Advancements in CRISPR Technology for Studying Antibiotic Resistance Mechanisms in Bacteria

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Abstract: Antibiotic resistance (AR) is a global threat in terms of public health that lowers the efficacy of antibiotics as well as raises disease loads. While traditional genomics-based strategies have worked in detecting resistance genes in bacteria, these strategies are not reliable in functional validation of genes. The emergence of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technologies has ushered in a very potent gene-editing tool in understanding resistance in bacteria. This paper is a discussion on recent advancements in CRISPR-Cas9, CRISPRi, and CRISPRa application in resistance gene research, in designing antibacterial strategies, as well as in modulating metabolic pathways in bacteria. Furthermore, it explores CRISPR application in phage therapy, novel antibiotic development, as well as resistance gene modification. Future research will involve improving CRISPR delivery methods, increasing specificity in CRISPR editing, as well as incorporating synthetic biology strategies toward more potent anti-resistance therapies.

1 INTRODUCTION

Antibiotic resistance (AR) poses a global threat to human health. The World Health Organization (WHO) projects that in 2050, over 10 million will be annually killed by antibiotic-resistant infections (WHO, 2020). Over recent years, multidrug-resistant (MDR) and extensive drug-resistant (XDR) bacteria have escalated the frequency at which antibiotics are becoming ineffective, with bacterial antimicrobial resistance (AMR) having 1.27 million reported deaths in 2019 alone. Resistance in bacteria is achieved in a variety of ways, including through enzymes that break down (degrade) antibiotics (e.g., β-lactamases); through efflux pumps (e.g., AcrAB-TolC), which pump out (actively remove from a bacterium) antibiotics (Du et al., 2018); and through target modification (e.g., 23S rRNA methylation), which lowers binding affinity between antibiotics and target (Wilson, 2014). The bacteria also lower membrane permeability, which lowers entrance into bacteria, as well as transfer resistance genes via horizontal gene transfer (HGT) pathways, which involve plasmid, as well as bacteriophage, mediated gene transfer. Genomesequence-based approaches as well as transcriptome wide sequencing have identified a vast array of resistance-coding genes; these approaches are correlation-based alone and lack a direct means of ascertaining gene functionality. Over recent years, CRISPR-Cas technologies have evolved as a highly targeted gene-editing platform that holds a new approach toward understanding antibiotic resistance.

The advent of CRISPR started with the discovery of short, repeated units in the genome of Escherichia coli by Ishino et al. subsequently established that these repeats can be utilized in order to store virus-derived DNA segments that facilitate bacteria in forming immunological memories that can be utilized in defense against bacteriophage infections. Barangou et al. proceeded with establishing that CRISPR-Cas is a defense mechanism that can specifically target as well as cleave invasive DNA. In 2012, CRISPR-Cas9 as a gene-editing platform was established by Doudna as well as Charpentier, followed by its utilization in mammalian cells by Zhang et al. (2013), which paved its way towards extensive utilization in genetic modification. CRISPR over a time developed into a range of forms, which are CRISPR-Cas9 (for gene modification as well as knocking out), CRISPRi (for inhibition in gene expression by binding with dCas9), as well as CRISPRa (for activation in gene expression with transcriptional activation factors) (Zhang et al., 2013). All these developments have immensely

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improved specificity as well as efficacy in research on antibiotic resistance.

