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Microbiome editing in infectious disease prevention and therapy: CRISPR Applications in Host-Microbe Interactions

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Abstract

The advent of CRISPR-Cas systems has redefined the frontiers of microbiome engineering, offering unprecedented precision in modulating host-microbe interactions for the prevention and treatment of infectious diseases. This article explores the revolutionary potential of microbiome editing, focusing on the deployment of CRISPR-based technologies to restore microbial balance, suppress pathogenicity, and enhance host immunity. It traces the limitations of traditional microbiome modulation methods—such as probiotics, prebiotics, and fecal microbiota transplantation—and contrasts them with the unparalleled specificity and adaptability of CRISPR-guided interventions. Key applications include the in situ editing of commensal and pathogenic bacteria, the development of programmable antimicrobials targeting antibiotic resistance, and the engineering of probiotics to deliver targeted therapeutic payloads. Through case studies involving *Clostridioides difficile* and *Salmonella* spp., the article demonstrates real-world feasibility and therapeutic promise. Additionally, it addresses the systemic influence of the gut microbiota on distant organ systems via the gut-lung, gut-brain, and gut-immune axes, underscoring the relevance of microbiome-targeted therapies in conditions such as sepsis, respiratory infections, and HIV. The paper critically evaluates the delivery mechanisms of CRISPR constructs—spanning phage vectors, conjugative plasmids, and nanoparticles—while navigating the ethical, ecological, and regulatory landscapes that frame this emerging field. By integrating recent scientific advances with translational insights, this review establishes microbiome editing not merely as a futuristic concept, but as a transformative strategy poised to redefine the clinical management of infectious and systemic diseases.

Keywords: CRISPR-Cas; Microbiome Editing; Antimicrobial Resistance; Engineered Probiotics; Pathogen Targeting; Gene Therapy; Gut Dysbiosis; Programmable Antimicrobials.

1. Introduction

1.1. Overview of the Human Microbiome and Its Role in Health and Disease

The human microbiome comprises trillions of microorganisms, including bacteria, viruses, fungi, and protozoa, residing in various body sites such as the gut, skin, oral cavity, and respiratory tract. These microbial communities play a pivotal role in maintaining physiological homeostasis by contributing to digestion, synthesizing essential vitamins, modulating

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the immune system, and protecting against pathogenic invasions [1,2]. The gut microbiota, in particular, has been extensively studied for its influence on metabolic processes, immune responses, and even neurological functions .

A balanced microbiome is integral to health, whereas disruptions in its composition, known as dysbiosis, have been linked to a myriad of diseases, including inflammatory bowel disease, obesity, diabetes, and certain cancer [3]. Advances in sequencing technologies have enabled a deeper understanding of these microbial communities, revealing their complexity and the intricate interplay between host and microbes. This growing body of research underscores the significance of the microbiome in health and disease, positioning it as a potential target for therapeutic interventions [3,4].

1.2. Infectious Diseases and Dysbiosis: Cause or Consequence?

The relationship between dysbiosis and infectious diseases is complex and bidirectional. On one hand, infections can disrupt the microbial balance, leading to dysbiosis; on the other, a dysbiotic microbiome can predispose individuals to infections. For instance, antibiotic-induced dysbiosis can diminish colonization resistance, making the host more susceptible to opportunistic pathogens like Clostridioides difficile [5]. Conversely, infections can alter the microbiota composition, further compromising the host's defense mechanisms.

Research has shown that dysbiosis can impair the gut barrier function and modulate immune responses, creating an environment conducive to pathogen colonization and persistence [6,7]. Moreover, certain pathogens can exploit dysbiotic conditions to establish infections, highlighting the intricate interplay between microbial communities and infectious agents. Understanding this relationship is crucial for developing strategies that restore microbial balance and enhance resistance to infections [7].

1.3. Limitations of Traditional Microbiome Modulation Methods

Traditional approaches to modulate the microbiome, such as probiotics, prebiotics, and fecal microbiota transplantation (FMT), have shown varying degrees of success. Probiotics involve the administration of live beneficial microbes, while prebiotics are non-digestible food components that promote the growth of beneficial bacteria. FMT entails the transfer of fecal matter from a healthy donor to a recipient to restore microbial balance. While these methods have demonstrated efficacy in certain contexts, they are not without limitations [8,9].

One major challenge is the lack of specificity; these interventions often result in broad changes to the microbiota, which can have unpredictable outcomes. Additionally, the long-term stability and integration of introduced microbes remain uncertain. There is also a risk of transferring undesirable traits or pathogens, particularly with FMT. These limitations underscore the need for more precise and controllable methods to modulate the microbiome effectively. Table 1 provides a foundational comparison of traditional microbiome modulation strategies, setting the stage for the subsequent discussion on the advantages of CRISPR-based approaches.

1.4. Rationale for Gene Editing in Microbiome Modulation

Gene editing technologies, particularly CRISPR-Cas systems, offer a promising avenue for precise microbiome modulation. Unlike traditional methods, gene editing allows for targeted manipulation of specific microbial genes or strains, enabling the correction of dysbiosis at a granular level. This precision can lead to more predictable and stable outcomes, minimizing unintended effects on the broader microbial community [10].

CRISPR-based tools have been employed to selectively eliminate pathogenic bacteria, modify metabolic pathways, and engineer beneficial traits into commensal microbes [11]. Such targeted interventions hold the potential to restore microbial balance, enhance resistance to infections, and improve overall health outcomes. As our understanding of the microbiome deepens, integrating gene editing technologies could revolutionize the prevention and treatment of infectious diseases by harnessing the microbiome's therapeutic potential [10,12]. The origins of CRISPR-Cas systems as bacterial immune defenses against viral infections are illustrated in Figure 1, providing context for their application in genome engineering.

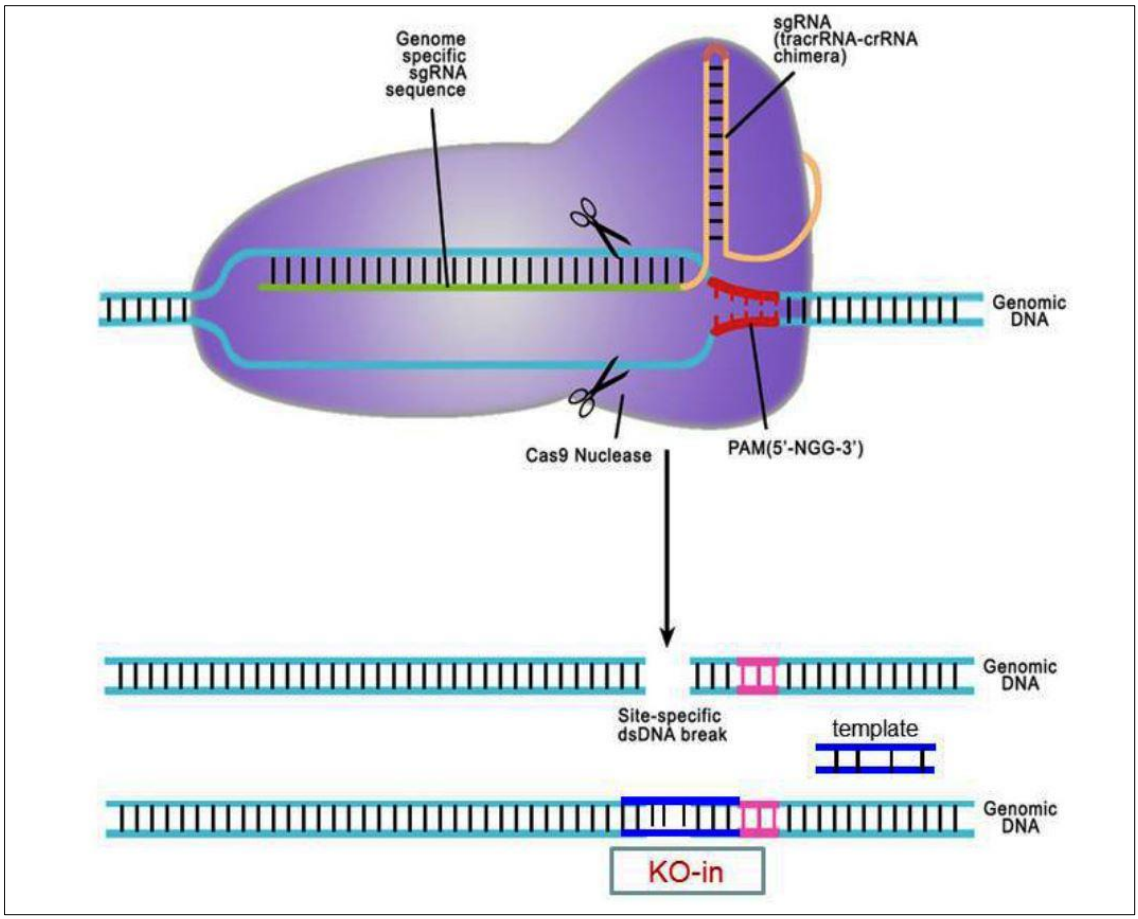

Figure 1 Overview of the natural CRISPR-Cas9 immune mechanism in bacteria. Bacteria capture snippets of viral DNA and integrate them into their genome, enabling the CRISPR-Cas9 system to recognize and cleave matching viral DNA during future infections. Reproduce with permission from Ref. [12]

Table 1 Comparative Overview of Traditional Microbiome Modulation Methods

Modulation Method	Mechanism	Target Specificity	Advantages	Limitations	Clinical Applications
Probiotics	Introduction of beneficial live microorganisms to restore microbial balance	Low; affects broad microbial communities	Generally safe; improves gut health	Variable efficacy; strain-specific effects	Gastrointestinal disorders, antibiotic-associated diarrhea
Prebiotics	Non-digestible food components that promote growth of beneficial microbes	Low; promotes general beneficial bacteria	Enhances growth of beneficial microbes; easy to administer	Non-specific; may also feed pathogenic microbes	Digestive health, immune modulation
Fecal Microbiota Transplantation (FMT)	Transfer of stool from healthy donor to patient to restore microbiota	Moderate; depends on donor microbiota	Effective for recurrent Clostridioides difficile infections	Risk of pathogen transmission; donor variability	Recurrent C. difficile infection, research in IBD and IBS

