next-generation antimicrobials contrast with traditional antibiotics by necessitating specific, targeted action against particular bacterial strains or species, thus preventing beneficial microbiota (Citorik et al., 2014; Dethlefsen et al., 2008). They should be able to remove resistance genes (Bikard et al., 2014), reduce selective pressures that promote additional resistance (Wright, 2010), and quickly adjust to new pathogenic challenges (Marraffini, 2015). CRISPR-based antimicrobials are currently among the most promising therapeutic, motivating development of versatile gene-editing platforms. CRISPR Cas systems, initially discovered as bacterial immune response, can be evolved into programmable tools designed for targeting nucleic acids with sequence specificity (Barrangou et al., 2007; Marraffini, 2015). Despite enormous versatility of CRISPR based antimicrobials, their major drawback is the difficulty of delivering effectively into host.

1.5 The Delivery Challenge

Nanotechnology plays a crucial role in enhancing the effectiveness of CRISPR-based antimicrobials, particularly because bacterial cell envelopes and biofilm matrices present significant barriers to delivery, especially in Gram-negative bacteria (van der Oost et al., 2014; Flemming et al., 2016). Nanocarriers address these challenges in several ways: they protect CRISPR components from degradation, improve penetration across bacterial membranes, and allow deeper infiltration into stubborn biofilms (Wang et al., 2016; Flemming et al., 2016). Additionally, these nanoscale delivery systems can be engineered for controlled and targeted release, ensuring that CRISPR payloads reach their intended bacterial targets efficiently (Patra et al., 2018; Zhou et al., 2018). Recent studies have highlighted the real-world potential of this approach, showing that Nano-CRISPR platforms can significantly enhance cellular uptake and antimicrobial activity, successfully eliminating MDR pathogens such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in vivo (Baig et al., 2025; de la Fuente Tagarro et al., 2024). This integration of nanotechnology with CRISPR tools represents a promising strategy to overcome some of the toughest obstacles in treating resistant bacterial infections.

2. Mechanisms of Antibiotics and Resistance

2.1 Classes of Antibiotics and Mechanisms of Action

Antibiotics can be broadly classified as bactericidal or bacteriostatic based on their effect on bacteria. Bactericidal antibiotics eliminate bacteria directly, usually by attacking crucial structures or functions. Cell wall inhibitors such as β-lactams and vancomycin hinder peptidoglycan production, resulting in osmotic lysis, while fluoroquinolones target DNA gyrase or topoisomerase IV, resulting in fatal DNA damage. Colistin and

daptomycin are examples of membrane disruptors that damage the integrity of bacterial membranes, causing rapid cell death (Brown, 2015; Hooper, 2001; Falagas & Kasiakou, 2006).

Bacteriostatic antibiotics, on the other hand, limit bacterial growth without actually destroying the cells, allowing the host's immune system to remove the infection. Inhibitors of protein synthesis, including tetracyclines and macrolides, disrupt ribosomal activity, which prevents essential protein formation, while antimetabolites like sulfonamides and trimethoprim obstruct folic acid synthesis, ultimately stopping nucleotide generation and bacterial reproduction (Wright, 2010). Both classes exert selective pressure that drives the evolution of resistance (Davies & Davies, 2010).

2.2 Drivers of AMR

The increase in antimicrobial resistance is caused by a mix of human, environmental, and systemic influences. In healthcare, improper use of antibiotics like overprescribing, dependence on empirical treatment, unfinished therapy courses, and lack of stewardship initiatives speeds up resistance emergence (Pulcini & Gyssens, 2013). In farming, the widespread application of antibiotics, with approximately 70% given to animals, leads to pools of resistant bacteria that can migrate to humans via the food chain or surroundings (Van Boeckel et al., 2015; Marshall & Levy, 2011). International travel and trade perpetuate the problem by promoting the cross-border transmission of hazardous bacterial strains (Kumarasamy et al., 2010; Tacconelli et al., 2018). Environmental pollution significantly contributes, as waste from hospitals, drug manufacturing, and farming releases antibiotics and resistant microorganisms into soil and water systems, perpetuating resistance reservoirs in ecosystems (Martinez, 2009; Berendonk et al., 2015; Martínez Quintela et al., 2024). Ultimately, diagnostic constraints, especially in low- and middle-income nations (Okeke et al., 2005) further contributes to issue of antimicrobial resistance.

