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UNLEASHING THE POTENTIAL OF CRISPR CAS9: REGULATION AND OVERCOMING ANTIMICROBIAL RESISTANCE

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ABSTRACT Globally, antibiotic resistance has grown to alarmingly high levels. In order to address this issue, researchers are focusing on Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing technology based research to make it possible to eliminate and combat antibiotic-resistant bacteria almost as rapidly as antibiotic-sensitive germs. Cas9 with nuclease activity, can be programmed with a particular target sequence. This defence system protects commensal bacteria while targeting pathogens present in the microbiome. The present article explores the current status of CRISPR antimicrobial research, the potency of CRISPR antimicrobials in infection and colonization models, and the diverse delivery mechanisms for these potentially useful therapeutic tools.

KEYWORDS :

INTRODUCTION:

Rapid advancements in gene editing technologies make precise and direct DNA editing a practical possibility. CRISPR, which stands for clustered regularly interspaced short palindromic repeats, was first used in 2002 (Gostimskaya 2022). The CRISPR-Cas system has been deemed an adaptive immune system that helps prokaryotes fight invasive genetic elements (mostly viruses and plasmids) by eliminating foreign DNA and RNA. CRISPR-Cas systems are divided into two groups (1 and 2) based on the variations in effecter module complexity (Makarova *et al.,* 2020). The class 2 type II, V, and VI systems use a single multi-domain effector to carry out target destruction, whereas the class 1 systems, which include the type I, III, and IV systems, use a multi-subunit effector complex in conjunction with an additional Cas nuclease to eliminate the target nucleic acids (Chaudhuri *et al.*, 2022). A CRISPR-Cas system typically consists of a CRISPR locus and its corresponding cas operon, which perform immunity in three stages: adaptation, synthesis of CRISPR RNA (crRNA), and interference (Fig.1).

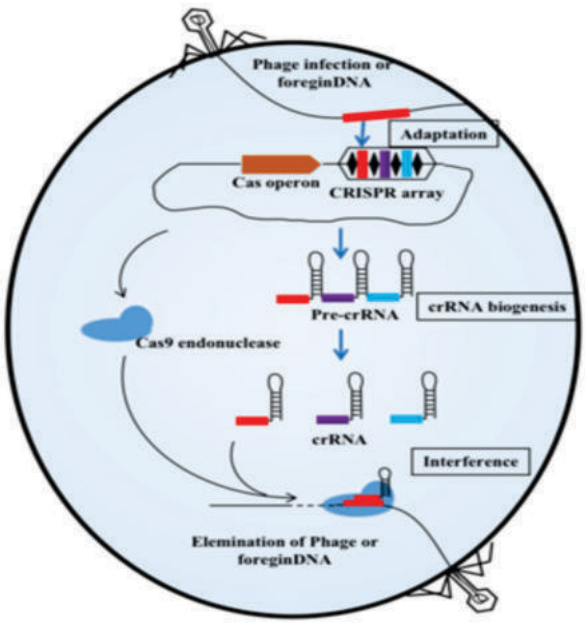

Fig.1: Phases of the CRISPR-Cas adaptive immunity. Three phases of CRISPR-Cas immunity: adaptation, crRNA biogenesis, and interference. A new spacer (red) is produced by the CRISPR array by the inclusion of a DNA fragment of the invasive genetic element—such as a phage genome—during adaptation. The whole CRISPR array is translated into a pre-crRNA during crRNA synthesis, which is then processed into mature crRNAs. By base pairing, the crRNA precisely identifies a target protospacer sequence in the invaders during interference, directing the Cas effector to destroy the targets.

Among the CRISPR types, many groups have since altered the CRISPR/Cas9 technology and used it for gene editing. Because of its high specificity, high effectiveness, and ease of reprogramming, the CRISPR system has been used in gene editing more frequently than transcriptional activator-like effectors (TALENs) and zinc finger nucleases (ZFNs) (Xu and Li., 2020). Since CRISPR technology alters the base sequence of a brief guide RNA segment to direct Cas proteins to a specific location in the genome, it improves gene editing efficiency and broadens the range of applications for gene editing, in contrast to ZFNs and TALENs, which target DNA strands using proteins (Li *et al.,* 2023).

The global health is being threatened by antimicrobial resistance (AMR). AMR pathogens are often endowed with complex intrinsic and adaptive resistance mechanisms in addition to the ability to readily obtain transmissible AMR genes from the environment (Preethi et al., 2017). This resistance gives the pathogens a great ability to withstand and grow in the face of regular antimicrobial chemotherapy treatments and to continue infecting people (WHO 2021). Other cutting-edge and potent antimicrobial techniques that can replace antibiotics are desperately needed to alleviate the worldwide crisis of antimicrobial resistance since the discovery of new antibiotics is significantly slower than the emergence of bacterial resistance to them. Notably, more research has revealed that in recent years, CRISPR-Cas systems have emerged as one of the most promising options to address antibiotic resistance (Chandran and Gopal, 2023). In this paper, we provide an overview of the latest developments in the CRISPR-Cas antimicrobials and the primary obstacles to their practical application as well as possible remedies.