2. CRISPR Tools for Microbiome Engineering

2.1. CRISPR-Cas Systems as Microbial Genome Editors

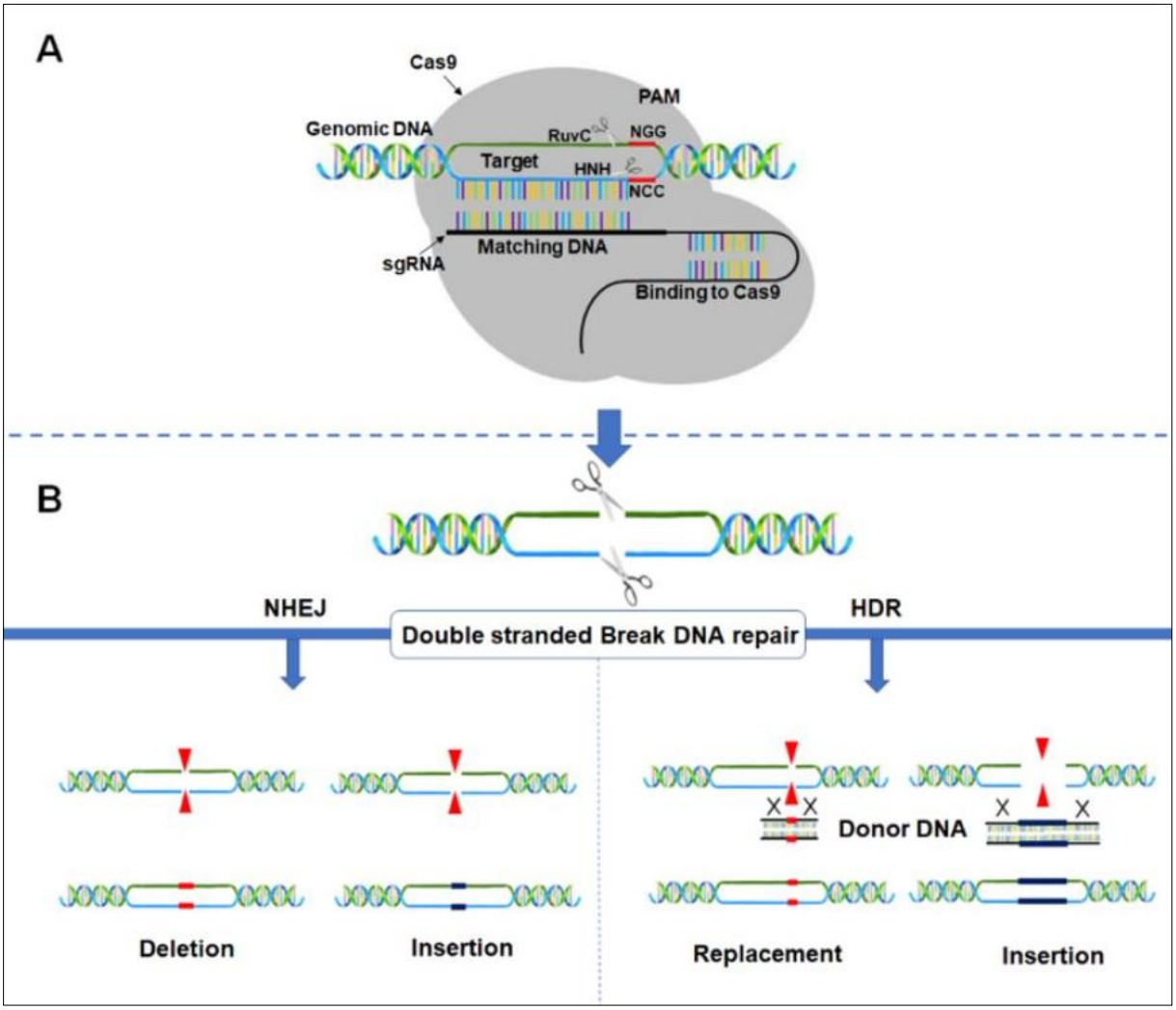

Figure 2 Schematic representation of the CRISPR-Cas9 system. The Cas9 protein, guided by a single-guide RNA (sgRNA), binds to the target DNA sequence adjacent to a protospacer adjacent motif (PAM) and introduces a double-stranded break, facilitating genome editing. Reproduced with permission from Ref. [17].

The CRISPR-Cas system, an acronym for "Clustered Regularly Interspaced Short Palindromic Repeats" and "CRISPR-associated proteins," is a revolutionary gene-editing tool derived from the adaptive immune system of bacteria and archaea. This system enables microorganisms to defend against invading genetic elements such as plasmids and phages by capturing snippets of foreign DNA and integrating them into their own genome as spacers. These spacers serve as a genetic memory, allowing the organism to recognize and mount a defense against subsequent invasions by similar genetic elements. The CRISPR-Cas system has been harnessed for precise genome editing due to its ability to target specific DNA sequences, making it an invaluable tool in microbiome engineering [13-15].

There are several types of CRISPR-Cas systems (see Table 2), broadly categorized into two classes based on their structural and functional characteristics. Class 1 systems utilize multi-protein effector complexes, while Class 2 systems rely on a single, multidomain effector protein. Among these, the Class 2 Type II CRISPR-Cas9 system from *Streptococcus pyogenes* has been the most extensively studied and widely adopted for genome editing applications. The Cas9 protein, guided by a single-guide RNA (sgRNA), introduces double-stranded breaks at specific genomic loci, facilitating targeted modifications through the cell's natural DNA repair mechanisms. Other notable systems include Cas12a (Cpf1) and Cas13, which have unique properties such as staggered DNA cuts and RNA targeting capabilities, respectively, expanding the toolbox for microbial genome editing [16,17]. To better understand the molecular precision and programmability of CRISPR-Cas9 systems, a schematic representation of its mechanism is presented in Figure 2.

In the context of microbiome engineering, CRISPR-Cas systems offer unparalleled precision in editing the genomes of microbial communities. This precision allows for the modification of specific genes within commensal or pathogenic microbes, enabling the study of gene function, metabolic pathways, and microbial interactions within complex ecosystems. For instance, CRISPR-Cas9 has been employed to knock out genes responsible for virulence factors in pathogenic bacteria, attenuating their pathogenicity without affecting beneficial microbes [18]. Additionally, CRISPR-based tools have been used to engineer probiotic strains with enhanced therapeutic properties, such as improved colonization abilities or the production of beneficial metabolites [17].

Despite the remarkable potential of CRISPR-Cas systems in microbial genome editing, several challenges persist. One significant hurdle is the delivery of CRISPR components into target microbes within complex communities, such as the human gut microbiome. Traditional transformation methods are often inefficient or inapplicable to many microbial species. To overcome this, researchers have explored alternative delivery mechanisms, including bacteriophage-mediated transduction, conjugative plasmids, and nanoparticle-based systems. Moreover, concerns regarding off-target effects, horizontal gene transfer, and the stability of edited traits necessitate the development of robust safety measures and regulatory frameworks to guide the responsible application of CRISPR technologies in microbiome engineering [19,20].

Table 2 Types and Classes of CRISPR-Cas Systems Used in Microbiome Editing

Class	Type	Effector Proteins	Targeting Mechanism	Application in Microbiome Editing	Example Studies
Class 1	I (A-F)	Cas3, Cas5, Cas7, Cas8	DNA interference via multi-protein complexes	Limited use due to complexity	Research in bacterial immunity
	III (A-D)	Cas10, Cas5, Cas7	DNA and RNA targeting	Potential for dual targeting	Studies in archaea and bacteria
	IV	Csf1, Cas5, Cas7	Unknown; plasmid-associated	Under investigation	Plasmid defense mechanisms
Class 2	II (A-C)	Cas9	DNA cleavage guided by sgRNA	Widely used for precise genome editing	Numerous studies in various organisms
	V (A-E)	Cas12a-e	DNA targeting with distinct PAM requirements	Alternative to Cas9 with unique features	Applications in plant and animal models
	VI (A-D)	Cas13a-d	RNA targeting	Emerging tool for RNA viruses and transcriptome engineering	Research in RNA virus detection and control

2.2. Editing Commensals and Pathogens In Situ

2.2.1. Overview of In Situ Microbiome Editing

In situ editing refers to the direct modification of microbial genomes within their native environments, such as the human gastrointestinal tract, without the need for ex vivo manipulation. This approach offers the advantage of preserving the complex interactions and ecological balance inherent in microbial communities [21,22]. Traditional genetic engineering techniques often require the isolation and cultivation of target microbes, which can disrupt their native functions and interactions. In contrast, in situ editing enables precise genetic modifications while maintaining the integrity of the microbial ecosystem.

The advent of CRISPR-Cas systems has revolutionized the field of microbial genome editing, providing tools for targeted and efficient genetic modifications. These systems can be programmed to recognize specific DNA sequences, allowing for the selective editing of genes within commensal and pathogenic bacteria. In situ application of CRISPR technologies involves the delivery of these systems directly into the microbial community, facilitating real-time genetic modifications within the host environment [21].

Recent studies have demonstrated the feasibility of in situ editing using engineered delivery vehicles, such as bacteriophages and conjugative plasmids. These vehicles can transport CRISPR components into specific bacterial

populations, enabling targeted gene disruption or modification. For instance, engineered phages have been utilized to deliver base editors to *Escherichia coli* strains residing in the mouse gut, achieving efficient gene editing without disrupting the overall microbial community [23].

2.2.2. Targeting Commensal Bacteria

Commensal bacteria play a crucial role in maintaining host health by contributing to nutrient metabolism, immune modulation, and protection against pathogens. However, certain commensals can harbor genes that contribute to disease under specific conditions. In situ editing of these bacteria allows for the modification or removal of deleterious genes while preserving beneficial functions [25].

One notable example involves the use of CRISPR-based base editors delivered via engineered bacteriophages to modify *E. coli* strains in the mouse gut. This approach achieved a median editing efficiency of 93% in the target population, with the edited bacteria remaining stable for at least 42 days post-treatment. Such precision editing enables the attenuation of harmful traits without compromising the overall microbial balance [23,26].

Furthermore, in situ editing can be employed to enhance the beneficial properties of commensal bacteria. For instance, engineering probiotic strains to produce therapeutic compounds or to outcompete pathogenic species offers a promising avenue for disease prevention and treatment. By harnessing the capabilities of CRISPR technologies, researchers can tailor the functions of commensal microbes to support host health [23].

2.2.3. Targeting Pathogenic Bacteria

In situ editing also holds significant potential for combating pathogenic bacteria within the microbiome. Traditional antibiotic treatments often lack specificity, leading to collateral damage to beneficial microbes and the development of resistance. CRISPR-based approaches offer a targeted alternative, enabling the selective disruption of virulence factors or essential genes in pathogens [27].

For example, researchers have engineered bacteriophages to deliver CRISPR-Cas systems that specifically target and disrupt antibiotic resistance genes in pathogenic *E. coli* strains. This strategy not only reduces the pathogenicity of the target bacteria but also minimizes the impact on non-target microbial populations. Such precision targeting is crucial for preserving the beneficial functions of the microbiome while addressing pathogenic threats [28].

Additionally, in situ editing can be utilized to sensitize pathogens to existing antibiotics, enhancing the efficacy of conventional treatments. By disrupting resistance mechanisms or restoring susceptibility genes, CRISPR-based interventions can rejuvenate the utility of antibiotics against resistant strains. This synergistic approach offers a multifaceted strategy for managing infections within the complex microbial landscape of the host [29].

2.2.4. Challenges and Future Directions

Despite the promising advancements in in situ microbiome editing, several challenges remain. Efficient and specific delivery of CRISPR components to target microbes within the dense and diverse microbial communities of the gut is a significant hurdle. Developing delivery systems that can navigate the complex environment and achieve high editing efficiencies is an ongoing area of research [30].

Moreover, ensuring the stability and persistence of edited traits within dynamic microbial populations poses additional challenges. Horizontal gene transfer, selective pressures, and microbial competition can influence the longevity and impact of genetic modifications. Addressing these factors requires a comprehensive understanding of microbial ecology and the development of strategies to maintain desired traits over time. Ethical considerations also play a role in the application of in situ editing technologies. Balancing the potential benefits with the risks of unintended consequences, such as off-target effects or ecological disruptions, is essential. Establishing regulatory frameworks and conducting thorough risk assessments will be critical for the responsible advancement of these technologies [31,32].

2.3. Programmable Antimicrobials vs. Conventional Antibiotics

The escalating crisis of antimicrobial resistance (AMR) has spotlighted the limitations of conventional antibiotics and underscored the urgent need for innovative therapeutic strategies. Traditional antibiotics, while historically effective, often exhibit broad-spectrum activity, indiscriminately targeting both pathogenic and beneficial microbes. This lack of specificity can disrupt the delicate balance of the human microbiome, leading to dysbiosis and associated health complications. Moreover, the overuse and misuse of antibiotics have accelerated the emergence of resistant bacterial strains, rendering many standard treatments ineffective [33,34].

In contrast, programmable antimicrobials, particularly those leveraging CRISPR-Cas systems, offer a paradigm shift in antimicrobial therapy. These tools enable precise targeting of specific bacterial genes, allowing for the selective elimination of pathogenic strains while sparing commensal bacteria. By programming CRISPR systems to recognize and cleave essential genes or resistance determinants within pathogens, researchers can design antimicrobials with unprecedented specificity and adaptability [35].