2.3 Molecular and Cellular Mechanisms of Resistance

Bacteria employ diverse strategies to withstand antibiotics. Certain bacteria possess intrinsic resistance, includes impermeable cell membranes prevent drug penetration, absence of essential molecular targets, or efflux pumps actively expel antibiotics from the cell, maintaining low internal drug concentrations (Nikaido, 2003). A different approach uses enzymes, as bacteria create substances such as β-lactamases that dismantle β-lactam antibiotics, rendering them ineffective (Bush & Jacoby, 2010; Ramirez & Tolmasky, 2010).

Efflux pumps like AcrAB-TolC in *E. coli*, MexXY-OprM in *P. aeruginosa*, and NorA in *S. aureus* function as pumps to remove antibiotics, whereas alterations in porins restrict the amount of drug that can penetrate the cell, resulting in a highly resistant setting. Target-site alterations represent an additional strategy: changes in genes such as *gyrA* and *parC* affect DNA gyrase and topoisomerase IV, leading to reduced fluoroquinolone binding; PBP2a in MRSA provides β-lactam resistance; and *erm* genes methylate ribosomal RNA, diminishing macrolide attachment (Blair et al. 2015).

Bacteria create biofilms and utilize quorum sensing for their protection. In biofilms, groups of bacteria are trapped in adhesive extracellular matrices that reduce antibiotic infiltration and form microenvironments where bacteria can thrive more effectively. Quorum sensing enables bacteria to communicate, synchronize gene expression, and more effectively exchange resistance genes (Hall & Mah, 2017; Flemming et al., 2016).

Ultimately, horizontal gene transfer disseminates resistance quickly. Bacteria can share resistance genes through plasmids, transposons, integrons, or through transformation and bacteriophages, allowing multidrug resistance to spread across species and ecosystems (von Wintersdorff et al., 2016; Mortreux et al., 2025).

Collectively, these mechanisms underscore the sophistication of bacterial defence and the necessity for innovative approaches such as CRISPR-based antimicrobials.

3. CRISPR-Cas Systems

3.1 Discovery and Conceptual Foundations

The function of CRISPR loci was initially discovered in *E. coli* in 1987, but their role as adaptive immune response was recognized only in the early 2000s. In 2007, Barrangou et al. demonstrated CRISPR-mediated adaptive immunity in *S. thermophilus* in 2007. CRISPR arrays act as genetic memory, directing Cas nucleases to complementary foreign nucleic acid sequences. These systems are present in about 40% of bacteria and 85% of archaea (Makarova et al., 2020; Koonin & Makarova, 2019).

3.2 Mechanistic Phases of CRISPR Immunity

Mojica and Jansen coined the term "CRISPR" in 2002 to refer to the unique family of interspaced repeat sequences. The CRISPR Cas system degrades the bacterium's genome in three steps: adaptation or acquisition of a spacer sequence, biogenesis or expression of CRISPR RNA (crRNA), and interference or targeting. In the first step, the Cas proteins of the CRISPR Cas system identify the protospacer adjacent motif (PAMs), which are 2–6 base pairs of nucleotide motifs that Cas proteins recognize. Once the foreign DNA is recognized, a few base pairs of nucleotides next to the PAM motif are incorporated into the pathogen's CRISPR loci. It causes the bacterium to start remembering the foreign DNA.

Now that the integrated foreign sequence is produced as RNA, it combines with tracrRNA (trans-activating CRISPR RNA) to form premature crRNA (pre-crRNA). Following processing, the premature crRNA processed into mature crRNA, which directs the Cas endonuclease to insert double-strand breaks into the incoming DNA sequence. These are also referred to as guideRNAs depending on the particular role of crRNAs. These double-strand breaks specifically cleave invading genome that is complementary to the fully developed crRNA. The final stage in which crRNAs recognize and create a complementary base pair unique to a foreign genome is called interference. As a result, the invading genome-crRNA complex is cleaved and destroyed. The cas protein functions as both a helicase and an endonuclease. The invading genome is cut at a particular DNA

sequence upstream of the proto-spacer adjacent motif (PAM) by the Cas nuclease in cas9. Cleavage is prevented and leads the susceptibility to infection if there is a mutation in the proto-spacer adjacent motif (PAM) or a difference in complementarity between the DNA of the invader and spacer (Barrangou & Marraffini, 2014). The modular nature of this process enables researchers to reprogram CRISPR-Cas systems to target bacterial genes for antimicrobial purpose.

