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Review article

Role of CRISPR-Cas systems and anti-CRISPR proteins in bacterial antibiotic resistance

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A B S T R A C T

The emergence and development of antibiotic resistance in bacteria is a serious threat to global public health. Antibiotic resistance genes (ARGs) are often located on mobile genetic elements (MGEs). They can be transferred among bacteria by horizontal gene transfer (HGT), leading to the spread of drug-resistant strains and antibiotic treatment failure. CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated genes) is one of the many strategies bacteria have developed under long-term selection pressure to restrict the HGT. CRISPR-Cas systems exist in about half of bacterial genomes and play a significant role in limiting the spread of antibiotic resistance. On the other hand, bacteriophages and other MGEs encode a wide range of anti-CRISPR proteins (Acrs) to counteract the immunity of the CRISPR-Cas system. The Acrs could decrease the CRISPR-Cas system's activity against phages and facilitate the acquisition of ARGs and virulence traits for bacteria. This review aimed to assess the relationship between the CRISPR-Cas systems and Acrs with bacterial antibiotic resistance. We also highlighted the CRISPR technology and Acrs to control and prevent antibacterial resistance. The CRISPR-Cas system can target nucleic acid sequences with high accuracy and reliability; therefore, it has become a novel gene editing and gene therapy tool to prevent the spread of antibiotic resistance. CRISPR-based approaches may pave the way for developing smart antibiotics, which could eliminate multidrug-resistant (MDR) bacteria and distinguish between pathogenic and beneficial microorganisms. Additionally, the engineered anti-CRISPR gene-containing phages in combination with antibiotics could be used as a cutting-edge treatment approach to reduce antibiotic resistance.

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1. Introduction

Sharing genetic material between organisms that are not in a parent-offspring relationship is known as horizontal gene transfer (HGT) [1]. Acquiring genetic material, such as antibiotic resistance, adaptation, and virulence genes through HGT, is crucial in bacteria adapting to diverse environments [2]. By exchanging genetic material across genera, HGT contributes to the spread of antibiotic resistance genes (ARGs), which increases the likelihood that a harmful, antibiotic-resistant bacteria will emerge [3]. A wide spectrum of environmental and commensal bacteria, as well as mobile genetic elements (MGEs), contain ARGs [4] that can be transferred among bacteria through different mechanisms of HGT, including transformation (uptake of free DNA), transduction (phage-mediated transfer), and conjugation (plasmid-mediated transfer) [5,6]. However, bacteria have developed numerous defense mechanisms under long-term selection pressure for genome protection that restrict both phage infection and HGT [7]. These include established systems such as CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated genes) and restriction-modification systems, and a wide range of recently discovered systems [8–10]. CRISPR-Cas systems could restrict the acquisition of MGEs and provide acquired and heritable immunity against foreign nucleic acids. A typical CRISPR-Cas system is comprised of three components: i) a CRISPR array ii) an operon containing a group of *cas* genes, and iii) a leading sequence. A CRISPR array typically consists of highly conserved and short (23–47 bp) direct repeats (DR) that are separated by non-repetitive DNA fragments complementary to the target site (spacers) acquired from MGEs [11–13]. The AT-rich leader sequence (100–350 bp) is commonly adjacent to the CRISPR array and acts as a promoter to initiate the CRISPR array transcription [14]. The number of *cas* genes in the CRISPR-Cas locus is variable, often located next to the CRISPR repeat-spacer and encodes corresponding Cas proteins. These proteins possess an analogous variety of enzymatic domains with polymerase, helicase, or nuclease activity that play a crucial role in the immune response of the CRISPR system [15,16]. On the other hand, in response to CRISPR defense, MGEs have evolved anti-CRISPR systems. Over 50 entirely distinguished anti-CRISPR (Acr) protein families, which can inhibit different CRISPR systems, have been identified in plasmids, phages, archaeal and bacterial genomes [17]. These proteins were first identified in the genome of phages infecting opportunistic pathogen *Pseudomonas aeruginosa*, which allowed phages to evade the CRISPR-Cas system [17].

Since their discovery in 1928, antibiotics have consistently been regarded as the preferred tool for treating common infections [18]. As the world progressed, antibiotics became increasingly important in agriculture, animal husbandry, food production, and the health care systems. One of the most significant worldwide crises, antibiotic resistance, is the result of the race between microorganisms and antibiotics [19,20]. Misuse and over-prescription of antibiotics, inadequate surveillance, and poorly controlled regulation have increased antibiotic resistance in the environment and medical facilities [21,22]. Antibiotic resistance development in bacterial

Table 1
Correlation between the CRISPR-Cas system and antibiotic resistance in bacteria.

Antibiotic resistance	Organism	CRISPR-Cas type	Strategy	Reference
Positive Correlation	*Campylobacter jejuni*	Type II CRISPR-Cas system	Regulating ribosomal proteins	[58]
	Francisella novicida	Type II CRISPR-Cas system	Increasing envelope integrity by regulation of a bacterial lipoprotein	[59]
	Salmonella enterica Serovar Enteritidis	Type I-E CRISPR-Cas system	Regulation of the genes involved in membrane integrity	[60]
	Vibrio cholerae	Type I-E CRISPR-Cas system	Facilitating the uptake of ARGs	[61]
	Neisseria meningitidis	Type II CRISPR-Cas system	Facilitating the uptake of ARGs	[61]
	Aggregatibacter actinomycetemcomitans	Type I–F CRISPR-Cas system	providing cells with a potent mechanism of HGT by the competence system	[62]
	Staphylococcus epidermidis	Type III-A CRISPR-Cas system	Generating mutations via nonspecific DNase activity	[63]
Negative Correlation	*Klebsiella pneumonia*	Type I-E CRISPR-Cas system	Inhibition of the transformation of *bla*^{KPC} plasmids	[49]
	Pseudomonas aeruginosa	Type I CRISPR-Cas system	Hindering the acquisition of ARGs	[64]
	Acinetobacter baumannii	Type I-Fb CRISPR-Cas system	Targeting mRNA of the quorum sensing master regulator *abaI*	[57]
	Escherichia coli	Type I–F CRISPR-Cas system	Interfering with antimicrobial resistance plasmids	[65]
	Enterococcus faecalis	Type II CRISPR-Cas system	Hindering the acquisition of ARGs	[51]
	Mycobacterium smegmatis	Type III-A CRISPR-Cas system	Reducing the drug-induced persistence	[66]
	Streptococcus pyogenes	Type II CRISPR-Cas system	Hindering the acquisition of ARGs	[67]
	Streptococcus pneumoniae	Type II CRISPR-Cas system	Hindering the acquisition of ARGs	[68]
	Staphylococcus aureus	Type III-A CRISPR-Cas system	Inhibition of the plasmid transfer in a transcription-dependent manner	[69]

pathogens has become a challenge worldwide that threatens human safety and health and causes considerable problems in treating infectious diseases [23,24]. The threat of antibiotic resistance is predicted to grow in the future decades, leading to more than 10 million deaths per year by 2050 [25]. In addition, conventional antimicrobials can cause substantial collateral damage to the commensal human microbiota because they target beneficial bacteria in addition to pathogenic bacteria. On the other hand, developing new antibiotics and proving their effectiveness against evolving drug-resistant pathogens is time-consuming and costly [18,26–29]. Consequently, new bactericidal strategies are needed to avoid the spread of antibiotic resistance and treat bacterial infections which can target detrimental bacteria and have minimum effect on beneficial bacteria and the patient. Phage therapy [30,31], zinc finger nucleases (ZFNs) [32,33], peptide nucleic acid (PNA) as an ultra-narrow-spectrum antibiotic [34–36], and CRISPR-Cas system [37] could be promising approaches against the growing challenges of antibiotic resistance prevalence. Among these, CRISPR-Cas systems have become the most popular genome editing technology in molecular biology labs worldwide because of their straightforward design, high efficiency, short-cycle, good repeatability, and low cost [18,38,39]. The evolving picture points to the possibility of using the CRISPR-Cas9 system in a sequence-specific manner to either re-sensitize bacteria to antibiotics or selectively remove individual bacterial strains from a mixed bacterial population [40–42]. Furthermore, the discovery of Acrs has attracted the attention of researchers as a promising therapeutic agent against multidrug-resistant (MDR) bacteria [43,44]. Acrs can broaden the host range of phage therapeutics by inhibiting the CRISPR-Cas system; therefore, they could be used to enhance phage therapy strategies [45]. It is now crucial to look at alternatives to antibiotics to treat MDR bacterial infections because conventional antimicrobials are no longer effective due to the rapidly spreading antibiotic resistance genes, and developing novel antibiotics is not financially viable. Therefore, in this review, we aimed to assess the relationship between the CRISPR-Cas systems and Acrs with antibiotic resistance. We then highlighted the CRISPR technology and Acrs to control and prevent antibacterial resistance.

