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CRISPR-ENABLED ANTIMICROBIAL STRATEGIES: REDEFINING PRECISION THERAPY AGAINST MULTIDRUG-RESISTANT PATHOGENS

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ABSTRACT

Antimicrobial resistance (AMR) has become one of the most serious global public health threats, driven by the rapid emergence and dissemination of multidrug-resistant (MDR) pathogens. The declining effectiveness of conventional antibiotics necessitates the development of innovative therapeutic strategies capable of selectively targeting resistant microorganisms while preserving beneficial microbiota. CRISPR-Cas technology has emerged as a promising precision antimicrobial platform with the potential to transform infectious disease management. This review summarizes the molecular mechanisms, delivery platforms, therapeutic applications, emerging innovations, safety considerations, translational barriers, and future prospects of CRISPR-enabled antimicrobial strategies for combating MDR bacterial infections. This review evaluated recent peer-reviewed literature on CRISPR-Cas systems, AMR, genome editing, precision medicine, delivery technologies, and translational research. The available evidence was synthesized to assess current advances, therapeutic potential, existing limitations, and future opportunities for CRISPR-based antimicrobial interventions. CRISPR-enabled antimicrobial strategies demonstrate remarkable specificity by selectively eliminating multidrug-resistant bacteria, disrupting resistance genes, targeting virulence factors, inhibiting biofilm formation, and restoring antibiotic susceptibility. Advances in bacteriophage-mediated delivery, nanoparticle-based carriers, programmable RNA-targeting systems, artificial intelligence-assisted guide RNA design, synthetic biology, and personalized precision medicine have further expanded their clinical potential. Nevertheless, challenges related to delivery efficiency, off-target editing, bacterial escape mechanisms, biosafety, regulatory approval, and large-scale manufacturing remain significant translational barriers. Overall, CRISPR-based antimicrobial therapy represents a transformative approach to precision infectious disease management, with continued technological innovation, interdisciplinary collaboration, and robust clinical validation expected to facilitate its safe, effective, and clinically accessible implementation against multidrug-resistant pathogens.

KEYWORDS: CRISPR-Cas systems; Antimicrobial resistance; multidrug-resistant pathogens; precision antimicrobial therapy; genome editing.

1. INTRODUCTION

Antimicrobial resistance (AMR) has become one of the biggest public health challenges worldwide, threatening the work that has taken place over the past 50 years to discover and use antibiotics in clinical practice. Most widely used antimicrobial drugs are becoming less effective, causing a significant increase in treatment failures, longer hospitalizations, health care costs, and death from infections. The use of antibiotics is ubiquitous and is frequently prescribed inappropriately in human and veterinary medicine, in agriculture and in the production of food, leading to the selection of resistant bacterial populations and the emergence of bacteria that are able to adapt quickly to antimicrobial pressure (Tarique, 2025). At the same time, the resistance determinants spread through plasmids, transposons, integrins, and other mobile genetic elements have led to the rapid spread of AMR among taxonomically diverse bacterial species making AMR a complex global ecological and clinical challenge (Wizrah, 2026). Thus, infections that can now be treated by conventional antimicrobial therapy are becoming difficult to manage, highlighting the need for new and sustainable treatment strategies.

AMR also presents a substantial socioeconomic burden, due to its direct clinical impact, long-term disability costs, and impact on healthcare resource utilization, as well as decreased productivity of the workforce and reduced effectiveness of complex medical interventions like organ transplantation, cancer chemotherapy and major surgical procedures. Resistant

pathogens are continually emerging and eroding the utility of current antimicrobial agents, thus undermining the basis of modern medicine. In addition, the resistance of bacteria to antibiotics has outpaced the discovery rate, resulting in a large gap between need and availability of effective treatment, and the need for precision-based antimicrobial technologies that could be used to selectively target resistant bacteria while reducing further evolutionary pressure (Wizrah, 2026; Tarique, 2025).

One of the most worrying symptoms of the AMR situation is the emergence of multidrug-resistant (MDR) pathogens. A variety of clinically important bacteria have acquired the ability to resist several classes of antibiotics with various molecular mechanisms, such as methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacterales, MDR Pseudomonas aeruginosa, Acinetobacter baumannii and vancomycin-resistant Enterococcus (VRE). These organisms have a number of mechanisms that help them resist the antimicrobials, such as enzyme deactivation, changing the target of the antimicrobials, decreasing membrane permeability, activating multidrug efflux pumps, and acquiring resistance genes via horizontal gene transfer (Tarique, 2025). These adaptive mechanisms may hinder the effectiveness of therapy and contribute to the spread and survival of resistant strains in healthcare settings and the community.These characteristics significantly enhance the survival of bacteria and are responsible for recurrent and chronic infections which are challenging to treat using standard treatment strategies (Yadav & Yadav, 2026). The interest in targeted therapeutic systems to overcome the challenges in current treatment modalities has been shown by the recent development of nanotherapeutic strategies that can deliver antimicrobial agents and penetrate biofilms, especially in resistant bacteria such as MRSA (Rajnani & Kurup, n.d.; Yadav & Yadav, 2026).

Antibiotics are a critical part of treating bacterial infections, but there are a number of inherent challenges to the effectiveness of long-term use. Most conventional antibiotics are broadly active meaning that they kill pathogens as well as members of their normal microbiota. This imprecision often leads to dysbiosis, opportunistic infections and the ongoing selection of resistant bacterial populations. Moreover, repeated exposure with antibiotic drugs forces strong evolutionary pressure, which increases the rate of emergence of new resistance mechanisms, thus reducing the clinical life of new antimicrobial drugs (Wizrah, 2026). While antimicrobial peptides, phage therapy, nanomedicine, and host-directed therapies have shown promising results in preclinical studies, each has a set of limitations with respect to stability, efficiency of delivery, manufacturing, or clinical implementation at a large scale (Rajnani & Kurup, n.d.). The use of nanomedicine-based antimicrobial formulations has enhanced targeted drug delivery and intracellular bacterial eradication, but challenges like long-term biocompatibility, biodistribution, toxicity evaluation, and regulatory standardization remain limitations in the broad clinical application of nanomedicine (Yadav & Yadav, 2026). These challenges all highlight the need for programmable antimicrobial technologies that selectively kill antimicrobial resistant bacteria while maintaining microbial homeostasis and minimizing selective evolutionary pressure.

The discovery and engineering of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins has revolutionized the field of molecular medicine by providing highly targeted genome-editing technologies. Initially thought to be an adaptive immune system that fights invading genetic elements in bacteria and archaea, CRISPR-Cas systems have since evolved into powerful tools for targeted genetic manipulation and precision antimicrobial therapy (Tarique, 2025). In contrast to traditional antibiotics that target susceptible bacteria, the CRISPR-based AMR can use guide RNAs to be customizable for each pathogen to target a specific DNA or RNA sequence, allowing for selective killing of pathogens with AMR genes or virulence factors, while leaving beneficial microorganisms (Wizrah, 2026) unaffected. At the same time, novel delivery systems that include genetically engineered bacteriophages have shown great potential for delivering specific CRISPR components into target bacteria in a highly specific manner. The current phage engineering approaches are well equipped with programmable targeting, host specificity, and delivery capabilities, forming the basis for precision treatment of MDR and extensively drug-resistant bacterial infections (Piracha & Saeed, 2026). The increasing interest in translating phage engineering to biomedical applications also underscores the platform's broader translation potential of biologically targeted therapeutics (Hsu et al., 2025). Molecular diagnostics based on Cas12 and Cas13 enzymes have been developed using CRISPR technology, which can be used for the rapid identification of clinically relevant resistance determinants, thus aiding in early diagnosis, antimicrobial stewardship and timely clinical intervention (Wang et al., 2025). A few recent diagnostic systems represented by programmable CRISPR have shown excellent performance in detecting uropathogens and AMR markers, highlighting the possibility of implementing precision diagnostic and targeted antimicrobial treatments in the same clinical context (Tiwari & Rathinasabapathi, 2026).