2.3.1. Mechanisms of Action: Precision Targeting

CRISPR-based antimicrobials operate by harnessing the sequence-specific DNA recognition capabilities of CRISPR-Cas systems. By designing guide RNAs (gRNAs) complementary to target sequences within bacterial genomes, these systems can direct nucleases like Cas9 to introduce double-stranded breaks at precise locations. When targeting essential genes, this results in bacterial cell death; when directed at plasmid-encoded resistance genes, it can lead to plasmid loss, thereby resensitizing bacteria to antibiotics [36,37].

This precision contrasts starkly with the broad-spectrum activity of conventional antibiotics, which often target conserved bacterial structures or processes, such as cell wall synthesis or protein translation. While effective against a range of bacteria, this approach lacks the specificity to distinguish between harmful pathogens and beneficial microbiota [36,38].

2.3.2. Advantages Over Traditional Antibiotics

The specificity of CRISPR-based antimicrobials offers several advantages over traditional antibiotics. By focusing on specific genetic sequences, these antimicrobials minimize collateral damage to the microbiome, thereby preserving beneficial bacterial populations. Additionally, their ability to target multiple genes simultaneously reduces the likelihood of resistance development, as bacteria would need to acquire several mutations at once to evade treatment. CRISPR systems are also highly adaptable, allowing for rapid reprogramming to target emerging pathogens or resistance genes, making them a flexible platform for addressing evolving microbial threats. These attributes position programmable antimicrobials as a promising alternative in the fight against antimicrobial resistance (AMR), with the potential to restore the efficacy of existing antibiotics and extend the lifespan of antimicrobial therapies [39,40].

2.3.3. Challenges and Considerations

Despite their potential, several challenges must be addressed before CRISPR-based antimicrobials can be widely adopted:

Delivery Mechanisms: Efficiently delivering CRISPR components to target bacteria within complex environments, such as the human gut, remains a significant hurdle. Strategies under investigation include bacteriophage vectors, conjugative plasmids, and nanoparticle-based systems [36].

Off-Target Effects: Ensuring the specificity of CRISPR systems is critical to avoid unintended genetic modifications in non-target organisms, which could have unforeseen consequences [36].

Regulatory and Ethical Considerations: The deployment of gene-editing technologies raises ethical questions regarding their use, particularly concerning unintended ecological impacts and the potential for misuse. Robust regulatory frameworks and thorough risk assessments are essential to guide responsible development and application [36,41].

2.3.4. Future Perspectives

Advancements in CRISPR technology continue to enhance the precision, efficiency, and safety of programmable antimicrobials. Ongoing research aims to refine delivery methods, improve targeting accuracy, and expand the range of treatable pathogens. As these tools progress toward clinical application, they hold the promise of transforming infectious disease management, offering targeted, adaptable, and sustainable solutions to combat AMR [42,43].

2.4. Specificity, Safety, and Ethical Considerations

The advent of CRISPR-Cas systems has revolutionized the field of genetic engineering, offering unprecedented precision in editing genomic sequences. In the context of microbiome modulation, this precision holds the promise of selectively targeting pathogenic microbes or undesirable genes within commensal populations, thereby restoring or enhancing host health. However, the application of such powerful tools necessitates a thorough examination of their specificity, safety, and the ethical implications of their use.

2.4.1. Specificity and Off-Target Effects

A cornerstone of CRISPR-Cas technology is its ability to target specific DNA sequences through guide RNAs (gRNAs) that direct the Cas nuclease to the desired genomic locus. Despite this high degree of specificity, off-target effects—unintended modifications at sites with partial sequence homology—remain a significant concern. These unintended edits can disrupt gene function, leading to unforeseen consequences such as the activation of oncogenes or the inactivation of tumor suppressor genes, thereby posing potential risks to host health [44].

In the complex milieu of the human microbiome, where microbial genomes are diverse and densely packed, the risk of off-target effects is amplified. The horizontal gene transfer prevalent among microbial communities further complicates the predictability of CRISPR interventions. To mitigate these risks, researchers are developing high-fidelity Cas variants and employing computational tools to design gRNAs with minimized off-target potential. Additionally, techniques such as whole-genome sequencing and GUIDE-seq are employed to detect and quantify off-target events, thereby informing the refinement of CRISPR systems for safer applications [45].

Moreover, the development of anti-CRISPR proteins, naturally occurring inhibitors of CRISPR-Cas systems, offers a potential fail-safe mechanism to control or halt CRISPR activity post-delivery. These proteins can be harnessed to temporally regulate gene editing, thereby reducing the window for off-target interactions and enhancing the overall safety profile of CRISPR-based therapeutics [46]

2.4.2. Safety Concerns in Microbiome Editing

The safety of CRISPR-mediated microbiome editing extends beyond genomic specificity to encompass the broader ecological impacts within the microbial community. The human microbiome is a complex and dynamic ecosystem, where microbial species interact in intricate networks that influence host physiology. Perturbations to this ecosystem, even if targeted, can have cascading effects that disrupt microbial balance, potentially leading to dysbiosis and associated health issues [47].

For instance, the elimination of a specific bacterial strain might inadvertently create a niche for opportunistic pathogens, or the disruption of metabolic pathways could affect the production of essential metabolites. Therefore, comprehensive assessments of microbial community structure and function are imperative before and after CRISPR interventions. Longitudinal studies and advanced metagenomic analyses are essential to monitor the resilience and adaptability of the microbiome post-editing, ensuring that therapeutic benefits are not offset by unintended adverse effects [48,49].

Additionally, the delivery mechanisms for CRISPR components, such as bacteriophages or conjugative plasmids, must be scrutinized for their own safety profiles. These vectors should be engineered to minimize immunogenicity and prevent horizontal gene transfer that could disseminate editing tools to non-target species. Rigorous preclinical testing and the development of controllable delivery systems are crucial steps toward the safe implementation of CRISPR-based microbiome therapies [50].

2.4.3. Ethical Implications of Microbiome Engineering

The ethical landscape of CRISPR-mediated microbiome editing is multifaceted, encompassing considerations of consent, equity, and the potential for unintended consequences. Unlike somatic gene editing, which affects individual patients, microbiome editing has implications that extend to the broader community and environment, given the transmissible nature of microbes. This raises questions about the scope of informed consent and the rights of individuals to alter microbial ecosystems that are shared among populations [51]. Equity in access to CRISPR-based therapies is another pressing ethical concern. The high costs associated with the development and deployment of these technologies may exacerbate existing healthcare disparities, limiting benefits to affluent populations while marginalizing underprivileged communities. Policymakers and stakeholders must work collaboratively to establish frameworks that promote equitable access and prevent the monopolization of advanced therapeutics [52].

Furthermore, the potential for dual-use applications of CRISPR technology necessitates vigilant oversight. While the primary intent may be therapeutic, the same tools could be repurposed for harmful purposes, such as the creation of antibiotic-resistant pathogens. Establishing robust regulatory mechanisms and fostering a culture of ethical responsibility among researchers are essential to mitigate the risks associated with the misuse of gene-editing technologies.

2.4.4. Regulatory and Governance Frameworks

The rapid advancement of CRISPR technologies has outpaced the development of comprehensive regulatory frameworks, leading to a fragmented landscape of guidelines and oversight mechanisms. International consensus on the governance of gene-editing applications, particularly those involving the microbiome, is lacking, resulting in disparities in ethical standards and safety protocols across jurisdictions [53]. To address this gap, there is a pressing need for the establishment of global regulatory bodies that can harmonize policies, facilitate information sharing, and oversee the ethical deployment of CRISPR-based interventions. Such bodies should include diverse stakeholders, including scientists, ethicists, policymakers, and representatives from affected communities, to ensure that multiple perspectives are considered in decision-making processes [54].

Moreover, adaptive regulatory frameworks that can evolve in response to emerging scientific insights are essential. These frameworks should incorporate mechanisms for continuous monitoring, post-market surveillance, and the integration of public feedback to maintain trust and accountability in the application of gene-editing technologies. By proactively addressing regulatory challenges, the scientific community can foster responsible innovation that maximizes benefits while minimizing risks [55].

3. Microbiome Editing for Preventing Pathogen Colonization

The human microbiome plays a pivotal role in maintaining health by providing colonization resistance against pathogenic organisms. This resistance is achieved through various mechanisms, including competition for nutrients and space, production of antimicrobial compounds, and modulation of the host immune system. However, disruptions to the microbiome, such as those caused by antibiotic use, can compromise this protective barrier, leading to increased susceptibility to infections [56].

Traditional approaches to restoring the microbiome, such as probiotic administration or fecal microbiota transplantation, have shown varying degrees of success and often lack specificity. The advent of CRISPR-Cas technology offers a novel avenue for precise microbiome editing, enabling targeted manipulation of microbial communities to enhance colonization resistance. This section explores the application of CRISPR-based strategies to engineer the microbiome for the prevention of pathogen colonization, focusing on competitive exclusion via engineered commensals, suppression of virulence factors, and real-world models involving pathogens like *Clostridioides difficile* and *Salmonella* [57].

3.1. Competitive Exclusion via Engineered Commensals

3.1.1. Engineering Commensals for Pathogen Exclusion

Competitive exclusion is a fundamental ecological principle where resident microbiota inhibits the establishment of pathogenic organisms by occupying ecological niches and utilizing available resources. Leveraging this concept, researchers have explored the engineering of commensal bacteria to enhance their competitive capabilities against pathogens. By introducing CRISPR-Cas systems into commensals, it is possible to endow them with the ability to target and suppress specific pathogens or their virulence factors [58]. For instance, studies have demonstrated the feasibility of engineering *Escherichia coli* Nissle 1917, a well-characterized probiotic strain, to express CRISPR-Cas systems targeting antibiotic resistance genes in pathogenic *E. coli* strains. This approach not only reduces the prevalence of resistant pathogens but also minimizes the impact on the overall microbiome composition [57,59].

Furthermore, engineered commensals can be designed to produce antimicrobial peptides or bacteriocins that specifically target pathogens. By integrating CRISPR-based regulatory elements, the expression of these antimicrobial compounds can be tightly controlled, ensuring their production only in the presence of specific pathogens, thereby reducing potential off-target effects on beneficial microbes [60].

3.1.2. Enhancing Colonization Resistance

Beyond direct antagonism, engineered commensals can be utilized to bolster the overall resilience of the microbiome against pathogen colonization. By modifying metabolic pathways, commensals can be tailored to outcompete pathogens for essential nutrients or to alter the local environment in ways that are unfavorable for pathogen survival. For example, engineering commensals to produce short-chain fatty acids can lower the gut pH, creating conditions that inhibit the growth of certain pathogens [61].

Additionally, CRISPR-based tools can be employed to modulate the expression of surface proteins on commensals, enhancing their adherence to mucosal surfaces and thereby occupying niches that might otherwise be exploited by pathogens. Such modifications can strengthen the physical barrier against pathogen invasion and promote the stability of the commensal population [62].

