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CRISPR/Cas9 Technology in Antimicrobial Resistance and Cardiovascular Therapeutics: Current Advances and Future Perspectives

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Abstract

Background

Antimicrobial resistance (AMR) and cardiovascular diseases (CVDs) represent two major global health challenges associated with high morbidity, mortality, and healthcare costs. The increasing prevalence of multidrug-resistant pathogens and the genetic basis of many cardiovascular disorders have highlighted the need for innovative therapeutic strategies. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) have emerged as powerful genome-editing technologies with broad biomedical applications.

Objective

This review aims to summarize the current advances, mechanisms, and therapeutic applications of CRISPR/Cas9 technology in combating antimicrobial resistance and cardiovascular diseases.

Methods

Relevant literature published in recent years was reviewed to evaluate the role of CRISPR/Cas9 in genome editing, antimicrobial resistance management, cardiovascular disease research, and emerging delivery technologies.

Results

CRISPR/Cas9 enables precise targeting of resistance-conferring genes such as mecA, vanA, blaKPC, and MCR-1, thereby restoring bacterial susceptibility to conventional antibiotics. Studies have demonstrated successful application of CRISPR-based approaches against multidrug-resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). Advances in delivery systems, particularly bacteriophage-mediated and nanoparticle-based platforms, have improved the efficiency of CRISPR-based antimicrobial therapies. In cardiovascular medicine, CRISPR/Cas9 has facilitated disease modeling and correction of mutations associated with familial hypercholesterolemia, cardiomyopathies, arrhythmias, and other inherited cardiac disorders. Emerging technologies such as CRISPR interference, base editing, and prime editing have further expanded therapeutic possibilities. However, challenges including off-target

effects, delivery limitations, immune responses, and ethical concerns remain significant barriers to clinical translation.

Conclusion

CRISPR/Cas9 represents a transformative genome-editing platform with substantial potential for addressing antimicrobial resistance and cardiovascular diseases. Continued advancements in precision editing and delivery technologies are expected to accelerate its integration into future clinical practice and personalized medicine.

Keywords

CRISPR/Cas9, Antimicrobial Resistance, Multidrug-Resistant Bacteria, Methicillin-Resistant Staphylococcus aureus, Genome Editing, Cardiovascular Diseases, Precision Medicine, Gene Therapy, Delivery Systems, Antibiotic Resistance

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges of the 21st century, significantly reducing the effectiveness of existing antimicrobial therapies and increasing morbidity, mortality, and healthcare expenditures worldwide (World Health Organization, 2023). The World Health Organization (WHO) recognizes AMR as a major threat due to the rapid global dissemination of multidrug-resistant microorganisms (WHO, 2023). Current estimates suggest that resistant infections are responsible for millions of deaths annually, and projections indicate that AMR-related mortality could reach nearly 10 million deaths per year by 2050 if effective interventions are not implemented (O'Neill, 2016). The excessive and inappropriate use of antibiotics in human medicine, agriculture, and animal husbandry has accelerated the development and spread of resistant pathogens, rendering many infections increasingly difficult to treat (Ventola, 2015).

Clinically significant multidrug-resistant pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus* species, which are associated with severe infections such as sepsis, pneumonia, meningitis, urinary tract infections, and endocarditis (Murray *et al*., 2022). These organisms employ multiple resistance mechanisms, including genetic mutations, enzymatic antibiotic inactivation, horizontal gene transfer, efflux pump activation, and biofilm formation (Blair *et al*., 2015). Consequently, there is an urgent need for innovative therapeutic strategies capable of selectively eliminating resistant pathogens while preserving beneficial microbiota (Bikard and Barrangou, 2017).

In response to these challenges, advanced therapeutic approaches such as bacteriophage therapy, antimicrobial repurposing, and genome engineering technologies are increasingly being investigated (Kortright *et al*., 2019). Among these, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) have emerged as revolutionary genome-editing systems with extensive biomedical applications (Jinek *et al*., 2012). Originally identified as adaptive immune defense mechanisms in bacteria and archaea against invading genetic elements such as bacteriophages and plasmids, CRISPR-Cas systems have transformed molecular biology because of their high specificity, programmability, and precision (Barrangou *et al*., 2007).