2. Role of CRISPR-cas systems in the bacterial antibiotic resistance

2.1. Negative correlation between the CRISPR-cas system and antibiotic resistance

The CRISPR-Cas system may affect antibiotic resistance among different bacterial species (Table 1). The potential of the CRISPR-Cas system to inhibit the uptake of beneficial MGEs (e.g. ARGs) has been extensively discussed [46–48]. There is evidence that the CRISPR-Cas system inhibits the spread of ARGs in some bacterial species. In this regard, Mackow et al. [49] demonstrated that the presence of CRISPR-Cas systems inhibits the transformation of *bla*^{KPC} plasmids in carbapenem-sensitive Klebsiella pneumonia (CS-Kp) strains. CRISPR-Cas systems are dramatically more common in CS-Kp strains than in Carbapenem-resistant K. pneumonia (CR-Kp) strains, leading to high pan-sensitivity to other antibiotics in CS-Kp strains. They also found that the CRISPR-Cas system was absent in most clinical *K. pneumonia* strains including the clinically important ST258 clone [49,50]. In addition, several studies have shown that CRISPR-Cas systems significantly impact the spread of ARGs in enterococci [51,52]. CRISPR1-Cas, CRISPR3-Cas, and orphan CRISPR2 (lack of cas genes) are three types of CRISPR systems found in the enterococci genome. Genetic analysis has shown that CRISPR3-*cas* could be a potent barrier to the horizontal acquisition of ARGs in *Enterococcus faecalis* [52,53]. Price et al. [51] demonstrated that MDR *E. faecalis* strains quickly acquire conjugative antibiotic resistance plasmids due to the lack of genome defense mechanisms. They confirmed that CRISPR-Cas and restriction-modification (R-M) systems, both individually and collectively, have considerable effects on conjugative plasmid transfer in *E. faecalis* [51]. Similarly, in *Acinetobacter baumannii*, the type I-Fb CRISPR-Cas systems can block the dissemination of ARGs [54–56]. Recently, Wang et al. [57] have suggested that the Cas3 nuclease of type I-Fb CRISPR-Cas system of *A. baumannii* targets mRNA of the quorum sensing (QS) master regulator *abaI* (QS synthase), leading to increasing bacterial antibiotic sensitivity. As a result of QS, synthase abaI is decreased, allowing efflux pumps to reduce, reactive oxygen species to generate, biofilm formation to become weaker, and drug resistance to reduce in response to the activity of the CRISPR-Cas system [57].

The negative impact of the CRISPR systems on antibiotic resistance has also been demonstrated in *Escherichia coli*. Two subtypes of CRISPR-Cas systems have been identified in *E. coli*, type I–F and type I-E [70,71]. Aydin et al. [65] revealed that type I–F CRISPR-Cas systems are potentially associated with antibiotic susceptibility in *E. coli*. They found that most isolates containing type I–F CRISPR-Cas system had spacer sequences matching IncI and IncF plasmids carrying ARGs. Additionally, the CRISPR-Cas systems widely exist in *Shigella* species and share homology with that in *E. coli* [72,73]. The results of a study showed that point mutations in the *cas1* and *cas2* genes enhance the degree of drug resistance in *Shigella* species [72]. These data indicate that pathogens containing CRISPR-Cas systems are less likely to carry antibiotic resistance genes than those lacking these defense systems. However, Touchon et al. [70] found no meaningful correlation between the presence/absence of CRISPR systems and the presence of integrons, plasmids, or antibiotic resistance in *E. coli*. These discrepancies in the results of different studies may be due to the presence of Acrs, the CRISPR locus generation via total or partial deletion in the *cas* genes cluster, and distinct evolutionary histories of the CRISPR-Cas system [70,74]. In addition, under strong selective pressure exerted by antibiotics, the CRISPR-Cas system may be lost or disabled and not be an effective barrier to spreading plasmid and drug resistance [75]. Therefore, analysis of the correlations between the CRISPR-Cas system and antibiotic resistance can help to identify and better understand antibiotic resistance mechanisms and provide new insights to prevent and treat bacterial antibiotic resistance.

2.2. Role of CRISPR-cas systems in increasing bacterial antibiotic resistance

The impact of CRISPR-Cas systems on antibiotic resistance may differ among different bacterial species. Several studies have demonstrated that the CRISPR-Cas system may be also involved in increasing antibiotic resistance with different approaches (Table 1). For example, Sampson et al. [59] confirmed the pivotal role of the CRISPR-Cas systems in antimicrobial resistance. They indicated that

the Cas9-dependent CRISPR-Cas system of *Francisella novicida* increases envelope integrity by regulating a bacterial lipoprotein. The Cas9 targets the endogenous transcripts for a bacterial membrane lipoprotein known as *FTN_1103* BLP, leading to promoting resistance to several membrane stressors such as membrane-targeting antibiotics. The Cas9 regulatory axis-mediated envelope enhancement is crucial during infection and promotes both evasion of the host's innate immune (inflammasome and Toll-like receptor-2) and virulence. Therefore, gene regulation mediated by the CRISPR-Cas system may contribute to the interaction of *F. novicida* with the host's eukaryotic cell [59]. Similarly, Shabbir et al. [58] demonstrated that the *cas9* gene is directly associated with antimicrobial resistance in *Campylobacter jejuni* by regulating the ribosomal proteins. In the absence of Cas9, the permeability of the *C. jejuni* envelope is increased, similar *to F. novicida*, leading to more susceptibility of the *C. jejuni* to erythromycin as the first-line treatment for campylobacteriosis [59,76].

Moreover, the CRISPR-Cas systems may facilitate the uptake of ARGs. In this regard, Shehreen et al. [61] demonstrated that the CRISPR-Cas system in *Neisseria meningitidis* and *Vibrio cholerae* is positively associated with some ARGs. It's possible that the apparent positive effects of the CRISPR-Cas system on ARGs in these strains could be due to the increased acquisition of ARGs through transduction [61]. In another study, Pursey et al. [63] demonstrated that type III-A CRISPR-Cas systems in *Staphylococcus epidermidis* are associated with increased numbers of ARGs compared to genomes lacking CRISPR-Cas systems. Recent research has indicated that type III-A systems' ability to generate mutations through nonspecific DNase activity may be a way to offset the constraints placed on