3.1.3. Safety and Biocontainment Considerations

While the engineering of commensals offers promising avenues for pathogen exclusion, safety and biocontainment are critical considerations. The potential for horizontal gene transfer of engineered elements to other microbes poses a risk of unintended consequences. To mitigate this, researchers have developed CRISPR-based kill switches that can be activated under specific conditions, ensuring that engineered commensals do not persist or disseminate beyond their intended environment [63]. Moreover, the use of inducible systems allows for temporal control over the expression of engineered traits, reducing the likelihood of adverse effects on the host or the native microbiota. Comprehensive risk assessments and regulatory frameworks are essential to guide the safe deployment of engineered commensals in clinical settings.[63]

3.2. CRISPR-Based Suppression of Virulence Factors

The advent of CRISPR-Cas systems has revolutionized the field of microbial genetics, offering unprecedented precision in gene editing. Beyond their initial applications in genome editing, these systems have been harnessed to modulate gene expression, particularly through CRISPR interference (CRISPRi), which employs a catalytically inactive Cas9 (dCas9) to repress transcription. This approach has opened new avenues for attenuating bacterial virulence without necessarily killing the organisms, thereby preserving the overall microbial community structure. In the context of microbiome engineering, CRISPRi presents a promising strategy to suppress specific virulence factors of pathogenic bacteria, mitigating their pathogenicity while maintaining ecological balance [59]

3.2.1. Targeting Biofilm Formation in Pathogens

Biofilms are structured communities of bacteria encased in a self-produced extracellular matrix, contributing significantly to bacterial persistence and resistance to antimicrobial agents. The formation of biofilms is a critical virulence factor for many pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. CRISPRi has been effectively utilized to suppress genes essential for biofilm formation, thereby attenuating bacterial virulence.

For instance, Azam et al. [59] demonstrated that CRISPRi-mediated suppression of the *fimH* gene in uropathogenic *E. coli* significantly reduced biofilm formation, highlighting the potential of this approach in mitigating urinary tract infections [59]. Similarly, targeting the *luxS* gene, which plays a role in quorum sensing and biofilm development, resulted in diminished biofilm formation, further underscoring the utility of CRISPRi in controlling bacterial virulence [59]. Moreover, CRISPRi has been employed to suppress the *csgD* gene in *E. coli* Nissle 1917, a probiotic strain known to form biofilms. The downregulation of *csgD* led to a significant reduction in curli amyloid fiber production and biofilm formation, suggesting that CRISPRi can be used to modulate the virulence potential of probiotic strains, enhancing their safety profiles [59].

3.2.2. Modulating Toxin Production

Toxin production is a hallmark of many pathogenic bacteria, contributing to disease severity and progression. CRISPRi offers a targeted approach to suppress toxin gene expression, thereby attenuating bacterial virulence without affecting bacterial viability. This strategy is particularly advantageous in preserving the beneficial aspects of the microbiome while mitigating pathogenic threats.

In *Clostridioides difficile*, a major cause of antibiotic-associated diarrhea, the expression of toxins A and B is central to its pathogenicity. CRISPRi-mediated suppression of the *tcdA* and *tcdB* genes has been shown to reduce toxin production, leading to attenuated virulence in vitro. This approach holds promise for developing targeted therapies against *C. difficile* infections, especially in the context of microbiome-sparing interventions [59].

Similarly, in *Staphylococcus aureus*, CRISPRi has been utilized to downregulate the expression of alpha-hemolysin, a key virulence factor responsible for host cell lysis. The suppression of this toxin gene resulted in decreased cytotoxicity, highlighting the potential of CRISPRi in modulating virulence factors to combat bacterial infections [64].

3.2.3. Engineering Probiotics to Counteract Pathogens

Beyond directly targeting pathogens, CRISPRi can be employed to engineer probiotic strains with enhanced capabilities to counteract pathogenic bacteria. By modulating gene expression in probiotics, it is possible to enhance their competitive fitness, antimicrobial production, and immunomodulatory properties. This strategy offers a dual benefit of suppressing pathogen virulence while promoting beneficial microbial functions [65].

For example, as briefly highlighted previously, *E. coli* Nissle 1917 has been engineered using CRISPRi to suppress genes associated with biofilm formation, such as *csgD*, thereby reducing its potential to contribute to pathogenic biofilms while retaining its probiotic functions. Such modifications enhance the safety and efficacy of probiotic strains in clinical applications [59].

Furthermore, CRISPRi has been applied to modulate metabolic pathways in probiotic strains, enabling them to produce antimicrobial compounds or compete more effectively with pathogens for nutrients and adhesion sites. This approach has the potential to fortify the microbiome against pathogenic colonization, offering a proactive strategy for infection prevention [59,66].

3.3. Real-World Models in Clostridioides difficile, Salmonella, etc.

The practical application of CRISPR-based microbiome editing has transitioned from theoretical frameworks to tangible models, particularly in addressing infections caused by *Clostridioides difficile* and *Salmonella* species. These pathogens are notable for their prevalence in healthcare-associated infections and foodborne illnesses, respectively. The utilization of CRISPR technologies in these contexts offers insights into the feasibility, efficacy, and challenges of microbiome editing in real-world scenarios. Table 3 summarizes key applications of CRISPR-based microbiome editing in infectious disease models, highlighting target organisms, editing strategies, and observed outcomes across experimental and preclinical studies.

3.3.1. Clostridioides difficile: A Model for CRISPR-Based Intervention

Clostridioides difficile (formerly *Clostridium difficile*) is a Gram-positive, spore-forming bacterium responsible for significant morbidity and mortality worldwide. Traditional treatments often involve broad-spectrum antibiotics, which can disrupt the gut microbiota and lead to recurrent infections. CRISPR-based strategies have been explored to specifically target and mitigate *C. difficile* infections without adversely affecting the broader microbial community [67].

According to Selle et al., phage-delivered CRISPR-Cas3 systems have been engineered to specifically target and kill *C. difficile* in vivo. This approach leverages bacteriophages as delivery vehicles for CRISPR components, enabling precise targeting of pathogenic bacteria while sparing beneficial microbes . The study demonstrated that this method could reduce *C. difficile* colonization in the gut, highlighting its potential as a therapeutic strategy [68]. From the findings of McAllister et al., CRISPR-Cas9-mediated genome editing was employed to generate *C. difficile* mutants defective in selenoprotein synthesis. The study successfully deleted the *selD* gene, which is essential for selenoprotein biosynthesis, resulting in attenuated virulence of the bacterium . This work underscores the utility of CRISPR-Cas9 in dissecting gene function and developing attenuated strains for potential vaccine development [68].

Furthermore, studies have utilized endogenous CRISPR-Cas systems within *C. difficile* for genome editing purposes. Maikova et al. demonstrated that redirecting the bacterium's native CRISPR-Cas system towards autoimmunity allows efficient genome editing, providing a tool for functional genomic studies and the development of novel therapeutic strategies [69,70].

3.3.2. Salmonella spp.: CRISPR Applications in Foodborne Pathogens

Salmonella species are leading causes of foodborne illnesses globally. The emergence of antibiotic-resistant strains has necessitated alternative approaches to control and prevent infections. CRISPR-based technologies have been investigated for their potential to specifically target and neutralize *Salmonella* pathogens [71].

Askoura et al. [72] highlighted the development of CRISPR-Cas systems designed to disrupt essential genes in *Salmonella enterica*, leading to reduced virulence and impaired survival. These systems can be delivered via bacteriophages or conjugative plasmids, ensuring specificity and minimizing off-target effects. The application of such targeted approaches offers a promising avenue for controlling *Salmonella* infections, particularly in agricultural settings.

Following the discussions raised by Zhang et al. [73], CRISPR interference (CRISPRi) has been employed to downregulate virulence genes in *Salmonella*, such as those involved in the type III secretion system. This repression

leads to attenuated pathogenicity, providing insights into gene function and potential targets for antimicrobial development. The use of CRISPRi allows for reversible and tunable gene regulation, which is advantageous for studying essential genes without inducing lethality.

Moreover, CRISPR-based antimicrobials have been explored for their ability to selectively kill *Salmonella* strains harboring specific resistance genes. By designing guide RNAs that target resistance determinants, these systems can eliminate resistant populations while preserving susceptible ones, thereby restoring the efficacy of existing antibiotics. This precision approach addresses the growing concern of antibiotic resistance in *Salmonella* and other pathogens [74].

Table 3 Applications of CRISPR-Based Microbiome Editing in Infectious Disease Models

Pathogen / Model	**CRISPR Strategy**	**Delivery Method**	**Target Genes / Functions**	**Observed Outcome**
Clostridioides difficile	CRISPR-Cas3 and CRISPR-Cas9	Engineered bacteriophages, gene knockouts	Toxin genes (tcdA, tcdB), selenoprotein synthesis (selD)	Reduced colonization; attenuated virulence; potential for vaccine development
Salmonella enterica	CRISPR interference (CRISPRi)	Phages, conjugative plasmids	Type III secretion system genes, invA	Decreased virulence and epithelial invasion; reduced pathogen burden in murine models
Escherichia coli O157:H7	CRISPR-Cas3 via engineered probiotic (*E. coli* Nissle)	Engineered probiotic strain	Genome-wide targeting of pathogenic strain	Selective degradation of pathogen DNA; prevented colonization
Staphylococcus aureus	CRISPRi	Engineered strains	Alpha-hemolysin toxin gene	Reduced cytotoxicity *in vitro*
Shigella flexneri	CRISPRi via probiotic delivery	Engineered probiotic (*E. coli* Nissle)	Toxin-encoding genes	Reduced epithelial damage in murine models

3.3.3. Challenges and Future Directions

While CRISPR-based microbiome editing holds significant promise, several challenges must be addressed to facilitate its clinical and environmental applications [75]. Delivery of CRISPR components to target bacteria within complex microbial communities remains a significant hurdle. Strategies such as phage-mediated delivery and conjugative plasmids are being optimized to enhance specificity and efficiency [75]. Nguyen et al. [76] emphasized the importance of understanding the ecological impacts of microbiome editing. Altering microbial populations can have unintended consequences on community dynamics and host health. Comprehensive studies are needed to assess the long-term effects of CRISPR interventions on the microbiome and to develop safeguards against potential dysbiosis [76].

From the findings of Jain et al. [77], regulatory and ethical considerations are paramount in advancing CRISPR-based therapies. Ensuring the safety, efficacy, and equitable access to these technologies requires collaborative efforts among scientists, clinicians, policymakers, and the public. Establishing clear guidelines and oversight mechanisms will be crucial for responsible development and deployment.

In essence, real-world models involving *Clostridioides difficile* and *Salmonella* demonstrate the potential of CRISPR-based microbiome editing in combating pathogenic bacteria. Continued research and interdisciplinary collaboration will be essential to overcome current challenges and harness the full potential of these innovative strategies.

4. Therapeutic Applications in Gastrointestinal Infections

The gastrointestinal tract harbors a dense and complex microbial ecosystem that plays a pivotal role in host physiology, immune modulation, and disease susceptibility [77]. Disruptions in this microbiota, often described as dysbiosis, have been implicated in a broad spectrum of gastrointestinal (GI) disorders ranging from inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) to infectious diarrheas and colorectal cancers [78]. In response to the limitations of

conventional treatments such as broad-spectrum antibiotics and immunosuppressants—which often exacerbate microbial imbalance—there has been a growing emphasis on microbiome-targeted therapeutic strategies. Among these, CRISPR-based technologies have emerged as a transformative approach, capable of precision-targeting pathogenic organisms or deleterious microbial genes while sparing beneficial taxa [78].

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems, originally discovered as adaptive immune mechanisms in bacteria and archaea, are now being repurposed for therapeutic microbiome modulation. In the context of gastrointestinal infections, these systems enable unprecedented specificity in targeting enteric pathogens, antimicrobial resistance genes, and virulence factors. Recent advances have also facilitated the integration of CRISPR constructs into probiotic delivery systems, allowing for the selective elimination of pathogens or the enhancement of host defenses. Such interventions are not only aimed at curbing infection but also at restoring microbiome equilibrium, thereby contributing to long-term gut health [79,80]. The therapeutic pipeline includes three major strategies: engineering probiotics with CRISPR-based effectors to suppress enteric pathogens, deploying CRISPR-guided systems to deliver antimicrobial payloads, and translating preclinical findings into clinical applications through case studies and synthetic biology pipelines. Together, these avenues represent a frontier in gastrointestinal therapeutics where precision microbiology intersects with clinical medicine [79].

