CRISPR-Cas Systems in the Detection and Treatment of Infectious Diseases: A Systematic Review

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ABSTRACT

The current systematic review aims to explore the clustered regularly interspaced short palindromic repeats (CRISPR) system's applications in detecting and treating infectious diseases. It was originally found as a part of bacterial adaptive immunity, where the system has been repurposed into powerful tools for precise genome editing. The review also included an explanation of the evolution and classification of the CRISPR-associated proteins (Cas) mechanism, and it highlights the increasing role in infectious disease diagnosis through Specific High-sensitivity Enzymatic Reporter unLOCKing, FELUDA, and DNA Endonuclease Targeted CRISPR Trans Reporter platforms. These tools are found with high specificity and a rapid detection ability, as assessed during the COVID-19 pandemic. CRISPR is assessed therapeutically for its effectiveness in the gene editing of viral genomes such as hepatitis B virus and HIV, and for its application in bacteriophage-based delivery to combat antibiotic resistance. Furthermore, preclinical and clinical research has highlighted promising outcomes, though not without challenges, such as off-target effects, immune responses, and limitations related to delivery. However, ethical concerns and dual-use research of concerns were addressed by focusing on regulatory importance. CRISPR-Cas systems were shown to hold significant potential for transforming infectious disease management, as long as scientific, ethical, and regulatory considerations are addressed.

Keywords: CRISPR-cas, SHERLOCK, DETECTR, Antibiotic resistance, Gene editing

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Introduction

Background

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) developed as revolutionary tools in microbiology that were also discovered in the form of adaptive immune mechanisms in archaea and bacteria.[1–3] The tool has the ability to target and modify particular sequences of nucleic acids, which has positioned the technique among leading diagnostic and gene editing innovations.[2,4] The field has expanded over the last 10 years beyond genome editing to detect and treat infectious diseases.[5] Such evolution is particularly timely, and given the global increase in antimicrobial resistance, like for the emerging viral pandemics of COVID-19, and increasing demand for sensitive, rapid, and accessible diagnostic platforms. Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) are CRISPR-based systems that have an exceptional potential for point-of-care diagnostics.[6] However, therapeutic approaches using CRISPR for editing viral genomes or modulating microbial gene expression are gaining traction in preclinical and clinical research.

Research Significance

The research outcomes of the current systematic review will indicate the need to improve infectious disease management through scalable, innovative, and precise technologies. CRISPR-Cas systems offer unparalleled adaptability and specificity, making them promising tools for therapeutic and diagnostic measures. Recurrent outbreaks, diagnostic delays, and antibiotic resistance have increased pressure on the global health system. Understanding the full scope of CRISPR's capabilities can help manage this pressure and inform future strategies in public health, biotechnology, and medical research. Furthermore, the study focuses on addressing the ethical and technical challenges of CRISPR use, which is important for the responsible implementation of tools within the health care system. The study implemented the TITAN guidelines, which indicate that the researcher has not used any AI tool to complete the current systematic review.[7]

Aim and Objectives

The current systematic review of literature aims to provide an inclusive overview of the CRISPR-Cas systems application to detect and treat infectious diseases.

The research objectives are:

1. To provide an overview of CRISPR-Cas biology, classification, and use of CRISPR-based diagnostic platforms, including SHERLOCK and DETECTR
2. To analyze CRISPR's potential as an antimicrobial and antiviral tool
3. To determine preclinical and clinical progress of CRISPR therapeutics in infectious disease contexts
4. To elaborate on technical limitations, ethical concerns, and regulatory challenges

Methods

The current systematic review followed PRISMA 2020 guidelines to ensure transparency and reproducibility (Figure 1). Literature was searched across PubMed, NCBI, Scopus, MDPI, Elsevier, Web of Science, and Google Scholar from January 2015 to May 2025. Search terms included *"CRISPR-Cas," "infectious diseases," "CRISPR diagnostics," "SHERLOCK," "DETECTR," "CRISPR therapy,"* and *"antibiotic resistance."* Boolean operators AND/OR were used for query refinement. The study-selection process involved title and abstract screening followed by full-text review by

two independent reviewers. A PRISMA flow diagram illustrates article selection. Inclusion criteria included peer-reviewed original research on CRISPR diagnostics/therapeutics in infectious diseases. Exclusion criteria included non-English articles, reviews, editorials, and studies unrelated to CRISPR in infectious disease. Risk of bias was assessed using the Cochrane ROB tool for interventional studies and the NIH Quality Assessment Tool for observational designs. Figure 1 presents studies conducted and accessed from various databases.

