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Review article

Gene-Targeted Antimicrobial Strategies Using CRISPR-Cas Systems Against Multidrug-Resistant Bacterial Pathogens

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Abstract: The rapid emergence of antibiotic-resistant bacteria has significantly outpaced the development of new antimicrobial agents, posing a global health threat. This review investigates the application of CRISPR-Cas9 and CRISPR-dCas9 systems as innovative strategies to combat resistance in key bacterial pathogens. A systematic literature search identified studies focused on methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*. In MRSA, the CRISPR-dCas9 system was used to suppress mecA gene transcription, leading to a 77% reduction in gene expression without compromising bacterial viability-an approach with implications for microbiome-safe interventions. In contrast, CRISPR-Cas9 targeting of conserved ESBL gene sequences in *E. coli* successfully cleaved resistance determinants, resulting in >99% re-sensitization to β-lactam antibiotics. The engineered pRESAFRESBL plasmid demonstrated cross-sensitivity restoration, suggesting plasmid-wide disruption of co-transferred resistance genes. Together, these findings highlight the transformative potential of CRISPR-based technologies in reversing resistance phenotypes through precise, gene-targeted interventions. This approach paves the way for next-generation antimicrobial therapies that either neutralize resistance while preserving beneficial flora or directly eradicate resistant clone

Keywords: CRISPR-Cas9, CRISPR-dCas9, antibiotic resistance, MRSA, ESBL-producing *E. coli*, gene editing, bacterial re-sensitization.

Introduction

The evolution of microorganisms resistant to antibiotics has been significantly hastened by the extensive use of antibiotics. Drug resistance in human pathogens is becoming more common, which is dangerous since it restricts treatments that were once effective for curable illnesses and raises the possibility of deadly consequences. [1, 2, 3]. Nevertheless, the rate at which drug-resistant bacteria emerge outpaces the rate at which new antibiotics are being developed . In the last five years, the Food and Drug Administration has only approved two systemic antibiotics: daptomycin and linezolid [4]. The first penicillin-resistant bacteria were found within ten years after Alexander Fleming's discovery of the antibiotic [5] and even before it was used in clinical settings . Third-generation cephalosporins, a class of extended-spectrum β-lactam antibiotics, were introduced in the early 1980s and initially represented a major advance in treating infections caused by β-lactamase-producing bacteria. Early β-lactamase inhibitors such as TEM-1, TEM-2, and SHV-1 were considered groundbreaking at the time. However, shortly after the clinical introduction of cephalosporins in the mid-1980s, a new group of enzymes—extended-spectrum β-lactamases (ESBLs)—was identified [6–8]. These enzymes significantly limited the efficacy of third-generation cephalosporins by hydrolyzing them, contributing to the rapid emergence of multidrug-resistant bacterial strains. In recent years, a growing concern in microbiology has been the slow pace of novel antimicrobial discovery compared to the rapid evolution of resistance in microorganisms, particularly bacteria, fungi, and viruses. This has spurred interest in innovative therapeutic approaches such as gene-editing-based antimicrobials. The CRISPR-Cas system, particularly CRISPR-Cas9, has emerged as a promising tool due to its RNA-guided DNA targeting mechanism. The specificity of CRISPR arises from RNA-DNA interactions, and because RNA can be synthesized cost-effectively, CRISPR-based systems offer a practical and scalable platform for antimicrobial development [9]. CRISPR-Cas systems can be employed in three main strategies to combat antibiotic-resistant bacteria: Species-specific gene targeting – selectively cleaving bacterial genes responsible for pathogenicity while preserving the host's natural microbiota [10]. Drug-resistance gene elimination – targeting and destroying resistance genes, thereby eliminating resistant strains and allowing susceptible wild-type bacteria to repopulate [11]. Gene suppression or modification – introducing targeted mutations or transcriptional repression to deactivate resistance genes without killing the host bacterium, a process known as re-sensitization [12]. Among the CRISPR systems, Type II CRISPR-Cas9 is the most widely utilized for

