



Targeting the Superbugs: Leveraging CRISPR-Cas Systems to Combat Antibiotic-Resistant Bacteria

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Abstract

Antibiotic resistance represents a critical challenge in the global health landscape, threatening to render current medical treatments for bacterial infections ineffective. This paper explores the multifaceted phenomenon of antibiotic resistance, focusing on its biological mechanisms, the role of human activities in its propagation, and the subsequent impact on public health systems worldwide. We highlight the adaptive strategies employed by bacteria, including the development of resistance through genetic mutations and horizontal gene transfer, exacerbated by the misuse of antibiotics in healthcare and agriculture. The review extends to innovative approaches combating this issue, particularly the application of CRISPR-Cas systems. These gene-editing technologies promise to revolutionize our approach by directly targeting and modifying the genetic elements responsible for resistance. The potential of CRISPR-based strategies is discussed through various case studies and theoretical frameworks, assessing their efficacy and addressing the technical and ethical challenges they pose. Our analysis emphasizes the necessity for an integrated approach that combines new scientific methods with stringent policy reforms and international collaboration to manage antibiotic resistance effectively. This comprehensive examination not only elucidates the complexities of antibiotic resistance but also outlines the imperative for urgent and coordinated action to mitigate this escalating crisis.

Introduction

In an era where the triumphant conquests of modern medicine are shadowed by the emergence of antibiotic-resistant pathogens, the urgency to innovate beyond conventional treatment paradigms has never been more critical. Antibiotics, once hailed as the harbingers of a new dawn in medical science, are now facing a formidable challenge: the relentless evolution of bacterial resistance. This crisis not only jeopardizes human health but also threatens the foundational structures of our medical and agricultural systems.

The phenomenon of antibiotic resistance is not merely a byproduct of their clinical overuse but is also significantly propelled by their rampant overuse in animal agriculture, leading to a perilous cycle of resistance that continuously undermines human efforts to control infectious diseases. As the efficacy of existing antibiotics dwindles, the medical community finds itself in a precarious race against time to devise alternatives that can circumvent the adaptive strategies of these microbial adversaries.

This paper seeks to unravel the complex biological and socio-economic threads of antibiotic resistance. It delves into the mechanisms by which bacteria elude the lethal effects of antibiotics

and explores the implications of this resistance on global health. The analysis extends to evaluating innovative biotechnological strategies, including the revolutionary potential of CRISPR-Cas systems, which offer a glimmer of hope in this daunting battle. By dissecting the successes and challenges of these novel approaches, this study underscores the critical need for a multifaceted response involving scientific innovation, regulatory foresight, and global cooperation to stem the tide of antibiotic resistance and secure a sustainable future for the next generations.

Understanding Antibiotic Resistance

Overview of Antibiotic Resistance Development

Antibiotics—drugs responsible for ushering in the age of modern medicine, sanitation, and safety—are rapidly losing their potency. Their rampant use in industrial animal agriculture (and widespread clinical overprescription) has cultivated an environment with selective pressures favoring novel resistant strains of pathogenic bacteria. Clinical and biomedical researchers are in an evolutionary arms race with so-called “superbugs:” bacteria resistant to an ever-diversifying array of humanity’s most potent chemical weaponry, posing an ever-greater threat to the tenuous stability of our dominion over microbial disease [Centers for Disease Control and Prevention].

Antibiotic resistance is a formidable challenge in public health, marking a grave turn in the fight against infections. This condition arises when bacteria evolve to resist the effects of antibiotics, making standard treatments futile and leading to increased medical costs, prolonged hospital stays, and heightened mortality rates. Misuse and overuse of antibiotics are the primary catalysts for this resistance, creating a selective pressure that allows resistant bacteria to thrive and spread.