3.3 CRISPR-Cas Variants

The two primary classes of CRISPR-Cas systems, each of which includes multiple types and subtypes, provide a comprehensive toolkit for creating novel antibacterial strategies (Makarova et al., 2020). Class 1 systems use multi-protein complexes to identify and cut genetic material. The most prevalent in nature are Type I systems, which are based on the Cascade complex and Cas3. They are particularly powerful because they can degrade long stretches of DNA, making them ideal for complete plasmid elimination or irreversible chromosomal disruption (Koonin & Makarova, 2019; Westra et al., 2012). Now, Type III systems, which center on the Cas10 protein, can target both DNA and RNA and activate cyclic oligoadenylate (cOA) signaling, giving them broad antiviral and antibacterial potential (Kazlauskiene et al., 2017; Niewoehner & Jinek, 2016).

In comparison, Class 2 systems rely on single, multifunctional effector proteins, greatly simplifying engineering and delivery. Type II systems, represented by Cas9, use an sgRNA and a PAM sequence to produce precise double-strand DNA breaks and have become essential tools (Jinek et al., 2012; Barrangou & Doudna, 2016). Type V systems, such as Cas12a and Cas12b, recognize T-rich PAMs and create staggered DNA cuts; their compact size makes them particularly suitable for delivery through nanoparticles and other therapeutic platforms (Zetsche et al., 2015; Teng et al., 2018). Type VI systems, including Cas13, uniquely target RNA rather than DNA, enabling temporary silencing of essential or regulatory RNAs without altering the genome (Abudayyeh et al., 2016; Cox et al., 2017). Together, the availability of CRISPR nucleases that act on both DNA and RNA provides a versatile and powerful foundation for designing highly targeted antimicrobial approaches.

3.4 CRISPR Tools for Antimicrobial Applications

Scientists can disrupt bacterial survival, virulence, or antibiotic resistance at the single-nucleotide level by using CRISPR-based antimicrobials, which target bacteria with remarkable precision using both natural and engineered Cas enzymes (Bikard & Barrangou, 2017; Rodrigues et al., 2019). Targeting essential genes can rapidly eliminate pathogens, while removing plamid targeting strategies remove resistance determinants such as *mecA, blaCTX-M, blaNDM, blaKPC, mcr* variations, and aminoglycoside-modifying enzymes (Hamilton et al., 2019; Wang et al., 2019).

Non-lethal approaches, including CRISPR interference (CRISPRi), which employs catalytically inactive Cas proteins or dead Cas to silence damaging genes involved in virulence, quorum sensing, biofilm formation, or

essential metabolic processes (Qi et al., 2013; Peters et al., 2019). On the other hand, CRISPR activation (CRISPRa) opens up new therapeutic possibilities by turning on bacterial genes that boost immune detection or increase antibiotic sensitivity (Liu et al., 2019; Dong et al., 2020). By targeting bacterial RNA instead of DNA, Cas13-based systems expand the capabilities even further by offering reversible, genome-safe approaches to inhibit essential transcripts or regulatory RNAs (Abudayyeh et al., 2016; Cox et al., 2017). These tools allow to modify microbial communities, offering selective removal of harmful species (Hsu et al., 2021; Lam et al., 2021).

3.5 Advantages of CRISPR-Based Antimicrobials Over Traditional Antibiotics

CRISPR has a number of benefits:

- Programmability (simple to redesign against emerging dangers)
- Strain-level specificity (minimizes dysbiosis)
- Targeting non-growing or biofilm-embedded bacteria
- Eliminating mobile resistance elements
- Modular compatibility with nanocarriers
- Low probability of resistance evolution

These advantages have resulted in increasing interest in Nano-CRISPR platforms.