Over recent years, CRISPR has experienced extensive application in research on resistance to antibiotics, in functional validation of resistance genes, research on antibacterial targets, as well as phage therapy. In functional validation, CRISPR-Cas9 is used in targeted deletion of resistance genes, making bacteria more susceptible towards antibiotics (Mascellino, 2024). CRISPRi can also be utilized in knocking down efflux pump genes (for instance, acrAB), which allows investigators to investigate its role in MDR strains. In research on antibacterial targets, CRISPR is applied in probing 23S rRNA mutations' impact on resistance in macrolide, as well as probing β-lactam antibacterial target impacts on bacteria survival, as in the case of penicillin-binding protein (PBP) mutations. CRISPR-phage is also a new strategy in combating resistant bacteria. CRISPR-phages have also been successfully engineered by investigators with specificity in lysing resistant species as well as eliminating MDR bacteria (Khan et al., 2021), whereas CRISPR-mediated targeting of resistance plasmids is used in halting resistance gene dissemination. Amidst these advancements, CRISPR is bedeviled with a series of challenges that include off-targeting that erodes specificity, bacteria-evoked development of anti-CRISPR (Acr) strategies that suppress CRISPR activity (Pawluk et al., 2016), as well as a need for refinement in delivery system in order to boost efficiency, particularly in bacteriophage as well as in nanoscale-based CRISPR delivery.

The objectives in this paper are to provide a comprehensive report on new advancements in CRISPR in research on antibiotic resistance with a focus on validation of gene functions, target profiling of antibiotics, as well as phage therapy. In addition, we discuss challenges that are attributed with CRISPR-based resistance management strategies as well as directions towards overcoming these challenges. In providing a theoretical understanding on mechanisms in antibiotic resistance, in turn, this research aims at fostering new strategies in antimicrobials as well as incorporating CRISPR into research on antibiotic resistance.

2 APPLICATION OF CRISPR IN BACTERIAL ANTIBIOTIC RESISTANCE RESEARCH

2.1 Functional Validation of Resistance Genes

CRISPR-Cas9 is a precise gene-editing tool that has been used to target and disrupt antibiotic resistance genes, making bacteria more susceptible to antibiotics. For example, Mascellino demonstrated that deleting blaTEM and blaCTX-M, which encode β-lactamases, using CRISPR-Cas9 significantly reduced bacterial resistance to penicillins and cephalosporins. Additionally, CRISPRi, by using dCas9 to bind specific promoter regions, can suppress the transcription of resistance genes, such as efflux pump genes (acrAB, mexAB-oprM), thereby increasing bacterial sensitivity to tetracyclines and fluoroquinolones (Qi et al., 2013).

2.2 Antibiotic Target Research

CRISPR has also been employed to investigate the mechanisms of antibiotic targets. For instance, researchers have used CRISPR-Cas9 to edit genes related to 23S rRNA methylation to examine how such modifications contribute to resistance against macrolide antibiotics, such as erythromycin and clarithromycin (Wilson, 2014). Furthermore, site-directed mutagenesis via CRISPR has been utilized to explore resistance mechanisms in β-lactam antibiotics, particularly by studying mutations in penicillin-binding proteins (PBPs). Studies have shown that certain pbp2a mutations can increase bacterial resistance to methicillin (Pandey et al., 2020).

2.3 CRISPR-Based Phage Therapy

CRISPR-based phage therapy is an emerging approach to combating antibiotic-resistant bacteria. Researchers have engineered bacteriophages to carry CRISPR-Cas components, allowing them to selectively target and degrade resistance genes in bacterial populations. This restores bacterial susceptibility to antibiotics (Khan et al., 2021). For example, a study on Klebsiella pneumoniae resistant to carbapenems demonstrated that CRISPR-phage therapy successfully eliminated resistance plasmids, reducing bacterial tolerance to these antibiotics. Furthermore, CRISPR-mediated plasmid degradation

has been used to decrease the spread of multidrug-resistant Escherichia coli, enhancing antibiotic efficacy (Mascellino, 2024).

2.4 CRISPR-Mediated Metabolic Regulation

CRISPR can also regulate bacterial metabolic pathways to enhance antibiotic effectiveness. CRISPRi can be used to silence key metabolic regulators, such as soxR and marA, thereby weakening bacterial resistance and increasing susceptibility to antibiotics. Additionally, CRISPR has been applied in metabolic pathway reprogramming to disrupt biofilm formation, which is a major contributor to antibiotic resistance. By modifying metabolic networks, researchers have improved antibiotic efficacy in treating chronic infections (Pawluk et al., 2016).