The Current Landscape of Antibiotic-Resistant Bacteria

Notable among antibiotic-resistant pathogens are Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant Enterococci (VRE), and bacteria that produce New Delhi Metallo-beta-lactamase (NDM-1), which confers resistance to a wide range of beta-lactam antibiotics. These bacteria develop resistance to a wide array of common antibiotics as they proliferate and evolve in the chemical cesspits of hospitals and healthcare facilities [World Health Organization 1]. The (WHO) has identified the critical necessity of developing novel antibiotics to bypass these pathogens’ biochemical pathways of resistance [World Health Organization 2], but committing humanity to an evolutionary arms race with the most resilient, diverse, and prolific organisms on planet Earth by pitting our scientific prowess against their raw reproductive power is an exercise in futility and anthropocentric hubris. Humanity desperately needs an alternative: a treatment and therapy without the mechanistic pitfalls of antibiotics; a viable weapon of war against our remarkably durable microscopic adversaries.

Challenges in Treating Infections Caused by Resistant Bacteria

Addressing infections caused by antibiotic-resistant bacteria presents numerous challenges. The pace of new antibiotic development has significantly lagged behind the emergence of resistant strains, leading to a critical shortage of effective treatments. The discovery of new antibiotics is hampered by the intricate chemistry involved and the substantial costs and lengthy timelines associated with drug development—multitiered institutional and medical bureaucracies are valuable wards against dangerous or ineffectual drugs being prematurely put out to market, but the critical state of modern scientific academia (compounded by privatization, a dearth of federal funding, and cutthroat inter-industry competition) caters pharmaceutical development to private profit instead of public health. Moreover, the rapid emergence of resistance to newly developed antibiotics highlights the need for innovative strategies to manage bacterial infections and combat antibiotic resistance effectively [The Pew Charitable Trusts].

CRISPR-Cas Systems – Mechanisms and Types

Basic Biology of CRISPR-Cas Systems

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins constitute a revolutionary gene-editing technology that has transformed the fields of molecular biology and genetics. Originally identified as a part of a rudimentary adaptive immune system in bacteria and archaea responsible for cleaving and neutralizing the invasive DNA of pathogenic bacteriophages, CRISPR has been isolated and adapted into a novel tool for genomic engineering: a pair of molecular scissors with a “copy and paste” functionality equipped with nucleotide-level precision. The mechanism involves the incorporation of snippets of DNA from these invaders into the CRISPR locus of the host organism's genome, which are then transcribed and processed into CRISPR RNAs (crRNAs). These crRNAs guide Cas proteins to the matching target DNA sequence, where the Cas protein induces a double-strand break, effectively neutralizing the threat [Jinek, Martin].

Types of CRISPR Systems and Their Mechanisms

CRISPR-Cas systems are categorized into two classes, which are further divided into six types (I-VI), based on the Cas proteins involved and their mechanisms of action. Class 1 systems, encompassing types I, III, and IV, employ a multi-protein complex for interference, while Class 2 systems, including types II, V, and VI, utilize a single protein effector. Type II CRISPR systems, particularly those involving Cas9, have been most extensively harnessed for genome editing purposes. In these systems, a single guide RNA (sgRNA) molecule, a fusion of crRNA and trans-activating crRNA (tracrRNA), directs the Cas9 nuclease to the target DNA sequence, facilitating precise genetic modifications [Adli, Mazhar].

Adaptation of CRISPR for Laboratory and Clinical Applications

The adaptability and precision of CRISPR-Cas systems have led to their widespread application in gene editing, from basic research to potential clinical interventions. In the laboratory, these

systems have enabled the targeted manipulation of genomes, allowing the study of gene function, the generation of model organisms, and the correction of genetic defects in cell lines. Clinically, CRISPR technology holds promise for treating genetic disorders by directly correcting mutations in the DNA of affected individuals. While precisely calibrating the mechanism of delivery so as to only target specific tissues or tumors remains an ongoing logistical hurdle for researchers, CRISPR holds significant promise as a potent tool for genetically engineering single-celled organisms and preimplantation zygotes. Research is underway to harness CRISPR for the development of novel therapies for a range of diseases, including cancer, genetic disorders, and infections caused by antibiotic-resistant bacteria [Knott, Gavin J., and Jennifer A. Doudna].