4.1. Engineered Probiotics Targeting Enteric Pathogens

The use of engineered probiotics as delivery vehicles for therapeutic agents has gained traction, particularly in the context of gastrointestinal infections. These beneficial microbes—commonly *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli* Nissle 1917—are genetically tractable and capable of colonizing the gut environment, making them ideal chassis for therapeutic interventions. Through the incorporation of CRISPR-Cas systems into probiotic genomes or plasmids, researchers have created living biotherapeutics that can identify and eliminate specific enteric pathogens or their associated virulence determinants [81].

4.1.1. CRISPR-Cas-Expressing Probiotics Against Escherichia coli and Salmonella

One of the most prominent applications of engineered probiotics involves targeting pathogenic *Escherichia coli* and *Salmonella* strains. In a landmark study by Bogut et al. [82], *E. coli* Nissle 1917 was engineered to carry CRISPR-Cas3 constructs targeting pathogenic *E. coli* O157:H7. Upon colonization of the host gut, the engineered strain selectively degraded the genomes of the pathogenic bacteria, significantly reducing colonization and preventing infection in murine models. Notably, this intervention exhibited minimal off-target effects on commensal microbial populations, affirming the specificity of CRISPR-based targeting.

A parallel study by Oh et al. [83] demonstrated the deployment of *Lactobacillus reuteri* engineered with CRISPR-Cas9 elements designed to target the *invA* gene in *Salmonella enterica*, a gene essential for epithelial invasion. The results showed a marked reduction in *Salmonella* burden in the mouse ileum and feces without impacting overall microbial diversity. This suggests that CRISPR-equipped probiotics could serve not merely as antimicrobial agents but as microbiota-sparing alternatives to antibiotics in managing enteric infections [83]. Moreover, the adaptability of the CRISPR system to multiplex targeting—where several guide RNAs are encoded within a single vector—further strengthens its therapeutic utility. This allows simultaneous targeting of multiple strains or resistance genes, thereby addressing the polyclonal nature of many gut infections and the challenge of horizontal gene transfer [83].

4.1.2. Probiotic Modulation of Virulence Gene Expression in Pathobionts

Beyond the outright elimination of pathogens, CRISPR-equipped probiotics have been employed to suppress the expression of virulence genes in resident or opportunistic pathobionts. Using CRISPR interference (CRISPRi), researchers have modulated gene expression at the transcriptional level without inducing double-strand breaks, thereby achieving reversible suppression of pathogenic traits. A study by Zuberi et al. [84] introduced CRISPRi components into *E. coli* Nissle 1917 to downregulate toxin-encoding genes in *Clostridioides difficile* and *Shigella flexneri*. The results indicated significant attenuation in toxin-mediated epithelial damage both in vitro and in murine models.

These interventions are particularly advantageous when dealing with facultative pathogens that coexist with the host in a commensal state under normal conditions but become pathogenic under stress or immunosuppression. By regulating virulence factors such as secretion systems, fimbriae, and hemolysins, engineered probiotics provide a nuanced approach to pathogen control, reducing the likelihood of resistance development compared to bactericidal therapies.

Additionally, the modularity of CRISPRi systems facilitates the exploration of essential genes whose complete knockout would be lethal. This enables the functional dissection of microbial gene networks and the identification of potential therapeutic targets without compromising bacterial viability—a crucial feature for studying slow-growing or unculturable gut microbes [82-84].

4.1.3. Synbiotic Integration and Host-Modulated Responses

The therapeutic efficacy of CRISPR-equipped probiotics is further enhanced when combined with synbiotic formulations—co-administration of prebiotics that support probiotic growth and function. In work by Rahman et al. [85], a synbiotic formulation consisting of *Bifidobacterium longum* engineered with CRISPR elements and inulin (a known bifidogenic prebiotic) showed superior suppression of *Campylobacter jejuni* in piglet models compared to probiotic alone. The synergy between targeted genetic editing and ecological support underscores the potential for integrated microbiome therapeutics in livestock and human medicine.

Host responses to engineered probiotics also warrant consideration. Evidence suggests that the release of pathogen-derived nucleic acids and cell debris following CRISPR-based cleavage may activate host pattern recognition receptors (PRRs), potentially enhancing mucosal immunity. For instance, a study by Mayorga-Ramos et al. [86] observed increased production of interleukin-22 (IL-22) and antimicrobial peptides in the intestinal mucosa of mice treated with CRISPR-equipped *Lactobacillus casei* targeting *Listeria monocytogenes*. These findings imply that engineered probiotics could exert both direct antimicrobial and immunomodulatory effects.

In terms of safety, most studies to date report minimal toxicity and immunogenicity, although the long-term ecological consequences of sustained probiotic colonization and CRISPR activity remain under investigation. The use of inducible promoters and kill-switch systems is being explored to mitigate potential risks, ensuring temporal control over CRISPR activation and containment of genetically modified organisms [87,88].

4.2. CRISPR-Guided Delivery of Antimicrobial Payloads

Table 4 Delivery Vectors for CRISPR Constructs in Microbiome Editing

Delivery Vector	Mechanism	Target Specificity	Stability	Advantages	Limitations	Representative Applications
Phagemid Systems	Bacteriophage capsids deliver plasmid DNA encoding CRISPR elements	High; species-specific	Moderate	High specificity; low off-target effects; effective in vivo	Limited host range; phage resistance; requires encapsulation for oral delivery	Targeting antibiotic-resistant *E. coli*, Klebsiella pneumoniae
Conjugative Plasmids	Bacterial conjugation transfers CRISPR constructs horizontally	Moderate; depends on plasmid compatibility	High	Broad dissemination in gut microbiota; persistent presence	Risk of horizontal gene transfer; biosafety concerns	Targeting vancomycin-resistant Enterococcus faecalis
Nanoparticles	Lipid or polymer carriers deliver CRISPR proteins or nucleic acids	Variable; depends on targeting ligands	High	Protects CRISPR from degradation; enables mucosal targeting	Complex manufacturing; limited targeting specificity	Targeting Bacteroides fragilis, RNA viruses in gut epithelium
Engineered Probiotics	Genetically modified bacteria express CRISPR systems in situ	High (programmable gRNA)	Moderate–High (colonizing)	Colonization capability; local and sustained CRISPR delivery	Long-term persistence concerns; need for biocontainment systems	Modulating virulence in C. difficile, Shigella, Salmonella

The CRISPR system's versatility extends beyond direct gene editing to the highly specific delivery of antimicrobial payloads within the gastrointestinal tract. Traditional antimicrobials, including antibiotics and bacteriocins, often exert broad-spectrum effects that disrupt commensal microbiota and facilitate antimicrobial resistance. By integrating CRISPR with bacteriophage vectors, nanoparticle carriers, or mobile genetic elements, researchers have developed delivery systems capable of targeting specific bacterial strains or genes without collateral damage to the microbial ecosystem [89]. These strategies offer not only targeted bacterial killing but also the suppression of resistance genes and virulence determinants in complex microbial communities. A variety of delivery vectors have been developed to facilitate the targeted application of CRISPR constructs within the gastrointestinal tract, each with distinct advantages and limitations in terms of specificity, stability, and applicability across microbial populations (Table 4).

CRISPR-guided antimicrobial delivery systems have demonstrated particular promise in addressing gastrointestinal pathogens such as *Clostridioides difficile*, *Salmonella enterica*, and multidrug-resistant *Enterobacteriaceae*. The approaches are diverse, ranging from conjugative plasmids engineered with CRISPR-Cas9 to phagemid constructs that deliver CRISPR components into target bacteria, inducing double-strand breaks in essential genes or resistance loci. These methods capitalize on the natural DNA delivery mechanisms of mobile elements or bacteriophages while harnessing CRISPR's precision and programmability [84]. Figure 3 outlines the general workflow of CRISPR-based gene editing, highlighting the sequential steps from guide RNA design to target modification, which underpins many microbiome editing strategies discussed herein.

Recent research has also explored the use of CRISPR-guided antimicrobials in polymicrobial settings, including the human gut, where interactions between microbes and the host complicate treatment. In these environments, CRISPR's ability to selectively modify or eliminate specific taxa offers a compelling alternative to broad-spectrum approaches, enabling precision microbiome engineering for both prophylactic and therapeutic purposes [86,90].

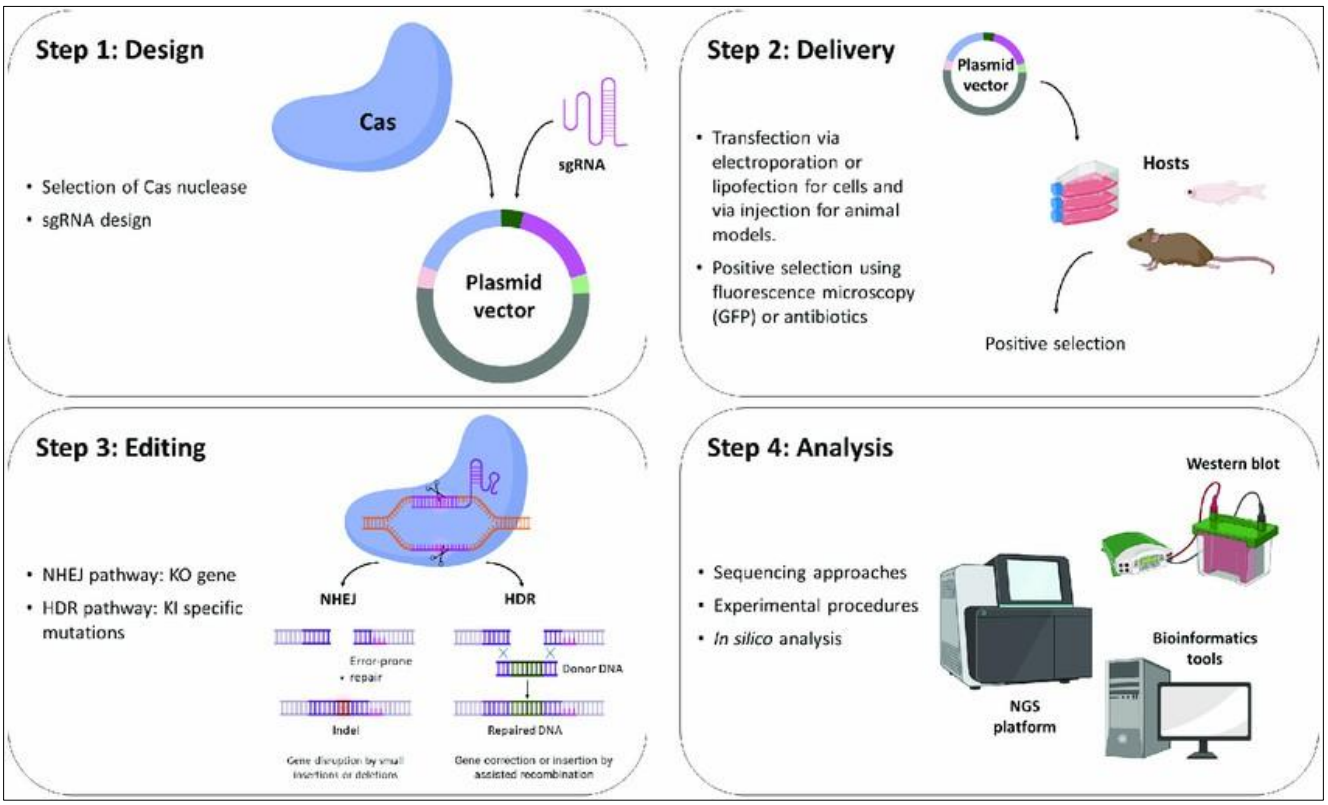

Figure 3 Step-by-step workflow of CRISPR-Cas9 gene editing. The process includes designing the sgRNA, delivering the CRISPR components into target cells, and facilitating genome modification through the introduction of double-stranded breaks and subsequent repair mechanisms. Reproduced with permission from Ref. [90].