Evolution and Functions of CRISPR-Cas

Growing Role in Infectious Disease Management

The CRISPR-Cas system was first identified in 1987 in *Escherichia coli* as a series of unusual DNA repeats.[8] However, its function remained unknown until the early 2000s, when it was found to be part of a prokaryotic immune system that defends against viral infections and plasmids.[9] Furthermore, CRISPR loci integrate snippets of viral DNA within the host genome in the form of "spacers," which enable the organisms to destroy and recognize analogous genetic elements in the future.[10] Cas (CRISPR-associated) proteins discovery, particularly Cas9, led to further development of programmable endonucleases that are also able to edit DNA precisely.[8] Moreover, CRISPR systems' evolution is also categorized into two main classes and various types (Type I–IV), and each class has distinct proteins and mechanisms.[11] Their adaptability and precision have since propelled CRISPR-Cas technologies into broader applications beyond microbial immunity, including genome editing, gene regulation, diagnostics, and therapeutic research for various human diseases.[9,10]

CRISPR in Pathogen Detection

The basic biological function of the CRISPR-Cas systems is the provision of adaptive immunity to archaea and bacteria against mobile genetic elements and invading viruses.[12,13] Moreover, after the survival of a microorganism from a viral attack, it participates in incorporating DNA fragments into its genome in the CRISPR array. This acts as a molecular memory, and after subsequent infection, the array is transcribed by the system into RNA, which is then applied as a guide for the direct Cas proteins to match sequences in invading DNA. It further allows neutralization and cleavage. Research indicates that CRISPR systems regulate gene expression and facilitate DNA repair.[14] These functions in biotechnology are adapted for gene editing, in which CRISPR-Cas9 is used to introduce the correction or targeted mutations in the genome. This capability of manipulating genetic material with unprecedented precision has opened novel avenues to treat genetic disorders, create transgenic organisms, and develop molecular diagnostic tools, significantly expanding the functional prospects of CRISPR technologies.[15,16]

SHERLOCK, DETECTR, FELUDA Platforms

The CRISPR-based diagnostic platforms, including DETECTR, SHERLOCK, and FELUDA, are developed for pathogen detection through accurate, rapid, and low-cost testing processes.[17,18] Furthermore, SHERLOCK applies Cas13 for RNA virus detection, which makes it highly suitable for diseases such as hepatitis B virus (HBV), as presented in Figure 2.[19] On the other hand, DETECTR implements Cas12 to target DNA, and it offers quick results for viruses such as HPV. Moreover, FELUDA was developed in India by using the dead Cas9 enzyme for precise detection through paper strip formats.[17] These platforms are useful due to the point-of-care diagnostics scalability, particularly in resource-limited settings, and represent a major lead in molecular testing innovations.