genetic engineering due to its simplicity. Unlike Class 1 systems, which require multi-protein complexes, Cas9 alone is sufficient for gene interference, as it contains two catalytic domains. The CRISPR-Cas9 system functions through a chimeric single-guide RNA (sgRNA), which combines the tracrRNA and crRNA into a single molecule. This sgRNA is designed to be complementary to the target gene sequence and must be adjacent to a protospacer adjacent motif (PAM), typically the "NGG" motif. To ensure specificity, the selected target must be unique and absent from other regions of the bacterial genome or mobilome. Once the sgRNA-Cas9 complex binds to its target, Cas9 introduces a double-stranded DNA break at the specified site, generating blunt ends [13]. This type of chromosomal damage poses a serious threat to bacterial viability and is typically repaired by non-homologous end joining (NHEJ), a repair process prone to errors. These errors can introduce insertions or deletions that disrupt gene function, often impairing protein synthesis or killing the cell [14]. This review compiles evidence from various studies on antibiotic-resistant bacterial species, demonstrating how CRISPR-Cas9 can be used to either deactivate resistance genes or revert bacteria to their drug-sensitive wild-type state, offering a powerful strategy for addressing the global antibiotic resistance crisis.

Methodology

2.1 Data collection procedures

A comprehensive literature search was conducted using the PubMed database to identify relevant studies on the use of CRISPR systems in combating antibiotic resistance. The search utilized the keywords “CRISPR,” “antibiotic,” and “resistance,” returning approximately 1,184 publications published between 2006 and 2025. To refine the results, filters were applied to focus specifically on studies involving ESKAPE pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—which are known for their role in multidrug-resistant infections and the other for "Cas9" to focus on studies utilizing the CRISPR-Cas9 system. The titles and abstracts of the retrieved articles were screened manually to identify studies reporting bacterial strains with mutations in genes associated with antibiotic resistance and involving the use of CRISPR-Cas9 either for therapeutic intervention or experimental analysis. If the abstract lacked adequate information for inclusion or exclusion, the full text was retrieved and assessed. In cases where only the abstract was available in PubMed, the article title was searched on Google Scholar to locate and download the complete study. The final date for data collection was March 21, 2025. All duplicate entries were removed, and only English-language studies were retained. Further exclusions were applied to studies that did not involve antibiotic-resistant bacteria, bacteria without relevant genetic mutations, or cases where the reported mutations were unrelated to resistance. Additionally, papers were excluded if they lacked sufficient information regarding CRISPR-Cas9 applications in resistant bacteria.

2.2 Data extraction and management

Data from the included studies were extracted manually and initially recorded in a Microsoft Word document before being transferred into an Excel spreadsheet for systematic organization and analysis. Bacterial strains were classified based on their designation as ESKAPE pathogens, with special emphasis on methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Escherichia coli, given their significant clinical importance and frequent representation in published studies. For each bacterial species, key information was extracted, including the type of CRISPR system used (with a focus on CRISPR-Cas9), the specific gene targets associated with antibiotic resistance, the mechanism of CRISPR action (e.g., gene disruption or editing), and the validation methods used to confirm gene editing or therapeutic efficacy, such as PCR, sequencing, or antibiotic susceptibility testing. This structured approach enabled the comparison of CRISPR applications across different bacterial species and resistance mechanisms, and the Excel database facilitated efficient data storage, retrieval, and cross-study analysis.

Table 1. *Application of CRISPR-dCas9 System for Targeted Suppression of the mecA Gene in Methicillin-Resistant Staphylococcus aureus (MRSA)*

System used	**CRISPR-dcas9 system.**
Study ID, references	[16]
Study type	Review.
Bacterial Strain	MRSA.
Resistance gene	Mec A methicilin resistant gene.
Codes for	Penicilin binding protein 2A (PBP 2A).
Antibiotic Resistance Profile (class)	Beta-lactam antibiotics.
Mechanism of action	Suppress the transcription of the gene.
Antibiotic test	-cefoxilin disk diffusion, -oxacillin microbroth serial dilution.
Validation method	RT-qPCR.

Table 2. CRISPR-Cas9-mediated re-sensitization of ESBL-producing E. coli K12 BW25113 by targeting TEM and SHV-type β-lactamase genes. Data from review study [8]; validation via PCR, CFU, and disk diffusion assay

System used	**CRISPR-cas9.**
Study ID, references	[8]
Study type	review
Bacterial Strain	E.coli K12 BW25113.
Resistance gene	Bla gene ESBL gene, TEM type ESBL & SHV-type ESBL.
Coding for	
Antibiotic Resistance Profile (class)	Ampicillin & Ceftazidime
Mechanism of action	Re-sensitization of E.coli strain by help of gene (cas9).
Antibiotic test	CFU (colony forming unit), Disk diffusion assay.
Validation method	PCR