4.2.1. Phagemid-Based CRISPR Delivery for Selective Pathogen Elimination

One of the most effective platforms for delivering CRISPR-based antimicrobials in the gastrointestinal tract is the phagemid system. Phagemids are plasmid-phage hybrids that utilize bacteriophage capsids to deliver plasmid DNA encoding CRISPR-Cas elements into target bacteria. From the findings of Citorik et al. [91], phagemid particles carrying

CRISPR-Cas9 constructs targeted at antibiotic resistance genes (e.g., *blaNDM-1*) successfully eliminated multidrug-resistant *E. coli* and *Klebsiella pneumoniae* strains in vitro and in mouse models without affecting sensitive strains. This level of specificity is critical in maintaining microbial diversity and preventing dysbiosis during treatment [91].

According to Yosef et al. [92], CRISPR-phagemid systems can be engineered to carry multiple guide RNAs, enabling simultaneous targeting of several resistance or virulence genes. In gastrointestinal applications, these phagemids can be administered orally, with encapsulation technologies protecting the phage particles from degradation in the acidic stomach environment. Following release in the intestine, the phages infect specific bacterial hosts, introducing the CRISPR machinery that cleaves target DNA sequences and ultimately results in bacterial cell death through the DNA damage response [92].

Beyond resistance genes, researchers have expanded the application of phagemid CRISPR delivery to pathogenicity islands and toxin genes. In a study by Steele et al. [93], CRISPR-guided phagemids targeting *C. difficile* toxin genes (*tcdA* and *tcdB*) significantly reduced disease severity and mortality in mouse models of C. difficile infection (CDI). These results demonstrate that CRISPR payload delivery via phagemids can both eliminate pathogens and modulate pathogenicity, offering dual benefits in infection control.

4.2.2. Conjugative Plasmid Delivery of CRISPR Constructs in Complex Microbiota

While phagemids offer high specificity, their host range is often limited by phage-host compatibility. To address this, conjugative plasmids have been employed as an alternative vehicle for CRISPR delivery, leveraging bacterial conjugation to propagate therapeutic genes among microbial populations. From the work of Rodrigues et al. [94], a self-transmissible plasmid carrying a CRISPR-Cas9 construct was introduced into *Enterococcus faecalis* populations to target and eliminate vancomycin-resistance genes. The plasmid spread horizontally among gut enterococci, significantly reducing the prevalence of resistant strains without requiring direct targeting of each bacterial cell.

Following the discussions raised by Tao et al. [95], conjugative plasmids offer the unique advantage of persistence within microbial populations and the ability to propagate under selective conditions. In the gut, this can translate into long-term carriage and propagation of CRISPR-based therapeutics, especially when paired with selective pressures such as dietary components or mild antibiotics that favor plasmid-containing strains. Importantly, researchers have employed kill-switch systems to control plasmid propagation and limit unintended ecological consequences, enhancing the safety profile of this approach.

In a complex microbiota such as the human gut, conjugative plasmids allow for broad dissemination of CRISPR constructs while maintaining strain-level specificity. Studies have shown that CRISPR-induced targeting of plasmid-borne resistance genes can restore susceptibility in enteric pathogens, thereby resensitizing them to conventional antibiotics. This opens the door for combinatorial therapies that integrate CRISPR delivery with conventional drug regimens, enhancing treatment efficacy and minimizing resistance selection [95].

4.2.3. Nanocarrier-Based CRISPR Delivery Systems for Mucosal Targeting

More recent developments have explored the encapsulation of CRISPR-Cas systems in nanoparticles designed for mucosal delivery. Nanocarriers such as liposomes, chitosan-based particles, and polymeric micelles can be engineered to protect CRISPR components from enzymatic degradation and facilitate targeted delivery to intestinal sites. According to Zhu et al. [96], a lipid nanoparticle system carrying Cas12a and guide RNAs was used to target enterotoxigenic *Bacteroides fragilis* in colitis-induced mice. The nanoparticles, functionalized with pH-sensitive coatings, released their payload in the colonic environment, leading to significant reductions in inflammation and pathogen burden.

From the findings of Zhang et al. [97], mucoadhesive nanoparticles loaded with CRISPR-Cas13a constructs were able to suppress RNA viruses that infect gut epithelium, highlighting the platform's adaptability beyond bacterial targets. These approaches are especially relevant in immunocompromised patients or in chronic infections where repeated dosing or localized targeting is necessary.

Nanoparticle-mediated CRISPR delivery also enables co-encapsulation of supportive agents such as immunostimulants, prebiotics, or anti-inflammatory compounds. This multipronged strategy not only eliminates pathogens but also facilitates microbiome recovery and epithelial repair. Moreover, the scalable synthesis and customizable surface chemistry of nanocarriers make them amenable to clinical translation, though regulatory and safety concerns remain ongoing challenges [97].

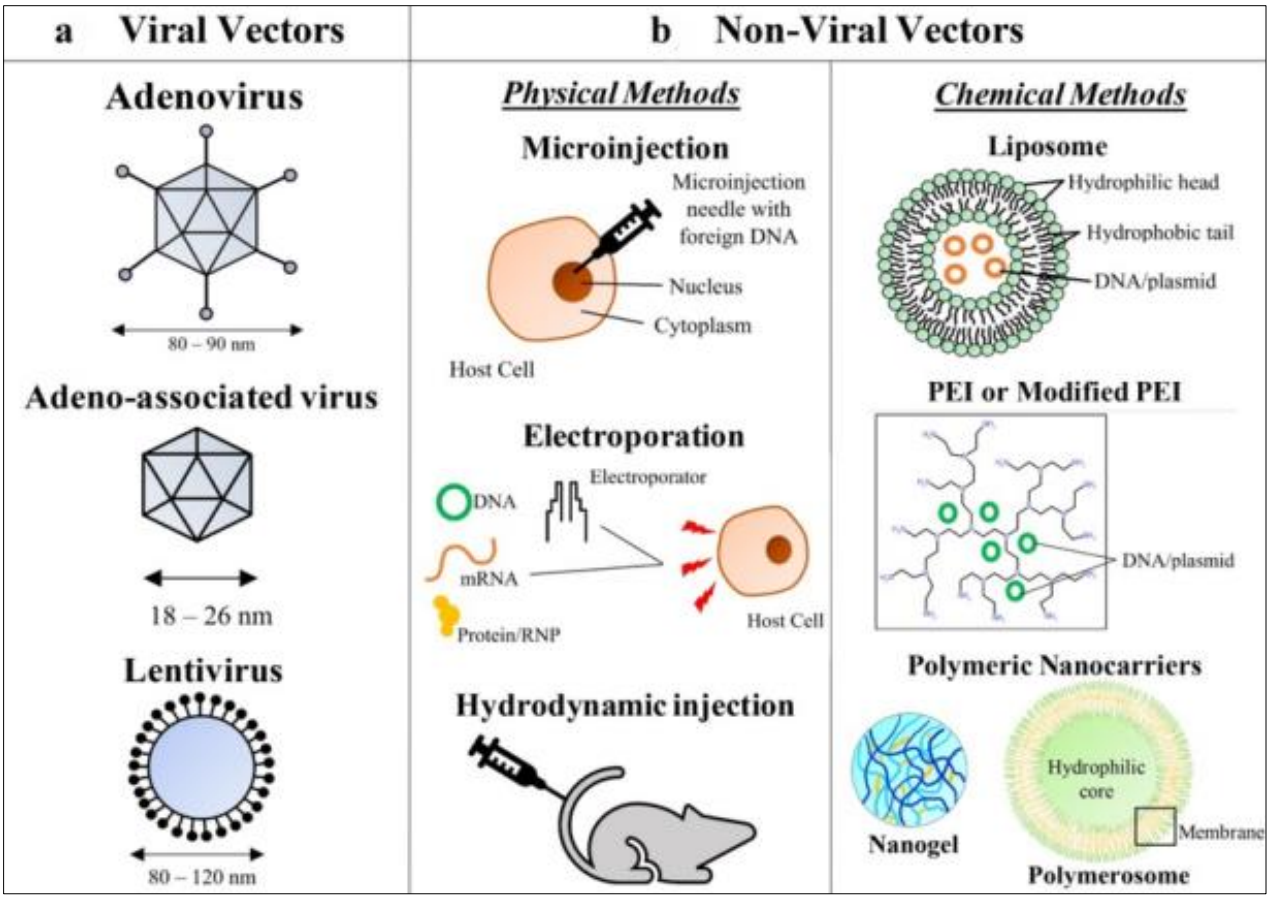

Figure 4 Comparison of CRISPR-Cas9 delivery methods. Various strategies, such as viral vectors, nanoparticles, and physical techniques like electroporation, are employed to introduce CRISPR components into target cells, each with specific advantages and challenges. Reproduced with permission from Ref. [90].

4.3. Case Studies and Translational Pipeline

While laboratory-based investigations and preclinical studies continue to demonstrate the potential of engineered probiotics and CRISPR-based systems in managing gastrointestinal (GI) infections, translating these technologies into clinical settings requires rigorous validation, safety profiling, and regulatory consideration. Case studies from experimental models and early-phase human trials highlight both the therapeutic promise and translational challenges of these microbiome-targeted interventions. From the findings of several investigators, strategic advancements in delivery methods, host compatibility, and regulatory navigation have begun to close the gap between bench and bedside.

Case reports and trials involving specific pathogen targeting, microbiome modulation, and CRISPR-mediated gene silencing offer a window into how these technologies can reshape the treatment paradigm for GI infections. At the same time, the translational pipeline reveals that success in humans hinges not only on microbial efficacy but also on immunological tolerance, mucosal biodistribution, manufacturing feasibility, and long-term microbiome equilibrium. As a result, regulatory frameworks must evolve alongside scientific innovation to accommodate the unique challenges posed by living or gene-editing therapeutics.

4.3.1. Clinical Case Study: Synlogic's SYNB1020 in Hyperammonemia and Microbiome Modulation

One of the earliest clinical-stage examples of engineered probiotic therapeutics is SYNB1020, developed by Synlogic, a synthetic biology company. Although not designed for infectious pathogens per se, SYNB1020—a genetically modified strain of *E. coli* Nissle—was engineered to consume ammonia in the gut and convert it into a nontoxic metabolite, thereby reducing systemic ammonia levels in patients with hyperammonemia [98]. According to Tan et al. [99], early-phase clinical trials demonstrated that SYNB1020 was well-tolerated, successfully colonized the gut, and reduced circulating ammonia levels in patients with liver dysfunction.

The trial outcomes also provided a roadmap for how engineered probiotics could function in more pathogen-focused settings. The live therapeutic was orally administered and remained confined to the GI tract, illustrating the potential for localized and controllable microbiome interventions. Following the discussions by Isabella et al. [100], the regulatory feedback for SYNB1020 stressed the importance of biocontainment features, such as auxotrophy for synthetic amino acids, which prevent environmental escape of the engineered strain—a principle that has since been applied to probiotic strains targeting pathogens such as *Salmonella* and *C. difficile*.

Importantly, the SYNB1020 trial underscored that engineered microbes must balance therapeutic efficacy with minimal disruption to host physiology and native microbial communities. Though designed for metabolic detoxification, SYNB1020's success laid foundational groundwork for next-generation microbial therapeutics targeting infectious disease. It also advanced discussions on dosing strategies, microbiota competition, and host-microbe interactions in the context of engineered bacterial interventions [100].

4.3.2. Preclinical Pipeline: Eligo Bioscience and CRISPR-Based Antimicrobials

Eligo Bioscience has been at the forefront of developing CRISPR-guided antimicrobial platforms, known as Eligobiotics™, which deliver precise CRISPR-Cas systems to eliminate antibiotic-resistant bacteria. According to Bikard et al. [101], these technologies use engineered bacteriophage particles to deliver DNA encoding CRISPR-Cas nucleases and specific guide RNAs to selectively kill bacteria harboring undesirable genes, such as β-lactamase or virulence genes. Preclinical studies using animal models of gut colonization showed high specificity and a reduction in resistance gene carriage without collateral damage to the overall microbiota.