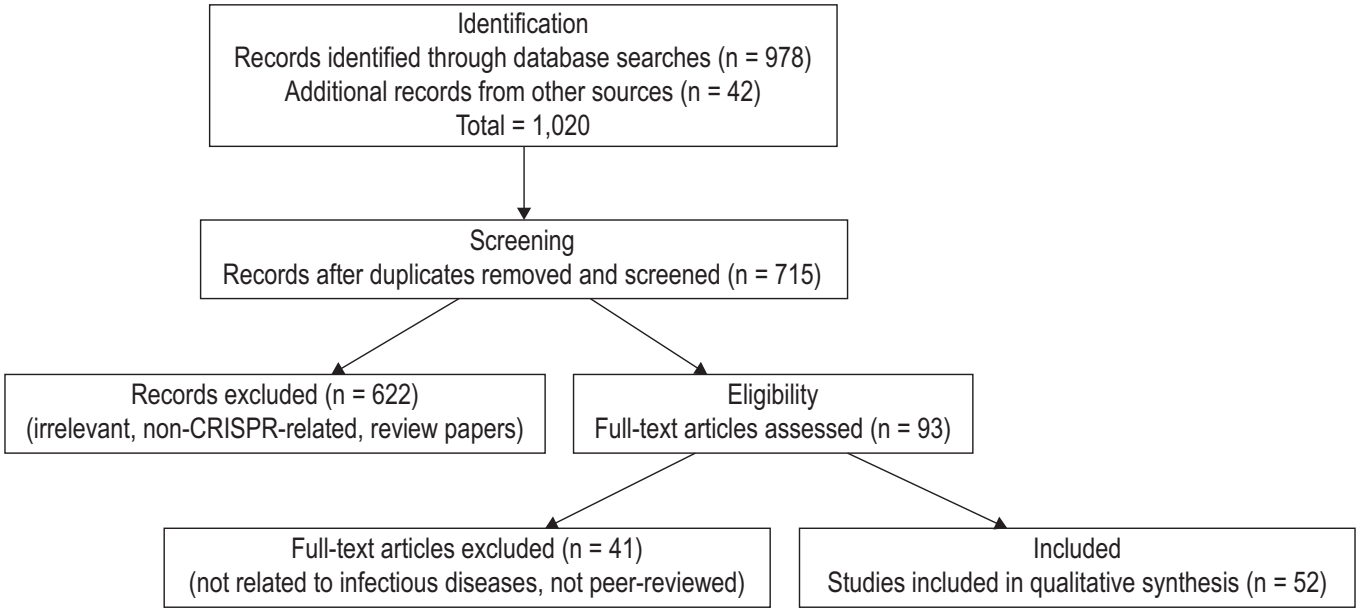

Fig 1 | PRISMA flow chart

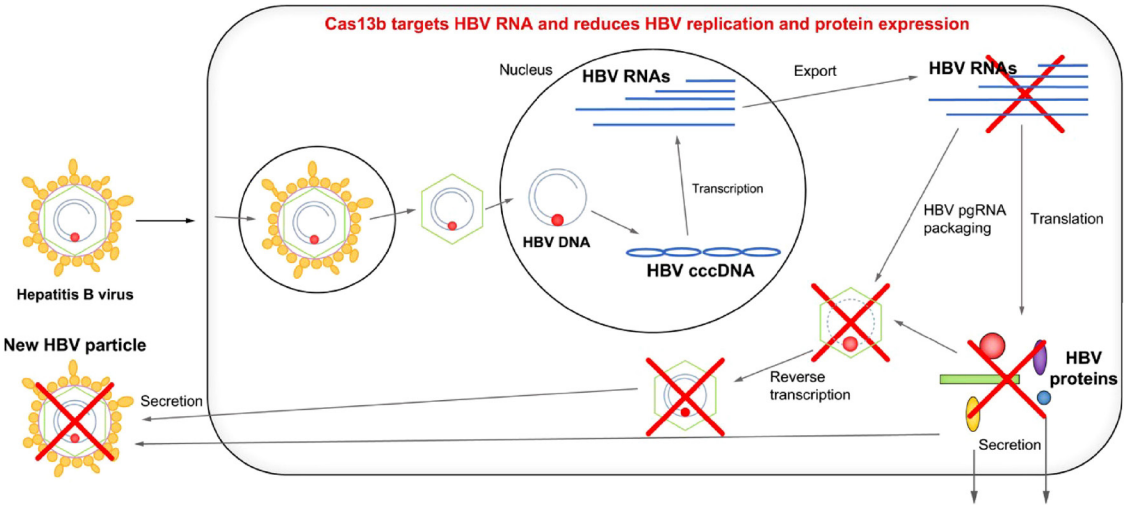

Fig 2 | CRISPR-Cas13b-mediated suppression of HBV replication[19]

Point-of-Care Diagnostics and Sensitivity

CRISPR as a Therapeutic Tool

CRISPR-Cas systems are showing immense potential as therapeutic tools to combat infectious diseases by targeting and modifying pathogen genomes with higher precision levels.[6] In connection with the programmable Cas enzyme nature, researchers can disrupt essential genes in the bacteria and viruses that inhibit the capability to replicate or cause infection.[9] Unlike traditional antimicrobials, CRISPR therapies can be designed to target specific strains, reducing the risk of off-target effects on beneficial microbiota.[20] This specificity is particularly valuable in treating antibiotic-resistant bacterial infections, where conventional treatments often fail.