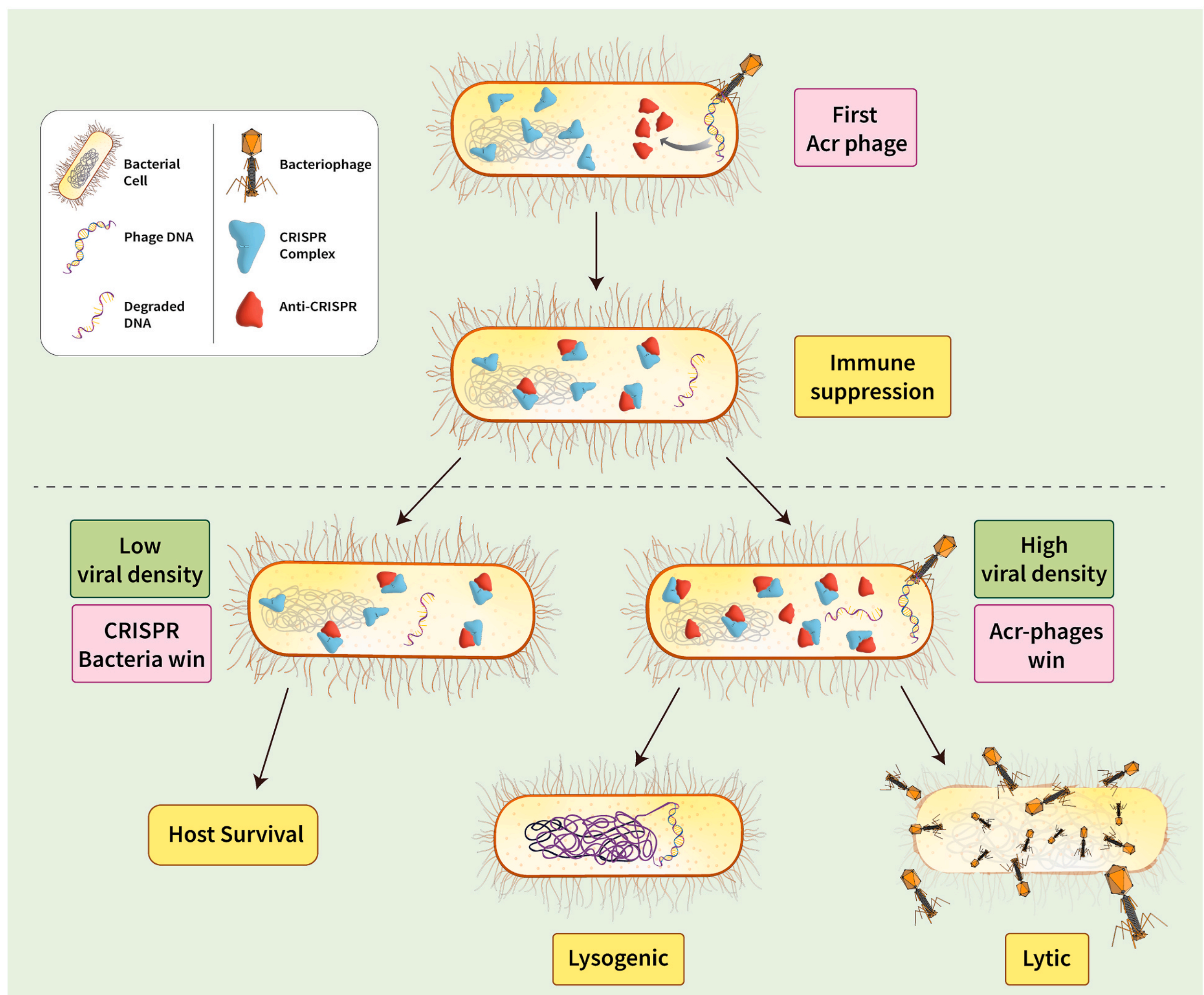

Fig. 1. Acr-phages cooperation to suppress CRISPR-Cas system immunity. Bacteria use CRISPR-Cas systems to protect themselves against bacteriophages (phages), and some phages produce anti-CRISPR proteins that inhibit immune system function. Acr-phages often need to cooperate to overcome CRISPR-Cas system resistance. During the initial stages of infection, anti-CRISPR proteins produced by phage genomes suppress the host bacterium's initial immune response, which predisposes the cell to successful infection by other phages in the population. When Acr-phages encounter CRISPR-Cas system immunity, the production of anti-CRISPR does not guarantee phage replication; but instead, if the number of Acr-phages falls below a critical threshold, the host bacterium survives. Viral replication occurs only if multiple Acr-phage genomes deliver a sufficient dose of anti-CRISPR to a single cell.

genome evolution by CRISPR systems [77]. Consequently, genomes with type III-A CRISPR-Cas systems may generate diversity through mutation rather than DNA acquisition. The other possibility is that these genomes contain *acr* genes that prevent CRISPR-Cas system activity [63]. These results indicate that the CRISPR-Cas systems can increase antibiotic resistance in addition to limiting the spread of ARGs. However, there is no single, agreed-upon explanation of the association between the CRISPR-Cas system and antibiotic resistance until now. This may be due to variations in factors such as strain isolation time, geographic location, host, bacterial species, and antibiotic species. Therefore, more researches are needed to evaluate the association between the CRISPR-Cas system and antibiotic resistance.

3. Acrs and antibiotic resistance

There are numerous CRISPR-Cas systems in bacterial genomes, and they provide a fitness advantage due to their common function in defense against foreign genetic elements like phages. The CRISPR-Cas system, however, might also stop the uptake of genes that could be beneficial. This is especially true in the context of antibiotic selection, where hindering the uptake of ARGs could be deleterious. The *acr* genes, which suppress the activity of various types of CRISPR-Cas systems, are recently discovered features in these evolutionary dynamics [61,78]. It has been found that HGT can occur with high frequency, despite the prevalence of CRISPR-Cas system defense mechanisms [79,80]. The widespread distribution and broad-spectrum activity of Acrs potently facilitate ARGs acquisition by inhibiting the CRISPR-Cas system activity [68]. It should be noted that the role of Acrs and CRISPR-Cas systems in spreading antibiotic resistance is very different in clinical environments and specific pathogenic species. In this regard, Shehreen et al. [61] demonstrated that the presence of CRISPR-Cas systems did not correlate with the presence of ARGs among 104947 reference genes, including 5677 different species. Nevertheless however, they observed a positive or negative correlation in some clinically important species such as *P. aeruginosa*. The presence of *acr* genes in *P. aeruginosa* was positively associated with the presence of ARGs for beta-lactam, aminoglycoside, fosfomycin, and phenicol drug classes while there was no association between CRISPR and ARGs [61]. Therefore, Acrs could potentially facilitate ARGs uptake in *P. aeruginosa* by inhibiting CRISPR-Cas systems. On the other hand, there was a meaningful negative association between *acr* genes and tetracycline and beta-lactam resistance genes in *Neisseria meningitidis*. However, there was no association between *acr* and ARGs in *Listeria monocytogenes* and *Ralstonia solanacearum* [61]. Therefore, even though Acrs restrict the activity of CRISPR systems against phages and other MGEs, they may provide the advantage of acquiring ARGs for bacteria [38,43].

Additionally, Acrs carried by MGEs such as conjugative plasmids and phages reveal a role in the HGT of different MGE-encoded traits. Acrs promote HGT by inhibiting CRISPR systems, and acr genes may be positively correlated to antibiotic resistance. Several studies have indicated that a critical threshold level of Acrs is required for CRISPR resistance and immune host infection. Indeed, Acr-phages frequently work together to get past the host's CRISPR-Cas immune system; the first phage blocks the immunity of the CRISPR-Cas system, allowing the second Acr-phage to replicate successfully. Infections fail if inadequate Acrs dose is delivered to a single cell by multiple phage genomes [81,82] (Fig. 1). However, Stanley et al. [82] pointed out that Acr-phages remain sensitive to CRISPR-Cas systems, indicating that the action of Acrs may be an imperfect process. Bacteria can upregulate the intracellular concentration of Cas proteins, when most vulnerable to phage infection, such as when they are present at high cell densities. This allows the CRISPR-Cas system to overcome Acrs and deactivate the Acrs function [82,83].