This review covers in detail the CRISPR based antimicrobial approaches as the new generation of precision therapeutics for MDR pathogens. It takes an in-depth look at the underlying principles of CRISPR-Cas systems, recent development of programmable antimicrobial mechanisms, targeted delivery platforms, diagnosis and therapeutic approaches to clinically relevant resistant bacteria. In addition, the current translational challenges, safety concerns, regulation aspects, and future research directions are discussed that could aid in the implementation of CRISPR technologies into precision antimicrobial medicine. This review seeks to bring together recent advances in these areas, outlining the potential of approaches enabled by CRISPR for redefining the next generation of antimicrobial therapy and countering the ever-growing global threat of AMR.

2. CRISPR-Cas Systems: Molecular Basis and Functional Diversity

2.1 Discovery and Evolution of CRISPR-Cas Systems

The CRISPR system, along with the associated (Cas) proteins, revolutionized understanding of bacterial adaptive immunity. CRISPR loci are first discovered as unusual repetitive sequences in prokaryotic genomes, then later found to be a component of the RNA-guided defense system that confers resistance against invading bacteriophages and plasmids

in bacteria and archaea. When microorganisms encounter foreign genetic material repeatedly, they add short bits of invader DNA into the CRISPR arrays to create an inherited molecular memory that allows them to quickly identify invaders during future attacks. The evolution of the host-microbial DNA element relationship has diversified CRISPR-Cas systems into several structural and functional variants to different ecological niches. This evolutionary plasticity has not only led to increased bacterial resistance, but it also offers the potential to build genome editing tools programmed by humans and tailor antimicrobial strategies with precision (Balcha & Neja, 2023). Moreover, the CRISPR systems are extremely adaptable and have been found to be useful for microbial engineering, antibiotic resistance research and therapeutic biotechnology (Chen et al., 2020).

2.2 Classification of CRISPR-Cas Systems (Class 1 and Class 2)

Based on the composition of the effector complexes, CRISPR-Cas systems can be classified into two major types. Class 1 systems recognize and degrade foreign nucleic acids, and include Types I, III, and IV. Class 2, by contrast, uses a single multidomain effector protein, to which the proteins are structurally simpler and more suited for genome engineering applications. These type II systems, which are highly programmable and precise, have been the most widely used genome editing system. The DNA- or RNA-targeting properties of types V and VI, which are mainly Cas12 and Cas13, respectively, add to the functional versatility of CRISPR technology. The plethora of these systems will allow researchers to choose the appropriate effectors based on the therapeutic, diagnostic, and antimicrobial goals, ultimately expanding the applications of precision medicine and microbial management (Chen et al., 2020). As their applications continue to grow, they also help develop novel strategies for controlling clinically relevant and food-borne pathogens (Elbehiry & Alajaji, 2026).

2.3 Molecular Mechanism of CRISPR-Mediated Immunity

The three steps of CRISPR-mediated immunity are adaptation, expression, and interference. In the adaptation phase, pieces of invading nucleic acids are trapped and added to the CRISPR array as new spacer sequences, thus creating a record of previous infections in the genome. The CRISPR locus is then transcribed into precursor CRISPR RNAs, which are processed into mature guide RNAs that bind to Cas proteins. During the interference phase, guide RNA leads the Cas effector to bind the complementary sequences, which allows for the highly specific degradation of foreign DNA or RNA to occur without affecting the host genome. This is a sequence-directed mechanism, which is much more precise than the conventional antimicrobial strategies, and is the molecular basis for genome editing and targeted bacterial elimination using the CRISPR system (Balcha & Neja, 2023). The programmability of guide RNA has also enabled studies of bacterial regulatory networks, AMR mechanisms, and functional genomics, which in turn has helped to speed the identification of novel therapeutic targets (Chen et al., 2020). The order of these events is the molecular basis for the extraordinary specificity of CRISPR-mediated immunity, and has led to the design of genome editing and precision antimicrobial technologies.

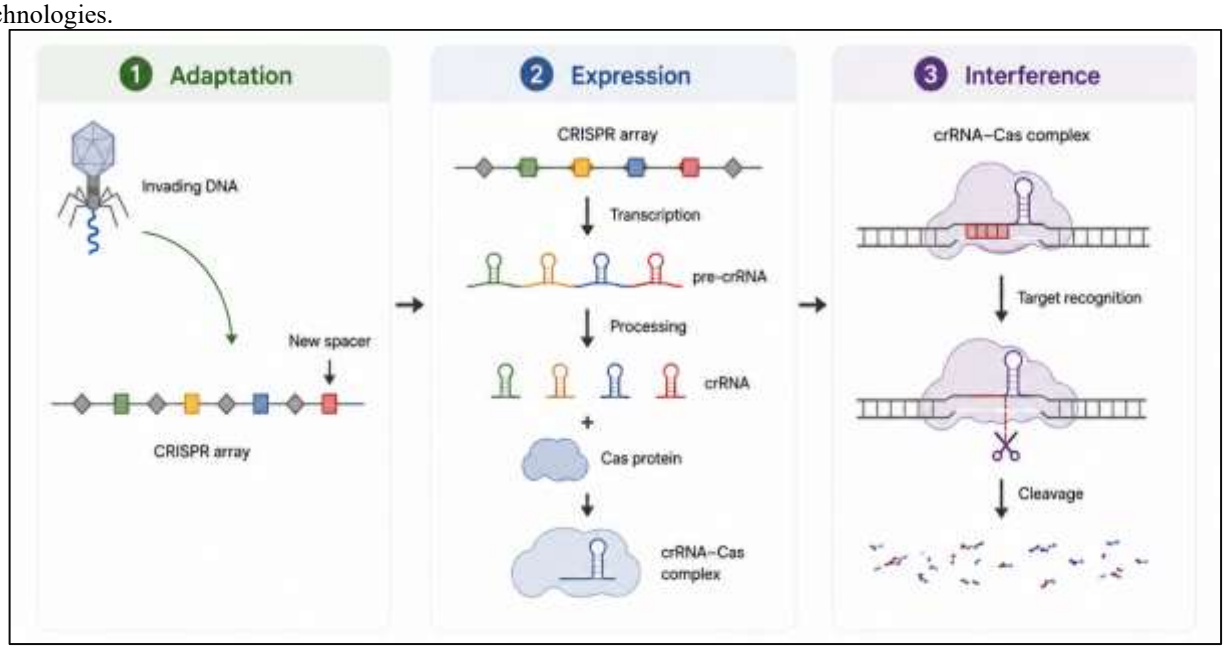

Figure 1. Molecular mechanism of CRISPR-mediated immunity

The concerted transition from acquisition of spacers to targeted nucleic acid cleavage is the basis for the extraordinary precision of the CRISPR-Cas systems (see Figure 1). As opposed to conventional antimicrobials which are broadly active against whole microbial populations, the recognition is programmed by RNA molecules, allowing sequence-specific targeting of resistance genes or virulence determinants, or even essential bacterial genomic regions.

2.4 Major Cas Effectors in Antimicrobial Applications (Cas9, Cas3, Cas12, Cas13 and Emerging Variants)

Cas9 is one of the most broadly used nucleases for sequence-specific DNA cleavage due to its simplicity, efficiency and programmability. Cas3 is also a processive degrader of DNA, which is very useful for removing large chunks of genomic DNA and resistance associated mobile genetic elements. In addition to its double-stranded DNA recognition property, Cas12 also has collateral single-stranded DNA cleavage capabilities that have been used to strengthen its antimicrobial and diagnostic applications. Compared to Cas13, which targets RNA molecules, Cas13 offers possibilities for transient

gene silencing, without permanent DNA editing, and for the control of RNA-based pathogens and the regulation of gene expression in bacteria. Recently, engineered Cas variants with enhanced specificity, minimal off-target activity, and greater targeting potential further bolster the therapeutic promise of CRISPR. From precision antimicrobials to engineered probiotics, to microbiome modulation and functional bacterial engineering, these effectors have diverse applications (Wang et al., 2025; Li et al., n.d.).

2.5 Advantages of CRISPR Compared with Conventional Antimicrobial Strategies

CRISPR-based antimicrobial strategies offer several important advantages over conventional antibiotics. Unlike broad-spectrum antimicrobial agents that indiscriminately eliminate both pathogenic and beneficial microorganisms, CRISPR systems can selectively target resistance genes, virulence determinants, or essential bacterial sequences with remarkable sequence specificity. This targeted approach minimizes disruption of the normal microbiota while reducing selective pressure that promotes resistance evolution. Furthermore, CRISPR technology can eliminate plasmid-mediated resistance, disrupt biofilm-associated pathogens, and restore bacterial susceptibility to existing antibiotics, thereby complementing rather than replacing current antimicrobial therapies (Elbehiry & Alajaji, 2026). Compared with traditional drug development, programmable guide RNAs also allow rapid redesign against newly emerging resistant strains, increasing adaptability in response to evolving pathogens. In addition, understanding bacterial genome plasticity and resistance evolution provides valuable insights for designing durable CRISPR-based interventions capable of overcoming the adaptive mechanisms commonly observed among ESKAPE pathogens (Das et al., 2022).