In viral infections, CRISPR can excise viral genomes integrated into host DNA, offering potential cures for chronic diseases such as HIV and hepatitis B.[21] Therapeutic applications are also being explored for emerging threats such as SARS-CoV-2. Despite challenges in delivery and immune response, advances in vector systems, such as lipid nanoparticles and viral vectors, are improving the feasibility of in vivo applications.[22] Overall, CRISPR offers a transformative platform for precision infectious disease therapy.

Gene Editing of Viral Genomes (HIV, HBV)

The rise of antibiotic resistance has prompted the exploration of alternative therapies, and CRISPR-based bacteriophage delivery systems are among the most promising innovations.[23] Engineered bacteriophages and viruses that infect bacteria can deliver CRISPR-Cas constructs directly into resistant bacterial cells, enabling targeted genome editing to kill or resensitize pathogens to antibiotics.[24] Unlike broad-spectrum antibiotics, this approach allows precise targeting of resistance genes such as *bla*, *mecA*, or *vanA*, thereby sparing beneficial bacteria and reducing selective pressure for resistance.[25] Early studies have shown success in restoring antibiotic susceptibility in *E. coli* and *Staphylococcus aureus*.

Bacteriophage vectors also offer a natural delivery mechanism, bypassing some of the toxicity and uptake issues associated with synthetic vectors.[26] However, challenges remain in ensuring phage-host specificity, immune responses, and regulatory approval. As phage therapy regains interest in the postantibiotic era, CRISPR-phage strategies represent a hybrid solution that could reshape the management of multidrug-resistant infections.[24]

Bacteriophage Delivery for Antibiotic Resistance

Gene editing with CRISPR offers a powerful approach to disrupt and deactivate persistent viral infections such as HIV and HBV.[26,27] Furthermore, in the case of HIV, the viruses penetrate the host genome and forms latent reservoirs that can evade therapies. Moreover, CRISPR-Cas9 is able to target and excise the segment of the HIV proviral DNA that has the potential to eradicate the infection at its source.[28,29] In the preclinical models, successful removal of the HIV-1 genome is demonstrated from the infected cells, and the application of CRISPR further sparks hope for a functional cure.[29]

In addition, in HBV, CRISPR is able to disrupt covalently closed circular DNA (cccDNA), and it is central to the chronic persistence of the virus.[30] It led to long-term clearance or suppression of the virus. With greater potential, these applications also face challenges such as efficiency of delivery, off-target editing, and viral escape mutations. However, RNA design and delivery vehicles are rapidly addressing such concerns. Furthermore, CRISPR-based genome editing can also become a foundation in its fight against chronic viral diseases. Table 1 presents a comparison of assay sensitivities and specificities versus legacy tests and turnaround time.

Targeting Latent Reservoirs

The greatest hurdle in reducing chronic infections such as HIV and tuberculosis is the latent reservoirs that harbor dormant pathogens evading immune detection

and treatment.[28] Another aspect is CRISPR technology, which offers a novel strategy for finding and eliminating hidden reservoirs. In the HIV context, CRISPR-Cas9 was used to edit integrated viral DNA from the previously infected cells, potentially eliminating the virus completely.[29] On the other hand, in the case of tuberculosis, CRISPR can be engineered to disrupt bacterial genes that are important for survival or latency under stress conditions. It also reactivates the bacteria, translates them, and exposes them to antibiotics.

Moreover, CRISPR can be employed for the modification of host genes by involving the latency maintenance that makes the host environment least conducive toward persistent infections.[30] However, in the experimental phase, targeting latent reservoirs with CRISPR can transform the treatment paradigm from viral suppression to true eradication. The approach also offers hope to achieve a cure for the disease that has also been challenged for complete elimination (Table 1).