4. CRISPR-cas system-based strategies to combat bacterial infection

4.1. Genome editing based on CRISPR-Cas9 system

In recent years, CRISPR-Cas systems have been efficiently used for specific gene regulation and genome editing [84,85], as well as promising applications such as the development of next-generation antimicrobials [85,86], genome engineering of bacteria, mammalian, and plant cells [87–89], reversing antibiotic resistance by targeting ARGs, treating genetic diseases, and molecular recording [90]. In the context of genome editing, class 1 CRISPR-Cas systems are scarcely used as a genome editing tool since these systems are difficult to construct and transform into target cells [91,92]. By contrast, in class 2 CRISPR-Cas systems, all domains indispensable for DNA cleavage are fused into an individual effector protein, making them a favorable choice for gene editing [40,93]. Because of its simplicity, effectiveness, adaptability, and specificity, the CRISPR-Cas9 system (a type II CRISPR-Cas system) has been extensively used to target virulence and antibiotic resistance genes in the bacterial genome [94]. In the CRISPR-Cas9 system, an associated *trans*-activating CRISPR RNA (tracrRNA) hybridizes with the repeat sequence in the crRNA through base pair complementary, forming a unique double-stranded RNA (dsRNA). The dsRNA is identified, cleaved, and processed by cellular non-Cas RNase III enzyme and other unknown nucleases. The tracrRNA-crRNA hybrid is named single guide RNA (sgRNA) and complexes with Cas9 nuclease. The sgRNA directs the Cas9 nuclease to the target site to mediate the cleavage of the foreign DNA fragments. Subsequently, Cas9 unwinds the DNA duplex and generates site-specific breaks in the double-stranded DNA target in 3 bp upstream of the protospacer-adjacent motif (PAM) sequence. It is worth noting that the presence of a PAM sequence (2–5bp) immediately downstream of the target region is indispensable for the selection and degradation of CRISPR targets since serves as a binding signal for Cas proteins [40,95–97].

With the ability to target almost any sequence of interest, the CRISPR-Cas9 system has sparked a revolution in genome editing. Researchers have adapted the CRISPR-Cas9 system to edit DNA by producing sgRNA that binds to a specific target sequence in the DNA of a cell. Cas9 enzyme then forms a stable ribonucleoprotein (RNP) complex with the sgRNA. When introduced into cells, sgRNA identifies the desired DNA sequence, and the Cas9 enzyme essentially cleaves the DNA at the target site. While the most frequently used

enzyme is Cas9, other enzymes, such as Cas12a, can also be employed. Following the cutting of the DNA, scientists use the cell's built-in DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by substituting a specific DNA sequence for an existing segment [98–100].

4.2. Employment of CRISPR-Cas9 system to combat bacterial infection

The CRISPR-Cas system can effectively mitigate the issues posed by drug-resistant pathogenic bacteria by targeting antibiotic resistance genes. Recent research has demonstrated that the CRISPR-Cas9 system has the potential to replace traditional antimicrobials. The power of nucleic acid destruction by RNA makes the CRISPR-Cas9 system a promising gene-editing tool for eliminating pathogenic bacteria with high precision and controlling the prevalence of ARGs among bacteria [18,101–103]. The CRISPR-Cas9 system could be utilized as an antimicrobial agent with two different approaches, a gene-focused approach and a pathogen-focused approach, depending on where the target gene is located (Table 2). The gene-focused approach involves targeting antibiotic resistance genes carried by episomal plasmids. Through the removal of the plasmid, this strategy causes bacteria to become re-sensitive to antibiotics. On the other hand, the pathogen-focused approach involves targeting bacterial chromosomes in specific regions, leading to the death of the targeted pathogenic strain [104–106] (Fig. 2). A sgRNA can be created specifically to target antibiotic resistance genes because doing so will cause a break in the dsDNA of MDR bacteria, turning them into antibiotic-sensitive ones. The study and development of these approaches may create new alternative treatments for MDR bacterial infections.

4.2.1. Gene-focused approach

The CRISPR-Cas systems could function as an antibacterial agent by restoring bacterial susceptibility to antibiotics by targeting antibiotic resistance genes on the plasmids of pathogenic bacteria. Since this method is very accurate and efficient, it could be used to control the prevalence of ARGs and eliminate the virulence genes in bacteria. The targeted elimination of antibiotic resistance genes by CRISPR-Cas systems can be an excellent approach to clinical control of ARGs transmission and drug-resistant bacteria. By designing new sgRNAs and using the CRISPR-Cas9 system, inserting or deleting a specific sequence at a genomic locus of interest with high precision is possible [104,121,122]. Kim et al. [107] demonstrated that the CRISPR-Cas9 system along with the specific sgRNA for a conserved target sequence in TEM- and SHV-type Extend-spectrum beta-lactamases (ESBLs) could be delivered into ESBLs-harboring *E. coli* by $pRESAFR_{bla}$ plasmid and restore their antibiotic susceptibility. The $pRESAFR_{bla}$ also disarmed resistance to other antibiotics in addition to re-sensitizing the bacterial cells [107]. In another study, Wu et al. [112] successfully re-sensitized the carbapenem-resistant *Shewanella algae* to imipenem, ampicillin, and sulfonamides by CRISPR-Cas9 system-mediated deletion of bla_{OXA-55}-like, *NmcR*-like, and *sul2* genes. In a typical gene-focused approach, Rodrigues et al. [110] engineered conjugative plasmid pPD1 with a complete, constitutively expressed CRISPR-Cas9 system that specifically targeted cassettes containing the erythromycin (*ermB*) and tetracycline (*tetM*) resistance genes, leading to reducing antibiotic resistance of the *E. faecalis* strains *in vivo* and *in vitro*.

Table 2
Genome editing based on the CRISPR-Cas system to combat bacterial infection.

Approach	Organism	Target gene	Method/Result	Study
Gene-Focused Approach	*E. coli*	bla_{TEM}, bla_{SHV}	Insertion of the CRISPR-Cas9 system into $pRESAFR_{bla}$ plasmid/Re-sensitization of *E. coli* ESBLs	[107]
	K. pneumoniae	*ramR*, *tetA*, *mgrB*	pSGKP-spe and pBECKP-spe plasmids engineered with the CRISPR-Cas9 system/Effect on bacterial susceptibility to tigecycline or colistin	[108]
	E. coli	*aacC1*	Broad host-range conjugative pKJK5 plasmid engineered with the CRISPR-Cas9 system/Destroying Gentamicin-resistance gene – harboring pHERD30T plasmid	[109]
	E. faecalis	*ermB*, *tetM*	Conjugative plasmid pPD1 engineered with the CRISPR-Cas9 system/Reducing antibiotic resistance of the *E. faecalis* strains	[110]
	E. coli	*mcr-1*	Insertion of the CRISPR-Cas9 system into pCas9 plasmid/Re-sensitization of *E. coli* to colistin	[111]
	Shewanella algae	bla_{OXA-55}, *NmcR*, *sul2*	A single plasmid containing CRISPR-Cas9 and recE/recT recombinase/Re-sensitization of *S. algae* to imipenem, ampicillin, and sulfonamides	[112]
	E. coli	bla_{NDM-1}, $bla_{CTX-M-15}$	Delivery of the programmed CRISPR-Cas9 system by λ phage/Destroying beta-lactamase resistance genes - harboring plasmids	[31]
	CRE	bla_{KPC}, bla_{NDM-1}, bla_{OXA-48}	Electrotransferred the pCasCure plasmid into CRE isolates/Re-sensitization of CRE to carbapenems	[113]
Pathogen-Focused Approach	*E. coli*	*fucP*, *ogr*, *groL*	Insertion of the type I-E CRISPR-Cas system into the pCRISPR plasmid/Selective removal of individual bacterial strains	[114]
	S. aureus	*nuc*, *esxA*	Delivery of the programmed CRISPR-Cas9 system by temperate bacteriophage ϕSaBov/Chromosomal DNA degradation	[115]
	E. coli, *S. aureus*	bla_{IMP-1}, *mecA*	Packaging the CRISPR-Cas13a into carrier phage capsid (CapsidCas13a)/Targeted elimination of the *E. coli* and *S. aureus* by the CRISPR-Cas system	[116]
	C. difficile	*RNase Y*	Recombinant bacteriophage ϕCD24-2 expressing bacterial genome-targeting crRNAs/Chromosomal DNA degradation by the CRISPR-Cas3 system	[117]
	S. aureus	*Aph-3*, *nuc*	Phagemid-delivered RNA-guided nuclease Cas9/Sequence-specific killing of *S. aureus*	[118]
	M. tuberculosis	chromosomal genes	Phagemid-delivered artificial mini-CRISPR array/Chromosomal DNA degradation by the subtype III-A CRISPR-Cas system	[119]
	E. coli	bla_{NDM-1}, bla_{SHV-18}	Recombinant bacteriophage ΦRGN expressing bacterial genome-targeting gRNAs/Removing of antibiotic-resistant bacteria	[120]