3. CRISPR-Based Precision Antimicrobial Strategies

The current use of CRISPR-Cas systems has revolutionized antimicrobial research by introducing programmable strategies that go beyond the traditional bactericidal methods. In addition to their antimicrobial effects on a wide spectrum of pathogens, CRISPR-based technologies can target MDR microorganisms, resistance genes, virulence genes, and genes associated with biofilm formation while maintaining beneficial bacteria. These strategies also enable pathogen re-sensitization to existing antibiotics and microbiome engineering, which together are a paradigm shift towards precision antimicrobial therapy. Table 1 provides a summary of key antimicrobial strategies based on CRISPR, their molecular targets, therapeutic aims and illustrative examples.

Table 1. Major CRISPR-Based Precision Antimicrobial Strategies Against MDR Pathogens

Strategy	Primary Molecular Target	Mechanism of Action	Therapeutic Outcome	Representative Application	Key Reference(s)
Selective elimination of MDR bacteria	Essential bacterial genes	CRISPR-mediated cleavage of essential genomic sequences	Specific eradication of resistant pathogens with minimal microbiome disruption	MRSA, Brucella spp.	Lau et al. (2025); Yadav & Yadav (2026)
Removal of antibiotic resistance genes	Resistance genes on chromosomes or plasmids	Targeted cleavage of resistance determinants (e.g., bla genes)	Restoration of antibiotic susceptibility and reduced resistance dissemination	Carbapenem-resistant bacteria	Sun et al. (2026); Pérez-Rodríguez et al. (2025)
Targeting virulence factors	Virulence genes and pathogenicity islands	Inactivation of toxin, adhesion, and secretion system genes	Reduced pathogenicity without extensive bacterial killing	Brucella and other bacterial pathogens	Lau et al. (2025)
Biofilm disruption	Biofilm regulatory genes and quorum-sensing pathways	Interference with biofilm formation and maintenance	Enhanced antimicrobial penetration and biofilm eradication	Chronic bacterial and Candida biofilm infections	Mallick et al. (2026); Tan et al. (2026)
Re-sensitization to antibiotics	Resistance-associated genes	Deletion or disruption of resistance mechanisms	Recovery of susceptibility to existing antibiotics	MRSA and other MDR bacteria	Yadav & Yadav (2026); Sun et al. (2026)
Combination CRISPR–antibiotic therapy	Resistance genes and bacterial defense pathways	CRISPR weakens bacterial defenses before antibiotic treatment	Synergistic antimicrobial activity and reduced antibiotic dosage	Precision combination therapy	Yadav & Yadav (2026); Pérez-Rodríguez et al. (2025)
Microbiome engineering	Species-specific genomic targets	Selective editing or removal of pathogenic	Preservation of beneficial microbiota and	Precision microbiome modulation	Pérez-Rodríguez et al. (2025); Sun et

		microorganisms	restoration of microbial balance		al. (2026)

Based on the summary in Table 1, the CRISPR-based precision antimicrobial strategies have several complementary mechanisms to combat the main drawbacks of traditional antimicrobial therapy.

3.1 Selective Elimination of MDR Bacteria

A major benefit of the antimicrobial therapeutic strategy using CRISPRs is that it can target any MDR (MDR) bacteria without harming beneficial microbial communities. CRISPR systems are distinct from conventional antibiotics which broadly target susceptible microorganisms, because they utilize programmable guide RNAs to guide Cas nucleases to species- or strain-specific genetic sequences. This precision allows for specific killing of resistant pathogens without killing commensal bacteria by disrupting essential chromosomal genes or resistance associated genetic elements. This selective activity brings about a microbial balance, and thus lessens the selection pressure that leads to further antimicrobial resistance. In recent years, there has been a growing number of research studies showing the potential of CRISPR-based strategies to control clinically relevant pathogens, such as Brucella spp. and methicillin-resistant Staphylococcus aureus (MRSA), as next-generation precision antimicrobials (Lau et al., 2025). In addition, combining the CRISPR therapeutics with targeted nanomedicine-based platforms can further improve the specificity of the bacterium targeted, deliver the therapeutics into the cell and provide better therapeutic efficacy against MDR pathogens (Yadav & Yadav, 2026).

3.2 CRISPR-Mediated Removal of Antibiotic Resistance Genes

In addition to killing resistant bacteria, CRISPR can also remove antibiotic resistance genes that cause antimicrobial resistance failure. The guide RNAs can be engineered to bind to a resistance determinant in the bacterial genome or in a plasmid, enabling Cas nuclease to selectively cut the plasmid. The removal of these genetic traits opens the door to the return of bacteria to their normal susceptibility to ordinary antibiotics and decreases the possibilities of horizontal transmission of resistance in microbial communities. This is a promising strategy over conventional antibiotics as it specifically targets the genetic basis of resistance and not just the bacteria viability. Furthermore, molecular platforms based on CRISPR technology have shown remarkable precision in detecting clinically relevant resistance genes, such as the genes encoding carbapenemases like bla_{NDM} for therapeutic guidance and swift molecular surveillance (Sun et al., 2026). In addition, the use of complementary biosensor technologies further improves early detection of AMR, aiding in the timely implementation of precision antimicrobial strategies (Pérez-Rodríguez et al., 2025).

3.3 Targeting Virulence Factors and Pathogenicity Islands

A lot of bacterial pathogens are not solely dependent on antibiotic resistance as a virulence pathway, but on virulence-associated genes. Selective inactivation of genes of toxins, adhesion proteins, secretion systems and other pathogenic traits using CRISPR-based precision antimicrobials can help reduce bacterial virulence but not necessarily kill the bacteria. Anti-virulence strategies have less selection pressure for the emergence of resistance than traditional bactericidal antibiotics and still have a beneficial effect on reducing disease severity. Bacteria are also prevented from co-ordinately expressing several virulence genes, thereby compromising their ability to colonize and infect by CRISPR mediated editing of pathogenicity islands. This highly selective strategy is especially appealing for the treatment of chronic infections wherein pathogen persistence depends on specific virulence mechanisms. More recently, studies of Brucella species have shown the increasing promise of CRISPR technologies to enable pathogen detection and specific disruption of virulence-associated genetic pathways (Lau et al., 2025).

3.4 Biofilm Disruption Using CRISPR Technologies

The presence of biofilms is one of the main reasons for the failure of antimicrobial treatment. Biofilms grow extracellular polymer matrix which protects the microorganism from the antibodies, immune system of the host and environmental factors. CRISPR technology offers a novel approach to disrupt the integrity of the biofilm by targeting adhesion, production of extracellular matrix, quorum sensing and biofilm maturation genes. Inactivation of these regulatory pathways disrupts biofilm architecture, increases antimicrobial penetration and increases bacterial eradication. These methods are especially useful for the management of chronic and device-associated infections that are highly tolerant to antimicrobials. With the recent progress in the study of antifungal and antibacterial biofilms, CRISPR-based approaches are emerging as promising targets to combat biofilm-associated resistance, especially in Candida infections (Mallick et al., 2026). Additionally, bibliometric analyses reveal growing global interest in the emerging strategy of controlling biofilms with CRISPR, which is being employed for combating biofilm-associated AMR in a wide variety of microbial pathogens (Tan et al., 2026).

3.5 CRISPR-Based Re-Sensitization of Resistant Pathogens to Antibiotics

One new use of CRISPR technology has been to make resistant populations of bacteria susceptible to antibiotics; this is not a direct elimination of the bacteria. CRISPR systems can reverse acquired resistance by selectively disrupting resistance-conferring genes and/or regulatory pathways, thus making the effectiveness of the existing antimicrobial agents once again possible. This re-sensitization strategy not only maintains the effectiveness of the older drugs, but also minimizes the need for constant discovery of new antimicrobial substances. In addition, susceptibility testing can be used to reduce costs of treatment and reserve "last line of defence" antibiotics for more severe infections. The combination of CRISPR-mediated genome editing with highly sophisticated drug delivery platforms such as nanoparticles has been shown to hold promising potential in increasing intracellular targeting and therapeutic efficacy for resistant strains like MRSA (Yadav & Yadav, 2026). At the same time, a quick identification of the resistance determinants by CRISPR technology allows the clinician to choose the best antibiotic after successful genetic re-sensitization (Sun et al., 2026).

