CRISPR Antibiotics: Turning Defence into Offence

CRISPR/CAS9 TO OVERCOME ANTIBIOTIC-RESISTANT AND PATHOGENIC BACTERIA

INTRODUCTION

Antimicrobials are commonly deployed in animal production all over the world, not only to improve animal health and welfare, but also to increase animal growth rates and productivity. Antimicrobial usage, on the other hand, can contribute to the formation of resistance as well as the spread of resistant genes and bacteria between species.

In just 2010, 57,334 metric tonnes of antibiotics were used in livestock production worldwide which is significantly more than the level of human consumption (15). This number is set to rise to over 180,000 tonnes by 2030. In addition to treating sick animals with antibiotics, antibiotics are commonly administered to healthy animal feed and drinking water to prevent illness.

Antimicrobials that are both efficient and cost-effective are crucial for animal health, animal welfare, and food security. Some implications of antibiotic resistance include reduced food security, increased food safety concerns, large economic losses for farm households, and especially pollution of the environment.

The amount of antibiotics used locally is reflected in the distribution of antibiotics in water sources, which varies dramatically across time of year and locality. The effluent from antibiotic manufacturers, large-scale animal farms, and hospitals is the primary cause of pollution in the aquatic environment. Water conditions are fairly conducive to resistant gene transfer (19).

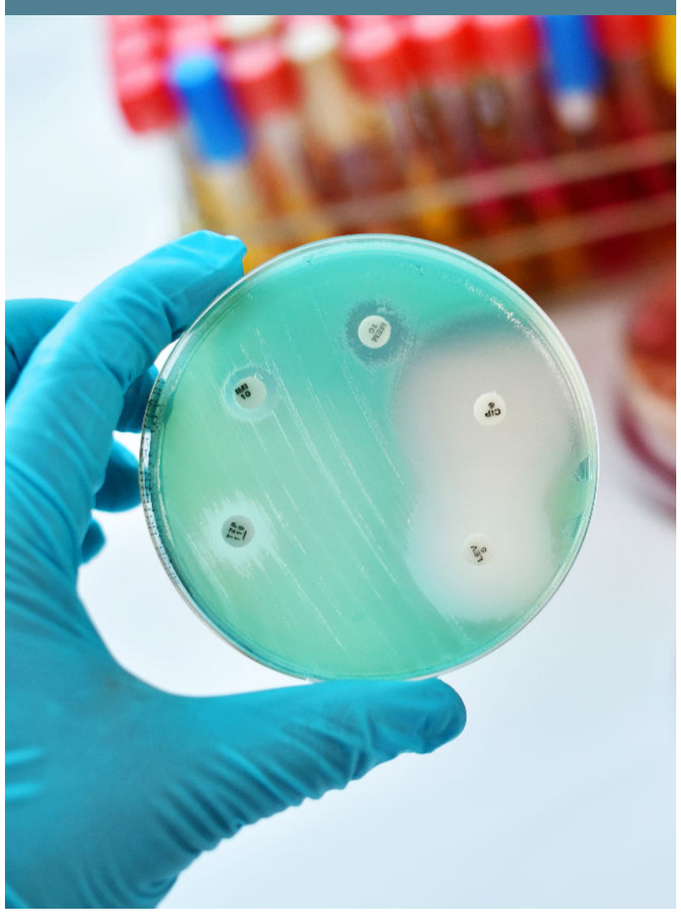
Despite the fact that drug-resistant microbes are more commonly observed in farms and hospitals, they can now be found in contaminated water sources and the environment. Antibiotics and antibiotic resistance genes from these bacteria have the potential to circumvent water treatment facilities and return to the environment and human bodies (19).

ANTIBIOTIC USAGE

Almost 80% of all antibiotics in the United States aren't taken by people.

Colistin, a key last-line antibiotic for treating serious infections in humans, is one of the most extensively used antibiotics in animal production across the world to enhance the development of livestock, particularly pigs (16, 17).

The CDC estimates that the cost of antimicrobial resistance is $55 billion every year in the United States alone, $20 billion for health care and about $35 billion for loss of productivity.