The CRISPR-Cas9 system primarily consists of a Cas9 nuclease and a guide RNA (gRNA) that directs the nuclease to a complementary DNA sequence adjacent to a protospacer adjacent motif (PAM) (Jinek *et al*., 2012). Following target recognition, Cas9 induces double-stranded DNA breaks that are subsequently repaired through non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling targeted insertion, deletion, or correction of genetic material (Doudna and Charpentier, 2014). Owing to its programmable gene-editing capability, CRISPR-Cas9 has become one of the most widely utilized tools in modern biomedical research (Hsu *et al*., 2014). Furthermore, advanced CRISPR-based platforms such as CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), and dead Cas9 (dCas9) have expanded its applications to transcriptional regulation, epigenome editing, and genomic imaging (Qi *et al*., 2013).

Recent studies have demonstrated that CRISPR-Cas systems can selectively target antimicrobial resistance genes including mecA, tetM, ermB, grlA, grlB, and MCR-1, thereby restoring bacterial susceptibility to antibiotics (Bikard *et al*., 2014; Wang *et al*., 2019). Unlike conventional antimicrobial agents, CRISPR-based antimicrobials provide highly selective targeting of pathogenic bacteria while minimizing disruption of normal microbiota (Rodrigues *et al*., 2019). This selective mechanism highlights the significant therapeutic potential of CRISPR technology in combating multidrug-resistant infections (Bikard and Barrangou, 2017).

Beyond antimicrobial applications, CRISPR-Cas9 has also demonstrated promising therapeutic potential in cardiovascular diseases (CVDs), which remain the leading cause of mortality worldwide (Roth *et al*., 2020). Cardiovascular disorders such as stroke, heart failure, hypertension, arrhythmias, and vascular diseases continue to impose a substantial burden on healthcare systems globally (Roth *et al*., 2020). CRISPR-Cas9 technology offers unique advantages in cardiovascular medicine because of its ability to precisely modify nuclear and mitochondrial DNA and regulate gene expression through epigenetic mechanisms (Musunuru, 2017). Compared with earlier genome-editing technologies such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas systems provide superior flexibility, accuracy, efficiency, and ease of design, making them highly valuable for personalized medicine and targeted therapeutics (Komor *et al*., 2017).

Despite its remarkable therapeutic promise, several challenges continue to limit the widespread clinical application of CRISPR-Cas technology. These include delivery inefficiency, off-target effects, immune responses, ethical considerations, regulatory concerns, and long-term safety issues (Fu *et al*., 2013; Cornu *et al*., 2017). Nevertheless, ongoing advancements in CRISPR engineering, delivery systems, and precision-editing technologies continue to enhance its therapeutic potential (Anzalone *et al*., 2020). Therefore, CRISPR-based genome-editing systems are expected to play a transformative role in the future management of antimicrobial resistance, cardiovascular diseases, and other complex human disorders.

Current Advances and Applications of CRISPR/Cas9

CRISPR/Cas9-Mediated Reversal of Antibiotic Resistance in MRSA

According to Ates *et al*., 2024, recent advances in CRISPR/Cas9 genome-editing technology have demonstrated significant potential in reversing antimicrobial resistance among multidrug-resistant bacterial pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA). A study conducted by Ates A., Tastan C., and Ermertcan S. investigated the use of CRISPR/Cas9 systems to selectively target and disrupt multiple antibiotic-resistance genes in clinical MRSA isolates. Clinical *Staphylococcus aureus* isolates obtained from hospital samples were identified using MALDI-TOF MS and VITEK-2 systems, while resistance-associated genes including *mecA*, *grlA*, *grlB*, and *aacA* were detected through polymerase chain reaction (PCR) assays. Antibiotic susceptibility testing confirmed the multidrug-resistant profiles of the isolates.