2.3.1 Data Analysis

A notable study titled "Suppression of Antimicrobial Resistance in MRSA Using CRISPR-dCas9" explored the application of a modified CRISPR-dCas9 system to inhibit the transcription of antibiotic resistance genes. The researchers developed two CRISPR-dCas9 constructs specifically targeting the promoter region of the mecA gene in methicillin-resistant Staphylococcus aureus (MRSA). By binding to this region, the CRISPR-dCas9 complex successfully obstructed transcription initiation, leading to a significant reduction in mecA expression. This approach highlighted the potential of CRISPR interference (CRISPRi) as a precise, non-lethal method to suppress resistance genes and manage antimicrobial resistance in MRSA [16].

2.3.2. The Role of the mecA Gene

The mecA gene is a key determinant of antibiotic resistance in methicillin-resistant Staphylococcus aureus (MRSA), as it encodes penicillin-binding protein 2a (PBP2a)—a transpeptidase that enables cell wall synthesis to continue even in the presence of β-lactam antibiotics, which typically inhibit native PBPs. This gene is often acquired via horizontal gene transfer and confers resistance to a wide spectrum of β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems. To identify suitable CRISPR target sites within mecA, the gene sequence (accession number KF058908.1) from S. aureus strain ATCC 43300 was obtained from the NCBI database. Potential target regions were prioritized based on their proximity to the gene's promoter and whether they were located on the coding or non-coding strand. Each selected target consisted of a 30-base pair sequence situated upstream of a Protospacer Adjacent Motif (PAM), specifically the 3-nucleotide NGG sequence required for Cas9-mediated recognition and binding. Prior studies have shown that Protospacer Adjacent Motif (PAM) sites located near the Pribnow Box—approximately 10 to 35 base pairs upstream of the transcription start site—are particularly effective for gene repression. Moreover, target sequences on the coding strand tend to yield stronger transcriptional suppression compared to those on the non-coding strand. Following this rationale, two target sites within the mecA gene were selected: Site 43 (S43) on the coding strand and Site 46 (S46) on the non-coding strand. BLAST analysis confirmed the specificity of both sites to mecA, reducing the risk of off-target effects. As a result, three CRISPR-dCas9 plasmids were constructed: one targeting S43, another targeting S46, and a control plasmid lacking a specific target sequence. Each construct, approximately 9,326 base pairs in length, included critical CRISPR elements such as the dCas9 gene, a tracrRNA-encoding region, a customizable CRISPR target flanked by repeat sequences, and a chloramphenicol resistance gene for selection [16]. In a related investigation, a study titled "CRISPR/Cas9-Mediated Re-Sensitization of Antibiotic-Resistant Escherichia coli Harboring Extended-Spectrum β-Lactamases" showcased the use of CRISPR-Cas9 to counteract resistance in ESBL-producing Escherichia coli. In this approach, the CRISPR system was designed to specifically target and disrupt genes responsible for extended-spectrum β-lactamase production. The targeted cleavage of these resistance genes successfully restored the bacterial strain's sensitivity to β-lactam antibiotics. This study highlights the broader applicability of CRISPR-based gene-editing technologies as precise and effective tools for tackling multidrug-resistant bacterial infections [8].

2.3.3 Role of ESBL Genes.

Extended-spectrum β-lactamases (ESBLs) are a rapidly growing group of plasmid-encoded enzymes that enable bacteria to inactivate a broad range of critical β-lactam antibiotics. These enzymes fall into several major families, including SHV, TEM, CTX-M, OXA, PER, and others. While the original forms of genes like SHV-1, TEM-1, and TEM-2 do not possess ESBL activity, point mutations have given rise to variants capable of hydrolyzing extended-spectrum β-lactams. Often, these ESBL-encoding plasmids also carry additional antibiotic resistance genes, contributing to the emergence of multidrug-resistant bacterial strains,