Critical Appraisal of Delivery Innovations Since 2023

Since 2023, delivery innovations in CRISPR therapeutics have advanced significantly, aiming to overcome immune barriers, tissue specificity, and scalability constraints. Traditional adeno-associated virus vectors, while efficient, pose risks such as immunogenicity, limited cargo capacity, and long-term genomic integration. In contrast, LNPs offer nonviral, transient delivery with improved biocompatibility. Recent LNP formulations have demonstrated enhanced delivery of mRNA-encoded Cas9/Cas12a along with guide RNAs to hepatocytes and pulmonary tissues.[34] These systems are now being tested for infectious diseases such as HBV and SARS-CoV-2, enabling in vivo gene editing without persistent vector presence.

CasMINI is a compact Cas12f-based editing enzyme, roughly half the size of Cas9, making it ideal for delivery via LNPs or even extracellular vesicles. Despite its reduced size, CasMINI retains editing activity and has been engineered for enhanced stability and targeting accuracy. Its compact nature allows multiplexing and delivery to hard-to-reach tissues, a significant advantage for targeting viral reservoirs or latent infections.

Preclinical and Clinical Progress

The translation of CRISPR-based technologies from the laboratory to the clinic has also gained significant

Table 1 | Comparative table, assay sensitivities/specificities vs. Legacy tests

Assay/Test	Target Pathogen	Sensitivity	Specificity	Turnaround Time	Comparison to Legacy Test	References
SHERLOCK (Cas13)	SARS-CoV-2 (RNA virus)	~96%	~100%	<1 hour	Comparable to RT-PCR, faster and portable	31,32
DETECTR (Cas12a)	HPV, SARS-CoV-2 (DNA/RNA)	~95%	~98%	~40 minutes	Faster than RT-PCR; suitable for point-of-care	33
FELUDA (FnCas9)	SARS-CoV-2 (RNA virus)	~85–90%	~98%	~60 minutes	Slightly less sensitive than RT-PCR but highly specific	34
RT-PCR (Legacy)	Broad (RNA/DNA targets)	~95–98%	~98–99%	3–6 hours	Gold standard: requires lab infrastructure	35