4. Nanocarrier-Based Delivery of CRISPR Antimicrobials

4.1 Structural and Biochemical Barriers

- Gram-negative dual membranes and the cell envelope obstruct large proteins like Cas9 (~160 kDa) (Silhavy et al., 2010; Delcour, 2009).
- Nucleases and anti-CRISPR proteins degrade unprotected genetic material (Dupuis et al., 2013; Stanley & Maxwell, 2018).
- Biofilm adds an additional layer of protection known as extracellular polymeric substance (EPS) that limits nanoparticle diffusion (Costerton et al., 1999; Flemming & Wingender, 2010).

Overcoming these barriers is essential for effective Nano-CRISPR therapy (Fig. 1).

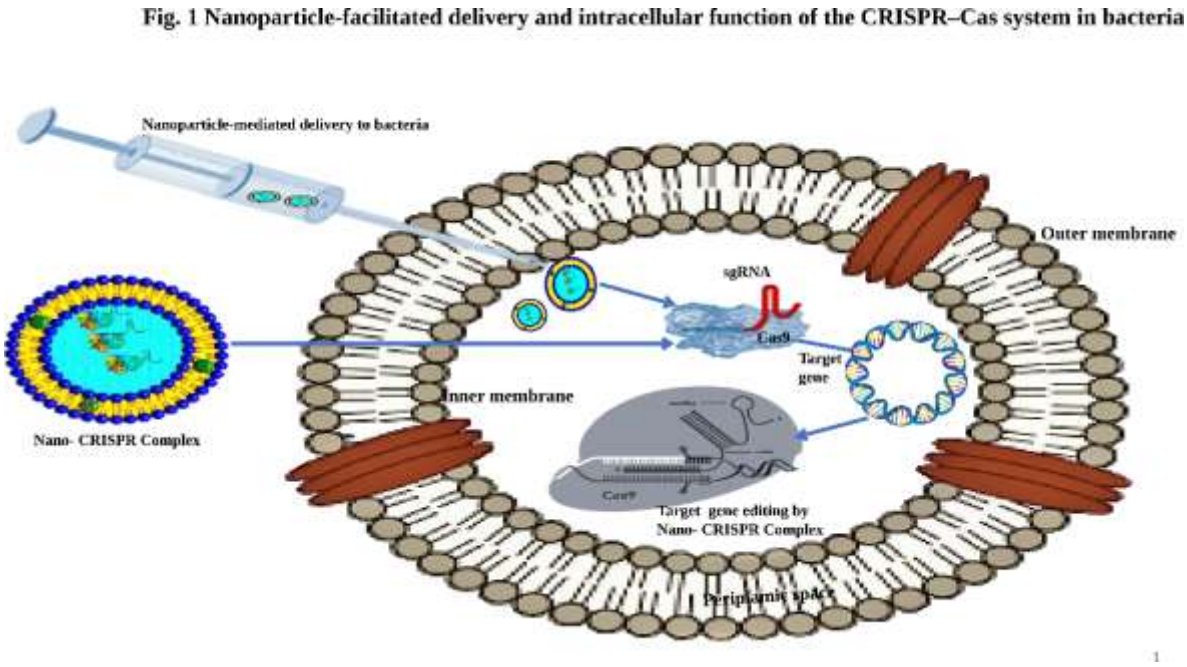


Fig. 1 Nanoparticle-facilitated delivery and intracellular function of the CRISPR–Cas system in bacteria

The figure summarizes the mechanism by which nanoparticle carriers deliver the CRISPR-Cas9 system into bacterial cells, from initial nanoparticle-envelope interaction through cytoplasmic release, target DNA binding, and Cas9-mediated cleavage.

4.2 Major Nanocarrier Platforms

Lipid Nanoparticles (LNPs)

Lipid nanoparticles were among the first non-viral delivery system found to be capable of co-delivering cas9 mRNA and sgRNA in vitro and in vivo. When administered locally, LNPs become unstable. But they are biocompatible and safe as long as they are formulated properly (Kulkarni et al., 2021; Gao et al., 2019).