which are increasingly difficult to treat. To counter this threat, CRISPR/Cas9 gene-editing technology has been investigated as a tool for selectively eliminating resistance genes. In one notable study, Escherichia coli was engineered to express Cas9 along with guide RNAs (gRNAs) that specifically targeted ESBL genes. The Cas9-induced double-stranded DNA breaks in these resistance genes led to targeted cell death, demonstrating that CRISPR/Cas9 can effectively re-sensitize antibiotic-resistant bacteria by disabling their resistance mechanisms. However, the extensive genetic diversity of ESBL genes poses a major challenge. With over 1,000 known ESBL gene variants and more than 200 variants within just the SHV, TEM, and OXA families—it is not feasible to design individual gRNAs for every mutation. To address this, researchers introduced a method called Re-Sensitization to Antibiotics from Resistance (ReSAFR). This approach focuses on identifying conserved regions shared across multiple ESBL variants—particularly within TEM and SHV families that can serve as universal targets for CRISPR/Cas9. To develop this strategy, all available ESBL gene sequences were sourced from the ESBL nomenclature database (maintained by George Jacoby and Karen Bush at www.lahey.org/studies) and the NCBI database. Using multiple sequence alignment tools such as ClustalW2, conserved DNA segments were identified and screened for the presence of the essential PAM sequence (NGG), which is required for Cas9 activity. Only regions with no mutations within the 20-nucleotide guide sequence upstream of the PAM were selected as suitable CRISPR targets. For instance, one such conserved 20-base sequence from the bla gene on the pUC19 plasmid followed by a PAM site was used as a model target to validate the ReSAFR approach. This strategy successfully disrupted both TEM and CTX-type ESBL genes, offering a promising avenue for reversing resistance in multi-drug-resistant bacteria.

Table X3 CRISPR strategies targeting β-lactam resistance in MRSA and *E. coli*.

ESKAPE	Strains	CRISPR Strategies	Construct	Target Gene	Virulence factor
S. aureus	MRSA	Suppress gene transcription	CRISPR associated gene+ Tracer CRISPR	*Mec*A	β Lactam
E.coli	K12BW25113	Re-sensitization ReSAFR system	Cas9+tracrRNA, +sgRNA, +crRNA	ESBL	β Lactam

3.Results

3.1 MIC TESTING

To assess whether the CRISPR-dCas9 system enhanced in vitro antibiotic susceptibility, minimum inhibitory concentrations (MICs) were measured for both the experimental and control MRSA strains. This evaluation was conducted using the oxacillin disk diffusion assay. According to the Clinical and Laboratory Standards Institute (CLSI) criteria, an MRSA strain is classified as susceptible to cefoxitin if the zone of inhibition measures 22 mm or greater, and resistant if the zone is 21 mm or less. The S43 group showed resistance to cefoxitin but displayed a larger inhibition zone than the control group (see Table 2a). Since the S46-transformed MRSA showed minimal effect on bacterial behavior, further analyses were limited to the S43 and control strains.

Table 4: Note: S43 is not susceptible to cefoxitin, although it has a wider zone of inhibition

Cefoxitin Sensitivity	
Sensitive/suspectable at 22mm	Resistance at 21 or less
Zone Of inhibition	
ATCC (mock plasmid)	14.33mm
S46 Plasmid	13.86mm
S43 Plasmid	18.75mm

3.1.1 *MEC*-A Expression Analysis

Following the observation of a partial decrease in β-lactam resistance during preliminary assays, quantitative reverse transcription PCR (RT-qPCR) was used to evaluate mecA gene expression. The assay produced consistent amplification curves and distinct melt peaks (Figure 1), indicating high specificity and reliability. Results from two independent experiments revealed an average fold reduction of mecA expression to 0.230—representing a 77% decrease relative to the mock-transformed control group ($p < 0.0005$; Figure 3). Amplification efficiencies were determined to be 67.6% for the 16S rRNA reference gene and 75.8% for mecA. These findings confirm that the CRISPR-dCas9 system effectively downregulated mecA transcription, although complete gene suppression was not achieved. The CRISPR-dCas9 platform was selected over CRISPR-Cas9 to silence gene expression without killing the bacteria, which allows for the possibility of creating a self-replicating, environmentally persistent

CRISPR-dCas9 system capable of sustained mecA repression. Importantly, bacterial viability was preserved in this study, offering a framework for further research aimed at optimizing target selection to achieve full mecA silencing. Future small-scale experiments could explore the stability and inheritance of the CRISPR-dCas9 plasmid during bacterial replication. This approach may be adaptable for broader use against other antibiotic-resistant pathogens. Because a single CRISPR-dCas9 construct can be designed to target multiple resistance genes, it offers the potential to simultaneously disrupt different resistance pathways within the same strain or across various bacterial species. In doing so, this strategy could help resensitize resistant bacteria to standard antibiotics, representing a promising avenue for combating antibiotic resistance.