To eliminate resistance determinants, guide RNAs specific for methicillin, ciprofloxacin, and gentamicin resistance genes were designed and cloned into CRISPR/Cas9 plasmids. These plasmids were amplified in *Escherichia coli* and subsequently introduced into MRSA isolates through electroporation. The CRISPR/Cas9 system induced targeted double-stranded DNA breaks within resistance-associated genes, thereby disrupting their functional activity and impairing resistance mechanisms.

Quantitative PCR analysis demonstrated substantial downregulation of resistance gene expression following CRISPR-mediated editing. Expression of the *mecA* gene was reduced by approximately 1.5-fold, while *grlA*, *grlB*, and *aacA* expression decreased by nearly 5.5-fold, 6-fold, and 4-fold, respectively. Importantly, these molecular alterations were associated with restoration of bacterial susceptibility to beta-lactam, quinolone, and aminoglycoside antibiotics. In addition, Western blot analysis revealed nearly 70% reduction in penicillin-binding protein 2a (PBP2a) expression, which is a critical determinant of methicillin resistance in MRSA. Sanger sequencing further confirmed successful mutations within the targeted resistance genes.

These findings strongly support the potential of CRISPR/Cas9 technology as a precision antimicrobial strategy capable of selectively removing resistance determinants while restoring antibiotic sensitivity in multidrug-resistant bacterial pathogens. Such targeted genome-editing approaches may provide an effective alternative to conventional antibiotics and could play an important role in addressing the growing global burden of antimicrobial resistance.

CRISPR-Cas Systems in the Management of Antimicrobial Resistance

As per the reviews of Ahmed M.M. *et al.*, 2024, antimicrobial resistance (AMR) has emerged as a major global healthcare crisis driven largely by the excessive and inappropriate use of antimicrobial agents in human medicine, agriculture, and animal husbandry. The rapid spread of multidrug-resistant pathogens has significantly increased morbidity, mortality, healthcare expenditure, and treatment failure worldwide. Common resistant organisms include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecium*, many of which exhibit resistance to critical antibiotics such as methicillin, ciprofloxacin, and colistin. These resistant pathogens possess multiple mechanisms of resistance including horizontal gene transfer, efflux pump activation, enzymatic antibiotic degradation, target modification, and biofilm formation.

In recent years, CRISPR-Cas systems have emerged as highly promising tools for combating antimicrobial resistance through precise genome editing. Unlike conventional antibiotics that broadly affect microbial populations, CRISPR-based systems enable highly selective targeting of resistance genes located on bacterial chromosomes or plasmids. CRISPR-Cas9 technology has demonstrated the ability to eliminate resistance-conferring genes such as *MCR-1*, thereby restoring bacterial susceptibility to antibiotics. This targeted approach offers significant therapeutic advantages by selectively eradicating resistant bacteria while preserving the normal microbiota.

Efficient delivery of CRISPR components into bacterial cells remains essential for successful therapeutic application. Several delivery strategies have been investigated, including phage-mediated delivery, plasmid-based systems, and nanoparticle-based delivery platforms. Bacteriophages engineered to carry CRISPR/Cas components have shown considerable success in selectively targeting multidrug-resistant bacteria, whereas nanoparticle systems improve the stability and intracellular transport of CRISPR machinery. These delivery approaches continue to evolve to enhance editing efficiency and bacterial targeting specificity.

Advanced genome-editing approaches such as base editing and prime editing have further expanded the potential applications of CRISPR technology. These newer editing systems provide improved precision with reduced off-target mutations compared to conventional CRISPR/Cas9-mediated double-strand DNA cleavage. Consequently, they offer safer and more efficient alternatives for correcting resistance-associated genetic mutations.

Despite its promising therapeutic potential, several limitations continue to challenge the widespread clinical implementation of CRISPR-based antimicrobial therapies. Major concerns include off-target genetic alterations, delivery inefficiency, variability among bacterial CRISPR systems, biosafety considerations, and ethical issues associated with genome editing. Nevertheless, ongoing advances in CRISPR engineering, delivery technologies, and precision-editing strategies continue to improve the feasibility of CRISPR/Cas systems as next-generation therapeutics against antimicrobial resistance.