Polymeric Nanoparticles

Biodegradable polymers are used for biofilm penetration and prolonged release. Because of their adjustable physicochemical characteristics, which allow for regulated drug release, longer circulation times, and better stability of delicate payloads, polymeric nanoparticles have become a multipurpose carrier. Their capacity to be designed with stimuli-responsive characteristics like pH, redox, or enzymatic triggered allows for administration. Targeted ligand surface modification has the potential to increase the selective uptake of bacterial cells and lessen off-target interactions. This method has benefits over the classic lipid-based delivery systems (Li et al., 2020; Beyer et al., 2022).

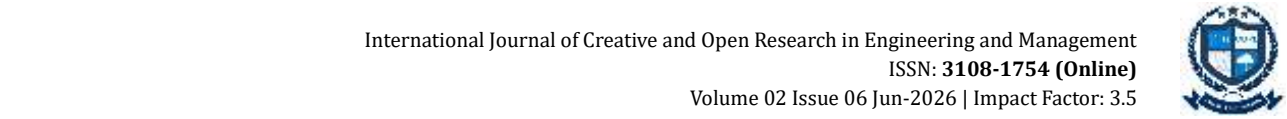
Biomimetic and Hybrid Nanocarriers

Biomimetic and hybrid nanocarriers are able to mimic important structural and functional aspects of natural biological systems has drawn a lot of attention. By adding elements like bacterial membranes, phage-derived elements, or extracellular vesicles, these carriers show improved cellular uptake, immune evasion, and biocompatibility; their innate recognition capabilities enable more effective targeting of particular tissues or

pathogenic cells, providing advantages over fully synthetic platforms; additionally, the hybrid design combines biological specificity with the stability and tunability of engineered materials, making them promising strategies for delivering nucleic acids and other therapeutic cargos (Gao et al., 2017; Kishimoto et al., 2017).

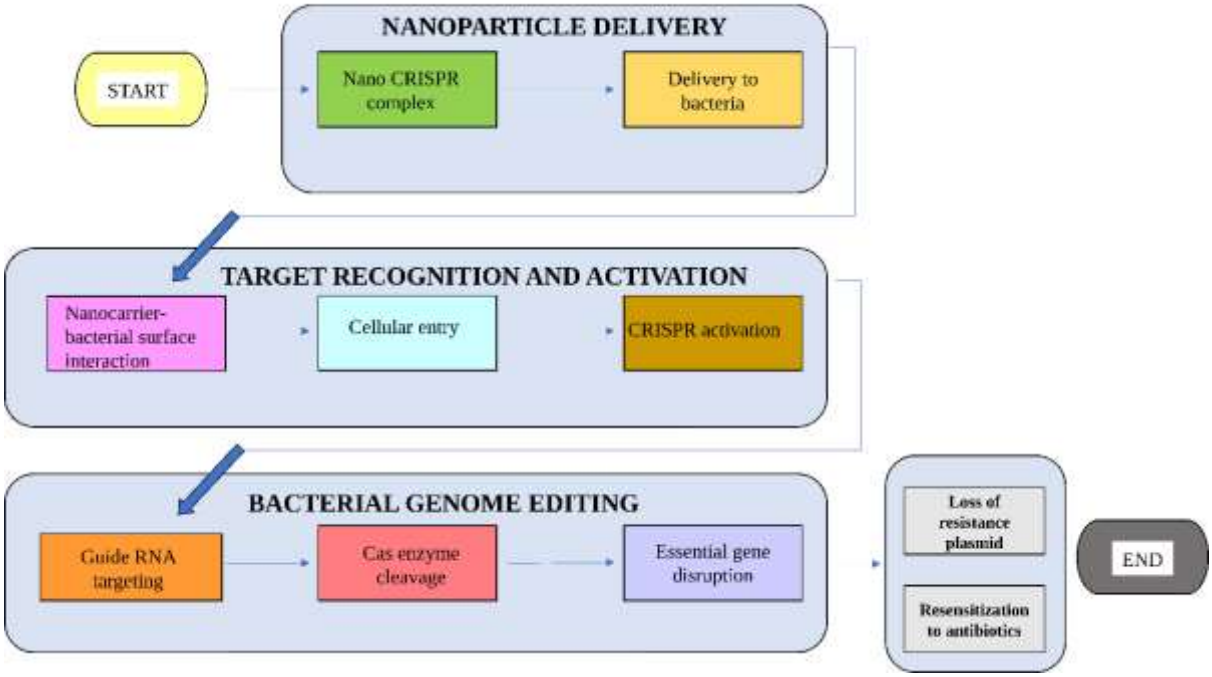
Table 1. Summary of Antibiotic Resistance, CRISPR Systems, and Nano-CRISPR Applications

