World Journal of Microbiology and Biotechnology (2025) 41:381
https://doi.org/10.1007/s11274-025-04608-z

REVIEW

CRISPR-Cas systems in combating antimicrobial resistance: which system to choose? A systematic review

Hannay Crystynah Almeida de Souza[1,2,3] · Pedro Panzenhagen[3] · Ana Beatriz Portes[1,2,6] · Anamaria M. P. dos Santos[1,2,5] · Juliana Fidelis[1,2,7] · Carlos Adam Conte Junior[1,2,3,4,5,7]

Received: 11 August 2025 / Accepted: 21 September 2025 / Published online: 14 October 2025

Abstract

Antimicrobial resistance (AMR) poses a growing threat to global public health, progressively compromising the efficacy of available antimicrobials. Technologies based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have emerged as promising tools for controlling resistant pathogens, offering high specificity and versatility. However, a comprehensive and systematic synthesis of CRISPR strategies applied to AMR remains limited. From February 12, 2025, we conducted a systematic review of the PubMed, Embase, and Scopus databases, using the following search strategy: Population (resistant bacteria or plasmid-mediated resistance), Intervention (CRISPR, including variants such as CRISPR-Cas9, Cas3, Cas12, Cas13, and CRISPR interference [CRISPRi]), and Outcomes (bacterial resensitization or plasmid curing). The CRISPR-Cas9 system was the most frequently employed (75.7%), with conjugation identified as the primary delivery method. We identified the advantages and limitations of each system, highlighting CRISPRi and CRISPR-Cas13a as alternatives to overcome the constraints of direct genome editing. Delivery efficiency remains a central challenge, although nanocarrier- and bacteriophage-based methodologies show promising potential. We also propose a decision map that guides the selection of the most appropriate CRISPR-Cas system and delivery strategy, considering factors such as therapeutic objective, gene location, methodology efficiency, application environment, and clinical feasibility. This review provides an updated and structured synthesis of CRISPR strategies applied to AMR, emphasizing their potential translational and clinical applications. The findings can inform the development of CRISPR-based therapeutics, guide the design of preclinical studies, and support future strategies for combating multidrug-resistant infections in clinical settings.

Keywords Drug resistance, microbial · Genetic therapy · CRISPR-Cas systems · Gene editing · Anti-infective agents

✉ Pedro Panzenhagen
panzenhagen@ufrj.br

[1] Center for Food Analysis (NAL), Technological Development Support Laboratory (LADETEC), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro 21941-598, Brazil

[2] Laboratory of Advanced Analysis in Biochemistry and Molecular Biology (LAABBM), Department of Biochemistry, Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro 21941-909, Brazil

[3] Graduate Program in Biochemistry (PPGBq), Institute of Chemistry (IQ), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ 21941-909, Brazil

[4] Analytical and Molecular Laboratory Center (CLAn), Institute of Chemistry (IQ), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro 21941-909, Brazil

[5] Graduate Program in Veterinary Hygiene (PGHIGVET), Faculty of Veterinary Medicine, Fluminense Federal University (UFF), Vital Brazil Filho, Niterói, RJ 24230-340, Brazil

[6] Laboratory of Microorganism Structure, Department of General Microbiology, Institute of Microbiology Paulo de Góes (IMPG), Federal University of Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

[7] Graduate Program in Food Science, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Introduction

Antimicrobial resistance (AMR) is one of the most serious public health challenges of the twenty-first century (World Health Organization 2022). The advancement of this problem has been increasingly accentuated by the indiscriminate and inappropriate use of antimicrobials in several sectors, including agriculture, veterinary medicine, and human health (Ferri et al. 2017; Vidović et al. 2022; Almansour et al. 2023). Furthermore, in some countries, the population uses antibiotics without a medical prescription, often unaware of the associated risks, contributing significantly to the selection pressure of resistant microorganisms (Musoke et al. 2021). It is estimated that, in 2021, AMR was directly responsible for approximately 1.14 million deaths and contributed to 4.71 million deaths worldwide, imposing a growing burden on health systems (Naghavi et al. 2024). From 2000 to 2023, a 65% increase in resistant infections was observed, with resistance rates 3 to 4 times higher in low- and middle-income countries compared to high-income countries (Alomari et al. 2024). If effective measures are not implemented, it is estimated that, by 2050, AMR could cause up to 10 million deaths annually worldwide and lead to a cumulative global economic impact of up to 100 trillion dollars (O'Neill 2016).

Beyond its established applications, the CRISPR-Cas-based technology has emerged as a promising approach to combat AMR (Okesanya et al. 2025; Souza et al. 2025). Originally described as adaptive immune mechanisms in bacteria and archaea, the CRISPR-Cas system enables the recognition and cleavage of foreign genetic elements such as phages and plasmids (Horvath and Barrangou 2010). This intrinsic capability has been explored not only for genome editing but also, more recently, for the specific elimination of resistance genes and the plasmids that carry them, representing an innovative and targeted strategy in the field of precision antimicrobial therapies (Pursey et al. 2018). In 2015, CRISPR systems were classified into five types and 16 subtypes, distributed across two classes (1 and 2), based on the genes encoding the effector modules involved in the crRNA processing and interference steps (Makarova et al. 2015).

Class 1 includes types I, III, and IV systems, characterized by effector modules composed of multiple subunits (Makarova et al. 2015). These proteins can act in a coordinated manner in the formation of complexes with crRNA, mediating both the processing of pre-crRNA and interference with the target genetic material (Huo et al. 2014). Type I CRISPR-Cas systems are characterized by the presence of the signature gene cas3, which encodes a bifunctional protein containing a helicase from superfamily 2 and a nuclease domain (Sinkunas et al. 2011). The helicase can unwind double-stranded DNA (dsDNA) and enable subsequent DNA strand cleavage by the nuclease (Gong et al. 2014). Yosef et al. (2015) demonstrated the effectiveness of a large type I-E CRISPR-Cas system that included five Cascade genes, and cas3 nuclease. This system was able to eradicate AMR plasmids carrying genes such as NDM-1 and CTXM-15, demonstrating its potential as a therapeutic tool against resistant bacteria (Yosef et al. 2015).

On the other hand, Class 2 systems, which comprise types II, V, and VI, are structurally more compact and functionally simpler because they use only a single multi-domain effector protein, such as Cas9, Cas12, and Cas13, responsible for both crRNA binding and interference activity, without the need for additional auxiliary nucleases (Makarova et al. 2015). Studies conducted by Bikard et al. (2014), Kim et al. (2016), and Wang et al. (2019a, b) demonstrate the broad therapeutic potential of this system in eliminating resistance genes in pathogenic bacteria. In addition to these systems, an important modification derived from the CRISPR-Cas type II system is CRISPR interference (CRISPRi), a transcriptional modulation approach that utilizes a catalytically inactive form of the Cas9 protein, known as dCas9 (Dominguez et al. 2016). Although unable to promote DNA double-strand breaks, dCas9 retains the ability to bind to crRNA-guided target sequences (Qi et al. 2013). By positioning itself over promoter regions or coding genes, it physically inhibits transcription initiation or elongation, resulting in the temporary and precise repression of gene expression (Qi et al. 2013). This approach has been increasingly explored in the fight against AMR, as it allows the selective inhibition of resistance-related genes without causing permanent damage to the bacterial genome (Frusteri Chiacchiera et al. 2025).

With the advancement of technologies based on CRISPR-Cas systems and the growing number of studies exploring this emerging approach, the scientific literature on the topic has expanded considerably. However, there is still no comprehensive review that critically synthesizes the data on the use of CRISPR-Cas technology in the re-sensitization of antimicrobial-resistant bacteria, providing an overall and concise view of the different CRISPR-Cas systems used, their intrinsic mechanisms of action, advantages, and limitations. Therefore, it is necessary to consolidate this knowledge to provide a concrete understanding that guides future research and contributes to the development of alternative strategies for AMR control. Unlike Pursey (2018), which presents only the state of the art without a critical comparative analysis, and McCarty (2020), which focuses on sgRNA multiplexing for gene editing, this systematic review compiles a structured synthesis that guides the choice of the most suitable CRISPR system for

practical and therapeutic applications against AMR. To our knowledge, this is the first systematic review to comparatively analyze the different CRISPR-Cas systems applied to AMR from both mechanistic and translational perspectives, highlighting their potential for bacterial re-sensitization and therapeutic innovation.

Methodology

Systematic review criteria

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al. 2021). An electronic database search was performed in PubMed, Embase, and Scopus on February 12th, 2025, covering all studies available from database inception to the search date. The search strategy was developed to cover all relevant terms associated with the PICO framework: Population (resistant bacteria OR drug-resistant bacteria OR plasmid-mediated resistance) AND Intervention (CRISPR OR CRISPR-Cas OR CRISPR-Cas9 OR CRISPR-Cas3 OR CRISPR-Cas12 OR CRISPR-Cas13 OR CRISPRi) AND Outcomes (bacterial re-sensitization OR plasmid curing). Two independent reviewers screened titles and abstracts, and any disagreements were discussed with a third reviewer, ensuring meticulous study selection and minimizing potential selection biases.

Focus questions

Research questions were developed to guide this systematic review, focusing specifically on using several CRISPR-Cas systems to combat AMR: (i) What CRISPR-Cas approaches have been used to combat AMR? (ii) What is the efficiency of different CRISPR-Cas systems in reversing AMR? (iii) What are the advantages and limitations of each CRISPR-Cas system when applied to combating AMR? (iv) Is there an ideal CRISPR-Cas system for combating AMR? (v) What challenges still hinder the clinical application of CRISPR-Cas systems in reversing AMR?

Selection criteria

Eligibility criteria

The inclusion criteria for this systematic review were designed to select studies that focus on the use of the CRISPR-Cas system in combating AMR. Only original experimental studies published in English were considered, including in vitro and in vivo approaches where CRISPR-Cas was applied as a gene-editing or gene-silencing strategy.

Exclusion criteria

Literature reviews, books, and incomplete articles were excluded from the study, as these do not provide original research data. Monographs and academic theses were also excluded because they might not have undergone rigorous peer review processes typical of journal publications. Additionally, studies published in languages other than English were not considered to ensure uniformity in analysis and interpretation.

Assessment of risk of bias

Several potential sources were identified and considered in assessing the risk of bias in this systematic review. These include the criteria for including and excluding studies, which might limit the breadth of data analyzed and potentially exclude relevant findings. The decision to restrict the review to studies published in English could also introduce language bias, potentially overlooking significant research published in other languages. Beyond methodological limitations inherent to experimental designs, other potential sources of bias were considered in this review, including publication bias, lack of standardization in defining editing success and antimicrobial re-sensitization, and the predominance of positive findings involving well-established CRISPR-Cas systems. Furthermore, studies employing non-randomized bacterial strains, variable delivery platforms, or lacking off-target assessment were flagged as having increased risk of bias. Table 1 summarizes the main domains, the type of bias, and the potential impact of each on the review:

Results

Systematic literature search

Initially, we identified a total of 932 articles from three databases: PubMed ($n = 414$), Embase ($n = 412$), and Scopus ($n = 106$). A preliminary assessment focused on reviewing titles and authors was conducted to identify duplicates, excluding 350 studies. Subsequently, the remaining 582 articles underwent a detailed analysis of titles and abstracts. Of these, 557 were excluded based on pre-established selection criteria or because they did not match the scope of this review. Finally, we selected 25 studies, and after including eight additional relevant articles, we reached a total of 33 articles used to develop this systematic review, as illustrated in the PRISMA flowchart (Fig. 1).