Structure and Mechanism of CRISPR-Cas9

Asif *et al*., 2024 and Motta *et al*., 2017 reported that CRISPR-Cas9 is a highly versatile genome-editing technology derived from the adaptive immune defense system of bacteria and archaea. The system primarily consists of two essential components: the Cas9 nuclease and a single-guide RNA (sgRNA). The sgRNA directs the Cas9 enzyme to a complementary target DNA sequence through base pairing, enabling site-specific genome editing. Cas9 functions as a molecular scissor that introduces double-stranded DNA breaks (DSBs) at targeted genomic locations. Structurally, Cas9 contains two major nuclease domains, namely the HNH and RuvC domains, which cleave the complementary and non-complementary DNA strands, respectively. Target recognition additionally requires the presence of a protospacer adjacent motif (PAM), commonly represented as the 5′-NGG-3′ sequence, which ensures specificity and prevents self-targeting within bacterial genomes.

In natural bacterial systems, CRISPR utilizes CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) to guide Cas9 activity. However, for simplified genome editing applications, these components are commonly fused into a single-guide RNA. Delivery of CRISPR-Cas9 components into target cells can be achieved through both viral and non-viral methods, including adenoviral vectors, electroporation, lipid nanoparticles, and microinjection techniques. Following DNA cleavage, endogenous cellular repair pathways repair the induced breaks through either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is an error-prone mechanism that frequently introduces insertions or deletions, making it useful for gene knockout studies, whereas HDR enables precise gene correction or insertion using homologous donor DNA templates.

Continuous optimization of CRISPR-Cas9 technology has focused on improving editing specificity, minimizing off-target effects, and enhancing guide RNA design. Novel CRISPR variants such as Cas12a (Cpf1) have expanded genome-editing capabilities by recognizing alternative PAM sequences and generating staggered DNA cuts. Additional modifications including high-fidelity Cas9 variants, CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), and dead Cas9 (dCas9) systems have further broadened the applications of CRISPR technology to transcriptional regulation, epigenome editing, live-cell genomic imaging, and functional genomics research. These advancements continue to improve the efficiency, versatility, and therapeutic potential of CRISPR-based genome engineering.

CRISPR/Cas9 Applications in Cardiovascular Diseases

According to Bonowicz *et al*., 2025, Motta *et al*., 2017 and Asif *et al*., 2024, CRISPR/Cas9 technology has emerged as a promising therapeutic and research tool in the field of cardiovascular medicine because many cardiovascular diseases possess strong genetic components. The CRISPR-Cas9 system, composed of the Cas9 nuclease and guide RNA, enables precise genome modification through targeted DNA cleavage and subsequent repair by non-homologous end joining (NHEJ) or homology-directed repair (HDR). This programmable editing capability has facilitated extensive investigation into the molecular mechanisms underlying cardiovascular disorders and has opened new opportunities for personalized therapeutic interventions.

Recent studies have demonstrated the application of CRISPR/Cas9 in several inherited and acquired cardiovascular diseases, including congenital heart disease, familial hypercholesterolemia, hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, hypertension, and ischemia-reperfusion injury. In familial hypercholesterolemia, CRISPR-mediated correction of mutations in the *LDLR* gene restored low-density lipoprotein receptor function and significantly reduced cholesterol accumulation and atherosclerotic plaque formation in experimental models. Similarly, editing of *PCSK9* and *APOC3* genes improved lipid metabolism and reduced cardiovascular risk by lowering low-density lipoprotein cholesterol and triglyceride levels.