3.6 Combination of CRISPR with Conventional Antibiotics

The use of CRISPR technology with traditional antibiotics is a complementary treatment approach that can overcome multiple mechanisms to combat AMR. CRISPR systems can first be used to inactivate resistance genes or reduce the strength of bacterial defense mechanisms, making the bacteria more susceptible to later treatment with antibiotics. This complementary approach helps to lower the concentration of antibiotic(s) needed to kill the bacteria, decrease toxicity and delay the development of new resistance. Combination therapy has several advantages over antibiotic monotherapy in the treatment of persistent infections of biofilms or MDR pathogens, which are less responsive to single agent therapy. Co-delivery of CRISPR components and antimicrobial agents is another promising approach to this strategy using nanomedicine-based drug delivery platforms, which enables a more precise treatment and the accumulation of drugs within cells where they are needed (Yadav & Yadav, 2026). The co-deployment of CRISPR diagnostics also enables personalized antimicrobial selection based on the identification of the resistance profile of the pathogen at a fast pace (Pérez-Rodríguez et al., 2025).

3.7 CRISPR-Driven Microbiome Engineering and Selective Pathogen Editing

The high specificity of CRISPR technology has opened up new application areas beyond pathogen destruction, such as microbiome engineering and selective microbial editing. Programmable CRISPR systems can selectively target the removal of pathogenic bacteria while maintaining a balance of beneficial microorganisms necessary for maintaining host health, rather than indiscriminately killing all bacteria. This is a directed manipulation which helps the restoration of microbial homeostasis and minimizes the negative side effects usually encountered with general antibiotic therapy. The removal of pathogens could also reduce the likelihood of antimicrobial-resistant pathogens colonizing the crop while not significantly changing the overall microbial diversity. By integrating CRISPR technology with molecular detection platforms with high sensitivity, the composition of microbial communities and the presence of resistance determinants can be monitored in real time, supporting precision-guided therapeutic interventions (Pérez-Rodríguez et al., 2025). Furthermore, the development of molecular diagnostics facilitated by CRISPR is ongoing, enabling further refinement of clinically important resistance genes and an integrated system of monitoring the microbiome and selectively intervening with antimicrobial treatment (Sun et al., 2026).

4. Delivery Platforms for CRISPR Antimicrobials

An ideal delivery platform should afford protection of CRISPR cargo from degradation, efficient delivery into pathogens, high host specificity, and minimal off-target effects. Several biologic and synthetic delivery systems have therefore been developed for enhanced therapeutic efficacy, which vary in their respective advantages based on the pathogen and clinical setting. Given the apparent complexity of the problem and the variety of approaches being taken, current delivery strategies for CRISPR antimicrobials have been summarized in Figure 2 and include: bacteriophage-based vectors; conjugative plasmids; polymeric and hydrogel systems; extracellular vesicles; and optimization factors which all aim to improve the delivery efficiency and precision of CRISPR antimicrobials.

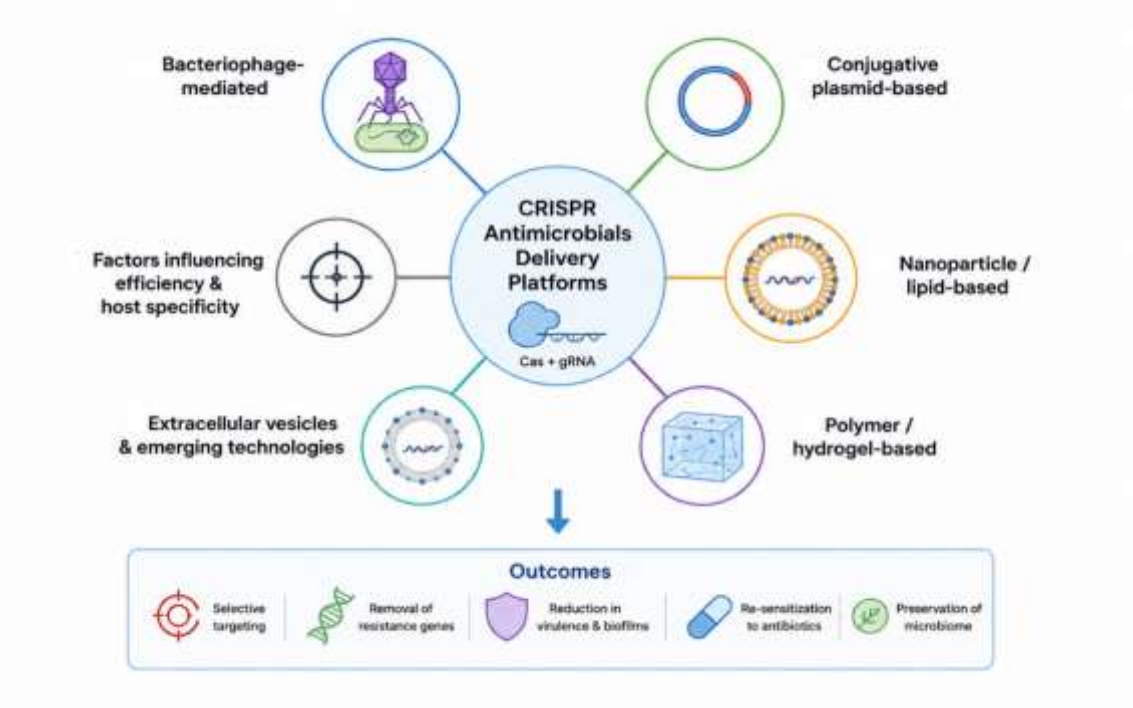

Figure 2. Delivery platforms for CRISPR-based antimicrobial therapeutics.

As shown in Figure 2, there is no single delivery system for all bacteria and no one delivery system will be universally applicable for all therapeutic goals.

4.1 Bacteriophage-Mediated CRISPR Delivery

One of the most promising delivery vehicles for CRISPR antimicrobials are the bacteriophages, as they are able to infect bacteria with an incredible host specificity. Engineered phages can be delivered directly to target pathogens, allowing for

sequence-specific cleavage of resistance genes or virulence determinants or essential chromosomal loci. Phages have the ability to naturally penetrate the cell walls of bacteria, enhancing both the efficiency of intracellular delivery with reduced off-target effects. They can also be programmed with a host range, further enabling precision antimicrobial therapy against MDR pathogens. Beyond therapeutics, phage engineering has opened up promising opportunities in the field of pathogen detection, such as creating highly sensitive detection systems for pathogens, and the development of integrated biosensing platforms that combine phage-based detection with therapeutic benefits (Wang et al., 2026). In addition, the molecular detection technologies which are phage-assisted have been shown to have significant potential in enhancing microbial surveillance and food safety monitoring (Mohammad et al., 2025).

4.2 Conjugative Plasmid-Based Delivery Systems

An alternative approach to delivering CRISPR components is through the natural phenomenon of bacterial conjugation using conjugative plasmids. Programmable CRISPR-Cas system components can be transmitted between bacterial cells and thus used to target editing of resistance genes in microbial communities. Conjugative plasmids preferentially spread between bacteria that are more closely related to each other, which allows for the dissemination of CRISPR antimicrobials within a local microbial community whilst minimizing unintended consequences on unrelated microbial communities. The delivery strategy is especially desirable for the eradication of plasmid-mediated AMR and for the reduction of horizontal gene transfer in clinical and environmental conditions. The bacterial conjugation and plasmid maintenance are also identified as promising therapeutic targets in recent studies for controlling AMR, thereby supporting the conjugation-based delivery platforms and the integration of CRISPR technology (Touati et al., 2026). All these strategies can significantly decrease the dissemination of resistance determinants among MDR pathogens.