Table 1 Risk of bias domains and categories considered in the systematic review

Domain	*Bias category*	*Description*
Search strategy	Selection bias	The use of a limited set of databases and search terms may exclude relevant studies
Search strategy	Language bias	Restricting to English-language studies may omit important regional findings
Study eligibility	Inclusion/Exclusion bias	When applied, overly restrictive criteria may omit relevant data, while unclear criteria may introduce inconsistency
Data extraction	Reporting bias	Many in vitro studies do not report replication, controls, or quantification methods in detail
Risk of bias assessment	Measurement bias	Differences in laboratory methods (e.g., CFU, MIC, qPCR) may affect comparability across studies
Risk of bias assessment	Performance bias	The absence of adequate controls or replication may overestimate the efficacy of CRISPR-Cas9
Risk of bias assessment	Detection bias	Lack of assessor blinding or failure to verify *off-target* effects may introduce bias
Synthesis	Publication bias	Excluding gray literature may favor studies with positive results

CFU, Colony Forming Units; *MIC*, Minimum Inhibitory Concentration; *qPCR*, Quantitative Polymerase Chain Reaction

Application of different CRISPR-Cas systems in combating AMR

Of the 33 studies analyzed in this systematic review, several CRISPR-Cas systems were employed to resensitize antimicrobial-resistant bacteria. The CRISPR-Cas9 system was the most predominant, utilized in 25 studies (75.7%), followed by the CRISPRi system in four studies (12.1%), CRISPR-Cas3 in three studies (9.0%), and CRISPR-Cas13a in one study (3.0%).

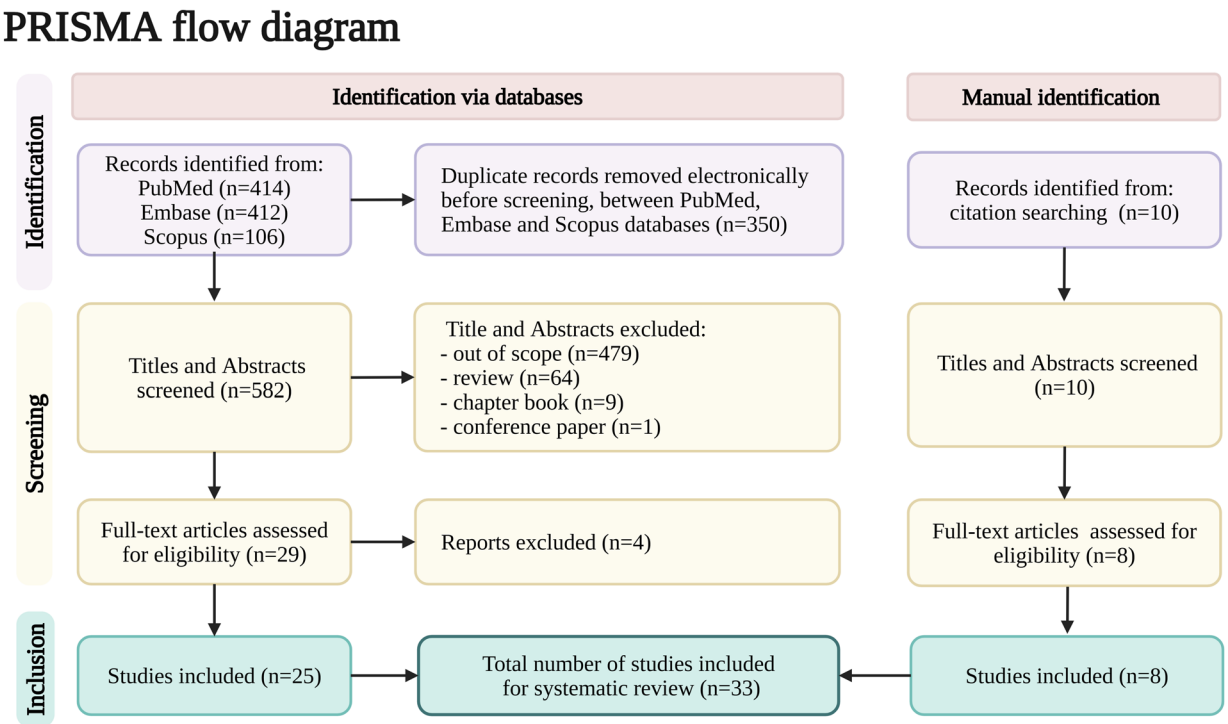

Fig. 1 PRISMA flow diagram displaying systematic search results during the identification phase, we retrieved 932 articles from electronic databases (PubMed, Embase, and Scopus), of which 350 were removed due to duplication. After screening 582 titles and abstracts, 553 studies were excluded for being out of scope, reviews, book chapters, or conference papers. Of the 29 articles evaluated in full, 25 were included. Additionally, 10 records were identified through manual searching, of which 8 were included after evaluation. A total of 33 studies were considered eligible and included in the systematic review

Mechanisms of action of CRISPR-Cas systems

The effectiveness of several CRISPR-Cas systems in combating antimicrobial-resistant bacteria is attributed to their different mechanisms of action (Fig. 2). Understanding the operational mechanisms of each CRISPR-Cas system allows us to differentiate how these genetic technologies are adapted and applied in different intervention contexts to address specific challenges related to AMR.

CRISPR-Cas9

The CRISPR-Cas9 system, frequently employed in the studies reviewed here, has well-explored mechanisms of action in combating AMR. It was observed that CRISPR-Cas9 is primarily used for two purposes: (i) the clearance of resistance plasmids, aiming to eliminate genetic elements that confer resistance to antimicrobials, and (ii) the elimination of resistant bacteria by targeting specific chromosomal genes.

Among the 25 studies that applied the CRISPR-Cas9 technology, 24 (95.45%) targeted curing plasmids containing different resistance genes (Table 2). Meanwhile, only three papers aimed to eliminate resistant bacteria through double-strand breaks in chromosomal genes (Table 2).

CRISPRi

Four studies applied CRISPR interference (CRISPRi) to reverse AMR, each using distinct approaches that illustrate the system's versatility. Overall, CRISPRi effectively increased bacterial susceptibility by targeting key genes involved in resistance mechanisms, such as efflux pumps, peptidoglycan biosynthesis, and specific resistance

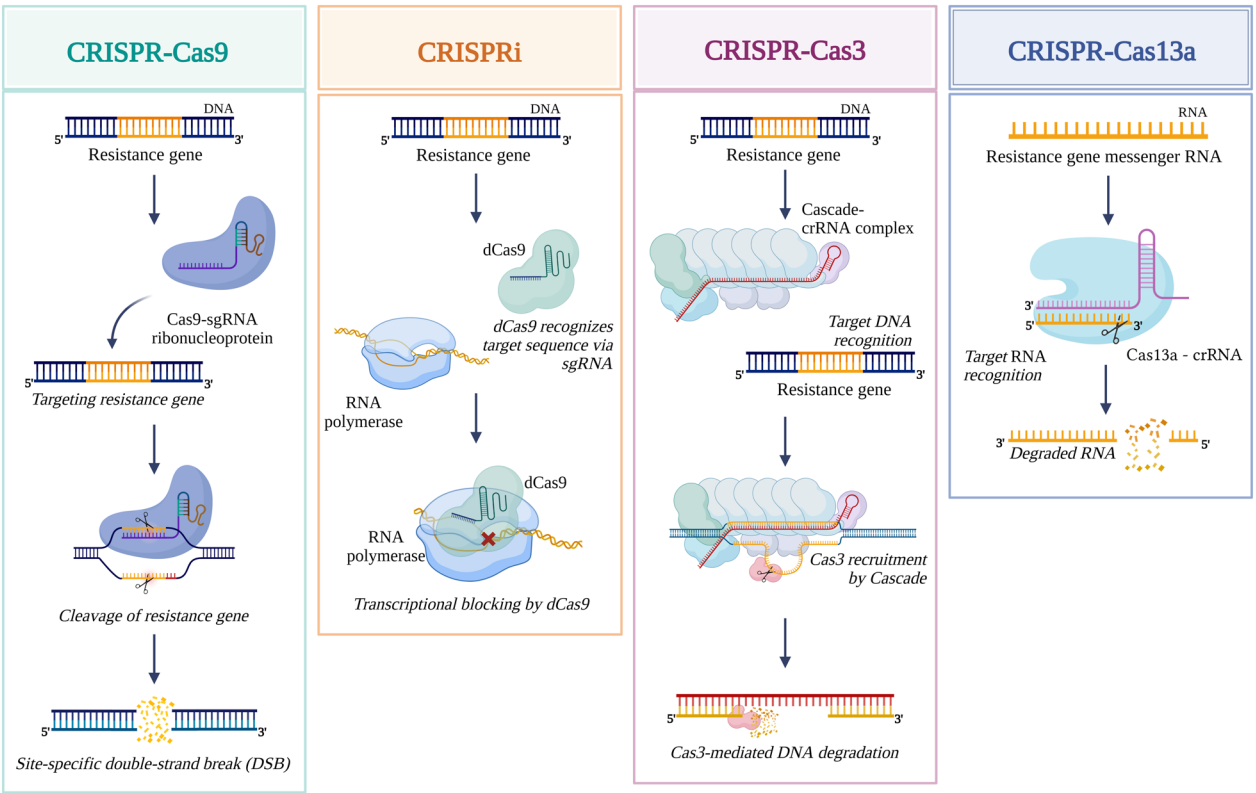

Fig. 2 Schematic representation of the mechanisms of action of the main CRISPR-Cas systems. CRISPR-Cas9: The ribonucleoprotein complex formed by the Cas9 endonuclease and guide RNA (sgRNA) recognizes and binds to the target sequence in the resistance gene. Cas9 promotes a specific double-strand break (DSB) in the DNA, leading to gene cleavage. CRISPR interference (CRISPRi): Uses a catalytically inactive version of Cas9 (dCas9), which, guided by the sgRNA, binds to the target sequence in the DNA without inducing cleavage. dCas9 physically blocks RNA polymerase, inhibiting transcription of the resistance gene (transcriptional silencing). CRISPR-Cas3: The Cascade-crRNA complex recognizes the target sequence in the DNA of the resistance gene. After recognition, the Cas3 nuclease-helicase is recruited by Cascade, promoting extensive degradation of the target DNA (DNA degradation). CRISPR-Cas13a: The Cas13a-crRNA complex specifically recognizes the messenger RNA (mRNA) of the resistance gene. Once bound, Cas13a cleaves the target RNA, leading to degradation of the molecule and inhibition of gene expression

Table 2 Efficiency of CRISPR-Cas systems in targeting antimicrobial resistance

CRISPR-Cas type	Target bacteria	Target (Gene/Plasmid/Other)	Reported Efficiency	MIC reduction (if applicable)	Reference
CRISPR-Cas9	E. coli	bla_{NDM-5}	98.11%	The MIC of meropenem decreased from 16 µg/mL to 0.06 µg/mL after the deletion of the bla_{NDM-5} gene	(Li et al. 2022)
	E. coli		99.88%		
	E. coli		87.00%		
	E. coli		95.83%		
	E. coli		99.00%		
CRISPR-Cas9	E. coli	mruby2	Loss of viability of ~ 4.5 log	N/A	(Djermoun et al. 2023)
	S. Typhimurium	Spacer 7 loci	Loss of viability of ~ 4 log		
	K. pneumoniae	Spacer 2 loci	Loss of viability of ~ 2 log		
	Vibrio cholerae	Six target loci	Loss of viability of ~ 3 log		
	P. aeruginosa	Spacer one locus	Loss of viability of ~ 4 log		
	E. coli	bla_{NDM-1}	100%	N/A	(Wongpayak et al. 2021)
CRISPR-Cas9	E. coli	bla_{KPC-2}	98.80%	The MIC of imipenem decreased from 5.55 µg/mL to 0.42 µg/mL after the elimination of the bla_{KPC-2} gene	(Tao et al. 2023a)
CRISPR-Cas9	Enterococcus faecium	vanA	80.00%	The MIC of vancomycin decreased from > 256 mg/L to 64 mg/L after the elimination of the vanA gene	(Tao et al. 2023b)
CRISPR-Cas9	E. coli	IncX4 gene	98.50%	N/A	(He et al. 2021b)
	E. coli	mcr-1	100%		
	E. coli	mcr-1	99.30%		
CRISPR-Cas9	E. coli	mcr-1	The fragment, indicating the presence of the mcr-1 gene, was not amplified in 18 out of 24 colonies transformed with pCas9-m1	The colistin MIC of the knockout strain was 0.25 mg/L, representing a sixfold reduction compared to the wild-type strain (2 mg/L)	(Wan et al. 2020)
	E. coli	mcr-1	> 80%		
	E. coli	mcr-1	> 80%		
CRISPR-Cas9	Klebsiella sp. Enterobacter cloacae	tmexCD-toprJ (Plasmids)	The pCas9Kill efficiently removed the tmexCD-toprJ gene from plasmids	In K. pneumoniae, E. cloacae, and Shewanella chilikensis strains, the deletion of the tmexCD-toprJ gene reduced the tigecycline MIC from up to 32 µg/mL to ≤ 2 µg/mL	(Xu et al. 2025)
	K. variicola Shewanella chilikensis	tmexCD-toprJ (Chromosome)	99.60%		
CRISPR-Cas9	E. coli	fosA3	The sgRNA named gRNA_195 demonstrated 100% efficiency in restoring fosfomycin sensitivity	N/A	(Walflor et al. 2022)
CRISPR-Cas9	E. coli	IncX3	99.3 ± 1.2	However, curing carbapenemase genes or plasmids, including the truncation of bla_{OXA-48} in 49210, successfully restored its susceptibility to carbapenems, with a > eightfold reduction in MIC values across all tested isolates	(Hao et al. 2020)
	E. coli	bla_{NDM-5}	97.2 ± 4.8		
	K. pneumoniae	IncL repA	100 ± 0		
	K. pneumoniae	bla_{OXA-48}	99.3 ± 1.2		
	K. pneumoniae	IncL repA and $bla_{OXA-48-like}$	100 ± 0		