CRISPR/Cas9 has also demonstrated considerable potential in correcting mutations associated with inherited cardiomyopathies and arrhythmias. Targeting mutations in genes such as *MYH6*, *MYBPC3*, *TTN*, *RBM20*, *RYR2*, and phospholamban (*PLN*) resulted in improved cardiac function, restoration of protein expression, and prevention of disease progression in various animal and cellular models. Furthermore, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have been extensively utilized in combination with CRISPR technology to model disorders such as long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and other inherited cardiac abnormalities. These models have enabled researchers to both introduce and correct disease-causing mutations, thereby improving understanding of disease mechanisms and therapeutic responses.

Beyond nuclear genome editing, CRISPR-based approaches have also been explored for mitochondrial genome modification in cardiovascular diseases associated with mitochondrial dysfunction. Correction of mitochondrial DNA mutations has demonstrated restoration of mitophagy and reduction of inflammatory responses linked to atherosclerosis progression. However, efficient delivery of guide RNA into mitochondria remains a major technical challenge.

Despite its significant therapeutic promise, several obstacles continue to limit the clinical application of CRISPR/Cas9 in cardiovascular medicine. Important concerns include off-target genomic alterations, low efficiency of homology-directed repair, immune responses against Cas proteins, genomic instability, delivery limitations, and ethical considerations related to human genome editing. To overcome these challenges, researchers are actively developing high-fidelity Cas9 variants, improved delivery systems, immune-orthogonal proteins, and advanced editing approaches such as base editing and prime editing. Continued advancements in these areas are expected to accelerate the translation of CRISPR/Cas9 technology into clinical practice for the treatment of cardiovascular diseases.

CRISPR/Cas9 as a Novel Strategy Against Antibiotic Resistance

Moitra *et al*., 2024 review that the rapid emergence of multidrug-resistant bacteria has intensified the global search for innovative antimicrobial strategies capable of overcoming conventional antibiotic resistance mechanisms. Among the most problematic pathogens are the ESKAPE organisms, which include *Enterococcus* species, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These pathogens possess multiple resistance mechanisms such as efflux pump activation, antibiotic inactivation, biofilm formation, target modification, altered membrane permeability, and horizontal gene transfer, enabling them to survive exposure to several classes of antimicrobial agents. Important resistance-associated genes identified in these organisms include *mecA*, *vanA*, *blaKPC*, *gyrA*, and *parC*, which contribute significantly to resistance against beta-lactams, glycopeptides, quinolones, and carbapenems.

CRISPR/Cas9 technology has emerged as a highly promising approach for selectively targeting antibiotic-resistance determinants in bacterial pathogens. Unlike traditional antibiotics, which often indiscriminately affect both pathogenic and beneficial microbiota, CRISPR-based antimicrobials enable highly specific targeting of resistance genes or essential bacterial genes. By inducing site-specific double-stranded DNA breaks within resistance-conferring genetic elements, CRISPR/Cas9 can either eliminate resistant bacterial populations or restore susceptibility to conventional antibiotics. This selective mechanism significantly reduces the likelihood of widespread microbiota disruption and may help limit the further spread of antimicrobial resistance.

Recent investigations have demonstrated the successful application of CRISPR/Cas9 systems against several clinically significant resistance genes. Targeted editing of genes such as *mecA*, *vanA*, and *blaKPC* has resulted in restoration of antibiotic susceptibility in multidrug-resistant bacterial strains. In addition, CRISPR-based strategies have shown potential in eliminating plasmids carrying resistance genes, thereby reducing horizontal transmission of resistance determinants among bacterial populations. The ability of CRISPR/Cas9 to selectively eradicate resistant bacteria while preserving susceptible strains highlights its considerable therapeutic value in antimicrobial resistance management.

Despite these promising findings, several challenges continue to limit the widespread clinical implementation of CRISPR-based antimicrobial therapies. Efficient delivery of CRISPR components into bacterial cells remains a major obstacle, particularly in complex infection environments. Additional concerns include off-target genetic modifications, bacterial escape mechanisms, immune responses, and biosafety considerations. Consequently, ongoing research is focused on improving delivery platforms, enhancing targeting specificity, reducing off-target effects, and establishing clinical safety profiles for future therapeutic applications.