4.3 Nanoparticle and Lipid-Based Delivery Platforms

Nanoparticle- and lipid-based delivery systems have recently garnered considerable attention due to their potential to deliver CRISPR parts into bacterial cells without a virus. Lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs) and hybrid nanocarriers (HNCs) can shield the CRISPR RNA from nucleases and increase uptake by cells and stability during systemic delivery. Selective targeting of bacterial populations or infected tissues is also made possible by surface functionalization, leading to higher therapeutic precision and less off-target interactions. Nanoparticle-based systems also have the advantage over biological vectors in that they are more flexible in their ability to carry a wide range of CRISPR payloads, such as DNA, mRNA, and RNP complexes. They are also compatible with point-of-care molecular technologies, which enables therapeutic delivery to be combined with pathogen identification and AMR profiling (Pan et al., 2026). The clinical translation of the antimicrobial therapeutics based on CRISPR technology should be further improved with continued optimization of nanocarriers composition and targeting ligands.

4.4 Polymer and Hydrogel-Based Delivery Approaches

Polymeric carriers and hydrogel matrices are versatile platforms for the localized and sustained delivery of the CRISPR antimicrobials. The components of CRISPR-Cas can be enclosed in biocompatible polymers which prevent their degradation and allow them to be released at the infection site in a controlled manner. Hydrogels also create a hydrated three-dimensional environment, which is beneficial for a longer retention time, which is especially suitable for chronic wounds, implant-associated infections, and biofilm-related diseases. Local targeted delivery reduces systemic exposure and increases the concentration of the antimicrobial agent at the infected tissues, which increases the antimicrobial effect and decreases adverse effects. Moreover, owing to the development of the smart biomaterials, stimuli-responsive hydrogels that release the CRISPR cargo upon the recognition of the environmental signals like pH, temperature, and bacterial enzyme have been developed. Such innovations complement molecular diagnostic technologies by providing support for localized precision therapy after quick pathogen identification (Pan et al., 2026).

4.5 Extracellular Vesicles and Emerging Delivery Technologies

Recently, the attractiveness of extracellular vesicles (EVs) as a biological vehicle for delivery of CRISPR was highlighted due to their properties of excellent biocompatibility, low immunogenicity, and intrinsic capability to inter-cellularly transport nucleic acids. Designed EVs can carry CRISPR-Cas components without those being destroyed extracellularly, and can be targeted to infected tissues. Apart from EVs, various emerging delivery technologies such as cell membrane-coated nanoparticles, bacterial membrane vesicles, and bioengineered microbial carriers are being explored to enhance the delivery efficiency and therapeutic specificity. The innovative platforms are believed to address some key challenges of viral vectors, such as cargo capacity, immune responses, and more. The promising integration of advanced molecular diagnostics could enhance the development of personalized antibiotic treatment by simultaneously identifying pathogens and delivering targeted antibiotic interventions (Hussain et al., 2026; Pan et al., 2026).

4.6 Factors Influencing Delivery Efficiency and Host Specificity

Effective delivery of an antimicrobial based on the CRISPR system and the correct targeting of the host are two critical factors in its therapeutic efficacy. The microbacterial cell wall composition, biofilm, vector stability, cargo size, recognition by the immune system, and microbial host range have a significant effect on delivery performance. Also crucial is the choice of highly specific guide RNAs with minimal off-target effects, yet strong cleavage of the desired genetic targets. Individual delivery platforms also are effective in different ways depending on bacterial genetic diversity and species-specific physiological characteristics. CRISPR interference-based functional genomic studies have yielded insightful findings on the essential genes and cellular pathways in bacteria that can inform better target selection and delivery optimization, especially for clinically relevant pathogens like Acinetobacter baumannii (Bai et al., 2021). With the development of delivery technologies, and molecular diagnostics and precision target identification, CRISPR-based antimicrobial therapies are anticipated to have an even more rapid transition through the clinical pipeline (Hussain et al., 2026).

5. Therapeutic Applications Against Major MDR Pathogens

CRISPR-Cas has been widely expanded to clinically relevant MDR (MDR) pathogens for therapeutic purposes. CRISPR's versatility of specificity allows for the disruption of resistance genes, virulence genes, and key genomic regions without affecting beneficial microbes. The therapeutic goals differ across the bacterial species depending on the resistance mechanisms and pathogenic properties of the bacteria, but the general aim of CRISPR-based interventions is to restore antimicrobial susceptibility, and to enhance the precision of treatment. Table 2 summarizes key properties of the major MDR pathogens, their common resistance mechanisms, the targets of CRISPR and the expected clinical impact.

Table 2. Therapeutic applications of CRISPR-based antimicrobial strategies against major MDR pathogens

MDR Pathogen	Major Resistance Mechanisms	Primary CRISPR Target(s)	Expected Therapeutic Outcome	Representative Reference(s)
Methicillin-resistant Staphylococcus aureus (MRSA)	mecA-mediated β-lactam resistance, virulence regulators	Resistance genes, virulence-associated genes, essential chromosomal loci	Selective pathogen elimination and restoration of antimicrobial susceptibility	Kushwaha (2026); Wu et al. (2022)
Vancomycin-resistant Enterococcus (VRE)	van resistance gene clusters, plasmid-mediated resistance	Vancomycin resistance genes and conjugative plasmids	Removal of resistance determinants and reduced horizontal gene transfer	Kushwaha (2026)
Carbapenem-resistant Enterobacterales (CRE)	Carbapenemase genes, plasmid-mediated resistance, multidrug efflux systems	Carbapenem resistance genes, plasmids, metabolic targets	Re-sensitization to carbapenems and suppression of resistance dissemination	Pines et al. (2018a); Camp et al. (2021)
MDR Pseudomonas aeruginosa	Biofilm formation, quorum sensing, efflux pumps	Biofilm-associated genes, quorum-sensing regulators, resistance genes	Reduced biofilm formation and enhanced antibiotic susceptibility	Wu et al. (2022)
MDR Acinetobacter baumannii	Stress adaptation, multidrug resistance determinants	Essential genes and resistance-associated loci	Targeted bacterial killing and improved therapeutic specificity	Kushwaha (2026)
Drug-resistant Mycobacterium tuberculosis	Mutations in drug-target genes, metabolic adaptation	Essential metabolic genes and persistence-associated pathways	Identification of novel therapeutic targets and precision antimicrobial intervention	Wu et al. (2022)
Other clinically relevant MDR pathogens	Diverse resistance genes, virulence determinants, microbiome imbalance	Species-specific resistance and virulence genes	Selective pathogen editing while preserving beneficial microbiota	Tayyab et al. (2025); Pines et al. (2018b)

Table 2 summarizes the biological targets of antimicrobial CRISPR therapy that can be specifically targeted by antimicrobials to the resistance genes, virulence determinant genes, genes involved in MDR pathogen biofilm formation, or essential genes of individual MDR pathogens.

5.1 Methicillin-Resistant Staphylococcus aureus (MRSA)

Despite the fact that methicillin-resistant Staphylococcus aureus (MRSA) has been identified as one of the most important causes of HAIs, it continues to be a problem because of its resistance to several β-lactam antibiotics and its ability to develop further resistance. A highly specific antimicrobial approach to tackle MRSA can be achieved using CRISPR-based technology, focusing on resistance-associated genes, virulence regulators, and essential gene regions, and avoiding disruption of beneficial microbial communities. The ability of the CRISPR-Cas system to be programmed also means that new MRSA strains can be adapted quickly, by redesigning the guide RNA. Moreover, the use of artificial intelligence (AI) for optimizing the guide sequence in CRISPR has enhanced the accuracy of target selection and the precision of therapy, helping to develop individualized intervention strategies against resistant S. aureus isolates (Kushwaha, 2026). Combination of CRISPR technology and adaptive microbial engineering methods could also speed up the improvement of precision therapies targeting emerging MRSA sub-populations (Wu et al., 2022).

5.2 Vancomycin-Resistant Enterococcus (VRE)

There are several reasons why Vancomycin-resistant Enterococcus (VRE) is a significant problem in the hospital setting: intrinsic resistance, and the ability to transfer resistance determinants to other Gram-positive organisms. CRISPR-based therapeutics have the potential to specifically disrupt the vancomycin resistance genes or to remove plasmids that enable dissemination of the resistance gene without compromising the susceptible populations. This focussed editing can help decrease reliance on last resort antibiotics and limit the selective pressure of traditional antibiotic use. With the improvement of computational biology, the design of the CRISPR guide and target prediction have also improved, which can better edit clinically important resistance determinants. The use of AI to optimize CRISPR will further improve future

antimicrobial interventions and precision, safety, and scalability against VRE and other MDR pathogens (Kushwaha, 2026).