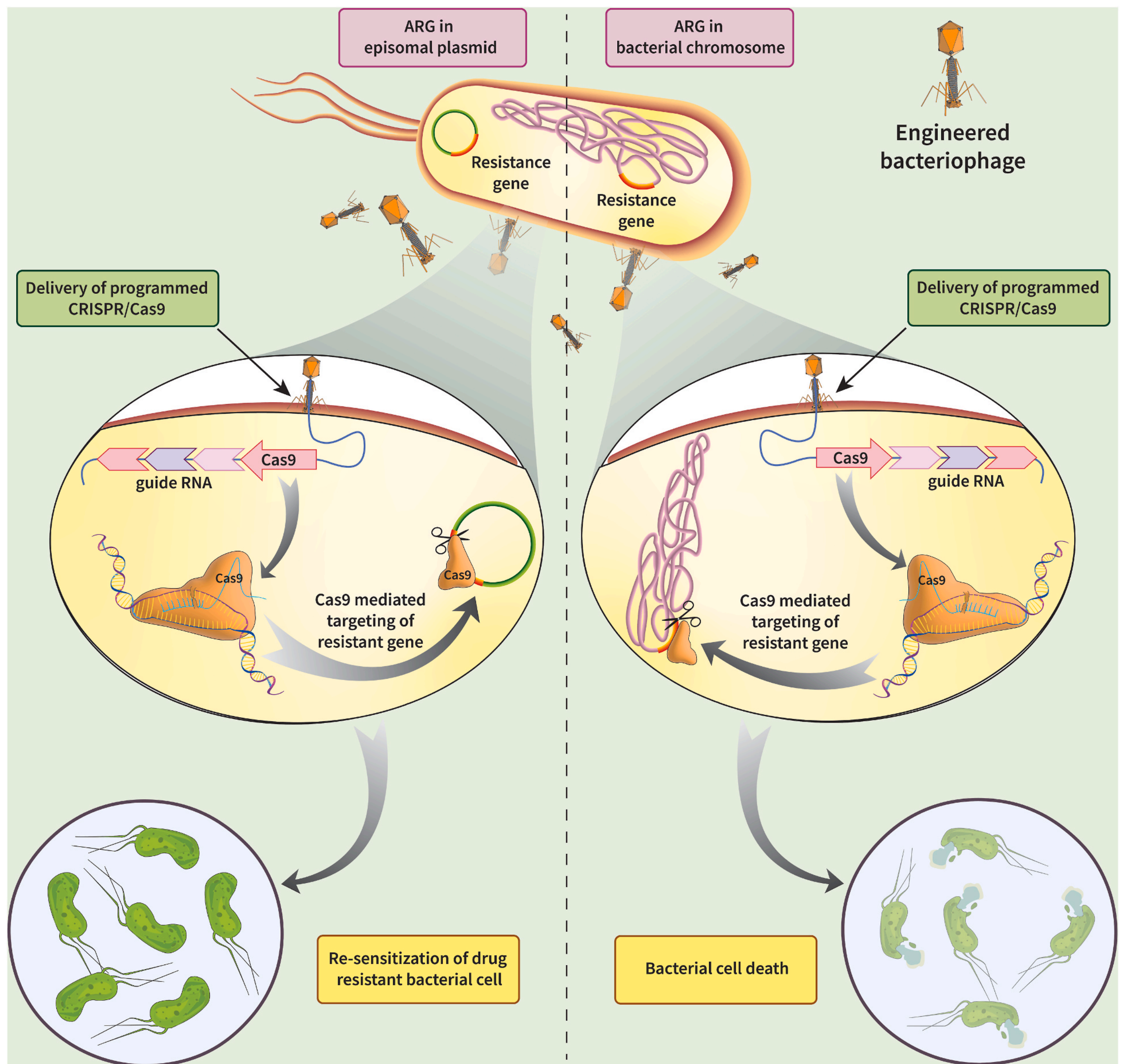

Fig. 2. CRISPR-Cas9 System-based Approaches to combat bacterial infection. ARGs can be carried on a plasmid and/or a chromosome, conferring resistance toward antibiotic treatment. The antibiotic-resistant bacteria are transduced with engineered phages carrying the CRISPR-Cas9 system. Identification and cleavage of the ARG sequence on a plasmid or chromosome by sgRNA/Cas9 complex causes bacterial cell re-sensitization or death, respectively.

Additionally, the engineered CRISPR-Cas9 system effectively and efficiently could be used to re-sensitize colistin-resistant isolates and reduce plasmid conjugation in *E. coli* [111,123,124]. The colistin resistance genes are located on *mcr-1* harboring plasmid pHNSHP45 and can be transferred horizontally [123,125]. Accordingly, Wan et al. [111] cloned two specific sgRNAs for the mcr-1 gene along with the CRISPR-Cas9 system into pCas9 plasmid, which could eliminate mcr-1 harboring plasmid in *E. coli*. Recently, CRISPR-Cas9-based systems are developed to eliminate several carbapenem-resistant plasmids in Carbapenem-Resistant *Enterobacteriaceae* (CRE) including the *bla*$_{KPC}$-harboring IncN pKp58_N and IncFIIK-pKpQIL plasmids, the *bla*$_{NDM}$-harboring IncX3 plasmid, and the *bla*$_{OXA-48}$-harboring pOXA-48-like plasmid [113,126]. pCasCure (CRISPR-Cas9-mediated plasmid-curing system) plasmid can effectively remove the epidemic carbapenem-resistant plasmids by targeting partitioning genes and therefore re-sensitize CRE to carbapenems [113]. On the other hand, the CRISPR-Cas9 system-based genome editing method could be successfully used to inactivate the *tetA* or *ramR* gene or the *mgrB* gene, which affect the susceptibility of carbapenem-resistant *K. pneumoniae* to tigecycline and colistin, respectively [127]. When the *mgrB* gene is inactivated, the PhoPQ two-component system is upregulated, leading to an increase in lipid A modifications and subsequent colistin resistance [127,128]. Sun et al. [127] demonstrated that *mgrB* deletion via

CRISPR/Cas9-based genome editing resulted in a marked increase in the minimum inhibitory concentration (MIC) of colistin. They also showed that the *ramA* gene was significantly overexpressed after deletion of the *ramR* gene, which may have increased the expression level of the AcrAB efflux pump and resulted in tigecycline resistance. These findings demonstrate that the CRISPR/Cas9-based genome editing method could be effective in the characterization of multidrug-resistant genes in *K. pneumoniae* [127].