From the findings of Shabbir et al. [102], Eligo's platform demonstrated effective elimination of *E. coli* carrying the *blaNDM-1* resistance gene in a murine gut colonization model, showcasing the translational relevance of CRISPR antimicrobials in complex intestinal environments. These findings have inspired similar efforts targeting *Enterococcus faecalis* and *Clostridioides difficile*, with studies incorporating improved delivery vectors and multiplexed gRNA systems to enhance targeting efficiency. As Eligo prepares for clinical entry, the company has emphasized scalable manufacturing and immune profiling of CRISPR delivery particles. A major translational milestone involved demonstrating the absence of significant inflammatory responses to the delivery vehicles in gut tissues—a critical concern given the immunogenicity of bacteriophages. Their current focus includes clinical development in indications such as *Staphylococcus aureus* nasal colonization, with GI-targeted applications likely to follow. These advancements point to a rapidly maturing translational pipeline for CRISPR antimicrobials with gastrointestinal applications [102].

Beyond *E. coli*, Eligo's Eligobiotic™ platform has expanded to tackle a range of pathogenic and antibiotic-resistant bacteria relevant to gastrointestinal infections. Following the discussions raised by Rodrigues et al. [94], researchers are now optimizing phage-derived delivery vehicles to carry multiplexed CRISPR systems targeting a wider array of resistance and virulence genes in the GI tract. This approach enables precise and combinatorial genome editing, allowing the eradication of bacterial strains harboring multiple resistance determinants without affecting commensals lacking these targets. Such fine-tuned specificity is especially important in the gut, where broad-spectrum antibiotics often cause dysbiosis and susceptibility to secondary infections like *Clostridioides difficile*.

Moreover, from the findings of Citorik et al. [91], phagemid-delivered CRISPR-Cas9 systems were shown to selectively eliminate plasmid-borne antibiotic resistance genes within a complex microbial environment, validating the feasibility of in situ genetic manipulation in gut ecosystems. These studies have catalyzed further refinements, including engineered phage capsids with gut-targeting ligands and encapsulation techniques that enhance oral stability and mucosal penetration. Eligo has reportedly integrated quorum-sensing circuits and kill-switches to ensure the self-limiting nature of their CRISPR delivery systems, addressing major biosafety concerns and facilitating eventual regulatory approval.

From a translational standpoint, Eligo's model has also emphasized the importance of preclinical data harmonization across murine, porcine, and ex vivo human gut models. This strategy aims to minimize discrepancies between model-specific microbial compositions and human gut ecology. According to Barrangou and Gersbach [103], early harmonization efforts improved the predictive power of pharmacodynamic responses and helped calibrate dosing regimens for future trials. Such rigorous modeling is instrumental in bridging the preclinical-clinical divide, especially when dealing with precision antimicrobials that interact dynamically with the host microbiome.

Collectively, Eligo Bioscience's preclinical trajectory provides a blueprint for the development and deployment of CRISPR-based antimicrobials for gastrointestinal use. Their innovations in delivery specificity, safety engineering, and translational modeling serve as a case study for how high-precision microbiome-targeted therapeutics can evolve from

concept to clinic. As their platform progresses toward first-in-human trials, it continues to influence broader scientific and regulatory discourse around the safe and effective implementation of genome-editing tools in the human gut.

4.3.3. Translational Challenges and Regulatory Considerations

Despite growing enthusiasm, the path to clinical translation for engineered probiotics and CRISPR-based antimicrobials in gastrointestinal infections is filled with complexities. Regulatory authorities such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have issued guidance on live biotherapeutic products (LBPs), emphasizing genomic stability, environmental containment, safety of genetic modifications, and manufacturing reproducibility. According to the International Council for Harmonisation (ICH) guidelines cited by Fekete et al. [104], engineered strains must undergo rigorous genotoxicity and off-target assessment, particularly for CRISPR-based interventions with the potential for unintended effects.

One major challenge is immunological: orally administered bacterial or phage vectors may induce local or systemic immune responses that diminish efficacy or introduce adverse events. From the review by Mimee et al. [105], strategies such as cloaking phages in PEGylated nanoparticles, using human-derived commensals as chassis, or integrating kill-switch systems have been proposed to mitigate these risks. Additionally, microbiome resilience and horizontal gene transfer remain unresolved concerns, particularly when genetic payloads interact with endogenous bacteria.

Manufacturing considerations also loom large. Engineered microbes and CRISPR-delivering vectors must be produced under strict GMP (Good Manufacturing Practice) conditions, with controls for plasmid stability, endotoxin levels, and microbial viability. Cold-chain storage, oral delivery formulation, and shelf-life stabilization are particularly challenging for live biotherapeutics intended for low-resource or outpatient settings. These barriers underscore the importance of interdisciplinary collaboration between microbiologists, synthetic biologists, clinicians, and regulatory experts to successfully navigate the translational pipeline [105].

5. Microbiome Editing and Systemic Infectious Diseases

The human microbiome, particularly the gut microbiota, plays a pivotal role in maintaining systemic health and modulating immune responses. Recent research has illuminated the intricate connections between the gut microbiota and various distant organs, leading to the conceptualization of axes such as the gut-lung, gut-brain, and gut-immune axes. These axes represent bidirectional communication pathways through which the gut microbiota can influence, and be influenced by, the physiological and pathological states of other organ systems [106].

Understanding these connections is crucial, especially in the context of systemic infectious diseases. Alterations in the gut microbiota, or dysbiosis, have been implicated in the pathogenesis and progression of respiratory infections, sepsis, and even neuroinflammatory conditions. Consequently, microbiome editing—through interventions like probiotics, prebiotics, and fecal microbiota transplantation—emerges as a promising therapeutic avenue to modulate these axes and improve clinical outcomes in systemic infections [106].

5.1. Gut-Lung, Gut-Brain, and Gut-Immune Axis

5.1.1. Gut-Lung Axis

The gut-lung axis refers to the bidirectional communication between the gastrointestinal tract and the respiratory system, mediated by the gut microbiota and its metabolites. This axis underscores how gut health can influence pulmonary immunity and vice versa [106]. Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, produced by the fermentation of dietary fibers by gut bacteria, play a significant role in this axis. SCFAs have been shown to enhance the function of alveolar macrophages and modulate inflammatory responses in the lungs, thereby providing protection against respiratory pathogens . Conversely, respiratory infections can lead to alterations in gut microbiota composition, indicating a feedback loop between these two systems [107].

Clinical studies have demonstrated that patients with respiratory infections, such as influenza and COVID-19, often exhibit gut dysbiosis characterized by reduced microbial diversity and an increased abundance of pathogenic bacteria . This dysbiosis can compromise gut barrier integrity, leading to systemic inflammation and exacerbation of pulmonary conditions. Therefore, maintaining a healthy gut microbiota is essential for optimal respiratory health [108,109].

5.1.2. Gut-Brain Axis

The gut-brain axis encompasses the complex communication network between the gastrointestinal tract and the central nervous system, involving neural, hormonal, and immunological pathways. The gut microbiota plays a central role in this axis, influencing brain function and behavior [110]. Microbial metabolites, such as SCFAs and neurotransmitter precursors, can cross the blood-brain barrier and modulate neuroinflammation, neurogenesis, and neurotransmission. Alterations in gut microbiota composition have been associated with various neuropsychiatric disorders, including depression, anxiety, and cognitive impairments. In the context of systemic infections, such as sepsis, dysbiosis can contribute to sepsis-associated encephalopathy (SAE), characterized by acute cognitive dysfunction [106,110].

The mechanisms underlying SAE involve the translocation of microbial products from the gut into the systemic circulation, triggering systemic inflammation and neuroinflammatory responses. Additionally, the vagus nerve serves as a critical conduit for gut-brain communication, transmitting signals from the gut microbiota to the brain and influencing neuroimmune interactions. Therapeutic strategies aimed at restoring gut microbiota balance may hold promise in mitigating neuroinflammation and improving neurological outcomes in systemic infections [110].

5.1.3. Gut-Immune Axis

The gut-immune axis highlights the integral role of the gut microbiota in shaping and modulating the host immune system. Approximately 70-80% of the body's immune cells reside in the gut-associated lymphoid tissue, underscoring the significance of gut health in immune function [111]. Commensal bacteria in the gut contribute to the development and education of the immune system, promoting the differentiation of regulatory T cells and the production of anti-inflammatory cytokines. Disruptions in the gut microbiota can lead to immune dysregulation, increasing susceptibility to infections and inflammatory diseases. For instance, gut dysbiosis has been linked to heightened inflammatory responses in the lungs during respiratory infections, as well as systemic inflammation observed in sepsis [111].

Interventions targeting the gut microbiota, such as probiotic supplementation, have shown potential in enhancing immune responses and reducing the severity of infections. By restoring microbial balance, these strategies aim to reinforce the gut barrier, modulate systemic immunity, and improve outcomes in systemic infectious diseases. The interplay between the gut microbiota and distant organ systems is mediated through distinct communication pathways known as microbiota-gut-organ axes. Table 5 summarizes the primary axes, their mechanisms of action, and their implications for systemic health.

Table 5 Gut Microbiota's Systemic Influence Through Microbiota-Gut-Organ Axes

Gut-Organ Axis	Affected System	Microbiota Mechanism	Associated Diseases	Relevance to CRISPR Therapy
Gut-Brain Axis	Central Nervous System	Production of neurotransmitters and modulation of inflammation	Depression, anxiety, neurodegenerative diseases	Potential to modulate neuroactive compound production
Gut-Lung Axis	Respiratory System	Immune system modulation via microbial metabolites	Asthma, COPD, respiratory infections	Alteration of immune-modulating microbial populations
Gut-Immune Axis	Immune System	Education and regulation of immune responses	Autoimmune diseases, allergies	Engineering of immune-modulatory microbes

5.2. Implications for Respiratory Infections, Sepsis, and HIV

5.2.1. Respiratory Infections

Respiratory infections, including those caused by bacterial, viral, and fungal pathogens, are major global health concerns, responsible for significant morbidity and mortality. The gut microbiota plays a pivotal role in modulating immune responses to respiratory pathogens, influencing both the prevention of infection and the severity of disease progression. Emerging evidence suggests that alterations in the gut microbiome can directly affect pulmonary immunity and contribute to the development of respiratory infections. Several studies have demonstrated that respiratory pathogens, such as influenza virus and Streptococcus pneumoniae, can disrupt the gut microbiota, leading to dysbiosis.

This disruption can impair the gut's ability to regulate immune responses, resulting in exaggerated inflammation and impaired pathogen clearance in the lungs. For example, research by Liu et al. [112] showed that gut microbiota depletion in animal models led to a heightened inflammatory response in the lungs during influenza infection, exacerbating the disease. Furthermore, respiratory infections such as COVID-19 have been shown to induce shifts in gut microbiota composition, with a reduction in beneficial microbial species like Lactobacillus and Bifidobacterium, alongside an increase in pro-inflammatory bacterial taxa [113]. These changes contribute to the development of gut inflammation, which in turn exacerbates systemic inflammation and worsens the outcome of respiratory infections. Thus, modulating the gut microbiota through dietary interventions, probiotics, or fecal microbiota transplantation (FMT) could represent a promising therapeutic strategy for preventing and managing respiratory infections [113].