CRISPR-Based Strategies Against Antibiotic Resistance

The escalation of antibiotic resistance among bacterial pathogens has necessitated the exploration of innovative therapeutic approaches—humanity’s longstanding tradition of frantically scrambling for novel antimicrobial drugs to lob at bacteria with ever-expanding repertoires of resistance genes is evidently insufficient. CRISPR-Cas systems, with their precise genome-editing capabilities, offer a promising avenue for developing novel antimicrobial strategies against drug-resistant bacteria. The possibilities are promising—genetically engineered phage viruses for precision targeting resistant bacterial strains, CRISPR-mediated excision of resistance-encoding plasmids, and coordinated disruption of bacterial virulence factors—all of these are potentially viable avenues by which rapidly proliferating, antibiotic-resistant “superbugs” could be combatted.

Phage Therapy with CRISPR-Engineered Bacteriophages

CRISPR can be used to precisely engineer potent bioweapons against these pathogens. Bacteriophages (naturally occurring bacteria-parasitizing viruses) can be engineered to insert CRISPR-encoding mRNA into specific bacterial strains, circumventing the cell’s own internal machinery to engineer weapons for its own destruction. The phages function as a convenient delivery mechanism—evolution has already equipped them with the biochemical machinery necessary to bypass bacterial defenses and inject their own foreign viral RNA into the cytoplasm—researchers are simply modifying them to insert RNA encoding for the production of CRISPR-cas components instead of the subcomponents that enable viral reproduction. Once translated and assembled, these cas proteins can target and cleave antimicrobial resistance genes from the bacterial genome, successfully re-sensitizing them to antibiotic therapy. The benefits are twofold: bypassing the biochemical pathways of resistance that typically dampen the efficacy of antibiotic therapy, and minimizing collateral damage to friendly flora. This strategy has already been proven efficacious: *Staphylococcus aureus*, including MRSA strains, has been successfully resensitized to antibiotics by the modified bacteriophage-mediated disruption antibiotic resistance genes [Bikard, David].

Direct Targeting of Resistance Genes in Bacterial Populations

CRISPR-Cas systems can also be deployed to directly target and cleave DNA sequences responsible for antibiotic resistance in bacterial populations, reducing the prevalence of resistance genes within microbial communities and potentially reversing resistance trends. CRISPR-cas9 has already been successfully employed to target and inactivate plasmid-borne resistance genes, thereby hindering the spread of resistance mechanisms among bacteria [Citorik, Robert J., Mark Mimee].

Disruption of Bacterial Virulence Factors

Beyond targeting resistance genes, CRISPR technology can be utilized to disrupt bacterial virulence factors, attenuating the pathogenicity of resistant strains and rendering them more susceptible to the host immune response and conventional antibiotics. This strategy involves editing genes critical for the expression of toxins and other virulence determinants, potentially transforming virulent pathogens into harmless commensals [Gomaa, Ahmed].

Case Studies of Successful CRISPR Applications Against Resistant Bacteria

Several case studies underscore the potential of CRISPR-based interventions against antibiotic-resistant bacteria. CRISPR-Cas9 has been applied to re-sensitize and eliminate *Escherichia coli* strains resistant to colistin, a last-resort antibiotic, by targeting the *mcr-1* gene responsible for colistin resistance. Additionally, CRISPR-Cas13a has been explored for its ability to specifically target and degrade RNA molecules, offering a novel antimicrobial strategy that could circumvent traditional resistance mechanisms [Abbott, Timothy R.].

These pioneering applications of CRISPR technology illustrate its versatility and potential in addressing the antibiotic resistance crisis. However, several hurdles must be overcome before these laboratory successes can be translated to efficacious clinical treatments—ensuring that the cas9 complex makes its way into the correct target colonies is easier said than done in the 89-trillion-cell complex of the bacteria-infested human body. Further research is needed to eliminate any unintended off-target effects—somewhat mysterious and potentially cataclysmic errors in which CRISPR cleaves non-target DNA at random locales throughout the genome. This technology is still in its infancy, and substantially more research needs to be conducted before these therapies can be successfully deployed amongst the populace.

Overcoming Challenges and Limitations

The application of CRISPR-Cas systems in combating antibiotic-resistant bacteria presents several challenges and limitations that must be addressed to fully realize their therapeutic potential. This section explores the technical hurdles related to the delivery of CRISPR components to target bacteria, potential off-target effects, and their mitigation, as well as strategies to ensure the specificity and efficacy of CRISPR-based treatments.