Table 2 (continued)

CRISPR-Cas type	Target bacteria	Target (Gene/Plasmid/Other)	Reported Efficiency	MIC reduction (if applicable)	Reference
CRISPR-Cas9	E. coli	bla_{TEM} and bla_{SHV}	99.90%	N/A	(Kim et al. 2016)
CRISPR-Cas9	E. coli E. coli	tet(X4) mcr-1	Over 90% resensitization in vitro and a reduction of resistant bacteria to 1% in vivo	N/A	(Zhang et al. 2024)
CRISPR-Cas9	Mycobacterium smegmatis	recA (DNA repair) and ilvH and argF (auxotrophic) The GFP gene in plasmids confers resistance to hygromycin	A 100-fold reduction in cell viability when targeting essential genes and efficient curing of plasmids in 90% of the transformed cells	N/A	(Sodani et al. 2023)
CRISPR-Cas9	E. coli	Plasmid replication genes	Removal efficiency > 90%	The removal of IncF, IncX3, and ColRNAI plasmids in K. pneumoniae resulted in a reduction of MICs for: Amikacin (≥64 mg/L to≤2 mg/L); Tobramycin (≥16 mg/L to≤1 mg/L); Cefotaxime (≥8 mg/L to≤1 mg/L); Ceftriaxone (≥16 mg/L to≤1 mg/L); and Aztreonam (≥64 mg/L to≤1 mg/L)	(Yen et al. 2024)
	K. pneumoniae	Resistance genes (plasmids)	Efficient curing of plasmids		
	Enterobacter hormaechei	Plasmid replication genes	Confirmation of plasmid removal by PCR and pulsed-field gel electrophoresis (PFGE)		
	Citrobacter freundii		Plasmid loss after CRISPR-Cas9 action		
	P. aeruginosa	Unspecific genes	Reduction of plasmid load		
CRISPR-Cas9	E. coli	Antibiotic resistance genes (tet, cat, aph(3′)-Ia)	CRISPR-Cas editing achieved 46.0% (tet), 27.1% (cat), and 36.2% (aph(3′)-Ia). Simultaneous editing improved efficiency (8.7–13.3%). In sterile soil, efficiencies reached 51.7% (tet/cat) and 45.7% (aph(3′)-Ia); in non-sterile soil, 49.6% and 39.4%	N/A	(Chen et al. 2024a)
CRISPR-Cas9	E. coli	aacC1 (gentamicin)	100% elimination of the target plasmid	N/A	(Sünderhauf et al. 2023)
	Pseudomonas putida		A 4-log reduction in plasmid acquisition		
	Kluyvera sp		Significant, but not total, reduction		
	Environmental isolates of coliforms		Variable efficiency between the samples, but a reduction was observed		
	Swine isolates (coliforms)		Partial removal of AMR plasmids		
	Pseudomonas spp.		Effective prevention of new plasmid acquisition		
CRISPR-Cas9	E. coli	$bla_{CTX-M-15}$ $bla_{CTX-M-55}$ $bla_{CTX-M-14}$ $bla_{CTX-M-27}$ $bla_{CTX-M-65}$ $bla_{CTX-M-90}$	Dose-dependent reduction in the viability of cefotaxime-resistant cells, with up to a 4 log10 decrease in viable cell count at a multiplicity of infection (MOI) of 100	N/A	(Nittayasut et al. 2024)

Table 2 (continued)

CRISPR-Cas type	Target bacteria	Target (Gene/Plasmid/Other)	Reported Efficiency	MIC reduction (if applicable)	Reference
CRISPR-Cas9	E. coli	nikA	88.5%±4.3%	N/A	(Wang et al. 2019a)
		hicB	64.6%±16.5%		
		sopA	15.9%±6.6%		
		hicB and sopA	7.8%±1.3%		
		mcr-1	Only one colony lost both plasmids. The other 11 colonies lost p14EC033b, but contained a variant of p14EC033a in which the mcr-1 region was truncated		
		mcr-1			
CRISPR-Cas9	E. coli (ExPEC)	repFIA and repFIB	Successful elimination of IncF plasmids in all treated strains	After plasmid removal, the strains became susceptible to ampicillin, cefazolin, gentamicin, tobramycin, tetracycline, and trimethoprim-sulfamethoxazole	(Chen et al. 2024b)
CRISPR-Cas9	E. coli 001	Replication gene of IncX4 plasmid	100±0	mcr-1: Reduction of colistin MIC from > 4 mg/L to 1 mg/L bla_{KPC-2} and bla_{NDM-5}: Reduction of meropenem MIC from 16 mg/L to < 0.25 mg/L	(He et al. 2021a)
	E. coli 001	mcr-1			
	E. coli 002	Replication gene of IncI2 plasmid			
	E. coli 003	Replication gene of IncHI2 plasmid			
	E. coli 004	mcr-1			
	E. coli 005	mcr-1			
	E. coli 006	bla_{KPC-2}			
	E. coli 007	bla_{NDM-5}			
CRISPR-Cas9	E. coli E. coli 180A E. hormaechei Klebsiella variicola	bla_{TEM-1}	In E. coli, deleting the bla_{TEM-1} plasmid restored β-lactam sensitivity. No significant MIC reduction occurred in E. hormaechei or K. variicola due to other resistance genes	There was no substantial reduction in MIC in E. hormaechei or K. variicola due to the presence of remaining resistance genes	(Tagliaferri et al. 2020)
CRISPR-Cas9	E. coli	bla	The traditional CRISPR system reduced E. coli colony-forming units (CFU) by ~ 100-fold. Pro-AG reduced CFU by ~ 100,000-fold	It was not directly reported, but there was efficient elimination of ampicillin resistance	(Valderrama et al. 2019)
CRISPR-Cas9	E. coli	bla_{NDM-1}, mcr-1	> 99.99% plasmid clearance in 8 h; > 6-log CFU reduction in vitro; > 5-log in vivo	N/A	(Liu et al. 2020)
CRISPR-Cas9	E. coli	bla_{NDM-1}, bla_{SHV-18}	2–3-log10 reduction in viable cells	N/A	(Citorik et al. 2014)
CRISPR-Cas9	S. aureus RN4220	Plasmid pG0400	Efficient plasmid cure; 0% resistant cells after treatment	N/A	(Bikard et al. 2014)
	S. aureus USA300	mecA	~10^4 reduction in viable cells		
CRISPR-Cas3	E. coli K-12	ndm-1 ctx_{-M-15}	Lysogenization by λ phage resulted in the elimination of resistance plasmids in E. coli	N/A	(Yosef et al. 2015)

Table 2 (continued)

CRISPR-Cas type	Target bacteria	Target (Gene/Plasmid/Other)	Reported Efficiency	MIC reduction (if applicable)	Reference
CRISPR-Cas3	E. coli	mcr-1 bla_{NDM-1} tet(X)	The conjugation frequencies of pHG101-mcr-1, pHG101 bla_{NDM-1}, and pHG101-tet(X) were reduced by 92.29%, 72.20%, and 74.62%, respectively	N/A	(Fang et al. 2024)
CRISPR-Cas3	K. pneumoniae	Various spacers corresponding to the IncFII plasmid	90–95%	N/A	(Zhou et al. 2023)
CRISPRi	Mycobacterium smegmatis	mmpL3	mmpL3 suppression reduced bacterial viability with sub-MIC rifampin, rifabutin, ceftriaxone, or isoniazid ($p<0.05$) but not with clarithromycin or amikacin	MICs decreased at one- to two-fold dilutions in the presence of 0.5 ng/mL ATc, possibly due to a growth defect from mmpL3 repression	(Yuliani et al. 2024)
CRISPRi	Mycobacterium abscessus	pbpB e cwlM	Suppressing pbpB (28%) and cwlM (36%) reduced CFU by 3 log_{10} with imipenem	N/A	(Kurepina et al. 2022)
CRISPRi	E. coli	AcrAB-TolC efflux system genes confer resistance to multiple antibiotics	Targeting acrB and tolC together led to the highest gene suppression: acrA (78.3%), acrB (90.0%), and tolC (65.4%)	MIC reduction: rifampicin (6 → 3.5 mg/L, 2x), erythromycin (32 → 2 mg/L, 16x), tetracycline (1.5 → 0.2 mg/L, 8x)	(Wan et al. 2022)
CRISPRi	E. coli	tetA	Reduction > 10 times	MIC reduction > 10 times, indicating high efficiency	(Frusteri Chiacchiera et al. 2025)
		bla_{NDM-1}	Reduced from > 500 to 200 µg/mL	Reduction of MIC by up to 4 times	
		mcr-1	Reduced from 8 to 4 µg/mL	Reduction of MIC by up to 4 times	
		$bla_{TEM-116}$	Inhibition of $bla_{TEM-116}$ by a gRNA targeting the bla promoter led to a MIC of 5000 µg/ml and a 6–7 h growth delay for AMP between 10 and 1000 µg/ml	Growth is still possible at high antibiotic concentrations	
		bla_{NDM-5} (isolated ST167)	MIC reduced by > 4 times	MIC reduced by > 4 times	
		mcr-1 (isolated ST617)	MIC reduced by 2 times	MIC reduced by 2 times	
		$bla_{CTX-M-14}$ (blood)	MIC reduced > 2 times	MIC reduced > 2 times	
		$bla_{CTX-M-15}$ (urinary)	There is no significant reduction in MIC but with a growth delay of up to 7 h	There is no substantial decrease in MIC but with a growth delay of up to 7 h	
CRISPR-Cas13a	E. coli	bla_{IMP-1}, bla_{OXA-48}, bla_{VIM-2}, bla_{NDM-1}, bla_{KPC-2}, mcr-1 and mcr-2	Reduction of bla_{IMP-1}-bearing E. coli by 2–3 orders of magnitude	N/A	(Kiga et al. 2020)
	S. aureus		Reduction of mecA-carrying S. aureus MRSA		

Data on the efficiency of the different CRISPR systems, including MIC reduction data, when available. *N/A*, Not analyzed; *MIC*, Minimum Inhibitory Concentration

determinants (Table 2). For example, in *E. coli,* suppression of the *AcrAB-TolC* efflux pump enhanced susceptibility to multiple antimicrobials, while in *Mycobacterium* spp., inhibition of *pbpB*, *cwIM*, and *mmpL3* genes led to re-sensitization to β-lactams and other antibiotics. Additionally, CRISPRi-mediated transcriptional blockade of multiple resistance genes demonstrated its potential in combined strategies to combat multifactorial resistance.

CRISPR-Cas3 and CRISPR-Cas13a

The CRISPR-Cas3 system was applied in three studies to tackle AMR, demonstrating its ability to efficiently eliminate resistance determinants. Applications included the targeted destruction of antibiotic resistance genes in *E. coli*, the curing of IncFII plasmids in *K. pneumoniae* with 90–95% efficiency, and innovative phage-mediated delivery strategies for plasmid elimination (Table 2). Similarly, the CRISPR-Cas13a system was employed to degrade RNA from multiple resistance genes in *E. coli* and *K. pneumoniae*, effectively inducing bactericidal effects through specific gene targeting (Table 2). These findings underscore the versatility of both CRISPR-Cas3 and Cas13a in addressing AMR via different molecular mechanisms.