5.3 Carbapenem-Resistant Enterobacterales (CRE)

While bacterial strains resistant to both multiple classes of antibiotics and carbapenems are present in nearly every healthcare setting, carbapenem-resistant Enterobacterales (CRE) are among the most alarming AMR pathogens as they often contain genes that encode plasmid-mediated carbapenemase (PMC) that can be readily transferred to other enterobacters, which use plasmid transfer mechanisms. CRISPR technology offers a potential approach to selectively target these resistance determinants and restore susceptibility to carbapenems and limit the spread of resistance genes among bacteria. CRISPR systems can be utilized to study metabolic pathways linked to antimicrobial susceptibility and adaptive evolution in addition to direct editing of resistance loci. CRISPR-based genome engineering has been shown to be a powerful tool for the study of resistance mechanisms and identification of therapeutic targets in experimental studies of mutations associated with fosmidomycin resistance in Escherichia coli (Pines et al., 2018a). Furthermore, knowledge of the mechanisms of resistance other than carbapenemases, such as the role of multidrug efflux systems within the clinically relevant Escherichia coli ST131 lineages, can help identify alternative CRISPR targets for precision antimicrobial intervention (Camp et al., 2021).

5.4 MDR Pseudomonas aeruginosa

Pseudomonas aeruginosa is one of the most resistant bacterial pathogens to eradicate due to its remarkable genomic plasticity, intrinsic AMR and its ability to form biofilms. CRISPR-based interventions have the potential to target genes associated with quorum sensing, biofilm formation, efflux systems and AMR, leading to lower populations of bacteria and increased susceptibility to currently available antibiotics. Systematic investigation of adaptive resistance mechanisms that develop over extended periods of exposure to antimicrobials is also possible with programmable genome editing. In highly adaptable bacterial species, recent progress in the field of adaptive laboratory evolution, in conjunction with genome engineering, has yielded important resources for identifying resistance-associated pathways and engineering optimized antimicrobial targets for CRISPR (Wu et al., 2022). The advances will help in the design of precision therapies that will overcome the remarkable evolutionary ability of P. aeruginosa.

5.5 MDR Acinetobacter baumannii

Acinetobacter baumannii is a significant cause of ventilator associated pneumonia, bloodstream infections and wound infections as a result of its resistance mechanism to multiple determinants and its resistance to harsh environmental conditions. CRISPR-based antimicrobial approaches allow precise disruption of key genes used for bacterial survival, stress response, and AMR to boost specificity of therapy. Systematic identification of essential bacterial genes has shown to be a valuable basis for developing highly effective antimicrobial interventions using the CRISPR technology. The addition of AI to microbial engineering and engineering of A. baumannii (AWBI) is anticipated to further optimize target selection and enhance the therapeutic results against extensively drug-resistant A. baumannii (Kushwaha, 2026). These precision strategies could be used alongside traditional antimicrobial treatments, and help to prevent the development of further resistance.

5.6 Drug-Resistant Mycobacterium tuberculosis

Despite the long duration of treatment and slow growth of the bacteria, Mycobacterium tuberculosis remains a threat to global efforts to control TB, as well as because of emerging resistance to first-line and second-line antitubercular drugs. CRISPR technology can be used to study vital genes involved in bacterial persistence, drug resistance and metabolic adaptations, and can be used to discover new drug targets. Precision genome editing can also be used to help develop targeted interventions that can overcome resistance without impacting non-pathogenic microbial populations. Adaptive laboratory evolution coupled with CRISPR functional genomics has emerged as a valuable approach to understanding bacterial adaptation to antimicrobial stress and to identifying mechanisms that lead to persistent drug resistance (Wu et al. 2022). These breakthroughs could hasten towards the precision therapies for MDR-TB.

5.7 Other Clinically Relevant MDR Pathogens

In addition to the major MDR pathogens, CRISPR technology has been shown to be applicable against a wide range of clinically relevant bacterial species that cause HAIs and CAIs. Precision genome editing allows targeted modification of resistance genes, metabolic pathways and virulence determinants in order to selectively target different populations of bacteria without killing desirable microorganisms. Prominent new applications are in the field of microbiome engineering, where specific pathogenic microorganisms can be targeted for elimination without adversely impacting the balance of the ecosystem. The use of such methods is now known to be a viable alternative to whole spectrum antimicrobial treatment. The innovations in microbiome engineering underscore the potential of programmable microbial editing in improving disease resistance and maintaining healthful microbiome, offering insights that could guide future precision antimicrobial strategies in various clinical contexts (Tayyab et al., 2025). In addition, genomic research focused on resistance associated mutations of fosmidomycin resistance in E. coli has continued to guide in the discovery of new CRISPR targets and in the development of new antimicrobial approaches (Pines et al., 2018b).

6. Emerging Innovations in CRISPR-Enabled Antimicrobial Therapy

The potential of CRISPR-based antimicrobial systems has been greatly expanded by recent technological advances beyond that of genome editing. Collectively, emerging innovations such as the optimization of guide RNA with the help of AI, programmable RNA-targeting, precision genome-editing technologies, synthetic biology, and tailored therapeutic approaches are contributing to the evolution of next-generation precision antimicrobials. The parallel progressions are likely to make a difference in diagnostic, therapeutic, and clinical specificity and translation. The key innovations for the future of antimicrobial therapy with CRISPR are concisely summarized in Figure 3.

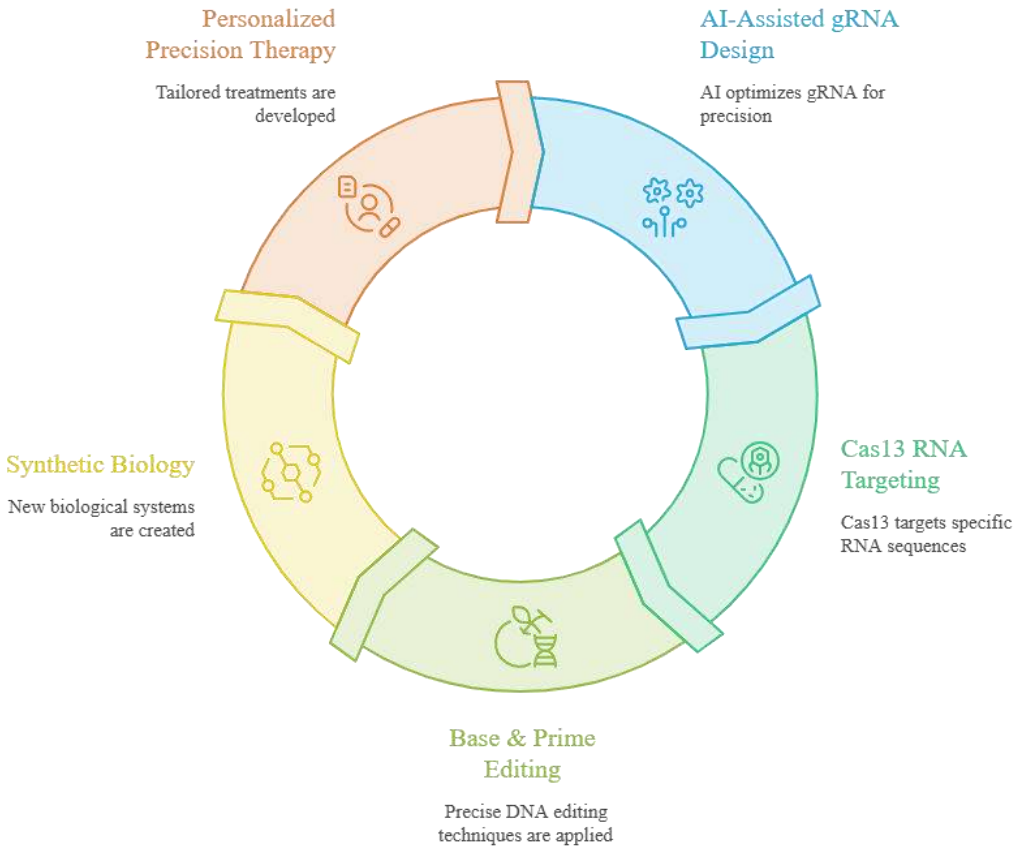

Figure 3. Emerging innovations driving the advancement of CRISPR-enabled antimicrobial therapy

The future of genome editing as an antimicrobial therapy is not only dependent on genome editing, but also multiple complementary technologies as shown in Figure 3. The power of AI, programmable RNA targeting, cutting-edge genome editing technologies, synthetic biology, and personalized precision medicine should enhance the precision of therapeutic interventions, mitigate off-target effects, and hasten the translation of research into clinical practice.