CRISPR Cas9 based Antimicrobial modules:

A single effector protein is all that is needed for the type II system, which is much more versatile. Studies show that the most popular and well-studied type II system, CRISPR-Cas9, can be designed to specifically eliminate the AMR *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp (ESKAPE) pathogens (Shetty *et al.,* 2023).

Studies on the delivery of Cas9 and mini-CRISPR components using phagemids have shown that CRISPR-Cas9 antimicrobials can both cure specific strains of AMR plasmids and selectively eliminate AMR pathogens in mixed populations. Additionally, the efficacy of the CRISPR-Cas9 antimicrobials was evaluated by looking at how well they eliminated *S. aureus* on mouse skin and enterohemorrhagic *E. coli* in Galleria mellonella larvae, respectively. This demonstrated the systems' enormous potential for use in clinical therapies (Bikard *et al.,* 2014; Citorik *et al.,* 2014).

Delivery Mechanism for CRISPR Cas9 Antimicrobials:

CRISPR-Cas9 delivery involves the CRISPR payload and the methods of delivering it into the cells. CRISPR-Cas9 can be delivered using :

Conjugative Plasmid mediated delivery:

The conjugative plasmid, which can transmit genetic material between bacterial cells, is one of the delivery vehicles. The plasmid uptake does

not require receptors during conjugation. As a result, resistance to delivery because of receptor modifications will not exist in plasmid-based delivery (Pereira *et al.,* 2021). However, some other difficulties, such as plasmid conjugation's limited host range and low delivery efficiency, exists (Wongpayak *et al*., 2021).

Phage mediated delivery:

Bacterial pathogens frequently have large phage populations adept at injecting DNA into host bacterial cells. Phages are thus viewed as the most potential technique for CRISPR-Cas antimicrobial module delivery.

Although increasing evidence proves that phage-based delivery of CRISPR-Cas antimicrobials can be used to eliminate AMR plasmids or AMR infections, there are several limits to the therapeutic applications of CRISPR-Cas antimicrobial modules via phage-based delivery strategy (Hua *et al*., 2017). The size of the phage capsid appears to be proportional to the size of the phage genome. Furthermore, Most phage species have limited host ranges because phage absorption, the initial stage in phage infection, is controlled by the interaction of a phage receptor-binding protein with a particular receptor on the host cell membrane. The occurrence of phage-receptor protein interaction suggests that a specific phage may be required to treat a specific disease. Understanding the mechanisms behind phage absorption and then altering existing phages that have already demonstrated a high ability to deliver CRISPR-Cas antimicrobials could potentially increase their host range. A further concern with phage delivery is that it may transfer not only the required CRISPR-Cas elements but also chromosomal segments from the host that serve for phage propagation into target cells, raising concerns about the safety of spreading virulence factor genes (Mayorga-Ramos *et al.*, 2023).

Nano-particle mediated delivery:

Another method is to use nanoparticles to deliver Cas effectors and crRNA molecules directly into the target bacterial cells. With the rapid development of nanotechnology, numerous nanoparticles, such as cationic polymer-based nanoparticles and inorganic nanoparticles, have become readily available for the transfer of CRISPR-Cas system components(Lee *et al*., 2017; Rahimi *et al*., 2020). It was demonstrated that a cationic polymer-based nanosized CRISPR complex containing the Cas9 protein and crRNA can be successfully introduced into MRSA in vitro and is functional to kill bacteria by targeting the methicillin-resistant gene (Kang et al., 2017). Yet, nanoparticle-based CRISPR-Cas delivery is still in its early stages, and many questions remain unresolved such as the way to enhance the encapsulation rate and achieve efficient delivery into unusual pathogens, such as Mycobacterium tuberculosis, which has exceptionally thick and extremely impermeable cell walls (Chiaradia et al., 2017).

Prospects for the future

CRISPR-Cas antimicrobial modules offer multiple potential advantages over traditional antibiotics. CRISPR-Cas systems are exceedingly diverse, with at least 33 subtypes (Makarova et al., 2020), and current exploitations are still in their early stages, with a focus on the most prevalent forms, such as type I-E and I-B, type II Cas9, type V Cas12 and type VI Cas13a systems.

More CRISPR-Cas forms, we believe, could be investigated in order to obtain varied antibacterial uses. Once the delivery and targeting efficiency challenges are overcome, we anticipate that CRISPR-Cas systems will be designed as the most effective antimicrobial module to control the composition of the gut microbiome by distinguishing pathogenic and beneficial bacteria, eradicate AMR pathogens, and prevent the spread of AMR genes in future medical applications.

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Disclosure Statement:

The authors declare no conflicts of interest.

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