Overall, CRISPR/Cas9 represents a powerful and innovative genome-editing platform with substantial potential to combat antimicrobial resistance. Continued advancements in CRISPR engineering and delivery technologies may ultimately facilitate the development of highly targeted next-generation antimicrobial therapies capable of addressing the growing global burden of multidrug-resistant infections.

Delivery Systems for CRISPR/Cas-Based Antimicrobial Therapy

As Carlos de la Fuente Tagarro *et al*., in 2024 said that the efficient delivery of CRISPR/Cas components into target bacterial cells is one of the most critical factors determining the therapeutic success of CRISPR-based antimicrobial strategies. Since bacterial cell walls and membranes act as natural barriers to foreign genetic material, multiple delivery systems have been developed to improve the transport, stability, and intracellular activity of CRISPR machinery. Among these, bacteriophage-mediated delivery and nanoparticle-based systems have emerged as the most promising approaches for antimicrobial resistance management.

Bacteriophages, or bacterial viruses, have been extensively engineered to function as delivery vehicles for CRISPR/Cas systems. These engineered phages can selectively infect pathogenic bacteria and introduce CRISPR-associated nucleases targeting antibiotic-resistance genes located on plasmids or bacterial chromosomes. Several studies have demonstrated successful phage-mediated elimination of resistance-conferring plasmids in multidrug-resistant *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. In some investigations, modified bacteriophages carrying anti-CRISPR proteins were also developed to overcome bacterial defense mechanisms and improve gene-editing efficiency. Despite these advantages, bacteriophage-based delivery systems face several limitations, including narrow host specificity, immunogenicity, potential bacterial resistance to phages, and regulatory challenges associated with clinical application.

Nanoparticle-based delivery systems provide an alternative non-viral strategy for transporting CRISPR/Cas components into bacterial cells. Various nanoparticle platforms, including liposomes, polymeric nanoparticles, lipid nanoparticles, and carbon quantum dots, have been investigated to improve the stability, protection, and intracellular delivery of CRISPR machinery. These systems have successfully targeted resistance-associated genes such as *papG* and *mecA*, resulting in restoration of antibiotic susceptibility in resistant bacterial strains. Compared with viral vectors, nanoparticle systems offer improved biocompatibility, reduced immunogenicity, and greater flexibility in cargo packaging. However, challenges such as toxicity, delivery efficiency, and large-scale manufacturing remain important considerations.

In addition to antimicrobial applications, optimized delivery platforms have also contributed significantly to antibiotic discovery and industrial antibiotic production. CRISPR/Cas systems have been widely utilized in *Streptomyces* species to activate silent biosynthetic gene clusters responsible for secondary metabolite production, leading to the discovery of novel antimicrobial compounds such as auroramycin. Gene-editing approaches have further enhanced the production of clinically important antibiotics including erythromycin, vancomycin, oxytetracycline, fengycin, penicillin, and cephalosporin-C through modification of regulatory genes and biosynthetic pathways.

Furthermore, advanced CRISPR-based platforms such as CAPTURE have enabled the identification and expression of previously uncharacterized biosynthetic gene clusters, facilitating the discovery of new antimicrobial compounds active against multidrug-resistant pathogens. These findings highlight the broad therapeutic and biotechnological potential of CRISPR/Cas systems not only in combating antimicrobial resistance but also in expanding antibiotic discovery and production.

Although significant progress has been achieved in CRISPR delivery technologies, several challenges still limit their widespread clinical implementation. Delivery efficiency, immune responses, biosafety concerns, host specificity, and regulatory issues remain major obstacles requiring further investigation. Nevertheless, continuous advancements in delivery strategies and genome-editing technologies are expected to accelerate the future clinical translation of CRISPR/Cas-based antimicrobial therapies.