Category	Types	Description	Salient features/ Examples	New findings	References
Antibiotic Resistance	Intrinsic Resistance	Natural abilities of bacteria to withstand certain antibiotics without prior exposure	Includes reduced drug uptake, efflux pumps, and naturally occurring enzymes	Understanding these barriers helps tailor CRISPR strategies to bypass innate bacterial defenses	Delcour, 2009;
	Acquired Resistance	Resistance gained through mutations or gene transfer	Point mutations or plasmids carrying genes like bla or mecA	Engineered CRISPR can selectively remove these resistance genes from harmful bacteria	Gomaa et al., 2014;
	Biofilm-Associated Resistance	Bacterial communities encased in protective matrices (biofilms)	Dense EPS, limited drug penetration, quorum sensing	Nano-CRISPR nanoparticles can penetrate biofilms and target genes that control community formation	Zuberi et al. 2024
	Enzymatic Antibiotic Inactivation	Bacteria produce enzymes that inactivate antibiotics	β-lactamases, aminoglycoside-modifying enzymes	CRISPR systems can disable these enzymes, restoring antibiotic effectiveness	Hao et al., 2020; Tao et al., 2023
CRISPR/Cas Systems	Type I (Cas3)	Multi-protein complex that destroys DNA	Cas3 helicase-nuclease can degrade large DNA regions	Useful for eliminating plasmids carrying resistance genes	He et al., 2020
	Type II (Cas9)	Well-known genome-editing tool	Cas9 introduces double-strand breaks in DNA	Efficiently targets resistance genes in lab and animal studies	Ahmed et al., 2024
	Type III (Cas10)	Can target DNA or RNA depending on subtype	Cas10 has multiple enzymatic domains; III-B targets RNA	Helps silence actively expressed resistance or virulence genes	Stella & Marraffini, 2023
CRISPR-Based Antimicrobials	Activating Native CRISPR	Stimulates bacteria's own CRISPR to target resistance	Uses carefully chosen protospacers	Can remove resistance from inside bacteria without adding foreign DNA	Jiang et al., 2013
	Exogenous CRISPR Delivery	Introduces engineered CRISPR tools into bacteria	Delivered as plasmids, RNA, or proteins	Allows precise targeting across different bacterial species	Citorik et al., 2014;
	CRISPR-Mediated Re-Sensitization	Cleaves resistance genes to make bacteria susceptible again	Example: Cas9 targeting mecA or blaNDM-1	Successfully restored antibiotic sensitivity in over 90% of treated bacteria	Zheng et al., 2020
	Gene Silencing (dCas9)	Represses gene expression without cutting DNA	Dead Cas9 blocks transcription of resistance or virulence genes	Reduces harmful traits while leaving beneficial bacteria intact	Liu et al., 2020
	Gene Silencing (dCas9)	Represses gene expression without cutting DNA	Dead Cas9 blocks transcription of resistance or virulence genes	Reduces harmful traits while leaving beneficial bacteria intact	Liu et al., 2020
Nano-CRISPR Delivery Systems	Lipid Nanoparticles (LNPs)	Tiny lipid vesicles carrying CRISPR machinery	Biocompatible, easily taken up by bacteria	Nearly complete removal of MRSA biofilms demonstrated	Zhang et al., 2021



	Polymeric Nanocarriers	Positively charged polymers protect and deliver CRISPR cargo	PEI, PLGA, chitosan	Improved stability and intracellular delivery in bacterial infections	Dong et al., 2021;
	Biomimetic Nanocarriers	Nanoparticles coated with bacterial or immune membranes	Outer-membrane vesicles or liposomes	Enhanced targeting of pathogens and evasion from host immunity	Li et al., 2020
	Phage-Derived Carriers	Engineered bacteriophages carrying CRISPR	Temperate or lytic phages	High species specificity, effective in removing plasmids from pathogens	Citorik et al., 2014; Gomaa et al., 2014

Fig. 2 Sequential steps involved in Nano-CRISPR antibacterial activity in bacterial cells



The Figure summarizes the overall sequence by which nanocarrier-delivered CRISPR systems achieve bacterial killing, from initial nanoparticle delivery through target recognition, genome editing, and bacterial growth suppression.