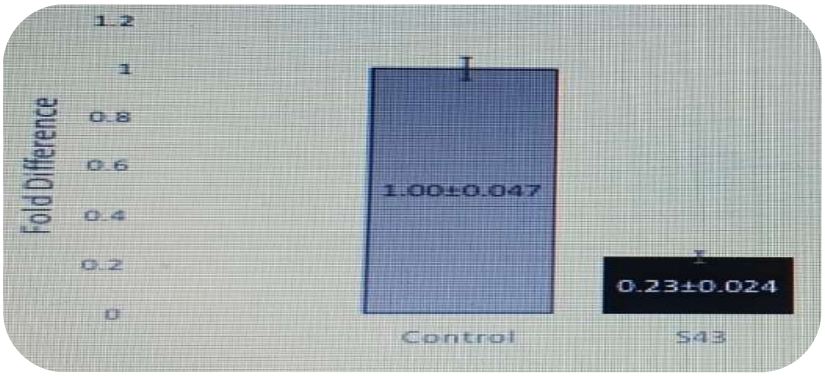

Figure. The CRISPR-dCas9 system led to a significant reduction in mecA mRNA expression in MRSA. In MRSA strains transformed with the S43 CRISPR-dCas9 plasmid, mecA expression levels dropped by approximately 77%, reaching 0.23 ± 0.024 relative to the mock-transformed control group (1.00 ± 0.047), with statistical significance ($p < 0.0005$). Fold change values were determined using the relative standard curve method. The results are presented as fold changes ± standard error of the mean.

3.2 Design of ReSAFR and Determination of the Target Sequence

This strategy was applied to re-sensitize Escherichia coli strains to antibiotics. The CRISPR-Cas9 system operates through the precise cleavage of DNA sequences, guided by RNA molecules. Its function depends on two core components: (i) short CRISPR-derived RNAs, specifically the CRISPR RNA (crRNA) and the trans-activating CRISPR RNA (tracrRNA), and (ii) the Cas9 protein, a double-stranded DNA endonuclease [2, 17]. The crRNA/tracrRNA/Cas9 complex locates the target DNA through base pairing between the crRNA and the complementary sequence. For Cas9 to bind and cleave effectively, the target region must be immediately followed by a Protospacer Adjacent Motif (PAM), typically the "NGG" trinucleotide sequence, located just downstream of the 20-base recognition site. To apply this mechanism, a specialized plasmid named pRESAFRESBL was constructed. This plasmid includes the Cas9 gene driven by the strong, constitutive synthetic promoter BBa_J23102, along with expression cassettes for tracrRNA and dual crRNAs specifically designed to target conserved regions of resistance genes. The structural and functional components of this plasmid are detailed in Table 5.

Table5: Component sequences targeting each antibiotic resistance genes.

Component	Sequence (5' to 3')
BBa_J23102	TTGACAGCTAGCTCAGTCCTAGGTACTGTGCTAGC
Cr-RNA-TEM	ATACGGGAGGGCTTACCATCTGG
Cr-RNA-SHV	GTCTGAGCGCCCGTTCGCAACGG
Sg-RNA bla	GCCATAACCATGAGTGATAACACTGGTTTTAGAGCTAGAAATAGCAAGTTAAAA-TAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTT

3.2.1 Re-Sensitization of Antibiotic-Resistant Cells

The study demonstrated that more than 99% of resistant E. coli cells containing the pRESAFRESBL plasmid were eliminated, indicating successful re-sensitization of the ESBL-producing strain to ampicillin (Amp) through targeted cleavage of a resistance gene sequence within the pESBL plasmid. Antibiotic susceptibility testing confirmed that while the original ESBL strain exhibited resistance to both Amp and cefotaxime (Cef), it remained sensitive to chloramphenicol (Clm) (Figure 2B). Importantly, disk diffusion analysis verified that the introduction of pRESAFRESBL restored susceptibility to Amp. Interestingly, the strain also regained sensitivity to Cef—despite Cef resistance typically being mediated by CTX-M β-lactamases, which are not direct targets of pRESAFRESBL. This unexpected outcome suggests that the plasmid may have facilitated the removal of

additional resistance genes that were co-located on the same plasmid, leading to a broader reversal of the multidrug-resistant phenotype. As an RNA-guided DNA endonuclease system, CRISPR/Cas9 offers a highly specific method for targeting and cleaving antibiotic resistance genes. In this case, the pRESAFRESBL construct not only neutralized Amp resistance but also appeared to indirectly affect resistance to other antibiotics. The likely explanation for the restored Cef sensitivity is the complete clearance of the pESBL plasmid, which carries both TEM and CTX-M resistance genes—the latter being the most prevalent ESBL gene in E. coli. Overall, the target sequence utilized in this study presents a promising avenue for addressing plasmid-mediated multidrug resistance, as resistance determinants are often transferred together on mobile genetic elements such as plasmids [11].