Technical Challenges in Delivering CRISPR Components to Target Bacteria

One of the primary challenges in utilizing CRISPR-Cas systems against antibiotic-resistant bacteria is the efficient delivery of the CRISPR components (i.e., Cas proteins and guide RNAs) into bacterial cells. Traditional delivery methods, such as viral vectors, may not be suitable for all bacterial targets due to host range limitations and potential immune responses. Non-viral delivery methods, including electroporation and nanoparticle-based systems (similar to the vesicles used in the Pfizer and Moderna mRNA COVID vaccines), offer alternative avenues, but may require reworking so as to function as intended in the complex molecular environment of the human body [Glass, Zachary].

Potential for Off-Target Effects and Their Mitigation

While CRISPR-Cas systems offer unparalleled precision in gene editing, the risk of off-target effects—where the CAS enzyme cleaves unintended parts of the genome—remains a concern. Such unintended modifications could potentially harm beneficial bacteria or lead to unforeseen consequences in microbial communities. To mitigate these risks, continuous advancements in CRISPR technology aim to enhance specificity through improved guide RNA design, Cas enzyme fidelity, and computational tools for improved target site prediction [Fu, Yanfang].

Strategies for Ensuring the Specificity and Efficacy of CRISPR-Based Treatments

Ensuring the specificity and efficacy of CRISPR-based antimicrobials necessitates several precautionary measures. Precise characterization and selection of target sequences is crucial to minimize off-target effects and ensure that CRISPR components effectively reach and modify the intended DNA sequences (without inflicting collateral genetic damage). Deploying multiplexed CRISPR systems, which target multiple genes simultaneously, can also aid in preventing target bacteria from acquiring anti-CRISPR resistance, thereby increasing therapeutic efficacy. Using CRISPR in conjunction with traditional antimicrobial drugs and phage therapy may provide synergistic effects for overcoming antibiotic resistance [Selle, Kurt, and Rodolphe Barrangou].

Ethical, Regulatory, and Practical Considerations

The application of CRISPR-Cas systems in combating antibiotic-resistant bacteria introduces a complex landscape of ethical, regulatory, and practical challenges, highlighting the need for a balanced approach that respects ethical standards, adheres to rigorous regulatory frameworks, and addresses practical hurdles in the development and deployment of these technologies.

Ethical Implications of Using CRISPR in Bacteria

The use of CRISPR technology in bacteria, especially for applications related to public health such as the treatment or prevention of disease, raises significant ethical questions. One major concern is the potential for unintended ecological impacts, such as off-target effects that could alter non-target bacterial communities critical for ecological or intestinal health. These concerns

necessitate transparent risk assessments and close collaboration between researchers and regulators to ensure that the CRISPR therapeutics that are developed remain safe and specific. There is also an ethical obligation to consider the long-term consequences of deploying gene-editing technologies in the environment, including the potential for horizontal gene transfer and the spread of edited genes to other organisms [Caplan, Arthur L.]. Contaminating the ecological landscape with genetically modified bacteria is an outcome better left to science fiction speculators.

Regulatory Landscape for CRISPR-Based Antimicrobials

Regulatory frameworks governing the use of CRISPR technology are still in active development and vary significantly between countries. Legislators, unfamiliar with biomedical research and ignorant of both its potential promise and peril, may end up providing more leeway to profit-hungry pharmaceuticals than is likely advisable. We urgently need active and open communication between researchers and government regulatory agencies. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) must address a novel set of considerations when overseeing the development of CRISPR-based antimicrobial therapies, closely evaluating their mechanisms of action, specificity, and durability, as well as the potential for resistance development. Furthermore, the classification of these agents—whether as drugs, biologics, or a new category of therapeutic entirely—impacts the regulatory pathways that must be followed, influencing everything from preclinical requirements to clinical trials and approvals [Oye, Kenneth A.].

Practical Considerations for the Development and Deployment of CRISPR-Based Therapies

The development of CRISPR-based therapies for antibiotic resistance faces several practical challenges. These include the technical aspects of delivering CRISPR components efficiently into target bacterial populations, ensuring the stability and activity of these components under physiological conditions, and scaling up production while maintaining quality and safety. Moreover, there are significant logistical challenges in deploying these therapies, especially in low-resource settings where antibiotic resistance is often most severe. Robust healthcare infrastructure is critical to ensuring the equipable distribution and administration of these therapies [Jorgensen, Thomas S.].