4.3 Computational Optimisation

Molecular docking improves design specificity and efficiency by predicting interactions among CRISPR components, bacterial surfaces, and nanocarrier (Sheikh et al., 2024; Lin et al., 2022). Applications include ligand selection, plasmid-curing design, surface engineering for enhanced biofilm/membrane penetration, sgRNA optimisation, and expedited preclinical screening. The accuracy and effectiveness of Nano-CRISPR platforms are improved by combining computational techniques with nanocarrier engineering (Park et al., 2023).

Why Docking Is Critical for Nano-CRISPR Drug Design

Docking completes the connection between experimental validation and computational prediction by:

1. Making sure CRISPR targeting is sequence-specific

2. Improving the surface chemistry of nanoparticles
3. Estimating the probability of membrane translocation
4. Improving the design of plasmid-curing
5. Quickening the cycles of therapeutic design

As a result, docking turns into a potent preclinical design tool that guarantees Nano-CRISPR creations connect accurately and successfully to their specified bacterial or genetic targets.

5. Nano-CRISPR Applications Against MDR Bacteria

5.1 In Vitro Efficacy

CRISPR–Cas systems can selectively eliminate plasmids encoding resistance genes, restoring bacterial sensitivity to drugs, according to in vitro research. By facilitating transport via membranes and biofilms, nanocarriers guarantee targeted, efficient CRISPR activity against strains resistant to several drugs (as discussed in 2.3).

5.2 In Vivo Studies

MDR pathogens like *A. baumannii* and *P. aeruginosa* can be reduced by nanocarrier-mediated CRISPR delivery while maintaining commensal microbiota, according to preclinical models. The requirement for consistent protocols prior to human translation is highlighted by variations in dosage, administration routes, and study design.

6 Comprehensive Scope

This review provides a comprehensive analysis of Nano-CRISPR antimicrobials by combining information from microbiology, CRISPR biology, nanotechnology, and computational design. The topics covered include antibiotic resistance mechanisms, CRISPR-Cas methodologies, nanocarrier delivery technologies, efficacy evidence, translational challenges, and novel advancements such as RNA-targeting effectors, AI-guided design, and microbiome modulation techniques. Understanding the ecological, evolutionary, and socioeconomic underpinnings of AMR highlights the need for precision treatments like Nano-CRISPR.

7 Challenges and Limitations

Despite promising preclinical results, a number of obstacles must be tackled before Nano-CRISPR medications may be widely employed in clinical settings.

7.1 Delivery and Uptake

Bacterial absorption varies even with sophisticated nanocarriers, especially in Gram-negative species with intricate outer membranes. Uniform delivery is further complicated by biofilm heterogeneity (Flemming et al., 2016; Silhavy et al., 2010).

7.2 Off-Target Effects and Resistance

CRISPR provides sequence-level accuracy, but off-target cleavage is still an issue. Unintentional gene disruption can result from mismatches between guide RNAs and non-target sequences, requiring careful computational design and validation (Lin et al., 2022).

7.3 Immune and Environmental Considerations

CRISPR proteins or nanocarriers may be recognized by the immune system, which could lead to inflammation or clearance. Biosafety assessments are necessary since the environmental discharge of Nano-CRISPR devices may also impact non-target bacterial populations (van der Oost et al., 2014; Dupuis et al., 2013).

7.4 Manufacturing and Regulatory Barriers

It is still difficult to produce Nano-CRISPR treatments in a scalable manner with reliable quality, stability, and repeatability. Clinical use of gene-editing antibiotics may be slowed by the ongoing development of regulatory mechanisms.