6.1 CRISPR-Based Antimicrobial Diagnostics and Companion Therapeutics

With the advent of targeted antimicrobial therapy alongside diagnostics, the new concept of companion therapeutics has been introduced, meaning that fast pathogen identification is directly connected to precise treatment. CRISPR diagnostic platforms can quickly detect single-stranded nucleic acid sequences of pathogens, AMR genes and clinically relevant mutations at high sensitivity, allowing for timely therapeutic intervention. The addition of programmable CRISPR antimicrobials allows for concurrent diagnosis and targeted elimination of resistant bacteria, minimizing the unnecessary use of antibiotics and enhancing clinical decision making. These are systems that can facilitate the use of therapeutic measures in a manner that is supportive of antimicrobial stewardship, by aligning therapeutic decisions with the genetic makeup of the organism causing the infection. The use of microbial engineering for diagnostics has been further reinforced by the development of multifunctional antimicrobial platforms that integrate molecular diagnostics with precision therapy, powered by advances in CRISPR-Cas9 genome editing technologies (Dindhoria et al., 2024). These innovations are anticipated to help speed the move towards personalized approaches to infectious disease care.

6.2 AI-Assisted CRISPR Guide RNA Design and Optimization

The application of artificial intelligence (AI) is rapidly cracking the code in designing guide RNA, predicting targets and enhancing editing efficiency in the context of CRISPR technology. Genomic sequencing can be used with machine learning algorithms to predict off-target interactions and to identify the most highly conserved regions of the bacteria genomes to increase the specificity and safety of antimicrobial interventions using CRISPR. Guide RNA optimization is also faster with AI, especially for newly identified MDR pathogens, fast-tracking therapeutic design. Moreover, computational modeling can be used to continually improve the strategies for CRISPR editing by incorporating genomic variation and evolutionary dynamics. The large numbers of genomes edited have resulted in huge datasets that serve as valuable training sets for predictive models developed using AI, which can be used to identify optimal antimicrobial targets and enhance genome-editing performance (Choudhury, 2019). This fusion between AI and CRISPR is a significant leap towards adaptive and data-driven precision antimicrobial treatment.

6.3 Programmable RNA-Targeting Systems (Cas13)

Cas13 is a type of programmable nuclease that targets RNA instead of DNA, potentially offering a platform for non-permanent regulation of bacterial gene expression. RNA-targeting systems allow for selective inhibition of resistance-associated transcripts, virulence factors, and regulatory RNA that is key to bacterial adaptation and pathogenicity. Unlike permanent changes to the genome, RNA modifications are reversible, providing another layer of therapeutic flexibility and mitigating potential concerns arising from permanent changes to the genome, which can be caused by Cas13-based approaches. In addition to antimicrobial therapy, programmable RNA targeting has also shown great promise for rapid

detection of pathogens and functional transcriptomic analysis, which are useful for therapeutic and diagnostic purposes. Microbial genome engineering has aided in deeper understanding of the CRISPR-associated nucleases and their programmability, leading to the design of more and more versatile RNA-targeting antimicrobial systems (Dindhoria et al., 2024).

6.4 CRISPR Base Editing and Prime Editing for Antimicrobial Applications

In summary, Base editing and Prime editing are next-generation CRISPR technologies that can introduce precise nucleotide modifications without creating DSBs. Such sophisticated editing systems enable editing of resistance-associated mutations without causing significant damage to the genome and with higher editing efficiency. They are used in antimicrobial research to accurately manipulate bacterial genomes for functional studies, validate resistance mechanisms and create engineered strains for therapeutic use. These technologies provide more flexibility than the standard CRISPR-Cas9 editing, allowing for the modification of individual nucleotides, introduction of specific genetic modifications with lower chances of unwanted mutations. This is of special importance for the study of necessary bacterial proteins linked to AMR and pathogenicity. Genome editing studies aimed at identifying novel antimicrobial targets in Acinetobacter baumannii highlight the need for precision genome editing to discover new antimicrobial targets and expedite therapeutic discovery (Bai, 2024).

6.5 Synthetic Biology and Next-Generation Programmable Antimicrobials

Synthetic biology has drastically broadened the application of the CRISPR technology by creating programmable microorganisms that can detect, respond, and destroy bacterial pathogens. A microbial platform can be engineered to express antimicrobial peptides, CRISPR cargo or to selectively inhibit resistance-associated genes within a complex microbial community. These programmable biological systems provide prolonged therapeutic activity and could represent a solution to some of the challenges of repeated use of traditional antimicrobial drugs. The development of microbial platforms for industrial and therapeutic applications has also been aided by the advancement of genetically engineered microorganisms (GEMOs) to enhance their biosafety, productivity, and precision (Vithalani & Bhatt, 2026). Simultaneously, the discovery of new bioactive molecules from endophytes of medicinal plants offers additional avenues to use CRISPR technology in conjunction with natural product discovery for the creation of new antimicrobial approaches (Madagundi et al., 2025).

6.6 Personalized Precision Antimicrobial Therapy

Personalized antimicrobial therapy has the objective of adapting the antimicrobial treatment to the genetic features of the pathogen, its AMR pattern and host specific features. CRISPR technology can be used to precisely target specific virulence determinants or resistance genes that were identified during pathogen characterization in order to enhance the therapeutic efficacy, while avoiding the disruption of beneficial microbiota. The future of treatments could involve a combination of fast molecular diagnosis, the optimization of guide RNA with AI tools, microbiome analysis, and programmable CRISPR antimicrobials forming customized treatment plans for each person. These comprehensive strategies will be expected to achieve better clinical results and decrease antimicrobial misuse and development of antimicrobial resistance. However, the contribution of next-generation probiotics that can aid in restoring health of the microbiome while simultaneously providing specific antimicrobial intervention, further reinforces the vision of personalized precision medicine (Jadhav et al., 2026). As a group, these innovations put CRISPR-based therapeutics on the cutting edge of the future of precision infectious disease therapy.

7. Safety, Challenges, and Translational Barriers

While the idea of antimicrobial therapy with CRISPR holds promise of unprecedented precision in fighting MDR pathogens, there are a number of scientific, technological, regulatory and manufacturing hurdles to overcome to make it ready for clinical translation. However, genome-editing safety is not limited to accuracy; the efficiency of delivery, the compatibility of the host immune system, the possibility of resistance evolution, the ethical governance, and large scale production are also safety issues. To translate platforms based on CRISPR into therapeutics for antimicrobial applications, a multidisciplinary approach to innovation will be key to overcoming these hurdles. The most important safety issues, potential consequences, existing mitigation measures, and implications for translation are summarized in Table 3.

Table 3. Major safety considerations, translational barriers, and mitigation strategies for CRISPR-enabled antimicrobial therapy

Challenge	Potential Impact	Current Mitigation Strategy	Translational Significance	Representative Reference(s)
Off-target genome editing	Unintended genetic modifications and reduced editing specificity	High-fidelity Cas variants, optimized guide RNA design, genome-wide validation	Improves therapeutic safety and editing precision	Li et al. (2026)
Clinical delivery limitations	Poor bacterial uptake, cargo degradation, reduced therapeutic efficiency	Bacteriophages, nanoparticles, lipid carriers, hydrogels, extracellular vesicles	Enhances targeted delivery and treatment efficacy	Fahim et al. (2026)
Horizontal gene transfer and CRISPR resistance	Dissemination of resistance genes and reduced CRISPR effectiveness	Multiplex CRISPR targeting, conserved genomic targets, continuous surveillance	Supports long-term antimicrobial effectiveness	Li et al. (2026)
Host immune	Immunogenicity,	Biocompatible delivery	Improves clinical	Fahim et al.

responses and biosafety	inflammatory reactions, vector-associated toxicity	systems, immune profiling, optimized biomaterials	safety and therapeutic tolerance	(2026)
Ethical and regulatory challenges	Delayed clinical approval and inconsistent governance	Standardized regulatory frameworks, biosafety assessment, ethical oversight	Facilitates responsible clinical implementation	Li et al. (2026)
Manufacturing and scalability	High production costs, formulation variability, limited accessibility	GMP-compliant manufacturing, scalable production platforms, process standardization	Enables commercialization and broader clinical adoption	Fahim et al. (2026)

To translate CRISPR-based antimicrobial therapy into clinical practice, multiple advances in genome editing precision, delivery, biosafety, regulatory oversight and scalable production must be made and coordinated (see Table 3). Great strides have been made toward enhancing the accuracy and therapeutic promise of CRISPR, however there are still biological and translational hurdles to overcome for wide-spread clinical use.