3 DISCUSSION

The fast development of CRISPR has provided a powerful tool for studying antibiotic resistance. Its high specificity, programmability, and broad applicability allow researchers to directly manipulate bacterial genomes to explore the gene functions, optimize antibiotic targets, and develop new anti-bacteria methods. However, CRISPR still faces several challenges. Which include off-target effects, bacterial anti-CRISPR mechanisms, and the need for improved delivery systems. To make CRISPR a viable tool against antibiotic-resistant bacteria, researchers must keeping refine its editing accuracy, enhance its efficiency in complex microbial communities, and surmount the bacterial defense mechanisms against CRISPR-Cas systems.

The precision of CRISPR largely depends on single-guide RNA (sgRNA) recognition of target sequences. However, the researchers have shown that Cas9 may introduce unintended cuts at non-target sites, leading to off-target effects (Slaymaker et al., 2016). This issue limits CRISPR's application in studying resistance genes and developing antibacterial strategies, as an unintended mutations could alter experimental outcomes or pose safety risks. To minimize off-target effects, researchers have developed several optimization strategies, including high-fidelity Cas9 variants such as eSpCas9 and HiFi-Cas9, which will improve DNA binding specificity (Kim et al., 2018). Additionally, using dual sgRNA strategies—where two sgRNAs target the same gene for precise editing—has been shown to reduce unintended modifications (Qi et al., 2013). With advancements in artificial intelligence (AI) and deep learning, computational models have been employed to predict potential off-target sites. For example, the "DeepCRISPR", is an AI-based tool, improves sgRNA design accuracy by analyzing large amount of CRISPR datasets, reducing the likelihood of off-target effects (Bengio et al., 2022).

Despite CRISPR-Cas9's high gene-editing efficiency, many bacteria have evolved anti-CRISPR (Acr) mechanisms to resist CRISPR-mediated genome modifications. These Acr proteins, often encoded by bacteriophages, inhibit CRISPR function by blocking Cas9 binding to DNA or degrading Cas protein complexes (Pawluk et al., 2016). Studies have shown that Acr systems significantly reduce CRISPR-Cas9 editing efficiency in certain multidrug-resistant bacteria, such as carbapenem-resistant Klebsiella pneumoniae and methicillin-resistant Staphylococcus aureus (Khan et al., 2021). To overcome this issue, researchers are developing novel CRISPR variants, such as Cas12 and Cas13, which function differently from Cas9 and may evade Acr-mediated inhibition. Additionally, AI-driven AcrFinder algorithms are being used to identify new anti-CRISPR proteins, helping scientists engineer improved CRISPR tools (Stanley et al., 2019).

Another critical challenge is CRISPR delivery systems, which play a crucial role in determining its effectiveness in real-world applications. Unlike laboratory experiments, clinical applications require CRISPR to be efficiently and precisely delivered to target bacterial populations. Existing delivery methods, such as electroporation, transformation, or plasmid transfection, are effective in controlled settings but have limitations in in vivo applications (Kim et al., 2018). To address this, researchers have developed bacteriophage-based CRISPR delivery systems, where engineered phages act as natural carriers to transport CRISPR components into resistant bacteria. This method has shown success in targeting carbapenem-resistant Klebsiella pneumoniae and multidrug-resistant Escherichia coli, effectively eliminating resistance plasmids (Mascellino, 2024). Additionally, nanoparticle-based delivery systems, such as lipid nanoparticles (LNPs) and polymer nanoparticles (PNPs), are being explored as non-viral alternatives to improve CRISPR stability and enhance its targeting efficiency in bacterial populations (Kim et al., 2018). Further optimization of these delivery systems, along with the integration of synthetic biology approaches to design

intelligent CRISPR carriers, will be essential for advancing CRISPR-based antibacterial therapies.