5.2.2. Sepsis

Sepsis, a life-threatening systemic inflammatory response to infection, is often triggered by the translocation of pathogens from the gut to the bloodstream. The gut microbiota is central to the development of sepsis, as alterations in its composition can disrupt the gut barrier function, facilitating the translocation of bacteria and their endotoxins into the bloodstream. Research has highlighted the critical role of the gut-immune axis in regulating systemic inflammatory responses during sepsis. In sepsis, gut dysbiosis is commonly observed, with an overgrowth of pathogenic bacteria and a decrease in beneficial microbes. According to studies by Zhou et al. (2019), dysbiosis in sepsis patients was associated with impaired immune responses, characterized by reduced regulatory T-cell (Treg) function and heightened inflammation in systemic tissues [114]. Furthermore, the loss of gut barrier integrity, caused by microbial imbalances, increases gut permeability, allowing harmful microorganisms and their products to enter the bloodstream, initiating a cascade of systemic inflammatory responses.

Therapeutic strategies targeting the gut microbiome, such as the administration of probiotics or antibiotics, have been explored as potential adjuncts to sepsis treatment. For instance, the administration of Lactobacillus and Bifidobacterium species has been shown to restore gut microbiota balance, improve gut barrier integrity, and reduce systemic inflammation in animal models of sepsis [114]. Moreover, FMT has demonstrated efficacy in restoring microbial diversity and enhancing immune responses in sepsis patients, providing a promising direction for clinical interventions aimed at modulating the microbiome to prevent sepsis-related complications.

5.2.3. HIV

Human immunodeficiency virus (HIV) infection is characterized by chronic immune activation and progressive immune dysfunction, leading to acquired immunodeficiency syndrome (AIDS). Recent studies have revealed a significant interaction between the gut microbiota and HIV pathogenesis. Gut dysbiosis in HIV-infected individuals has been linked to increased systemic inflammation, impaired immune responses, and an accelerated progression of the disease. Research by Nwosu et al. [115] highlighted that HIV infection is associated with reduced microbial diversity in the gut, with an overrepresentation of pro-inflammatory bacterial taxa, such as Enterococcus and Streptococcus, and a depletion of beneficial microbes, including Firmicutes and Bifidobacterium [115,116]. This microbial imbalance contributes to the disruption of the gut barrier, leading to the translocation of microbial products into the bloodstream, which exacerbates systemic inflammation and immune activation in HIV-infected individuals. Furthermore, gut dysbiosis in HIV is associated with poor responses to antiretroviral therapy (ART) and an increased risk of opportunistic infections and comorbidities, such as cardiovascular disease and metabolic disorders. Restoring gut microbiota balance through interventions like probiotics, prebiotics, and FMT has shown promise in reducing systemic inflammation and improving immune function in HIV patients, suggesting that microbiome modulation could complement ART and improve patient outcomes [116].

5.3. Animal and Early Human Studies

5.3.1. Animal Studies in Microbiome Modulation

Animal studies are fundamental in understanding the impact of microbiome modulation on systemic infections. Research in animal models has provided significant insights into the mechanisms through which the gut microbiome influences systemic diseases such as respiratory infections, sepsis, and HIV. Various animal models, including mice, rats, and non-human primates, have been used to examine how changes in microbiota composition affect immune responses and disease outcomes. In particular, mouse models have been extensively utilized to investigate the role of gut microbiota in respiratory infections. For instance, studies by Huang et al. [117] demonstrated that the transfer of specific microbiota from healthy mice to antibiotic-treated mice could restore lung immunity and reduce susceptibility to respiratory infections, such as pneumonia and influenza. This finding highlights the profound influence of gut microbiota on pulmonary immune function, underscoring the therapeutic potential of microbiome modulation for respiratory diseases [117].

Additionally, the role of gut microbiota in sepsis has been examined in animal models where interventions such as probiotic supplementation or fecal microbiota transplantation (FMT) have been shown to mitigate the severity of sepsis. In a study by Piccioni et al. [118], mice treated with probiotics exhibited a reduction in systemic inflammation and an improvement in survival following sepsis induction, demonstrating the potential of microbiome-targeted therapies for managing sepsis [116-118].

5.3.2 Early Human Studies in Microbiome Therapeutics

The translation of microbiome-based therapies from animal models to human studies is a crucial step in evaluating the clinical efficacy of these approaches. Several early human studies have explored the potential of microbiome modulation for the treatment of systemic infectious diseases, particularly through interventions like probiotics, prebiotics, and FMT. These studies have laid the groundwork for future clinical trials aimed at utilizing microbiome-based therapies to prevent or treat infections. One of the earliest clinical studies to examine the role of probiotics in modulating the human microbiome during infection was conducted by Zhang et al. [119]. Their study demonstrated that the administration of Lactobacillus and Bifidobacterium strains to patients with respiratory infections resulted in improved immune responses, including enhanced production of interferon-γ and reduced bacterial load. These results supported the idea that manipulating the gut microbiome with probiotics could enhance the body's defense against respiratory pathogens [119].

In another early human study, a cohort of HIV-infected patients was treated with FMT to restore gut microbial diversity. According to the findings of Xiao et al. [120], FMT resulted in significant improvements in gut microbiome composition, with enhanced immune responses and decreased microbial translocation, suggesting that microbiome restoration can complement traditional antiretroviral therapy (ART) in managing HIV. These findings have paved the way for future clinical trials investigating the use of microbiome modulation to improve ART efficacy and mitigate inflammation in HIV patients [120].

5.3.2. Challenges and Considerations in Translating Animal Findings to Humans

While animal studies have provided valuable insights into the potential of microbiome-targeted therapies for systemic infections, several challenges remain in translating these findings to humans. One of the major challenges is the complexity and variability of the human microbiome, which differs significantly from that of animal models. This variability makes it difficult to predict the success of microbiome-modulating interventions in humans based on animal data alone [121].

Moreover, the safety and long-term effects of microbiome-based therapies in humans remain uncertain. For example, although FMT has shown promise in treating conditions like Clostridioides difficile infection, there are concerns about the potential transmission of unknown pathogens or the induction of harmful immune responses following microbiome transplantation [122]. As such, rigorous clinical trials and long-term monitoring are needed to assess the safety and efficacy of these therapies before widespread adoption. Another challenge is the standardization of microbiome-based interventions. While studies have demonstrated the efficacy of probiotics and FMT in small cohorts, there is significant variability in the strains used, the dosages administered, and the methods of microbiome restoration. According to the research conducted by Khoruts et al. [123], future studies must address these variables to optimize the clinical applications of microbiome therapies. A more standardized approach to microbiome modulation could help ensure more consistent outcomes across diverse patient populations.

6. Future Perspectives and Emerging Trends

The field of microbiome-targeted gene editing is rapidly evolving, with novel concepts and interdisciplinary strategies paving the way for more precise, responsive, and individualized therapeutics. As fundamental tools such as CRISPR-Cas systems continue to mature, their integration into synthetic ecology, artificial intelligence-driven design, and personalized medicine offers a transformative outlook for managing both infectious and non-infectious diseases.

6.1. CRISPR-Based Synthetic Ecology

One of the most promising directions is the development of CRISPR-based synthetic ecologies—engineered microbial communities equipped with programmable genetic circuits capable of dynamically responding to environmental and host cues. Unlike traditional mono-strain interventions, synthetic ecologies can perform coordinated tasks such as biosensing, pathogen suppression, or metabolite production in a distributed fashion. This approach allows for the establishment of resilient, self-regulating consortia that interact symbiotically with the native microbiome [124]. By programming these consortia with CRISPR tools, researchers can ensure modular control over gene expression, quorum

sensing pathways, and interspecies communication. For example, CRISPR interference (CRISPRi) modules have been used to fine-tune metabolic outputs in synthetic gut consortia, reducing toxicity and improving community stability [124,125].

Such frameworks could ultimately enable the creation of "living therapeutics" that act more like adaptive biological devices than static treatments. However, realizing this potential will require overcoming challenges in microbial compatibility, gene circuit stability, and biocontainment—necessitating collaboration between synthetic biologists, microbiologists, and systems engineers.

6.2. Integration with AI for Microbial Circuit Design

Artificial intelligence (AI) is increasingly being leveraged to accelerate the design and optimization of microbial gene circuits. Machine learning algorithms can predict functional relationships between genes, metabolites, and environmental inputs, allowing for the de novo design of CRISPR-based constructs with minimal trial-and-error [126]. In microbiome editing, AI tools are already being used to model community responses to perturbations, identify editing targets based on ecological importance, and simulate long-term effects of interventions. These capabilities are especially critical for designing multi-layered genetic programs that interact with complex and variable host environments [126].

Furthermore, AI-driven tools are proving useful in metagenomic analysis and functional annotation of novel microbial genes, which expands the toolbox available for editing and synthetic biology applications. With advances in natural language processing and large biological language models, it is now feasible to automatically generate hypotheses for gene function or predict CRISPR guide efficacy across diverse microbial taxa. In the future, AI and CRISPR could form a powerful closed-loop system—where data-driven models not only guide the initial design of gene edits but also adaptively refine them based on real-time host and microbiome feedback [127].

6.3. Towards Personalized Microbiome Therapeutics

The move toward personalized microbiome editing is gaining momentum as researchers recognize the vast inter-individual variability in microbiota composition, function, and interaction with host immunity. Personalized approaches involve tailoring gene editing tools and microbial chassis to the specific microbial landscape and health status of each patient. Techniques such as strain-resolved metagenomics and single-cell microbiome analysis are enabling high-resolution mapping of patient-specific microbiota, facilitating the selection of optimal editing targets and delivery strategies [128].

Moreover, individualized gene therapies may benefit from personalized delivery vehicles such as custom phage cocktails or engineered probiotics designed to colonize and persist within a host-specific microbial context. Early trials in microbiome transplantation and targeted bacteriotherapy are already hinting at the clinical feasibility of such approaches. In the near future, it is conceivable that diagnostic microbiome profiling will be routinely used to guide therapeutic editing regimens, much like pharmacogenomics guides cancer therapy today [129].

However, implementing personalized microbiome therapeutics on a large scale will require not only robust regulatory frameworks and standardized diagnostics but also ethical considerations regarding privacy, equity, and consent. As technological capacity grows, the convergence of CRISPR, AI, and precision microbiome medicine may redefine how we treat infectious diseases, inflammatory disorders, and even neurodegenerative conditions—ushering in a new era of living, programmable medicine [128].

7. Conclusion

The emergence of precision microbiome editing represents a transformative shift in how infectious diseases are understood, diagnosed, and treated. By leveraging tools such as CRISPR-Cas systems, engineered probiotics, synthetic gene circuits, and phage-based vectors, researchers have unlocked new possibilities for modulating microbial ecosystems with high specificity. This progress has enabled the development of novel strategies to suppress pathogenic organisms, restore healthy microbial balance, and enhance host immunity—especially in the context of gastrointestinal and systemic infections. The integration of real-time sensing, dynamic response circuits, and AI-guided design has further accelerated the translation of these technologies from the bench to preclinical and early clinical stages.

Despite these promising advances, significant challenges remain. One of the foremost concerns is the safe and efficient delivery of editing tools to target microbial populations in vivo without off-target effects or disruption of beneficial species. Barriers such as phage-host specificity, immune clearance, and ecological unpredictability complicate deployment. The risk of horizontal gene transfer and unintended ecological shifts also raises biosafety and regulatory

questions that must be carefully addressed. Additionally, much of the current work remains confined to proof-of-concept studies in controlled settings, with limited data on long-term efficacy and safety in complex, real-world microbial communities.

Looking forward, the vision for precision microbiome editing is one in which programmable microbial therapeutics are personalized to each patient's microbiota, responsive to disease states, and dynamically modulated by host-microbe interactions. As our understanding of microbial ecology, host physiology, and gene-editing mechanics deepens, this field is poised to reshape infection control from a one-size-fits-all antibiotic paradigm to a finely tuned, systems-level approach. With interdisciplinary collaboration, robust regulation, and ethical foresight, microbiome-targeted gene editing holds the potential to redefine not only infectious disease therapeutics but also the broader landscape of preventive and precision medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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