It is worth noting that phage-mediated delivery is the more suitable than plasmid-mediated delivery method due to the natural infection of the phage with bacteria; for instance, it can be thought of as a superior therapeutic approach during acute infection [129]. Yosef et al. [31] delivered a programmed CRISPR-Cas9 system into the genome of antibiotic-resistant *E. coli* using λ phage as a vector, which could actively destroy beta-lactamase resistance genes ($bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$)- harboring plasmids. The delivered CRISPR-Cas9 system destroys ARGs-harboring plasmids and genetically modified lytic phages, leading to reverse drug resistance and eliminating the horizontal transfer of ARGs between strains. These recombinant phages could also be used in hand and hospital surface sanitizers that replace antibiotic-resistant bacteria with susceptible ones instead of killing the bacteria directly. As a result, patients infected by these sensitized bacteria can be treated with conventional antimicrobials [31]. Moreover, an engineered CRISPR-Cas9 system has been used to re-sensitize *S. aureus* to methicillin [130,131] and kanamycin [132] antibiotics. These studies indicate that the gene-focused CRISPR-Cas approach could potentially eliminate antibiotic resistance genes from diverse bacterial species and treat undefined bacterial infections [38,104]. However, this strategy has drawbacks because plasmid targeting can result in unfavorable recombination events in the targeted region. Moreover, the toxin-antitoxin system encoded by the targeted plasmid may unintentionally result in cell death after plasmid curing [18,133].

4.2.2. Pathogen-focused approach

Chromosomal genes are targeted in the pathogen-focused approach resulting in the bacteria death. This approach could be used to treat certain infectious diseases because the CRISPR Cas system selectively eliminates pathogens from the microbial community. A phage or plasmid containing a programmed CRISPR-Cas9 system is delivered to the target bacteria, specifically targeting a specific sequence found only in resistant strains [18,31,110]. Accordingly, modified exogenous CRISPR-Cas systems have been used for the sequence-specific killing of bacteria. Park et al. [115] exploited a programmed CRISPR-Cas9 system to target the *nuc* gene (encoding staphylococcal thermostable nuclease) in *S. aureus*. With the delivery of the CRISPR-Cas system to the target bacteria by temperate bacteriophage ϕSaBov, Cas9 identifies the target sequence in the chromosomal DNA, leading to DNA cleavage and eventually bacterial cell death with high efficiency [115]. In addition, endogenous CRISPR Cas systems could be repurposed for killing bacteria. In this regard, the endogenous type I–B CRISPR-Cas system of *Clostridioides difficile* has been used as an antibacterial agent by the expression of a self-targeting CRISPR that redirects the activity of endogenous CRISPR-Cas3 system against the bacterial chromosome. By comparing the killing efficacy of *C. difficile* bacteriophages with or without a CRISPR-Cas system, it has been determined that adding a CRISPR-Cas system improved bacterial killing *in vivo* and *in vitro* [117]. Furthermore, Gomaa et al. [114] demonstrated that the *E. coli* genome could be targeted by the type I-E CRISPR-Cas system. For the targeted elimination of pathogenic bacteria, they designed and inserted the CRISPR spacers into the CRISPR plasmid, which targeted *fucP* and *ogr* genes in *E. coli* K-12 and *E. coli* B, respectively. Using a similar approach, Kiga et al. [116] confirmed the targeted elimination of methicillin-resistant *S. aureus* and carbapenem-resistant *E. coli*, although they used the CRISPR-Cas13a system to eliminate the bacteria. The Cas9-based bactericide is limited to eliminating bacteria that carry the target gene on their chromosome. In contrast, the Cas13a-based agent eliminates bacteria with the target gene on both their plasmid and chromosome. In Kiga et al.'s study [116], the CRISPR-Cas13a system outperformed the CRISPR-Cas9 system in terms of bactericidal rate using a carbapenem-producing *E. coli* model with *blaIMP-1* gene located on a plasmid or chromosome. In addition, the CRISPR-Cas13a system does not cleave bacterial DNA directly. However, this system targets bacterial mRNA, which has a lower mutation activity. It suggests that CRISPR-Cas13a has great potential for development and application as an antibacterial agent [104,116]. In another study, Song et al. [134] developed the CRISPR Cas13a-based killing plasmids (CKPs), a *trans*-conjugative delivery system, that targeted endogenous transcripts of *Salmonella enterica* serovar Typhimurium. They designed five crRNAs targeting these endogenous transcripts, including one for *hilA* gene (CKP-*hilA*, encoding transcriptional regulator HilA), two for *dnaA* gene including CKP-*dnaA1* and CKP-*dnaA2* (involved in chromosomal replication initiator proteins), two for *katG* gene including CKP-*katG1* and CKP-*katG2* (encoding the catalase), and one crRNA for the nontarget (CKP-nontarget). *DnaA* is required for *S. typhimurium* growth, while the other two are nonessential. The CRISPR-Cas13a system has demonstrated bactericidal effects against *S. typhimurium* in mixed microbial flora. In addition, this system in the mouse infection model is effective when delivered by a donor *E. coli*. *S. typhimurium* colonization in the intestinal tract was greatly diminished by the CRISPR-Cas13a system. The system is also adaptable to target a variety of pathogens and can be easily delivered by conjugation. It is possible to use the system for biotherapy and microbial community modification with additional optimization and improvement [134]. Overall, the selective advantage of the pathogen-focused CRISPR-Cas approach over conventional antimicrobials is that it straightly and selectively targets antibiotic resistance genes and eliminates closely related bacterial strains without affecting other species in a bacterial complex. Therefore, the targeted elimination of antibiotic resistance genes by the CRISPR-Cas system may become a potential tool for the clinical control of ARGs transmission and drug-resistant bacteria. However, these approaches are still in their infancy and require further investigations and research efforts to determine therapeutic efficacy.

5. The limitations, challenges, and prospects of the CRISPR-Cas9 system

The CRISPR-Cas technologies have advanced dramatically in the last few years and have demonstrated remarkable potential in several life sciences research fields. CRISPR technologies could be successfully used in a wide range of genome engineering

technologies owing to rapid action, low cost, straightforward design, high editing efficiency, versatility, and low cytotoxicity [40,135]. Along with favorable outcomes, several limitations and concerns to implementing successful genome editing need to be addressed and resolved [136–138]. Some of the major challenges of the CRISPR Cas technology include off-target effects, PAM sequence requirement, complexity of microbial communities, delivery mechanisms, and resistance to the CRISPR Cas system, which are discussed below.

5.1. Off-target effects

The relatively high frequency of off-target effects (OTEs), which have been observed at a frequency of $\geq$50 %, is a major concern for applying the CRISPR-Cas9 system for gene therapy. Exceptional online gene-editing platforms have been developed and successfully used to identify and predict off-target cleavages in silico. These tools are largely based on sequence homology with the on-target site, including the position, the number, and the mismatched nucleotide's nature relative to the on-target sequence. Technical advances such as high throughput genome-wide next-generation sequencing can also play a significant role in minimizing off-target effects [135,139]. In addition, developing a well-optimized and engineered CRISPR system can remarkably decrease the off-target effects. On the other hand, limiting the duration of Cas9 activity can mitigate off-target effects induced by the CRISPR system. For instance, the CRISPR-Cas9 system delivered by electroporation had shown a shorter half-life compared to those delivered by other vector systems like lentiviral or plasmid vector system-based cargo delivery methods [140,141]. Various Cas9 variants such as SpCas9-HF1, eSpCas9, Sniper Cas9, evoCas9, HypaCas9, Cas9_R63 A/Q768A, and Cas9 nickases (Cas9n) have also been developed, which exhibit improvements in on-target specificity [142–146]. For example, Cas9n induces single-stranded breaks (SSBs), and when combined with a sgRNA pair targeting both strands of the DNA at the target site, produces a double-strand break (DSB). A pair of sgRNA-Cas9n complexes can cleavage both strands concurrently [137,147]. Using paired nicking, Ran et al. [147] demonstrated that off-target activity in cell lines can be reduced by 50–1500 fold.