Efficiency of CRISPR-Cas systems in targeting AMR

The efficiency of CRISPR-Cas system applications depends on several variables, including the characteristics of the target bacterial species, the chosen delivery strategy, and the specific gene or region targeted. However, understanding how these efficiency rates vary among different types of CRISPR-Cas systems is essential for optimizing their application (Table 2). Reported efficiency rates among the studies included in this review varied substantially depending on the CRISPR-Cas system employed, the bacterial species, and the experimental context. CRISPR-Cas9-based strategies consistently demonstrated high genome-editing efficiency, with reported success rates often exceeding 90% in plasmid clearance or gene disruption assays. CRISPRi systems have demonstrated moderate to high efficiency, particularly in targeting chromosomal genes, although variability in performance has been observed, often due to challenges in delivering the dCas9 and guide RNA components. CRISPR-Cas13a, targeting RNA molecules, achieved notable bactericidal effects, with some studies reporting complete elimination of target gene expression and significant reduction in bacterial viability. In contrast, systems such as CRISPR-Cas3, although mechanistically promising due to their processive DNA degradation, were underrepresented and exhibited more variable outcomes. These findings underscore the need to tailor CRISPR system selection to specific genetic targets, delivery vectors, and host bacterial features in order to maximize functional efficiency.

Vectors used for delivery of CRISPR-Cas systems

In general, the delivery of CRISPR-Cas systems in the 33 studies on bacterial resistance has been primarily based on plasmids ($n = 26$), followed by phages ($n = 6$), and, to a lesser extent, emerging vectors such as nitrogen-doped carbon dots (NCDs) ($n = 1$). The choice of vector for CRISPR-Cas system delivery is influenced by factors such as the type of system used and the specific characteristics of the target bacterium.

Among the 25 studies that employed CRISPR-Cas9-based technology, 80% ($n = 20$) used plasmids as vectors for delivery. Additionally, 16% ($n = 4$) explored phages as vectors, while 4% ($n = 1$) investigated the application of nitrogen-doped carbon dots (NCDs) for this purpose. In the four studies utilizing the CRISPRi, all authors selected plasmids as delivery vectors to target resistant bacteria. Regarding the three studies that employed the CRISPR-Cas3 system, 66.7% ($n = 2$) used plasmids, while 33.3% ($n = 1$) opted for temperate phages. The only study that employed the CRISPR-Cas13a system for this purpose chose phages as the delivery method.

Delivery methodologies of different CRISPR-Cas systems

The efficient delivery of the CRISPR-Cas system is crucial for successfully applying this technology to resistant bacteria. Therefore, we compiled and analyzed data from all studies included in this systematic review to identify potential trends in the delivery methodologies chosen for different CRISPR-Cas systems.

In general, conjugation is the commonly used delivery strategy among the analyzed studies, employed in 14 out of 33 studies (42.4%). Electroporation ranks second, used in 8 out of 33 studies (24.2%), followed by transformation, which appears in 7 out of 33 studies (21.2%). Phage-mediated delivery was used in 6 out of 33 studies (18.2%), while nanoparticle-based delivery was adopted in only 1 study (3.0%).

Among the studies that employed the CRISPR-Cas9 system, conjugation was the most used delivery methodology, applied in 12 out of 25 analyzed studies. Electroporation was the second most adopted approach, appearing in six studies (24%), followed by chemical transformation, used in five studies (20%). Temperate phage-mediated delivery was employed in four studies (16%), while the nanoparticle-based approach was reported by only one author (4%). In the studies that employed the CRISPRi system, electroporation

was the most commonly used delivery methodology applied in two of the four analyzed studies (50%) (Kurepina et al. 2022; Yuliani et al. 2024). Additionally, one study (Frusteri Chiacchiera et al. 2025) adopted conjugation as a delivery strategy, while another used transformation (Wan et al. 2022). Regarding the CRISPR-Cas3 system, its delivery was quite diverse, with each of the three analyzed studies adopting a different strategy: phage-mediated delivery (Yosef et al. 2015), chemical transformation (Fang et al. 2024), and conjugation (Zhou et al. 2023). In the study with the CRISPR-Cas13a system, delivery was carried out using phages (Kiga et al. 2020).

Advantages and limitations of CRISPR-Cas systems in combating AMR

CRISPR-Cas systems have been explored as promising tools for combating AMR, offering targeted gene editing to eliminate resistance determinants and/or resistant bacteria. However, understanding the strengths and inherent limitations of each CRISPR-Cas system is essential. Figures 3 and 4 summarize the main strengths and challenges of the different CRISPR-Cas systems, respectively (Supplementary Table 1).

Advantages of different CRISPR-Cas systems

The data analysis enabled the synthesis of several advantages associated with CRISPR-Cas systems (Fig. 3). The CRISPR-Cas9 system was the most widely reported in the literature within the scope of this review. Among its main strengths is its high efficiency, with multiple studies demonstrating high rates of resistant plasmid elimination, reaching up to 100% (Table 1). Additionally, it exhibits high specificity, being capable of targeting and acting on specific sequences. Another relevant point is its ability to promote bacterial re-sensitization, restoring the susceptibility of previously resistant strains to antimicrobials. CRISPR-Cas9 also has broad applicability, proving effective in various bacterial species and functional against different AMR profiles. Finally, several studies highlight its potential to prevent the horizontal transfer of resistance genes, blocking the spread of plasmids among bacteria.

Regarding the strengths of the CRISPRi system, several aspects stand out. Firstly, the technology enables precise modulation of gene expression in mycobacteria, making it a valuable tool for functional studies and the investigation of metabolic pathways (Kurepina et al. 2022). Another important point is the increased susceptibility to antimicrobials, as demonstrated in studies that used CRISPRi to inhibit essential genes such as *mmpL3*, resulting in greater bacterial sensitivity to different classes of antimicrobials and opening novel therapeutic possibilities (Yuliani et al. 2024). The system has also been employed to experimentally confirm the essentiality of genes such as *pbpB* and *cwlM*, which are critical for bacterial viability, further reinforcing its relevance in functional research (Kurepina et al. 2022). Additionally, CRISPRi has proven effective in inhibiting resistance efflux systems, such as the *AcrAB-TolC* system, outperforming previous approaches based on small RNAs (sRNAs) (Wan et al. 2022). Despite the still limited number of studies utilizing the CRISPR-Cas3 system, the results obtained so far indicate promising potential in various experimental contexts. Among its main strengths is the efficient elimination of plasmids carrying AMR genes, as demonstrated by several studies (Yosef et al. 2015; Zhou et al. 2023; Fang et al. 2024). Additionally, its application is associated with lower cellular toxicity than the CRISPR-Cas9 system due to its ability to promote progressive DNA degradation, which helps reduce the risk of unwanted mutations and resistance escape. Another aspect is the lower potential for inducing adversarial immune responses, especially when the endogenous CRISPR-Cas3 system is used, thus minimizing the activation of the host immune system.

Regarding the CRISPR-Cas13a system, which remains relatively little explored in the literature, several advantageous points stand out regarding its potential application. One of its main advantages is its high specificity for resistance genes, allowing the precise manipulation of these genes' messenger RNA (mRNA). This approach significantly reduces unwanted effects and contributes to the preservation of beneficial microbiota. In addition, studies have already been conducted regarding the use of bacteriophages as vectors for the efficient delivery of the CRISPR-Cas13a system (Kiga et al. 2020). This approach allows the targeted introduction of the system into specific bacteria, promoting a highly effective and selective strategy to combat resistant microorganisms.

Limitations of different CRISPR-Cas systems

CRISPR-Cas systems have limitations that can influence the choice of the most suitable system, depending on the research target. Among the 33 studies, we identified the limitations associated with each CRISPR-Cas system (Fig. 4).

The CRISPR-Cas9 system presents several limitations in its application to combat antimicrobial-resistant bacteria. First, the conjugation efficiency was considered low in some studies, showing the need for improvements in administration methods. Furthermore, the impact of biofilm structure on the transfer of the system remains poorly explored, representing a gap in current knowledge. Another challenge is the variability in efficiency between different bacterial species, particularly among those phylogenetically distant.

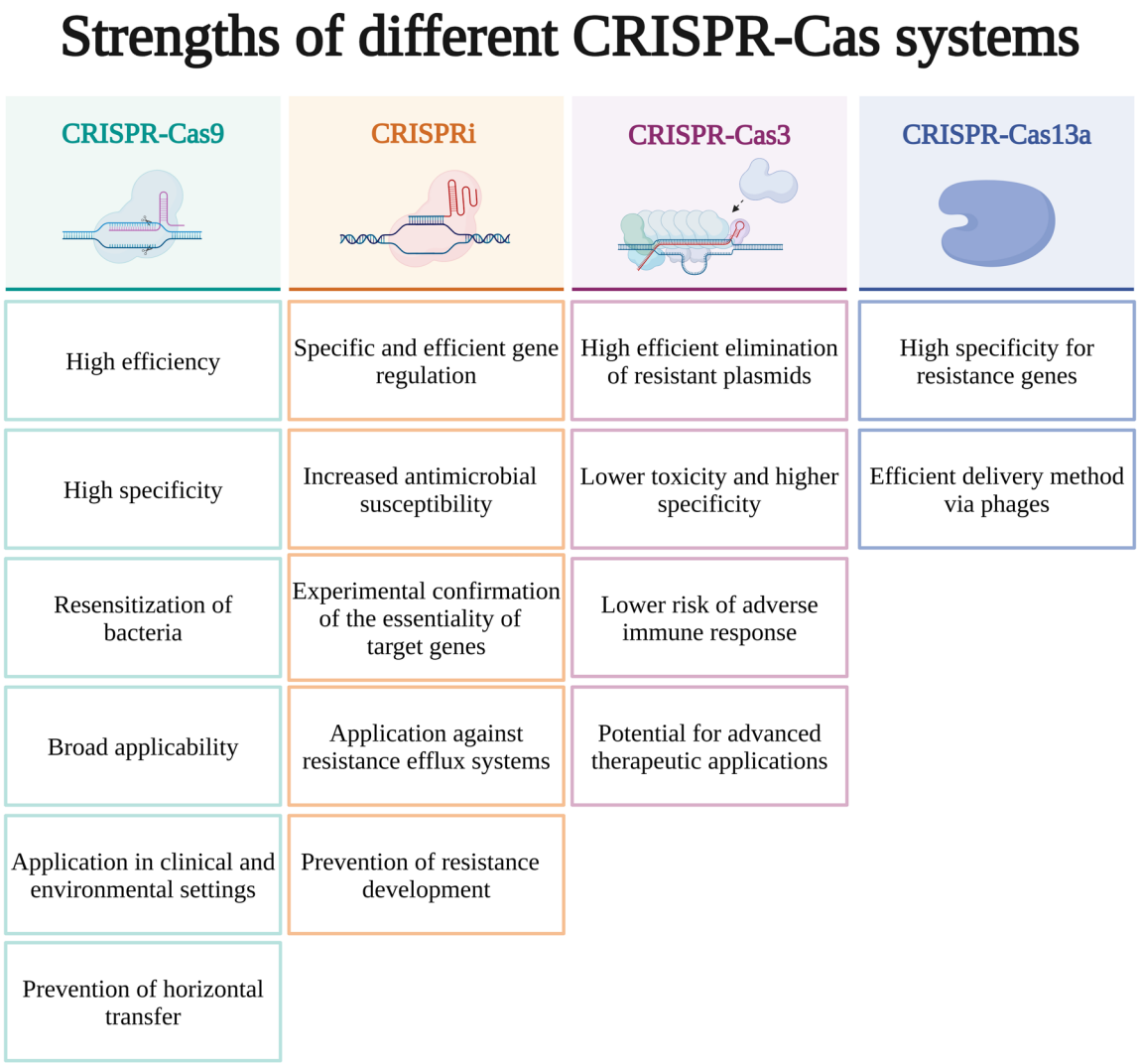

Fig. 3 Strengths of different CRISPR-Cas systems. Comparison of the main advantages of different CRISPR-Cas systems (Cas9, CRISPRi, Cas3, and Cas13a). Each system presents individual characteristics that favor specific applications, such as high specificity, delivery efficiency, or low immunological risk, making them suitable for diverse purposes

Most studies are limited to *E. coli*, without evaluations in complex microbiomes or other clinically relevant species. Finally, the emergence of escape mutants, often resulting from deletions in the CRISPR-Cas9 system, compromises the efficacy of the tool and requires complementary strategies to overcome this problem.