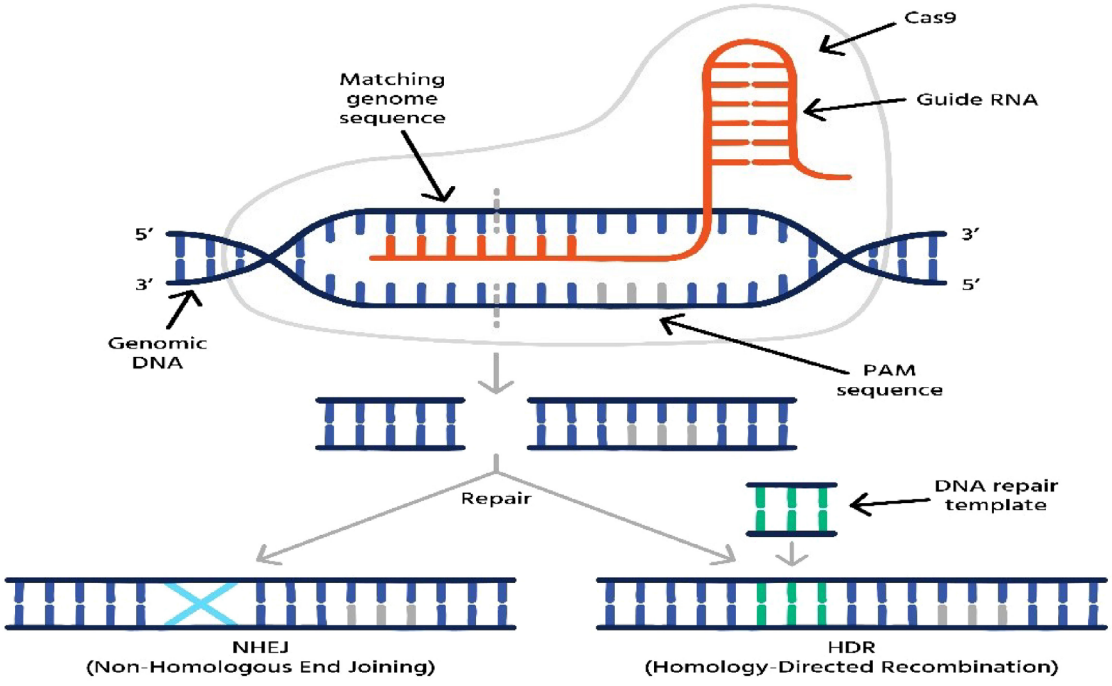

Fig 3 | Schematic overview of CRISPR-Cas9 gene editing

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potential, specifically in infectious disease treatment.[36] CRISPR has demonstrated efficacy in targeting a wide range of pathogens, including latent viruses, antibiotic-resistant bacteria, and newly emerging infectious agents.[37] Furthermore, in the mouse models, CRISPR-Cas9 also successfully employed HIV proviral DNA that disrupts the cccDNA and resensitizes drug-resistant bacteria toward antibiotics.[29] Additionally, animal studies using CRISPR-loaded nanoparticles and bacteriophages have shown improved specificity and delivery to infected tissues with minimal toxicity. Figure 3 presents how CRISPR works based on target identification, DNA cutting, and gene modification.

Clinical translation is underway, although more cautiously. One of the most notable advances includes the use of CRISPR in ex vivo editing of T cells for HIV therapy, currently in early-phase clinical trials.[36] While most CRISPR applications in clinical settings have focused on genetic diseases, recent trials are exploring its use in viral infections such as HPV and herpesviruses. For instance, the DETECTR system has been clinically validated for rapid COVID-19 detection, with emergency use authorization granted during the pandemic.[19] However, clinical use of therapeutic CRISPR in infectious diseases is still in its infancy due to concerns about off-target effects, delivery challenges, and immune responses.

Nonetheless, the rapid evolution of CRISPR tools, such as high-fidelity Cas variants and tissue-specific delivery methods, is paving the way for safer and more effective treatments.[38] Ongoing trials and regulatory frameworks will determine how quickly CRISPR moves into routine clinical use, but current preclinical and clinical progress underscores its transformative potential in combating infectious diseases.

Case Studies and Trial Updates

Applications in COVID-19 Detection Through DETECTR

During the COVID-19 pandemic, the CRISPR-based DETECTR platform was developed for rapid SARS-CoV-2 detection. DETECTR combined RT-LAMP and Cas12 to detect viral RNA with high specificity in under 40 minutes.[39] The platform demonstrated a sensitivity of 95% and specificity of 100% compared to RT-PCR, making it a valuable point-of-care diagnostic tool.[40,41] The U.S. FDA has granted it Emergency Use Authorization and made the first CRISPR diagnostics that were clinically applied for the first time. The highlights show the adoption rate of CRISPR for addressing global health crises with the help of decentralized and accessible testing.

CRISPR-Phage Therapy for Antibiotic-Resistant *E. coli*

Research conducted in 2024 has demonstrated the engineered bacteriophages for delivering the CRISPR-Cas3 systems that target the resistance genes in the multidrug-resistant *E. coli*.[42] The phages disrupted the blaNDM-1 gene that was responsible for carbapenem resistance. In vitro and mouse infection models have indicated a widespread control of the bacterial load without impacting commensal bacteria.[43] Such a targeted approach has eliminated the resistant strains but also minimized the chances of resistance transfer. Furthermore, the feasibility of CRISPR-phage therapy is assessed as a precision antimicrobial strategy and gives rise to further clinical trials of other antibiotics.

Technical and Ethical Challenges

Off-Target Effects, Immune Responses

The risk of off-target effects is found to be the most technical challenge of CRISPR-Cas application for infectious disease treatment.[44] Such intended genetic modifications occur when the Cas enzyme cuts the sequence of similar DNA that is not identical to the intended target. This off-target editing can result in mutations within essential host genes. It further causes harmful effects such as oncogenesis or dysfunction of the immune system. Further advanced techniques such as high-fidelity Cas9 variants such as eSpCas9 and SpCas9-HF1 have been developed for reducing the risks, but their complete elimination is a challenge.[45] The guide RNA design also needs to be optimized to improve efficacy and specificity.