Challenges and Limitations of CRISPR/Cas9 Technology

Despite the remarkable progress and therapeutic potential of CRISPR/Cas9 genome-editing systems, several important limitations continue to restrict their widespread clinical application. One of the primary concerns associated with CRISPR-based genome editing is the occurrence of off-target effects, in which unintended genomic regions are accidentally modified. Such unintended alterations may lead to genomic instability, abnormal gene expression, cellular dysfunction, or potential oncogenic transformations. Although high-fidelity Cas9 variants such as SpCas9-HF1 and engineered guide RNAs have been developed to improve targeting specificity, complete elimination of off-target editing remains a major scientific challenge (Ahmed *et al*., 2024).

Another significant obstacle involves the efficient delivery of CRISPR/Cas components into target tissues and cells. Viral vectors such as adenoviruses and adeno-associated viruses have demonstrated high transfection efficiency; however, they may induce immunogenic responses, insertional mutagenesis, and limitations related to cargo capacity. Non-viral delivery systems including lipid nanoparticles, electroporation, polymers, and microinjection techniques offer safer alternatives but often demonstrate lower delivery efficiency and reduced tissue specificity. In cardiovascular and antimicrobial applications, achieving targeted delivery to infected tissues or cardiac cells remains particularly challenging (Asif *et al*., 2024).

Immune responses against Cas proteins also represent a critical limitation for therapeutic implementation. Since Cas nucleases are primarily derived from bacterial species, preexisting immunity against these proteins may trigger inflammatory reactions and reduce therapeutic efficacy in humans. Additionally, repeated administration of CRISPR-based therapies may further increase the risk of immune-mediated complications. Researchers are therefore investigating immune-orthogonal Cas proteins, transient delivery approaches, and improved biomaterials to minimize immunogenicity (Moitra *et al*., 2024).

Ethical and regulatory concerns further complicate the clinical translation of CRISPR technology. Genome editing involving germline cells raises significant ethical issues because heritable genetic modifications may permanently alter future generations. Concerns regarding unintended genetic consequences, misuse of gene-editing technologies, and unequal accessibility to advanced therapies have prompted strict international regulatory oversight. Consequently, extensive preclinical validation, biosafety assessment, and ethical evaluation are essential before routine clinical implementation can be achieved (Motta *et al*., 2017).

Additional limitations include low efficiency of homology-directed repair (HDR), variability in editing outcomes among different cell types, bacterial escape mechanisms in antimicrobial applications, and technical challenges associated with mitochondrial genome editing. Moreover, long-term safety data for CRISPR-based therapies remain limited, necessitating continued investigation into potential delayed adverse effects and genomic stability following treatment (Klaudia Bonowicz *et al*., 2025).

Despite these challenges, continuous advancements in genome-editing precision, delivery technologies, guide RNA optimization, and novel CRISPR variants such as base editors and prime editors continue to improve the safety and effectiveness of CRISPR/Cas systems. Ongoing multidisciplinary research integrating molecular biology, nanotechnology, bioengineering, and clinical medicine is expected to address many of the current limitations and facilitate the successful translation of CRISPR-based therapies into clinical practice (Carlos de la Fuente Tagarro *et al*., 2024).

Conclusion

CRISPR/Cas9 technology has emerged as a revolutionary genome-editing tool with significant applications in both antimicrobial resistance management and cardiovascular medicine. Its ability to precisely target resistance-associated genes offers a promising alternative to conventional antimicrobial therapies and may help address the growing global burden of multidrug-resistant infections. In cardiovascular research, CRISPR/Cas9 has facilitated disease modeling, functional genomic studies, and the correction of pathogenic mutations associated with inherited cardiac disorders. Advances in delivery systems, including bacteriophage- and nanoparticle-based platforms, have further enhanced the therapeutic potential of CRISPR-based interventions. However, challenges such as off-target effects, delivery inefficiency, immune responses, and ethical concerns continue to limit widespread clinical application. Ongoing developments in high-fidelity nucleases, base editing, and prime editing are expected to improve safety and precision. Overall, CRISPR/Cas9 represents a powerful platform with the potential to transform future approaches to infectious diseases, cardiovascular disorders, and precision medicine.

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