8. Future Perspectives and Emerging Strategies

Nano-CRISPR antimicrobials represent a frontier in precision infectious disease therapy. Several innovative directions are likely to shape the next decade:

8.1 Multiplexed CRISPR Systems

Targeting several resistance genes or virulence factors at once may lessen the chance of bacterial escape and increase the effectiveness of treatment (as discussed in 3.4).

8.2 RNA-Targeting CRISPR (Cas13)

Direct targeting of bacterial mRNA is possible using Cas13-based methods, providing temporary and reversible suppression of harmful genes without long-term genomic alterations (Wang et al., 2016).

8.3 Stimuli-Responsive Nanocarriers

At infection sites, nanoparticles that react to pH, redox potential, or bacterial enzymes can deliver regulated CRISPR release, improving specificity and minimizing side effects (Patra et al., 2018; Zhou et al., 2018).

8.4 AI-Assisted Design

By optimizing sgRNA sequences, nanoparticle formulations, and targeting ligands, artificial intelligence and machine learning can speed up preclinical development and enhance safety profiles (Sharma et al., 2020; Li & Wang, 2022).

8.5 Hybrid Therapeutic Approaches

By combining Nano-CRISPR with immunomodulators, phage therapy, or traditional antibiotics, synergistic effects may be achieved, overcoming the drawbacks of individual treatments and lowering the emergence of resistance.

8.6 Clinical Translation Outlook

Although first findings are encouraging, comprehensive in vivo validation, toxicity testing, and regulatory approval procedures are necessary. To fully realize the potential of Nano-CRISPR antimicrobials, cooperation between microbiology, nanotechnology, computational biology, and clinical medicine will be essential.

9. Conclusion

A paradigm change in antimicrobial medicine is presented by the combination of CRISPR–Cas gene editing with sophisticated nanocarriers. MDR pathogens can be precisely targeted at the strain level using nano-CRISPR, which effectively breaks down bacterial barriers and biofilms while maintaining commensal microbiota. Achieving scalable production, reducing off-target and immunological effects, and guaranteeing safe, targeted distribution are still challenges. Promising answers are provided by emerging approaches such as multiplexed CRISPR-Cas systems, Cas13-based RNA targeting, stimuli-responsive nanocarriers, AI-assisted design, and hybrid treatments. Nano-CRISPR creates a next-generation, precision antibacterial platform that may modify microbial populations and eradicate resistant infections by combining programmable genome editing with tailored delivery. Transforming Nano-CRISPR from experimental innovation into clinically feasible medicines and ushering in a new era of precision-guided antimicrobial interventions will require ongoing interdisciplinary research, rigorous validation, and regulatory monitoring.

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Figure Legends

Fig. 1 Nanoparticle-facilitated delivery and intracellular function of the CRISPR–Cas system in bacteria (end of 4.1)

This diagram illustrates how CRISPR-Cas9 is delivered into bacterial cells via nanoparticle carriers to initiate targeted genome editing. Cas9–sgRNA complex-loaded nanoparticles are injected and aimed at the bacterial surface, where they interface with and pierce the outer envelope. The Cas9–sgRNA complex can enter the cytoplasm and attach to its complementary DNA sequence on the bacterial chromosome or plasmid once the nanoparticles release their CRISPR cargo within the cell. After then, Cas9 creates a precise double-strand break, rendering important genes like those needed for survival or antibiotic resistance inoperable.

Fig. 2 Sequential steps involved in Nano-CRISPR antibacterial activity in bacterial cells (end of 4.2)

The main phases by which Nano-CRISPR systems combat bacterial infections are depicted in this figure.

(1) Nanoparticle Delivery: CRISPR components, including guide RNAs and Cas enzymes, are packaged and delivered to the bacterial target site by specially made nanoparticles.

(2) Target Recognition and Activation: The nanoparticles' surface characteristics allow them to attach to bacterial cells, penetrate the cell envelope, and once inside the cell, activate the CRISPR machinery.

(3) Bacterial Genome Editing: After entering the bacterium, the Cas–guide RNA complex finds the corresponding DNA sequence and cleaves it, interfering with vital genes or markers of antibiotic resistance.

(4) Bacterial Growth Suppression or Death: The ensuing gene damage causes cell lysis or growth inhibition, which enables the targeted eradication of harmful bacteria while substantially sparing non-target microbiota.