Another important concern is the host's immune response to CRISPR components, particularly Cas proteins that are developed from bacteria such as *Streptococcus pyogenes*.[46] In this case, preexisting immunity can be triggered for inflammatory responses or minimize the effectiveness of the therapeutic interventions. It is also assessed that many people already have T cells or antibodies that are reactive to the commonly applied Cas enzymes.[47] This also shows the barriers to in vivo applications and increases the concerns about repeat doses and safety. Moreover, alternatives such as Cas proteins from the least common bacterial species and humanized delivery vectors are also explored.[28,40] The management of these risks is important to advance CRISPR therapies for clinical viability, such as in systemic infectious disease treatments.

Delivery Systems and Specificity

Precise and efficient delivery of the CRISPR components to infected target cells remains a basic challenge, specifically in the infectious disease treatment context.[44] The therapeutic success of CRISPR is also dependent on its capability to reach the correct cells, tissues, or organs without off-target distribution of degradation. Delivery systems include viral vectors (adeno-associated viruses), lipid nanoparticles, electroporation, and engineered bacteriophages.[47] While viral vectors offer high efficiency, they may induce immune responses and have packaging size limitations. In contrast, nonviral methods such as lipid nanoparticles are the least immunogenic but usually exhibit lower rates of transfection in tissues.

Another challenge is specificity, as in infectious diseases, pathogens can reside in various tissue compartments or in the latent forms that make the targeted delivery complicated. In addition, the interaction between host and pathogens can impact CRISPR access for infected cells. For instance, viral infections such as HIV and latent reservoirs are not easy to manage, and these are difficult to reach because of deep integration

within host DNA. Improvement of both specificity and delivery is important to minimize off-target effects and increase therapeutic benefits.

Bioethics and Dual-Use Research of Concerns (DURC)

CRISPR-Cas technologies' advancement raises critical bioethical and dual-use research of concerns, particularly in the infectious disease treatment context. A primary issue is the potential misuse of CRISPR to develop biological weapons or increase pathogenic virulence, which falls under the category of DURC. These possibilities indicate the need for strict oversight and international regulatory frameworks for the prevention of bioterrorism and ensuring the safety of scientific conduct.

Furthermore, ethically, the capability of editing the microbial genomes or human host factors is also related to an increased rate of infections. This directly raises concerns regarding unintended ecological consequences, concerns, and equity of access that specifically lower the setting where CRISPR-based treatments and diagnostics cannot afford availability.[5,11,28] In addition, genetic discrimination or misuse of the gene editing data can be carefully considered. Public engagement, ensuring transparency, and ethical governance are important as CRISPR is useful for clinical and global health applications.

Future Directions

CRISPR-Cas system's future in infectious disease management is based on its precision, integration, and accessibility with advanced technologies. A promising direction is the development of smart diagnostic technologies that are real-time devices and portable that apply CRISPR for pathogen detection at the point-of-care with a few pieces of equipment. These tools could revolutionize disease surveillance and outbreak response, especially in remote or resource-limited regions. Integrating CRISPR with nanotechnology or AI further expands its capabilities. Nanoparticles can enhance delivery and sensitivity, while AI can optimize guide RNA design, predict off-target effects, and interpret complex diagnostic data with greater speed and accuracy. Robust regulatory framework research is required to assess the widespread use of CRISPR, ensuring safety, ethical usage, and efficacy. Future research can also observe the equitable access to CRISPR-based tools, which is a challenge without deliberate policies for promoting affordability and distribution.

Conclusion

A rapid transformation is observed in the adoption of the CRISPR systems due to their power to fight against infectious diseases. It directly offers unprecedented precision in both therapeutics and diagnostics. From viral RNA detection through SHERLOCK and DETECTR to target genome editing for diseases such as HIV and HBV, these technologies are reshaping global health interventions. However, preclinical and early clinical research has also indicated the potential results of CRISPR that indicate how it overcomes the limitations of traditional treatments and diagnostic methods. It focuses on the potential in the era of antibiotic resistance and emerging pandemics. With its greater potential in health care, challenges persist related to off-target effects, delivery limitations, and immune responses. The ethical aspects are related to equitable and timely delivery. Furthermore, CRISPR tools are moved to clinical and public health implementation, and a robust regulatory framework and international collaboration become important. Furthermore, nanotechnology integration, AI usage, and smart diagnostic systems can amplify CRISPR's effectiveness and reach. However, advanced CRISPR technologies are guided by scientific and ethical stewardship.

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