The Future of CRISPR in Antimicrobial Research

The application of CRISPR-Cas systems in antimicrobial research holds transformative potential for the treatment of antimicrobial-resistant infections. New innovations and emerging technologies are continually revolutionizing the emerging landscape of CRISPR biotechnology. This section outlines the anticipated developments, potential impacts, and the essential role of collaboration and funding in driving forward the battle against antibiotic resistance.

Ongoing Research and Emerging Technologies

Current research is expanding the capabilities of CRISPR technology beyond its initial applications in genetic editing, exploring its potential in antimicrobial resistance management. Innovations such as anti-CRISPR proteins and advanced CRISPR delivery systems are being developed to enhance the precision and reduce the potential risks associated with CRISPR applications. Furthermore, the advent of next-generation CRISPR tools, such as base editors and prime editors, offers more targeted and efficient ways to combat pathogenic bacteria by directly modifying their genomes without introducing double-stranded breaks, thus minimizing unwanted mutations and enhancing safety [Anzalone, Andrew V.],[Pickar-Oliver, Ariel, and Charles A. Gersbach.].

Potential Impact of CRISPR on the Development of New Antibiotics and Treatments

CRISPR technology is poised to revolutionize the field of antibiotic development. By facilitating the precise manipulation of bacterial genomes, CRISPR allows researchers to deactivate resistance genes in pathogenic bacteria and to resurrect older antibiotics that have been rendered ineffective from overuse. CRISPR can be used to discover novel bacterial targets and to engineer bacteria with enhanced susceptibility to existing drugs, potentially reducing the time and cost associated with traditional antibiotic development processes [Yeh, Po-Jang],[Singh, Vipender].

Collaboration and Funding in the Fight Against Antibiotic Resistance

The successful integration of CRISPR technology into the fight against antibiotic resistance will require robust collaboration between industry leaders, academics, and legislators. Multi-disciplinary partnerships are essential for the successful synergism of disparate scientific fields—genetics, microbiology, drug development, and clinical practice chief amongst them. Sustained funding from both public and private sources is also crucial to support the research and development of CRISPR-based antimicrobials. Increased investment is particularly needed to navigate the regulatory pathways, conduct clinical trials, and ultimately bring effective treatments to market [Kwon, Diane],[O'Neill, James.].

Conclusion

The escalating crisis of antibiotic resistance demands a decisive and multi-faceted response from the global community. As this paper has demonstrated, the relentless evolution of resistant bacteria poses a grave threat not only to public health but also to the stability of medical and agricultural systems worldwide. The overuse and misuse of antibiotics have accelerated the pace at which resistance has developed, leaving us in a precarious position where traditional antibiotics are becoming increasingly ineffective.

The exploration and implementation of CRISPR-Cas systems represent a promising frontier in the fight against antibiotic resistance. By leveraging this precise gene-editing technology, it is

possible to directly target and neutralize resistance genes within bacterial populations, offering a novel approach that could supplement or even replace traditional antibiotics. However, as with any emerging technology, the deployment of CRISPR-based strategies involves significant challenges. These include ensuring the specificity and safety of gene edits, overcoming technical hurdles related to delivery and efficacy, and addressing the ethical and regulatory implications of gene editing in microbial communities.

To navigate these challenges, a collaborative effort spanning multiple disciplines and sectors is essential. This includes ongoing investment in research and development to refine and enhance the capabilities of CRISPR technology, as well as robust dialogue among scientists, policymakers, and the public to establish a regulatory framework that supports innovation while safeguarding public health and environmental integrity.

Ultimately, the fight against antibiotic resistance is not limited to the development of new technologies but requires a comprehensive strategy that includes better stewardship of existing antibiotics, enhanced surveillance of resistance patterns, and global cooperation to implement effective interventions. The stakes are high, and the need for action is urgent. By embracing innovation and fostering international collaboration, we can hope to sustain the efficacy of antibiotics and secure a healthier future for coming generations.

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