7.1 Off-Target Effects and Genome Safety

Although CRISPR-Cas systems are very precise, off-target cleavage of non-target genomic DNA is a significant concern for clinical use in antimicrobial applications. Off-target editing could affect the dynamics of microbial communities, therapeutic specificity or key bacterial genes. These unanticipated changes may also make efficacy testing and biosafety testing in the preclinical phase difficult. The optimization of gRNAs, high fidelity variants of Cas, and the prediction tools that guide the selection of gRNAs have significantly enhanced the accuracy of editing, but there is a still significant challenge to abolish all off-target activity. Therefore, before clinical application it is necessary to comprehensively validate and monitor the genome. With precision medicine becoming a major emphasis, there is an increased need for highly precise genome editing methods that can maximize the therapeutic potential with minimum unintended genetic modification (Li et al., 2026).

7.2 Delivery Challenges in Clinical Settings

One of the key challenges for the clinical translation of antimicrobials using the CRISPR platform is the need for efficient delivery. Delivery vehicles need to protect the CRISPR cargo from degradation, need to get into the bacterial cells in an efficient way, need to keep the CRISPR cargo stable, and need to selectively target pathogens, while not degrading or hurting healthy tissues or the beneficial bacteria. Other parameters related to infection sites, bacterial biofilms and host physiology further affect delivery efficiency. Nanotechnology-based delivery systems have shown significant potential for increasing the stability of the cargo, its controlled release, targeting to tissues and decreasing the systemic toxicity. It is expected that the therapeutic activity and clinical applicability of CRISPR antimicrobial interventions (Fahim et al., 2026) will become improved with continuous refinement of the engineering of nanoparticles.

7.3 Horizontal Gene Transfer and Resistance to CRISPR Systems

Horizontal gene transfer is still a big challenge for long-term antimicrobial control as it allows for rapid spread of resistance determinants within bacterial populations. Likewise, bacteria could develop resistance to CRISPR targeting by changing their target sequence, by producing anti-CRISPR proteins, and/or by mutating the sequence. If such adaptive responses occur, they may impact therapeutic durability and require constant redesigning of guide RNAs. To overcome these problems, surveillance of the evolution of bacterial genomes, identification of well-conserved regions and creation of multiplex CRISPR systems able to target multiple resistance determinants are needed. Precision genomic analysis of CRISPR may provide increased resilience against bacterial adaptation and long-term effectiveness of programmable antimicrobial therapies (Li et al., 2026).

7.4 Host Immune Responses and Biosafety Considerations

Another significant point to consider for clinical translation is the recognition of CRISPR associated proteins and delivery vectors by the host immune system. If the delivery platforms are already known to induce immune activation or pre-existing immunity to Cas proteins of the bacteria may decrease therapeutic efficacy or increase the risk of inflammatory responses. Proper biosafety testing should thus include immunogenicity, tissue compatibility, vector persistence as well as potential systemic toxicity. The recent developments in nanotechnology have enabled the creation of delivery platforms that are biocompatible, have an improved safety profile and exhibit controlled release characteristics and lesser immunogenic potential. These innovations not only enhance safety in the administration of CRISPR therapeutics but also aid in their wider clinical use (Fahim et al., 2026).

7.5 Ethical and Regulatory Considerations

Ethical, legal and regulatory implications must be taken into account for the clinical use of CRISPR-based antimicrobial therapy. Before the widespread application of genome editing in the clinic, regulatory bodies need to develop consensus standards for assessing genome editing accuracy, biosafety, manufacturing quality, environmental risk, long-term therapeutic effects, and so on. Other ethical questions involve unintended ecological effects, responsible utilization of programmable genetic technologies, equitable distribution of advanced therapeutics and the communication of risks in an understandable way. In the ever-changing landscape of precision medicine, the regulation should strike a balance between technological advancement and patient safety, environmental protection, and responsible clinical practices. For the implementation of standard procedures for CRISPR-based antimicrobial therapeutics, international research collaboration between researchers, regulators and healthcare agencies will be needed (Li et al., 2026).

7.6 Manufacturing, Scalability, and Cost Constraints

Scale-up manufacture and commercialization of CRISPR antimicrobial products is still a challenge. The production processes should produce consistent quality, stable formulations, effective delivery systems, conform to good manufacturing practice (GMP), and be cost-effective. The current manufacturing costs are high, and the wide availability of genome-editing technology is still restricted by complex delivery vehicles, specialized genome-editing components, and strict quality-control procedures. The ability to scale up production, to standardize formulation processes and to automate manufacturing will be essential to making laboratory innovations a reality in the clinic. Future developments of nanotechnology based formulation and precision delivery systems are likely to further enhance production efficiency, lower manufacturing costs, and enable wider application of antimicrobial therapy using CRISPR in clinical practice (Fahim et al., 2026).

8. Future Perspectives

The next generation of genome editing systems will be more widely applicable to a variety of MDR pathogens, more efficient, more specific, and with less off-target effects, and therefore be more suitable for future CRISPR-based drug development for antimicrobial purposes. The development of new Cas variants, RNA-targeting systems, multiplex editing approaches, and other delivery technologies is likely to enhance the therapeutic precision and clinical safety of these approaches even more. The combination of these with precision medicine, AI, speed of molecular diagnosis, microbiome profiling and the identification of host characteristics will enable personalised approaches to treatment that are based on the genotype of the pathogen, its resistance pattern and the host. At the same time, clinical trials are increasing in size and scope to prove efficacy, biosafety, optimal doses and therapeutic benefits in a variety of infectious diseases and patient populations for a longer period of time. Harmonised regulatory frameworks, harmonised manufacturing processes, scalable manufacturing technologies and cost-effective commercialisation routes that guarantee quality and access will also be important to progress towards routine clinical implementation. The future research priorities should include overcoming delivery constraints, reducing bacterial escape mechanisms, enhancing host compatibility, creating integrated diagnostic–therapeutic platforms, and investigating combination therapies of synthetic biology, nanotechnology and engineered probiotics. These advances in combination will pave the way for the next generation of antimicrobials based on CRISPR to be more exact, clinically translatable, and sustainable, and to help overcome the growing global threat of AMR.

9. CONCLUSION

The emergence of MDR pathogens has underscored the need for new antimicrobial approaches that go beyond the use of traditional antibiotics. CRISPR-Cas technology has proven to be a paradigm shift that can deliver highly-specific, programmable, and adaptable antimicrobial solutions by enabling targeted eradication of resistant bacterial strains, the removal of antibiotic resistance genes, disruption of virulence determinants, and precise modulation of the microbiome. The delivery systems such as bacteriophages, nanoparticles, conjugative plasmids, hydrogels, and extracellular vesicles have also been developed to enhance the potential of bringing CRISPR-based therapeutics into the clinic. New innovations like aiRNA optimization of guide RNA, programmable RNA targeting systems, base and prime editing, synthetic biology and personalized precision medicine further broaden the potential applications for antimicrobials that leverage the power of the CRISPR effect. However, a range of issues such as unintended genome editing, efficient delivery into the clinic, escape capabilities in bacteria, biosafety concerns, regulatory approval and scaling up of manufacture are still major hurdles to broader implementation. Collaboration across disciplines, strong clinical validation, and standardized regulatory approaches will be key to successful clinical translation of this solution to overcome these limitations. In summary, the development of antimicrobial therapy using CRISPR is a paradigm shift, from broad-spectrum antimicrobial treatment to precision management of infectious diseases. Moving forward, it is likely that CRISPR-based therapeutics will become a key component of next-generation approaches to tackling AMR and ensuring global public health.

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