Despite these challenges, CRISPR has vast potential in antibiotic resistance treatment, gene regulation, and personalized medicine. Future research should focus on AI-driven sgRNA optimization to improve editing specificity and efficiency (Bengio et al., 2022). Additionally, further exploration of alternative Cas proteins, such as Cas12 and Cas13, could help bypass Acr-mediated CRISPR inhibition (Pawluk et al., 2016). Moreover, the development of more efficient delivery systems, including bacteriophages, nanoparticles, and synthetic biology carriers, will further enhance CRISPR's clinical applicability (Kim et al., 2018).

4 CONCLUSION

This study evaluated CRISPR-Cas application in research on antibiotic resistance genes, antibiotic targets, CRISPR-phage therapy, as well as in metabolic pathways in bacteria. CRISPR is a very potent tool in resistance research as well as in new therapies design, although challenges do still lie in its way. Such challenges are off-targeting, CRISPR defenses in bacteria, as well as effective CRISPR delivery. Upcoming research will entail improving CRISPR delivery (for instance, bacteriophages, nanoparticles), making gene editing more targeted, as well as synergistically coupling CRISPR with synthetic biology in order to design new antibacterial therapies. CRISPR can be a key remedy in tackling antibiotic resistance, designing customized antibacterial therapies, as well as in phasing out classical antibiotics as CRISPR technologies mature.

REFERENCES

Bengio, Y., Bertin, P., Bauer, S., Aliee, H., & Krishnakumar, R. 2022. Causal machine learning for single-cell genomics. arXiv preprint arXiv:2310.14935.

Du, D., Wang, Z., James, C. E., & Venter, H. 2018. Structure of the AcrAB-TolC multidrug efflux pump. Nature, 558(7704), 60-64.

Khan, M., Abbas, A., Ahmad, T., & Nadeem, M. 2021. CRISPR-Cas System: Biological Role in Bacterial Virulence, Genome Editing, and Antimicrobial Resistance. Punjab University Journal of Zoology, 36(1), 85-96.

Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J. S. 2018. A highly efficient and biocompatible delivery system for CRISPR-Cas9 ribonucleoproteins. Genome Research, 28(8), 1175-1184.

Mascellino, M. T., Oliva, A., & Biswas, S. 2024. New therapeutic strategies against carbapenem-resistant gram-negative bacteria. Frontiers in Microbiology. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1513900/full

Pandey, S. D., Jain, D., Kumar, N., & Adhikary, A. 2020. MSMEG_2432 of Mycobacterium smegmatis mc²155 is a dual-function enzyme that exhibits DD-carboxypeptidase and β-lactamase activities. Microbiology Research, 166(6), 412-421.

Pawluk, A., Staals, R. H. J., Taylor, C., Watson, B. N. J., Saha, S., Fineran, P. C., Maxwell, K. L., & Davidson, A. R. 2016. Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species. Nature Microbiology, 1, 16085. h

Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell, 152(5), 1173-1183.

Slaymaker, I. M., Gao, L., Zetsche, B., Scott, D. A., Yan, W. X., & Zhang, F. 2016. Rationally engineered Cas9 nucleases with improved specificity. Science, 351(6268), 84-88.

Stanley, S. Y., Borges, A. L., Chen, K. H., Swaney, D. L., Krogan, N. J., & Bondy-Denomy, J. 2019. Anti-CRISPR-associated proteins are crucial modulators of phage biology. Nature Microbiology, 4(4), 556-566.

Wilson, D. N. 2014. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nature Reviews Microbiology, 12(1), 35-48.

World Health Organization (WHO). 2020. Antibiotic resistance: A global threat. Retrieved from https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Zhang, G., Xie, H., & Dai, X. 2024. DeepIndel: An Interpretable Deep Learning Approach for Predicting CRISPR/Cas9-Mediated Editing Outcomes. International Journal of Molecular Sciences, 25(20), 10928.