5.2. PAM sequence requirement

The need for a PAM sequence near the target site in the desired gene loci is another impediment to using the CRISPR-Cas system. In the absence of the PAM sequence, the Cas9 protein does not know where to bind and where to cleave the DNA strand [137,148]. Cas9, originating from *Streptococcus pyogenes* (SpCas9), is a highly employed Cas9 variant processing a comparatively concise canonical PAM recognition site of 5′NGG3', where N can be any nucleotide [149,150]. Recently, a near-PAMless SpCas9 variant named SpRY has been engineered. Base-editor variants and SpRY nuclease can target nearly all PAMs; they show strong activities on a broad range of sites containing NRN PAMs in human cells, and a lesser but significant activity on sites containing NYN PAMs. Now that SpRY allows editing of numerous sites with NRN > NYN PAMs, almost the majority of the genome can be targeted [151]. In addition, Cas9 variants that target RNA have been developed, which increase the gene targeting spectrum by reducing the limitations imposed by PAM requirements. A short oligonucleotide with a PAM sequence, referred to as a PAMmer, can be used to manipulate SpCas9 to target RNA, thereby removing the requirement for a PAM sequence within the target site [152,153]. There are additional subsets of Cas enzymes, like Cas13 d, that naturally target RNA without the need for a PAM sequence. After this effector was further engineered, CasRx was successfully developed for effective RNA-guided RNA targeting in human cells [154,155]. Though RNA-targeting CRISPR-Cas system advances offer a therapeutic option without the risk of DNA-damage toxicity, they do not allow the possibility of editing a perennial correction into the genome [137].

5.3. Complexity of microbial communities

The microbial community available in the environment, within humans or animals is incredibly complex and diverse. These natural communities are a component of different microbiomes, which are made up of billions of bacterial cells from over a thousand different species found in each gram sample of the matrix. When the CRISPR-Cas system is employed to treat antimicrobial resistance, this level of complexity may prove to be a hindrance. Despite the enormous potential that the CRISPR-Cas systems have demonstrated for killing bacteria or re-sensitizing antimicrobial resistance bacteria to antibiotics, all of the studies that have been conducted thus far have only evaluated the system's activity in near clonal bacterial populations [6,18,156]. Very few *in vivo* studies have been conducted using mouse models to target a Gram-negative bacterial pathogen to inhibit their colonization in the gut [134,157]. In addition, predicting the microbial population's response following treatment with the CRISPR-Cas antimicrobials is another challenge. For example, when a strain is eliminated from a microbial population, it can lead to dysbiosis and promote the growth of undesirable bacteria within the community. Therefore, the effects of removing antimicrobial resistance from complex microbial communities must be evaluated before using the CRISPR-Cas antimicrobials [18,158].

5.4. Delivery mechanisms

Other major challenges in genome engineering based on CRISPR-Cas systems include delivery efficiency and systemic delivery. Viral vectors or plasmids, such as adeno-associated viruses and species-specific phages, have been exploited for CRISPR-Cas systems delivery. However, due to their low loading and packaging efficiency, risk of carcinogenesis, immunogenicity, and narrow host range, both methods have limited practical applications [40,159,160]. To circumvent the limitations of viral vectors, researchers have focused a great deal of attention on nonviral vectors to deliver the CRISPR-Cas system, mainly through nanoparticles (NPs)-based delivery like polymeric nanoparticles, lipid nanoparticles, and gold nanoparticles (AuNPs) [40,161]. Non-viral nano-vectors have the

following benefits over viral vectors: cost-effectiveness, lower immunogenicity, ease of chemical modification, scale-up production, large packaging capacity, and better protection of CRISPR-Cas9 genome editing system from degradation *in vivo* [162]. With the assistance of NPs, the delivery of Cas9 and sgRNA can be accomplished through either mRNA or Cas9-sgRNA ribonucleoprotein complexes. Lipid nanoparticles serve as amphiphilic compounds that assist in encapsulating negatively charged CRISPR plasmid DNA and mRNA, thereby directing and shielding RNA from crossing the cell membrane [40,163]. Polymeric NPs are another significant approach for CRISPR delivery because of their high biocompatibility and low immunogenicity [40,164]. In addition, due to their distinct controllable traits, accurate modification, and relative safety compared to polymeric and lipid NPs, AuNPs are perfectly suitable for CRISPR ribonucleoprotein complex delivery [165]. Despite significant advancements in designing these NPs to optimize the impact of the CRISPR-Cas systems, more research is required to achieve safer delivery and higher efficiency.

5.5. Resistance to the CRISPR-cas system

Another underlying challenge for the broad utilization of the CRISPR-Cas systems as antimicrobials is the development of resistance. Bacterial resistance to CRISPR-Cas antimicrobials is evolving at an accelerating rate [18]. According to Uribe et al. [166], *in vitro* experiments with CRISPR-Cas antimicrobials have demonstrated both inherent and acquired resistance in bacteria. This is due to various parameters that must be properly studied and applied for the successful re-sensitization or killing of target bacteria [166]. Studies on phage-CRISPR interactions have shown that obtaining specific mutations in the CRISPR-Cas system's target sequence is the most frequent mechanism of resistance [18]. It was observed that after the occurrence of DNA damage, the ubiquitous Rec-A system uses an intact chromosomal copy of the cleaved DNA strand to repair the break. Consequently, if the expression of CRISPR Cas9 is at low or moderate levels, the rate of RecA-mediated DNA repair will surpass that of Cas9-induced double-stranded breaks, thereby resulting in an increased survival rate [18,166]. Moreover, resistance to CRISPR can emerge through Acrs, which deactivate significant constituents of the CRISPR-Cas system [18]. As certain *acr* genes are found in virulent *P. aeruginosa* strains and can be transferred to other *P. aeruginosa* strains through conjugation, these Acrs can suppress the activity of the CRISPR Cas antimicrobial. Thus, the possibility of employing CRISPR Cas against pathogenic bacterial strains will be hampered [167].

6. Acrs as antibacterial compounds (phage therapy)

Phage therapy is defined as the utilization of bacteriophages to prevent the growth and proliferation of bacteria. Bacteriophages employ a variety of strategies to counteract various bacterial defense mechanisms. They can deactivate the bacterial CRISPR-Cas system by producing a variety of Acrs. This approach allows the phages to continue with the infection cycle and eventually lead to the lysis of bacterial cells, hence infection. Mutation in the target sequences for the CRISPR-Cas system and mutation in restriction enzyme sites are other strategies phages implement to overcome the bacterial CRISPR-Cas system [30]. Recently, multiple approaches