The CRISPRi system has some limitations. Studies have been restricted to laboratory models such as *Mycobacterium smegmatis* and *E. coli* HB101, demonstrating the need for experiments using clinically relevant strains such as *Mycobacterium tuberculosis* and complex resistant isolates from clinical infections, as suggested by Yuliani et al. (2014). The efficacy of the system has been tested against a limited range of antimicrobials, and there is a need to expand these analyses to include a broader spectrum of antimicrobials, especially those most used in clinical settings. Another important problem is the scarcity of in vivo tests; no experiments have yet been conducted in animal models or more complex microbiomes. Furthermore, there is significant variability in the resensitization of resistant bacteria, with some highly resistant isolates showing no significant reduction in the minimum inhibitory concentration (MIC), indicating the presence of additional resistance mechanisms. Finally, the impact of resensitization on the microbiota remains unknown.

The limitations of the CRISPR-Cas3 system are mainly related to its host specificity. This is particularly evident

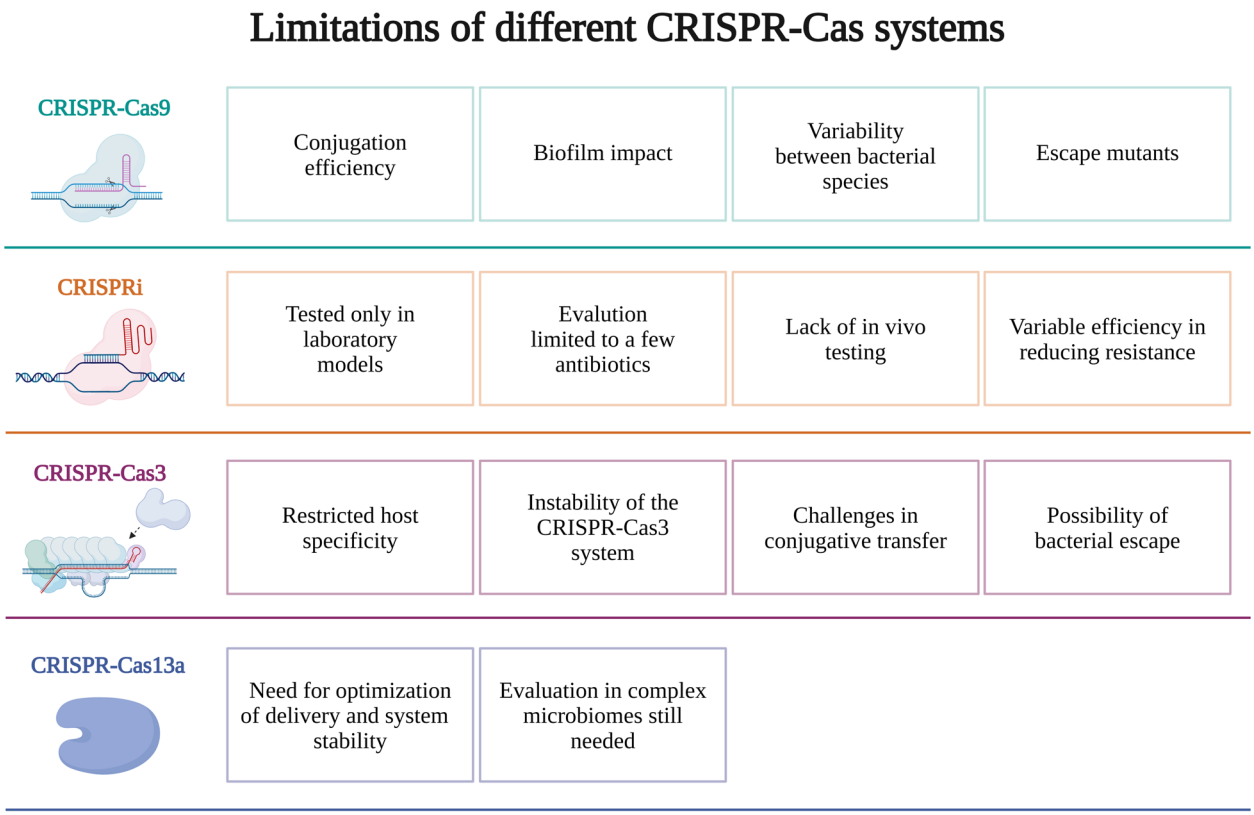

Fig. 4 Comparison of the main limitations of different CRISPR-Cas systems (Cas9, CRISPRi, Cas3, and Cas13a) used in the fight against AMR. Each variant of the CRISPR-Cas system—including CRISPR-Cas9, CRISPRi, CRISPR-Cas3, and CRISPR-Cas13a—presents specific challenges that limit its effectiveness and applicability

because one of the few studies that used this system was either delivered via bacteriophages or protectively restricted the range of host bacteria. Furthermore, the instability of the system has been observed depending on the bacterial context. Finally, bacterial escape is possible, and some strains may develop mechanisms that allow them to escape the system, reducing its effectiveness.

Currently, one of the main challenges in using the CRISPR-Cas13a system is the efficient and stable delivery of the system into bacterial cells. Furthermore, although the results in controlled laboratory environments are promising, their efficacy in more complex microbiomes and in vivo models still needs to be evaluated.

Decision-making map for selecting CRISPR-Cas systems to combat AMR

A simple synthesis of information alone rarely effectively guides the rational choice of the most appropriate system for combating AMR. To address this complexity, we propose a decision-making framework that synthesizes available evidence and supports the optimal selection and deployment of CRISPR platforms for AMR control (Fig. 5).

Discussion

Efficiency and limitations of CRISPR systems

The technology based on CRISPR-Cas systems has emerged as an alternative therapy to conventional antibiotics, whose effectiveness has been progressively compromised by the alarming rise in bacterial resistance (Gomaa et al. 2014). This advancement marks a milestone in transitioning to the era of precision antimicrobials (Okesanya et al. 2025). The CRISPR-Cas platform stands out for its specific targeting potential, enabling the selective elimination of pathogenic bacteria without affecting the commensal microbiota (Citorik et al. 2014). This specificity reinforces the technology as an innovative solution to tackling antimicrobial resistance. Through this Systematic Review, we critically assessed the functional outcomes,

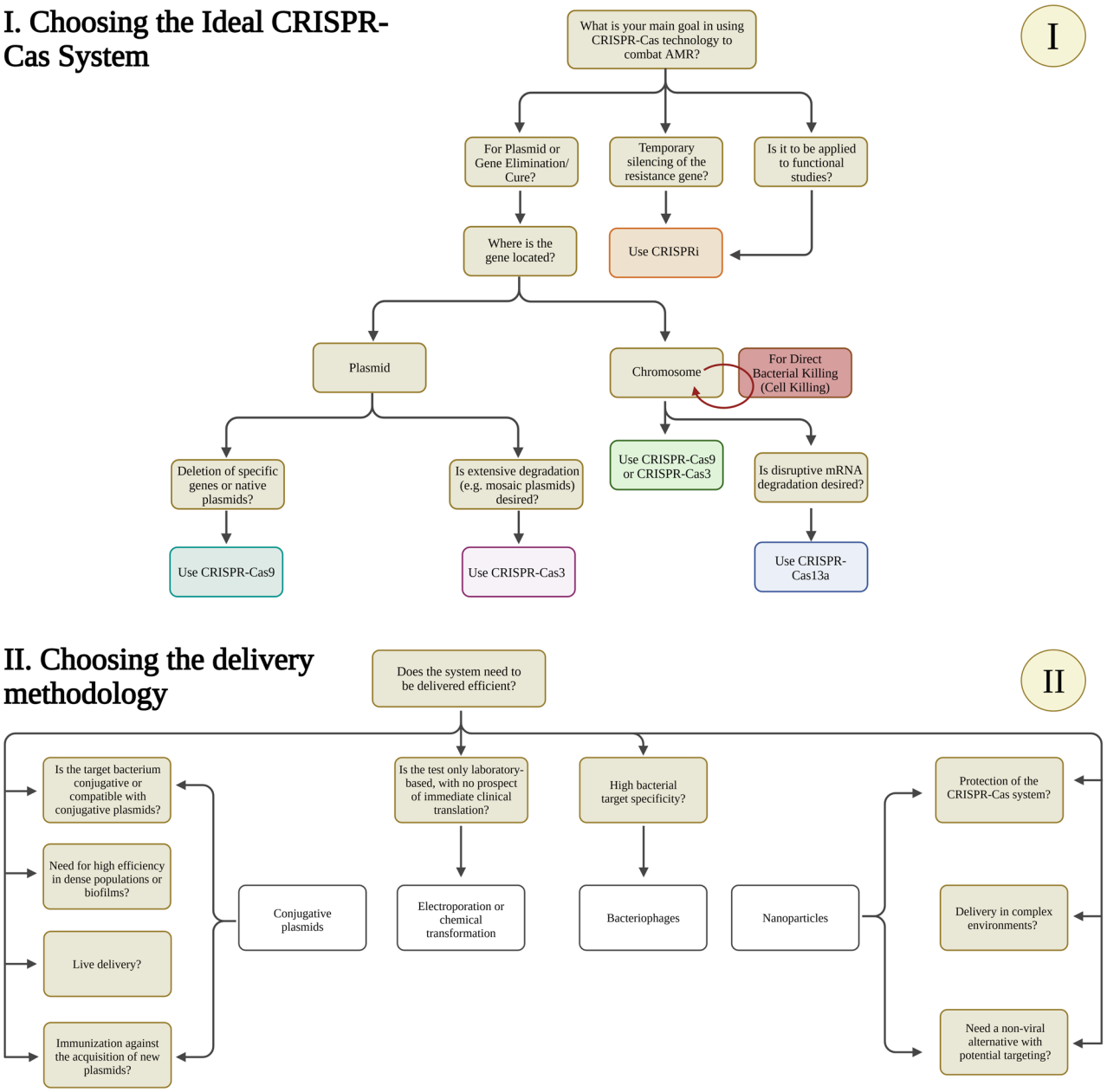

Fig. 5 Decision map for selecting the CRISPR-Cas system and delivery methodology for application in combating antimicrobial resistance (AMR). Section I guides the selection of the most appropriate CRISPR-Cas system based on the primary objective (e.g., plasmid elimination, temporary silencing, gene degradation, or functional application) and the target gene location (plasmid or chromosomal). Different variants are recommended: CRISPR-Cas9, CRISPR-Cas3, CRISPRi, or CRISPR-Cas13a, depending on the application purpose. Section II guides the selection of the most appropriate delivery strategy, depending on factors such as delivery efficiency, application environment (biofilms, complex microbiota), bacterial target, and clinical viability. Among the methodologies analyzed are conjugative plasmids, electroporation, chemical transformation of bacteriophages, and nanoparticles

delivery challenges, and comparative strengths of distinct CRISPR-Cas platforms applied to AMR. In the following sections, we review the efficiency, limitations, and potential applications of the main CRISPR-Cas systems explored for combating AMR.

CRISPR-Cas9

Among the various systems explored, CRISPR-Cas9 predominates due to its well-established use and high efficiency in inducing double-strand breaks (DSBs), leading to gene

inactivation and bacterial resensitization. Two main factors may justify the predominance of studies using the CRISPR-Cas9: (i) the first factor is related to the high efficiency of the Cas9 nuclease, whose ability to induce DNA double-strand breaks (DSBs) confers precision to the CRISPR-Cas9 system (Garneau et al. 2010; Doench et al. 2014). This action can lead to the inactivation of resistance genes and, consequently, bacterial resensitization to antimicrobials (Citorik et al. 2014). In many studies, Cas9 demonstrated success rates above 95%, with specific applications achieving 100% efficiency in eliminating resistance genes such as $bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$, and *mcr-1* (Kim et al. 2016; Wongpayak et al. 2021; He et al. 2021a; Li et al. 2022; Walflor et al. 2022; Djermoun et al. 2023; Yen et al. 2024; Xu et al. 2025); (ii) Its widespread adoption is also historically grounded, as Cas9 was the first CRISPR-based system widely implemented in gene editing (Jinek et al. 2012).