Table 6: Different valves of the zone of inhibition against the antibiotics

Diameter Of inhibition zone (mm)		
Antibiotics	**E.coli BW25113**	**PRESAFR ESBL**
Ceftazidime (1ug)	18	20 ± 0.65
Ampicillin (10ug)	15	16.6 ± 1.02

Discussion

Antimicrobial resistance (AMR) poses a significant global health challenge, particularly due to the increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing bacteria such as Escherichia coli and Klebsiella species. These pathogens can inactivate a broad range of antibiotics, making infections more difficult to treat. In this study, we investigated the potential of the CRISPR-dCas9 system as an innovative approach to counteract antibiotic resistance. CRISPR-dCas9 is a modified version of the well-known CRISPR-Cas9 gene-editing system. Unlike Cas9, which introduces double-stranded breaks in DNA, the dCas9 variant lacks nuclease activity and instead binds to specific DNA sequences to block gene expression. This allows for targeted silencing of antibiotic resistance genes without destroying the bacterial cell. Such a non-lethal strategy is particularly advantageous in complex microbial ecosystems—like the human gut—where maintaining the balance of beneficial bacteria is essential. A 2023 study by Sünderhauf et al. used an active CRISPR-Cas9 system to cut and remove AMR plasmids. They engineered a broad-host range plasmid, pKJK5::csg, to deliver the Cas9 system to many types of bacteria, including environmental and clinical strains. Their approach successfully removed resistance plasmids but may lead to bacterial death or microbiome disruption.Our approach, using dCas9, avoids this by simply silencing the resistance gene. This means we can potentially treat resistant infections while preserving the normal bacterial community. However, a current limitation is that our delivery system may not reach as many bacterial species as the broad-host range plasmid used by Sünderhauf et al. In the future, combining our dCas9 approach with a broad-host range plasmid could improve delivery and effectiveness. This would allow us to repress resistance genes in more types of bacteria across different environments. While dCas9 does not kill bacteria, it can make them sensitive to antibiotics again. This is especially useful against ESBL-producing bacteria. Our system could work alongside Cas9-based strategies—Cas9 for removing dangerous plasmids, and dCas9 for safely silencing genes. Some challenges remain, such as ensuring dCas9 works well in real-life settings like the human gut and making sure the bacteria keep the dCas9 plasmid. More research, including animal model testing, will be needed to move this technology forward.CRISPR-dCas9 offers a safe and targeted way to reverse antibiotic resistance. It may be an important part of future treatments, especially when used with improved delivery tools like those in the Sünderhauf study[18,19,20,21]. This review looks at the potential of the CRISPR-dCas9 system as an innovative strategy to reverse antibiotic resistance while preserving the viability of host bacteria. Unlike CRISPR-Cas9, which induces double-stranded DNA breaks that can lead to cell death, CRISPR-dCas9 functions through targeted gene repression. This allows for the silencing of resistance genes without disrupting the overall microbial population critical advantage in environments such as the human gut, where maintaining microbial balance is essential. By restoring resistant bacteria to their original, drug-sensitive state, CRISPR-dCas9 opens the door to non-lethal therapeutic interventions. This approach is particularly relevant for addressing infections caused by extended-spectrum β-lactamase (ESBL)-producing organisms, such as Escherichia coli and Klebsiella species, which are resistant to a broad spectrum of β-lactam antibiotics, including penicillin and cephalosporins. Previous studies using CRISPR-Cas9 have already demonstrated the feasibility of re-sensitizing such strains by specifically targeting and cleaving resistance genes. Together, these findings highlight the growing promise of CRISPR-based tools especially CRISPR-dCas9 as part of a new generation of antimicrobial strategies. The findings in this study suggest that specific target sequences identified for CRISPR-dCas9-mediated suppression of resistance genes could be extended to other plasmid-borne multidrug-resistant bacteria. Since multiple resistance genes are often carried on the same plasmid, disrupting one or more of them could significantly weaken or reverse the resistant phenotype. This approach offers a novel, gene-specific strategy for combating the growing threat of antimicrobial resistance.

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