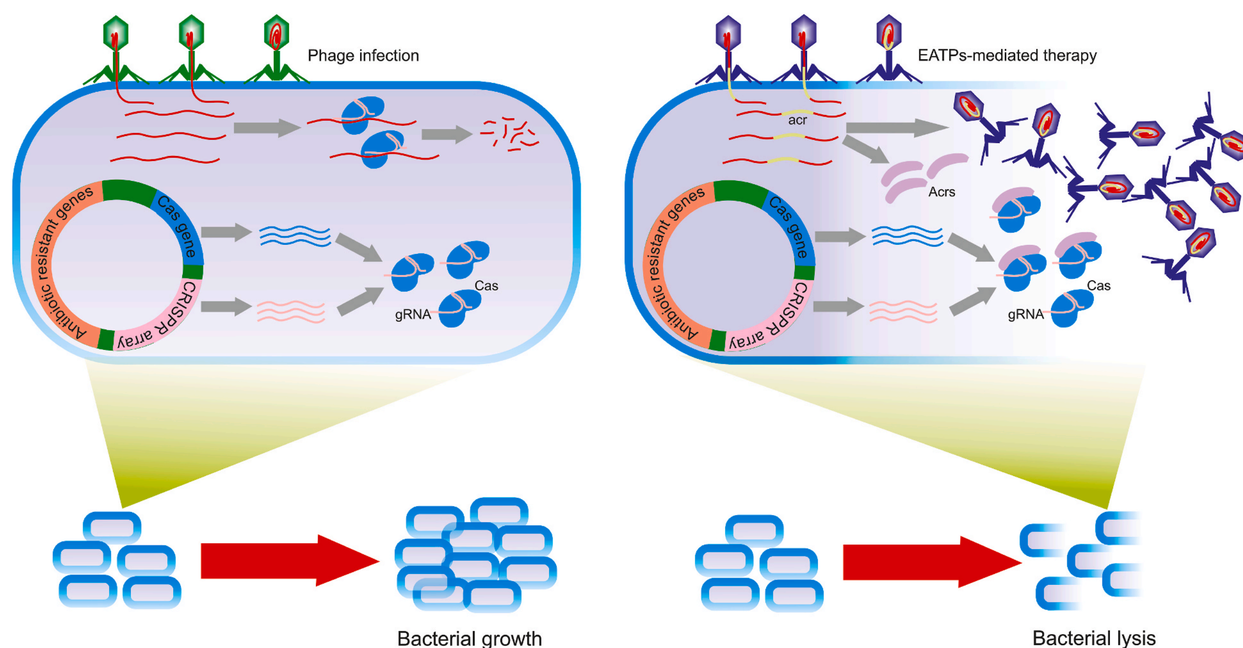

Fig. 3. Schematic illustration of engineered anti-CRISPR gene-containing phages in suppressing bacterial infections. Normally, phage infection activates the bacterial CRISPR-Cas system, which prevents phage replication by cleavage of phage genomes, resulting in preserving bacterial homeostasis, and eventually bacterial growth. EATPs suppress the bacterial CRISPR-Cas system-mediated adaptive immunity to protect their associated phage genomes by producing anti-CRISPR proteins, resulting in a large quantity proliferation of phages and, eventually, host bacteria's lysis.

have been developed to eradicate MDR bacteria using phage components, including single phage therapy, phage-derived protein therapy, phage cocktail therapy, combined use of phage with antibiotics, and phage anti-CRISPR-based therapy [45]. Among these approaches, Acrs-mediated phage therapy has received much attention due to recent biotechnological advances in the field of CRISPR systems. Bacteriophages containing some Acrs can inhibit the bacterial CRISPR-Cas system-mediated adaptive immunity; therefore, they successfully continue with the lytic cycle and cause the host bacteria's lysis (Fig. 3). Accordingly, bacteriophages harboring Acrs could be used as antimicrobial or antibacterial compounds with minimal side effects against pathogens like *Mycobacterium* species, *Streptococcus* species, *Staphylococcus* species, *Salmonella* species, *C. difficile*, *P. aeruginosa,* and *E. coli*, etc [168]. In a study, a three-phage cocktail was administered intravenously to a 15-year-old cystic fibrosis patient who had a disseminated *Mycobacterium abscessus* infection. The cocktail included one naturally occurring lytic phage (Muddy) and two bioengineered phages (ZoeJΔ*45* and BPs*33*ΔHTH-HRM10). Using genome engineering and forward genetics, effective lytic phage derivatives were created which effectively lysis the *M. abscessus* strain. For 32 weeks, phage treatment was administered by intravenous injection twice a day. After 9 days of treatment, the patient's clinical symptoms significantly improved and the intravenous phage treatment was well tolerated [169]. Zhu et al. [170] confirmed the presence of Acrs in several foodborne pathogens such as *Streptococcus pyogenes*, *Streptococcus thermophilus*, and *Listeria monocytogenes*, which cause human infections like pharyngitis, vaginosis, and listeriosis, respectively. These Acrs hold great promise for treating severe cases of food poisoning brought on by MDR pathogens [170]. Moreover, phage Acrs might be the only antibacterial therapy available for treating secondary bacterial infections associated with or followed by severe acute respiratory syndrome coronavirus (SARS-CoV) and coronavirus disease 2019 (COVID-19). The cause of this is the growing number of antibiotic-resistant bacteria as a result of the excessive use of antibiotics during the previous exposure [44,171,172]. Recently, Qin et al. [173] have demonstrated that engineered anti-CRISPR gene-containing phages (EATPs) exhibit potent antibacterial activities along with high safety against antibiotic-resistant *P. aeruginosa* through an *in vitro* and *in vivo* anti-CRISPR immunity mechanism (Fig. 3). They also found that EATPs significantly reduce the level of antibiotic resistance brought on by the highly antibiotic-resistant PA14 infection. Indeed, EATPs have great potential for treating antibiotic-resistant *P. aeruginosa* infection by lysing bacteria. EATPs combined with antibiotics could be used as a cutting-edge approach to reduce bacterial antibiotic resistance [173]. These findings suggested that engineered phages can be a feasible and alternative approach to treating patients with intractable MDR bacterial infections that are unresponsive to conventional antimicrobial therapy.

It should be noted that the discovery of Acrs is a relatively recent development in the field of CRISPR systems. Therefore, there are numerous obstacles and unanswered questions surrounding the existence and investigation of Acrs for their therapeutic application. The largest challenge is discovering new Acrs and clarifying their mechanism of action against various CRISPR-Cas systems. A thorough understanding of the CRISPR-Cas attenuation process will lead to a more effective use of Acrs in CRISPR-Cas system-mediated gene editing. Discovering new anti-CRISPR proteins in novel archaeal and bacterial sources will be facilitated by developing a new Acrs identification strategy. Additionally, developing an efficient system to deliver Acrs in the desired cells will be useful to reduce the "off-target" event during genome editing. In addition to the molecular mechanisms of Acrs, understanding of phage-phage interaction, phage biology, and bacterial strategies to counteract the effect of Acrs are other important questions that require careful attention and investigation to develop Acrs-mediated phage therapeutics.

7. Conclusion

The effect of the CRISPR-Cas system on antibiotic resistance varies in specific pathogenic species. According to the studies, bacterial pathogens containing the CRISPR-Cas system are less likely to carry antibiotic resistance genes than those without this defense system. These findings have promising implications for delivering cutting-edge technologies to combat antibiotic resistance such as CRISPR-Cas antimicrobials and phage therapy, which rely on evading CRISPR-Cas system defense mechanisms to kill cells. Indeed, they might be perfect for this purpose, if most MDR strains are also the most immunocompromised. The CRISPR-Cas system can target nucleic acid sequences with high accuracy and reliability; therefore, it has become a novel gene editing and gene therapy tool to prevent the spread of antibiotic resistance and control bacterial infections. We speculate that the CRISPR-Cas system will make it possible to eliminate pathogenic bacteria without targeting beneficial bacteria, features that conventional antimicrobials lack. Other applications of the CRISPR-Cas system, such as suppression of the ARGs expression and targeted removal of genes from bacterial pathogens, need further development and research efforts. In addition, Acrs-containing phages represent a promising alternative therapeutic approach for patients, especially in severe infections caused by MDR strains. By gaining a more comprehensive understanding of Acrs, their extensive utilization as accurate regulators of CRISPR-Cas system-mediated genome editing might be promoted. This could ultimately aid in tackling human diseases and yield benefits for humanity. However, an extensive experimental effort is needed to advance these approaches for clinical trials.

Ethics approval and consent to participate

This study was approved by the local ethics committee with reference number IR.TBZMED.VCR.REC.1400.096.

Consent for publication

All authors declare agreement and consent for publication.

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Declaration of competing 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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