However, despite its high effectiveness, it is essential to acknowledge the inherent limitations of the Cas9 system, such as (i) the activation of DNA repair pathways that may lead to unintended mutations, a critical limitation, particularly when considering genomic editing in clinical settings (Hamilton et al. 2019; Reuter et al. 2021); (ii) the potential emergence of escape mutants (Tagliaferri et al. 2020; Djermoun et al. 2023; Sodani et al. 2023; Frusteri Chiacchiera et al. 2025); (iii) the induction of the SOS response (Mayorga-Ramos et al. 2024); and (iv) low delivery efficiency, especially in environments with biofilm presence or complex microbiome samples (Mayorga-Ramos et al. 2024), which are significant concerns.

Nonetheless, other CRISPR systems, such as CRISPRi, CRISPR-Cas3, and CRISPR-Cas13a, have potential features that remain underexplored in the context of AMR.

CRISPRi

CRISPRi-based technology is derived from the CRISPR-Cas9 system and differs from the native system by using the dCas9 protein, a catalytically inactive variant that cannot cleave DNA (Razavi et al. 2024). Instead, it acts as a physical transcriptional blocker by specifically binding to the target region of the genome, thereby promoting transcriptional modulation (Karlson et al. 2021). Using dCas9 represents an alternative to overcome the limitations associated with native Cas9, particularly the risk of inducing mutations in the target sequence resulting from DNA strand (Razavi et al. 2024). Furthermore, since it does not cause DNA breaks, dCas9 does not activate cellular repair pathways such as non-homologous end joining (NHEJ) and homologous recombination (HR), drastically reducing the emergence of gene variants of resistance genes (Karlson et al. 2021). Its utility in silencing resistance genes and efflux pumps has been demonstrated in *E. coli* and mycobacteria, though its application remains limited by the small number of experimental models (Wan et al. 2022; Frusteri Chiacchiera et al. 2025).

CRISPR-Cas3

Cas3, a helicase-nuclease, enables the degradation of long stretches of DNA after being recruited by a multiprotein complex known as Cascade (Morisaka et al. 2019). This characteristic enables the progressive removal of large DNA segments, allowing for the deletion of entire genes or even multiple adjacent genes (Yosef et al. 2015). Unlike Cas9, which acts like molecular scissors by making precise cuts and sometimes allowing functional religation of the target gene, the continuous and progressive action of Cas3 significantly reduces the chance of the gene becoming functional again (Loeff et al. 2018). The CRISPR-Cas3 system has efficiently eliminated resistance genes such as *mcr-1*, $bla_{\text{NDM-1}}$, and *tet*(X) in the *E. coli* strain (Fang et al. 2024), and has also demonstrated the ability to cure multidrug-resistant plasmids (Zhou et al. 2023; Fang et al. 2024). Additionally, another study reported efficiency rates between 90 and 95% for eliminating IncFII family plasmids in *K. pneumoniae* strains (Zhou et al. 2023). This latter finding represents progress in combating AMR, considering that IncF family plasmids are widely disseminated among several bacterial genera and are heavily involved in spreading resistance determinants (Carattoli 2009). The ability of these plasmids to promote cointegration and form mosaic structures further facilitates the acquisition of resistance determinants (Villa et al. 2010). However, as observed with interference-based CRISPR systems (CRISPRi), the scarcity of studies involving Cas3 restricts its use in more diverse contexts.

CRISPR-Cas13a

Although limited information is available about the CRISPR-Cas13a system, it has demonstrated unique advantages in targeting messenger RNA (mRNA), resulting in the specific degradation of resistance gene transcripts. This RNA-based approach gives Cas13a a distinct mechanism of action compared to Cas9, which acts directly on DNA, reducing the likelihood of unwanted genetic mutations (Fang et al. 2024). Target recognition via the CRISPR-Cas13a system triggers non-specific cleavage activity against bacterial RNAs, often inhibiting bacterial growth (Kiga et al. 2020). Interestingly, Cas13a demonstrated bactericidal activity regardless of the target gene's location, whether plasmid-borne or chromosomal. This was exemplified in the inactivation of the carbapenem resistance gene $bla_{\text{IMP-1}}$ using LshCas13a, which resulted in a two-to-three-log reduction

in bacterial cell count in isolates carrying bla_{IMP-1} either chromosomally or on plasmids (Kiga et al. 2020). The ability to efficiently interfere with the expression of resistance genes grants CRISPR-Cas13a a significant advantage for developing broad-spectrum antimicrobial therapies independent of their genomic location, including those targeting chromosomal resistance mechanisms.

Delivery strategies and challenges

Despite the therapeutic potential of CRISPR-Cas systems in combating resistant bacteria, their practical application still faces challenges, particularly regarding the efficiency of delivery methods. According to our data, bacterial conjugation is the main strategy used (42.4%), followed by electroporation (24.2%) and chemical transformation (21.2%). Although conjugation enables horizontal gene transfer even in complex communities, its low transfer efficiency ($<10^{-1}$) limits its impact at the population level (Hao et al. 2020; Li et al. 2022; Chen et al. 2024b). Although optimization strategies, such as increasing the donor-to-recipient ratio, can elevate conjugation rates to near 100%, such adaptations may compromise the system's specificity, potentially leading to off-target effects in beneficial bacteria (Zhang et al. 2024).

In addition to the intrinsic challenges of conventional delivery methods, several factors can influence the efficient delivery of CRISPR systems into target bacteria. Aspects such as bacterial cell morphology and external conditions like biofilms can hinder system internalization (Wongpayak et al. 2021). One alternative to overcome this limitation is using bacteriophages as delivery vectors (Yosef et al. 2015). Unlike traditional methods, phages are naturally adapted to recognize, bind to, and inject their genetic material directly into specific bacterial populations (Yosef et al. 2017). Moreover, phages can penetrate physical barriers that typically prevent the entry of plasmids or nanoparticles, making them effective in hard-to-reach environments (Mayorga-Ramos et al. 2024). On the other hand, the narrow host range of phages remains one of the main limitations in their therapeutic application (Jia et al. 2023; Kim et al. 2024). To address these limitations, Yosef et al. (2017) developed an innovative approach to create hybrid particles with different tail fiber proteins. This approach enables an expanded host range and allows genetic material transfer to a broader spectrum of bacteria. Nonetheless, the strict dependence of transduction on the compatibility between host cell surface proteins and the phage-encoded tail fiber proteins remains a limiting factor (Kim et al. 2024). This specificity allows for the emergence of bacterial resistance to phages through modifications in surface proteins, which hinders phage recognition and infection (Le et al. 2013; Azam and Tanji 2019).

Nanotechnology-based delivery strategies represent potential alternatives to improve the delivery of CRISPR systems (Duan et al. 2021; Chen et al. 2024a). The use of nanomaterials as non-viral carriers has attracted significant interest from the scientific community due to their potential to ensure stable system delivery (Lee et al. 2017; Tang et al. 2021, p. 2022; Hu and Chen 2022). Several nanoscale delivery systems have been explored, including gold nanoparticles, nitrogen-based particles, graphene oxide, and lipid nanoparticles (Lee et al. 2017; Shahbazi et al. 2019; Wei et al. 2020; Chen et al. 2024a). In this review, the only study that used nanotechnology for the delivery of the CRISPR-Cas9/sgRNAs system mediated by nitrogen-doped carbon dots (NCDs) in *E. coli* proved to be effective, achieving about 50% efficiency even in complex environments such as soil (Chen et al. 2024a). Despite the promising advances observed in initial studies, using nanomaterials integrated with CRISPR-Cas systems still represents a frontier of scientific knowledge, with multiple technical challenges yet to be overcome.

Target-specific CRISPR applications

Considering the advances and limitations of current delivery strategies, the choice of the most appropriate CRISPR-Cas system should be guided by multiple factors. These include the specific therapeutic goal of each study, the location of the resistance gene (plasmid-borne or chromosomal), the target bacterium's characteristics, and the intervention environment's complexity (Walflor et al. 2022).

For example, when seeking to eliminate plasmids carrying resistance genes, the CRISPR-Cas9 and CRISPR-Cas3 systems stand out as viable alternatives, although they present significant functional differences (Zhou et al. 2023; Fang et al. 2024). In contrast, when resistance genes are located at the chromosomal level, as is often the case with vancomycin resistance (*vanA*) (Handwerger and Skoble 1995; Haas et al. 2023), methicillin resistance (*mecA*) (Noto et al. 2008; MacFadyen and Paterson 2024), or carbapenem resistance (bla_{IMP}) (Yano et al. 2024), the CRISPR-Cas13a and CRISPRi systems stand out as up-and-coming tools (Kiga et al. 2020; Yuliani et al. 2024; Frusteri Chiacchiera et al. 2025). Unlike Cas9, which depends on plasmid localization for efficient elimination, the CRISPR-Cas13a system acts with high specificity on messenger RNA, promoting selective degradation without directly affecting the genome (Zhang et al. 2024). CRISPRi effectively silences resistance genes and is also valuable for functional genomic studies (Zhang et al. 2021). Its mechanism of operation allows for reversible and less disruptive control of gene expression (Qi et al. 2013). This characteristic makes CRISPRi particularly advantageous when the goal is transiently modulating resistance,

enabling antimicrobials to regain effectiveness without causing permanent changes to bacterial genetic material (Wang et al. 2019b). Moreover, genomic analyses of surviving ("escaper") bacteria treated with CRISPRi confirmed the absence of mutations in the target sequence, demonstrating CRISPRi's non-mutagenic role in the selection of new resistance traits (Frusteri Chiacchiera et al. 2025).

Strategic and translational perspectives of CRISPR antimicrobials

The development of CRISPR-based antimicrobials has opened new frontiers in combating multidrug resistance (MDR). Strategies that integrate distinct CRISPR platforms have shown particular promise (Duan et al. 2021). For example, the combination of Cas13a with Cas9 allows the simultaneous targeting of RNA and DNA. Cas13a acts at the transcriptional level, enabling the degradation or modulation of specific transcripts, while Cas9 acts at the genomic level, inducing permanent DNA modifications. This complementary activity broadens the therapeutic spectrum of CRISPR technologies. Furthermore, multiplexed gRNA strategies allow the concomitant silencing or deletion of multiple resistance genes, thus addressing the multifactorial nature of AMR (McCarty et al. 2020). Such multiplexing is achieved through arrays of sgRNAs transcribed from unique promoters, incorporation of self-cleaving ribozymes, or other RNA-processing tools, including tRNA-based strategies (McCarty et al. 2020).

In addition to its therapeutic potential in reversing AMR, the CRISPR system also stands out as a highly sensitive and specific diagnostic tool for detecting resistant bacterial strains (Abavisani et al. 2023). Among the tools discussed in this review, the CRISPR-Cas13 system enables the targeted identification of pathogens or genetic determinants of resistance by binding to a specific target RNA (Huang et al. 2022). Its collateral cleavage activity degrades nearby reporter molecules, which can be fluorescent or colorimetric (Yan et al. 2018; Huang et al. 2022). This enables the generation of a highly sensitive signal based on the presence of the target RNA. The SHERLOCK (for specific high-sensitivity enzymatic reporter unlocking) platform exemplifies this approach (Kaminski et al. 2021). It integrates CRISPR-Cas13a-based detection with isothermal amplification methodologies such as RPA or RT-RPA, enabling portable, rapid, and accurate diagnostics, with significant potential for clinical and epidemiological surveillance of AMR (Kaminski et al. 2021).

Complementary RNA-targeted strategies are also gaining traction as alternatives to conventional antimicrobials (Abavisani et al. 2025). Antisense oligonucleotides (ASOs) can be rationally designed to recognize and bind to resistance-associated transcripts, blocking their translation or promoting degradation (Roberts et al. 2020; Hammond et al. 2021). Similarly, riboswitches, natural regulatory elements located primarily in mRNA, can modulate gene expression in response to specific ligands, altering transcription, translation, or RNA stability, offering precise control over target genes, including AMR determinants (Ellinger et al. 2023). These RNA-based approaches offer complementary mechanisms to CRISPR, acting primarily at the transcriptional or post-transcriptional level, and can be integrated with CRISPR strategies to enhance the inhibition of resistant pathogens.

Despite the impressive precision and effectiveness exhibited by CRISPR antimicrobials in various in vitro and in vivo models, substantial translational and regulatory challenges still exist. A primary concern is the potential for *off-target* effects, especially in larger genomes that often harbor multiple regions that are identical or highly homologous to the intended target (Kalter et al. 2025). To mitigate this limitation, several strategies have been proposed, including refined guide RNA design (Naeem et al. 2020) and the development of high-fidelity Cas9 variants (Zuo et al. 2022). Given these challenges, transcriptional regulatory platforms such as CRISPRi and CRISPR-Cas13a may offer a relative advantage, as they minimize the risk of permanent genomic alterations and thus facilitate regulatory acceptance and clinical implementation.

Conclusion

Currently, different CRISPR systems, such as CRISPR-Cas9, Cas3, Cas13a, and CRISPRi, are available, offering distinct mechanisms of action, enabling personalized interventions based on genetic location, bacterial species, and therapeutic targets. The successful application of this technology is closely linked to the rational selection of the appropriate CRISPR system, which must consider multiple factors, including administration efficiency, the risk of adverse effects, and the preservation of beneficial microbiota. Therefore, this study not only synthesizes current knowledge on the use of CRISPR-Cas technologies in combating AMR, but also introduces a novel perspective: that the effectiveness of these tools depends on the rational and strategic selection of each system based on genetic targets, bacterial context, and therapeutic targets.

Acknowledgements The authors acknowledge all individuals and institutions that contributed to this study.

Authors' contributions H.C.A.S. conceptualization, data curation, investigation, methodology, writing—original draft, writing—review and editing, and visualization, P.P. conceptualization, investigation, methodology, writing—review and editing, A.B.P. investigation, writing—review, A.M.P.S. writing—review and editing, J.F. writing—review and editing, and editing, and visualization, C.A.C.J. funding acquisition, project administration, supervision, and writing—review and editing.

Funding This study was supported by the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) Brazil—grantnumber [E26/202.227/2018] and [E26/204.078/2022], the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—grantnumber [313119/2020–1], and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Brazil—FinanceCode001.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Abavisani M, Khayami R, Hoseinzadeh M (2023) CRISPR-cas system as a promising player against bacterial infection and antibiotic resistance. Drug Resist Updat 68:100948

Abavisani M, Sajjadi SM, Ebadpour N et al (2025) RNA-based antibacterial agents: mechanisms, functional insights, and challenges in therapeutic development. Chem Eng J 520:165777. https://doi.org/10.1016/j.cej.2025.165777

Almansour AM, Alhadlaq MA, Alzahrani KO (2023) The silent threat: antimicrobial-resistant pathogens in food-producing animals and their impact on public health. Microorganisms 11:2127. https://doi.org/10.3390/microorganisms11092127

Alomari R, Abdel-Razeq A, Shamiah H (2024) Comprehensive assessment of the global burden of antimicrobial resistance: trends and insights from 2000 to 2023. Am J Biomed 12:151–168. https://doi.org/10.18081/2333-5106/2024.12/151

Azam AH, Tanji Y (2019) Bacteriophage-host arm race: an update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy. Appl Microbiol Biotechnol 103:2121–2131. https://doi.org/10.1007/s00253-019-09629-x

Bikard D, Euler CW, Jiang W et al (2014) Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol 32:1146–1150. https://doi.org/10.1038/nbt.3043

Carattoli A (2009) Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 53:2227–2238. https://doi.org/10.1128/AAC.01707-08

Chen F, Du H, Tao M et al (2024a) Nitrogen-doped carbon dots facilitate CRISPR/Cas for reducing antibiotic resistance genes in the environment. J Agric Food Chem 72:3397–3405. https://doi.org/10.1021/acs.jafc.3c08558

Chen L, Peirano G, Yen K et al (2024b) CRISPR-Cas9-mediated IncF plasmid curing in extraintestinal pathogenic *Escherichia coli*. Microbiol Spectr 12:e03692-e3723. https://doi.org/10.1128/spectrum.03692-23

Citorik RJ, Mimee M, Lu TK (2014) Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat Biotechnol 32:1141–1145. https://doi.org/10.1038/nbt.3011

Djermoun S, Reuter A, Derollez E (2023) Reprogramming targeted-antibacterial-plasmids (TAPs) to achieve broad-host range antibacterial activity. Plasmid 126:102680. https://doi.org/10.1016/j.plasmid.2023.102680

Doench JG, Hartenian E, Graham DB (2014) Rational design of highly active sgRNAs for CRISPR-Cas9–mediated gene inactivation. Nat Biotechnol 32:1262–1267. https://doi.org/10.1038/nbt.3026

Dominguez AA, Lim WA, Qi LS (2016) Beyond editing: repurposing CRISPR–Cas9 for precision genome regulation and interrogation. Nat Rev Mol Cell Biol 17:5–15. https://doi.org/10.1038/nrm.2015.2

Duan L, Ouyang K, Xu X et al (2021) Nanoparticle delivery of CRISPR/Cas9 for genome editing. Front Genet 12:673286. https://doi.org/10.3389/fgene.2021.673286

Ellinger E, Chauvier A, Romero RA et al (2023) Riboswitches as therapeutic targets: promise of a new era of antibiotics. Expert Opin Ther Targets 27:433–445. https://doi.org/10.1080/14728222.2023.2230363

Fang M, Zhang R, Wang C et al (2024) Engineering probiotic *Escherichia coli* Nissle 1917 to block transfer of multiple antibiotic resistance genes by exploiting a type I CRISPR-Cas system. Appl Environ Microbiol 90:e00811-e824. https://doi.org/10.1128/aem.00811-24

Ferri M, Ranucci E, Romagnoli P, Giaccone V (2017) Antimicrobial resistance: a global emerging threat to public health systems. Crit Rev Food Sci Nutr 57:2857–2876. https://doi.org/10.1080/10408398.2015.1077192

Frusteri Chiacchiera A, Casanova M, Bellato M et al (2025) Harnessing CRISPR interference to resensitize laboratory strains and clinical isolates to last resort antibiotics. Sci Rep 15:261. https://doi.org/10.1038/s41598-024-81989-5

Garneau JE, Dupuis M-È, Villion M et al (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468:67–71. https://doi.org/10.1038/nature09523

Gomaa AA, Klumpe HE, Luo ML et al (2014) Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. mBio 5:e00928-13. https://doi.org/10.1128/mBio.00928-13

Gong B, Shin M, Sun J et al (2014) Molecular insights into DNA interference by CRISPR-associated nuclease-helicase Cas3. Proc Natl Acad Sci U S A 111:16359–16364. https://doi.org/10.1073/pnas.1410806111

Haas W, Singh N, Lainhart W et al (2023) Genomic Analysis of Vancomycin-Resistant *Staphylococcus aureus* Isolates from the 3rd Case Identified in the United States Reveals Chromosomal Integration of the *vanA* Locus. Microbiol Spectr 11:e04317-e4322. https://doi.org/10.1128/spectrum.04317-22

Hamilton TA, Pellegrino GM, Therrien JA et al (2019) Efficient inter-species conjugative transfer of a CRISPR nuclease for targeted bacterial killing. Nat Commun 10:4544. https://doi.org/10.1038/s41467-019-12448-3

Hammond SM, Aartsma-Rus A, Alves S (2021) Delivery of oligonucleotide-based therapeutics: challenges and opportunities. EMBO Mol Med 13:e13243. https://doi.org/10.15252/emmm.202013243

Handwerger S, Skoble J (1995) Identification of chromosomal mobile element conferring high-level vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 39:2446–2453. https://doi.org/10.1128/AAC.39.11.2446

Hao M, He Y, Zhang H et al (2020) CRISPR-Cas9-mediated carbapenemase gene and plasmid curing in carbapenem-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother 64:e00843–20. https://doi.org/10.1128/AAC.00843-20

He Y-Z, Kuang X, Long T-F et al (2021a) Re-engineering a mobile-CRISPR/Cas9 system for antimicrobial resistance gene curing and immunization in *Escherichia coli*. J Antimicrob Chemother 77:74–82. https://doi.org/10.1093/jac/dkab368

He Y-Z, Yan J-R, He B et al (2021b) A transposon-associated CRISPR/Cas9 system specifically eliminates both chromosomal and plasmid-borne *mcr-1* in *Escherichia coli*. Antimicrob Agents Chemother 65:e01054–21. https://doi.org/10.1128/AAC.01054-21

Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 327:167–170. https://doi.org/10.1126/science.1179555

Hu T, Chen X (2022) Nano for CRISPR. ACS Nano 16:8505–8506. https://doi.org/10.1021/acsnano.2c05431

Huang Z, Fang J, Zhou M et al (2022) CRISPR-Cas13: a new technology for the rapid detection of pathogenic microorganisms. Front

Microbiol 13:1011399. https://doi.org/10.3389/fmicb.2022.1011399

Huo Y, Nam KH, Ding F et al (2014) Structures of CRISPR Cas3 offer mechanistic insights into Cascade-activated DNA unwinding and degradation. Nat Struct Mol Biol 21:771–777. https://doi.org/10.1038/nsmb.2875

Jia H-J, Jia P-P, Yin S et al (2023) Engineering bacteriophages for enhanced host range and efficacy: insights from bacteriophage-bacteria interactions. Front Microbiol 14:1172635. https://doi.org/10.3389/fmicb.2023.1172635

Jinek M, Chylinski K, Fonfara I et al (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821. https://doi.org/10.1126/science.1225829

Kalter N, Fuster-García C, Silva A et al (2025) Off-target effects in CRISPR-Cas genome editing for human therapeutics: progress and challenges. Mol Ther Nucleic Acids 36:102636. https://doi.org/10.1016/j.omtn.2025.102636

Kaminski MM, Abudayyeh OO, Gootenberg JS (2021) CRISPR-based diagnostics. Nat Biomed Eng 5:643–656. https://doi.org/10.1038/s41551-021-00760-7

Karlson CKS, Mohd-Noor SN, Nolte N, Tan BC (2021) CRISPR/dCas9-based systems: mechanisms and applications in plant sciences. Plants 10:2055. https://doi.org/10.3390/plants10102055

Kiga K, Tan X-E, Ibarra-Chávez R et al (2020) Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. Nat Commun 11:2934. https://doi.org/10.1038/s41467-020-16731-6

Kim J-S, Cho D-H, Park M et al (2016) CRISPR/Cas9-mediated re-sensitization of antibiotic-resistant *Escherichia coli* harboring extended-spectrum β-lactamases. J Microbiol Biotechnol 26:394–401. https://doi.org/10.4014/jmb.1508.08080

Kim MK, Chen Q, Echterhof A et al (2024) A blueprint for broadly effective bacteriophage-antibiotic cocktails against bacterial infections. Nat Commun 15:9987. https://doi.org/10.1038/s41467-024-53994-9

Kurepina N, Chen L, Composto K et al (2022) CRISPR inhibition of essential peptidoglycan biosynthesis genes in *Mycobacterium abscessus* and its impact on β-lactam susceptibility. Antimicrob Agents Chemother 66:e00093–22. https://doi.org/10.1128/aac.00093-22

Le S, He X, Tan Y et al (2013) Mapping the tail fiber as the receptor binding protein responsible for differential host specificity of *Pseudomonas aeruginosa* bacteriophages PaP1 and JG004. PLoS ONE 8:e68562. https://doi.org/10.1371/journal.pone.0068562

Lee K, Conboy M, Park HM et al (2017) Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA *in vivo* induces homology-directed DNA repair. Nat Biomed Eng 1(11):889–901. https://doi.org/10.1038/s41551-017-0137-2

Li P, Wan P, Zhao R et al (2022) Targeted elimination of bla_{NDM-5} gene in *Escherichia coli* by conjugative CRISPR-Cas9 system. Infect Drug Resist 15:1707–1716. https://doi.org/10.2147/IDR.S357470

Liu H, Li H, Liang Y et al (2020) Phage-delivered sensitisation with subsequent antibiotic treatment reveals sustained effect against antimicrobial resistant bacteria. Theranostics 10:6310–6321. https://doi.org/10.7150/thno.42573

Loeff L, Brouns SJJ, Joo C (2018) Repetitive DNA reeling by the Cascade-Cas3 complex in nucleotide unwinding steps. Mol Cell 70:385-394.e3. https://doi.org/10.1016/j.molcel.2018.03.031

MacFadyen AC, Paterson GK (2024) Methicillin resistance in *Staphylococcus pseudintermedius* encoded within novel staphylococcal cassette chromosome *mec* (SCC*mec*) variants. J Antimicrob Chemother 79:1303–1308. https://doi.org/10.1093/jac/dkae096

Makarova KS, Wolf YI, Alkhnbashi OS et al (2015) An updated evolutionary classification of CRISPR–Cas systems. Nat Rev Microbiol 13:722–736. https://doi.org/10.1038/nrmicro3569

Mayorga-Ramos A, Carrera-Pacheco SE, Barba-Ostria C, Guamán LP (2024) Bacteriophage-mediated approaches for biofilm control. Front Cell Infect Microbiol 14:1428637. https://doi.org/10.3389/fcimb.2024.1428637

McCarty NS, Graham AE, Studená L, Ledesma-Amaro R (2020) Multiplexed CRISPR technologies for gene editing and transcriptional regulation. Nat Commun 11:1281. https://doi.org/10.1038/s41467-020-15053-x

Morisaka H, Yoshimi K, Okuzaki Y et al (2019) Crispr-cas3 induces broad and unidirectional genome editing in human cells. Nat Commun 10:5302. https://doi.org/10.1038/s41467-019-13226-x

Musoke D, Namata C, Lubega GB et al (2021) Access, use and disposal of antimicrobials among humans and animals in Wakiso district, Uganda: a qualitative study. J Pharm Policy Pract 14:69. https://doi.org/10.1186/s40545-021-00361-4

Naeem M, Majeed S, Hoque MZ, Ahmad I (2020) Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing. Cells 9:1608. https://doi.org/10.3390/cells9071608

Naghavi M, Vollset SE, Ikuta KS (2024) Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet 404:1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1

Nittayasut N, Yata T, Chirakul S et al (2024) Non-replicative phage particles delivering CRISPR-Cas9 to target major bla_{CTX-M} variants. PLoS ONE 19:e0303555. https://doi.org/10.1371/journal.pone.0303555

Noto MJ, Fox PM, Archer GL (2008) Spontaneous deletion of the methicillin resistance determinant, *mecA*, partially compensates for the fitness cost associated with high-level vancomycin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 52:1221–1229. https://doi.org/10.1128/AAC.01164-07

O'Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations. London: HM Government and Wellcome Trust; 2016. Review on Antimicrobial Resistance, chaired by Jim O'Neill. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Accessed 12 June 2025

Okesanya OJ, Ahmed MM, Ogaya JB et al (2025) Reinvigorating AMR resilience: leveraging CRISPR–Cas technology potentials to combat the 2024 WHO bacterial priority pathogens for enhanced global health security—a systematic review. Trop Med Health 53:43. https://doi.org/10.1186/s41182-025-00728-2

Page MJ, McKenzie JE, Bossuyt PM et al (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev 10:89. https://doi.org/10.1186/s13643-021-01626-4

Pursey E, Sünderhauf D, Gaze WH (2018) Crispr-cas antimicrobials: challenges and future prospects. PLoS Pathog 14:e1006990. https://doi.org/10.1371/journal.ppat.1006990

Qi LS, Larson MH, Gilbert LA et al (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152:1173–1183. https://doi.org/10.1016/j.cell.2013.02.022

Razavi Z, Soltani M, Souri M, Van Wijnen AJ (2024) Crispr innovations in tissue engineering and gene editing. Life Sci 358:123120. https://doi.org/10.1016/j.lfs.2024.123120

Reuter A, Hilpert C, Dedieu-Berne A et al (2021) Targeted-antibacterial-plasmids (TAPs) combining conjugation and CRISPR/cas systems achieve strain-specific antibacterial activity. Nucleic Acids Res 49:3584–3598. https://doi.org/10.1093/nar/gkab126

Roberts TC, Langer R, Wood MJA (2020) Advances in oligonucleotide drug delivery. Nat Rev Drug Discov 19:673–694. https://doi.org/10.1038/s41573-020-0075-7

Shahbazi R, Sghia-Hughes G, Reid JL et al (2019) Targeted homology-directed repair in blood stem and progenitor cells with CRISPR nanoformulations. Nat Mater 18:1124–1132. https://doi.org/10.1038/s41563-019-0385-5

Sinkunas T, Gasiunas G, Fremaux C et al (2011) Cas3 is a single-stranded DNA nuclease and ATP-dependent helicase in the CRISPR/Cas immune system: Cas3 nuclease/helicase. EMBO J 30:1335–1342. https://doi.org/10.1038/emboj.2011.41

Sodani M, Misra CS, Rath D, Kulkarni S (2023) Harnessing CRISPR-Cas9 as an anti-mycobacterial system. Microbiol Res 270:127319. https://doi.org/10.1016/j.micres.2023.127319

Souza HCA, Panzenhagen P, Dos Santos AMP (2025) Unravelling the advances of CRISPR-Cas9 as a precise antimicrobial therapy: a systematic review. J Glob Antimicrob Resist 42:51–60. https://doi.org/10.1016/j.jgar.2025.02.002

Sünderhauf D, Klümper U, Pursey E et al (2023) Removal of AMR plasmids using a mobile, broad host-range CRISPR-Cas9 delivery tool. Microbiology 169:001334. https://doi.org/10.1099/mic.0.001334

Tagliaferri TL, Guimarães NR, Pereira MDPM et al (2020) Exploring the potential of CRISPR-Cas9 under challenging conditions: facing high-copy plasmids and counteracting beta-lactam resistance in clinical strains of *Enterobacteriaceae*. Front Microbiol 11:578. https://doi.org/10.3389/fmicb.2020.00578

Tang H, Zhao X, Jiang X (2021) Synthetic multi-layer nanoparticles for CRISPR-Cas9 genome editing. Adv Drug Deliv Rev 168:55–78. https://doi.org/10.1016/j.addr.2020.03.001

Tao S, Chen H, Li N et al (2023a) Elimination of bla_{KPC-2}-mediated carbapenem resistance in *Escherichia coli* by CRISPR-Cas9 system. BMC Microbiol 23(1):310. https://doi.org/10.1186/s12866-023-03058-7

Tao S, Hu C, Fang Y et al (2023b) Targeted elimination of Vancomycin resistance gene vanA by CRISPR-Cas9 system. BMC Microbiol 23:380. https://doi.org/10.1186/s12866-023-03136-w

Valderrama JA, Kulkarni SS, Nizet V, Bier E (2019) A bacterial gene-drive system efficiently edits and inactivates a high copy number antibiotic resistance locus. Nat Commun 10:5726. https://doi.org/10.1038/s41467-019-13649-6

Vidović J, Stojanović D, Cagnardi P et al (2022) Farm animal veterinarians' knowledge and attitudes toward antimicrobial resistance and antimicrobial use in the Republic of Serbia. Antibiotics 11:64. https://doi.org/10.3390/antibiotics11010064

Villa L, García-Fernández A, Fortini D, Carattoli A (2010) Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother 65:2518–2529. https://doi.org/10.1093/jac/dkq347

Walflor HSM, Lucena ARC, Tuon FF et al (2022) Resensitization of fosfomycin-resistant *Escherichia coli* using the CRISPR system. Int J Mol Sci 23:9175. https://doi.org/10.3390/ijms23169175

Wan P, Cui S, Ma Z et al (2020) Reversal of *mcr-1*-mediated colistin resistance in *Escherichia coli* by CRISPR-Cas9 system. Infect Drug Resist 13:1171–1178. https://doi.org/10.2147/IDR.S244885

Wan X, Li Q, Olsen RH et al (2022) Engineering a CRISPR interference system targeting AcrAB-TolC efflux pump to prevent multidrug resistance development in *Escherichia coli*. J Antimicrob Chemother 77:2158–2166. https://doi.org/10.1093/jac/dkac166

Wang P, He D, Li B et al (2019a) Eliminating *mcr-1*-harbouring plasmids in clinical isolates using the CRISPR/Cas9 system. J Antimicrob Chemother 74:2559–2565. https://doi.org/10.1093/jac/dkz246

Wang T, Wang M, Zhang Q et al (2019b) Reversible gene expression control in *Yersinia pestis* by using an optimized CRISPR interference system. Appl Environ Microbiol 85:e00097. https://doi.org/10.1128/AEM.00097-19

Wei T, Cheng Q, Min Y-L (2020) Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. Nat Commun 11:3232. https://doi.org/10.1038/s41467-020-17029-3

Wongpayak P, Meesungnoen O, Saejang S, Subsoontorn P (2021) A highly effective and self-transmissible CRISPR antimicrobial for elimination of target plasmids without antibiotic selection. PeerJ 9:e11996. https://doi.org/10.7717/peerj.11996

World Health Organization (2022) Global antimicrobial resistance and use surveillance system (GLASS) Report 2022

Xu L, Lu X, Li Y et al (2025) Eliminating the tigecycline resistance RND efflux pump gene cluster *tmexCD-toprJ* in bacteria using CRISPR/Cas9. Int J Antimicrob Agents 65:107390. https://doi.org/10.1016/j.ijantimicag.2024.107390

Yan WX, Chong S, Zhang H et al (2018) Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. Mol Cell 70:327-339.e5. https://doi.org/10.1016/j.molcel.2018.02.028

Yano H, Hayashi W, Kawakami S et al (2024) Nationwide genome surveillance of carbapenem-resistant *Pseudomonas aeruginosa* in Japan. Antimicrob Agents Chemother 68:e01669–23. https://doi.org/10.1128/aac.01669-23

Yen KK, Terlecky AJ, Hao M et al (2024) Curing of common plasmids in gram-negative bacteria using a Cas9-based conjugative vector. J Microbiol Methods 226:107047. https://doi.org/10.1016/j.mimet.2024.107047

Yosef I, Manor M, Kiro R, Qimron U (2015) Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. Proc Natl Acad Sci U S A 112:7267–7272. https://doi.org/10.1073/pnas.1500107112

Yosef I, Goren MG, Globus R et al (2017) Extending the Host Range of Bacteriophage Particles for DNA Transduction. Mol Cell 66:721-728.e3. https://doi.org/10.1016/j.molcel.2017.04.025

Yuliani Y, Ilmi AFN, Petsong S et al (2024) CRISPR interference-mediated silencing of the *mmpL3* gene in *Mycobacterium smegmatis* and its impact on antimicrobial susceptibility. Antibiotics 13:483. https://doi.org/10.3390/antibiotics13060483

Zhang R, Xu W, Shao S, Wang Q (2021) Gene silencing through CRISPR interference in bacteria: current advances and future prospects. Front Microbiol 12:635227. https://doi.org/10.3389/fmicb.2021.635227

Zhang H, Chen B, Wang Z et al (2024) Resensitizing tigecycline- and colistin-resistant *Escherichia coli* using an engineered conjugative CRISPR/Cas9 system. Microbiol Spectr 12:e03884–23. https://doi.org/10.1128/spectrum.03884-23

Zhou Y, Yang Y, Li X et al (2023) Exploiting a conjugative endogenous CRISPR-Cas3 system to tackle multidrug-resistant *Klebsiella pneumoniae*. EBioMedicine 88:104445. https://doi.org/10.1016/j.ebiom.2023.104445

Zuo Z, Babu K, Ganguly C et al (2022) Rational engineering of CRISPR-Cas9 nuclease to attenuate position-dependent off-target effects. CRISPR J 5:329–340. https://doi.org/10.1089/crispr.2021.0076