“We typically think of antibiotics used as performance enhancers as being fed at a somewhat lower dosage and for a longer period of time than for disease treatment... Of course, it raises concerns about resistance issues” - Gay Miller: Veterinarian, University of Illinois

ANTIBIOTICS & RESISTANCE:

After Fleming, Florey and Chain's discovery and research into penicillin, antibiotics soon became widespread in the veterinary and livestock world. However, antibiotic misuse in these fields has aided the growth of multidrug-resistant microorganisms (2).

Antimicrobial resistance is not a new phenomenon, with methicillin resistant Staph Aureus (MRSA) first making its appearance in the 1960s. However it is a problem that until recently has been sorely overlooked. Newly antibiotic-resistant strains of bacteria are rapidly emerging, whilst progress in new antibacterial compounds is continuing at an astonishingly slow pace. This poses a huge danger to veterinary medicine and staple livestock, as modern medicine's capacity to cure and prevent diseases is becoming fruitless against resistant pathogens.

Furthermore, many antimicrobial drugs lack specificity, causing antibiotic-associated illnesses by killing pathogenic and useful non-pathogenic bacteria alike (3,4). This emphasizes the crucial need for new therapies that avoid existing medication resistance while also increasing selectivity (5). CRISPR/Cas9 systems that target bacteria and antibiotic resistance genes are being investigated as exciting novel prospective antimicrobials to achieve this. As aforementioned, antibiotics also have a large toll on the environment from their release through agricultural and medical effluent, however this affect isn’t seen with CRISPR/Cas9 based antimicrobials. As the CRISPR/Cas9 complex is released into the host upon bacterial cell death, it is recognised as foreign by Cas9 specific antibodies and degraded by the hosts immune system, never leaving the animal or individual being treated (20).

CRISPR/CAS9

CRISPR/Cas systems can be programmed to target practically any DNA sequence of interest. CRISPR/Cas9 has the potential to be utilized as an antimicrobial, with the goal of removing undesired genetic characteristics in microbes (6).

CRISPR/Cas9 is known for its target specificity, which can allow it to distinguish between commensal and harmful microorganisms, something modern antibiotics cannot do. Antibiotic resistance, pathogenicity, and critical pathogen genes may all be selectively targeted with guide RNAs (8).

The aim of a CRISPR/ Cas9 antimicrobial can be to:

- Induce bacterial cell death
- Suppress the growth of a targeted bacterial species
- Delete functional genes from pathogens
- Delete or alter mobile elements like plasmids responsible for transfer of antibiotic resistance

CRISPR/CAS9 METHODOLOGIES

CRISPR/Cas9 is currently being utilised as an antimicrobial through:

1. Recruiting a bacteria's endogenous systems to deliver a self-targeting CRISPR array. Essentially programming the bacteria to edit its own disease causing traits on its own chromosome or on a plasmid.
2. Delivering an engineered whole external targeting system to bacteria to remove resistance genes or pathogenic traits (7).

SELF-TARGETING:

The host CRISPR/Cas9 system of one bacterial strain (Clostridioides difficile) was recently programmed for self-targeting. This was done by delivering a chromosome-targeting CRISPR complex using a recombinant bacterial virus. This study showed that through adding a self-targeting CRISPR payload, an increase in bacterial cell death was observed in vitro. When mice were given this CRISPR therapy, C. difficile populations decreased roughly 10 times in the intestines (10).

TARGETING BACTERIAL RESISTANCE GENES ON THE CHROMOSOME:

Often, the genetic information responsible for antibiotic resistance is encoded on the chromosomes. CRISPR/Cas9 was effectively used to target chromosomally encoded antibiotic resistance genes in E. coli and S. aureus, triggering cell death in both in vitro and in vivo models (9,11).

TARGETING BACTERIAL PLASMIDS:

Plasmids are an appealing target for drug resistance gene removal as they are often responsible for the shared transfer of resistance within a strain population (9). Engineered CRISPR/Cas9 has been used in ground-breaking research to target antibiotic resistance plasmids, revealing that doing so does not result in direct bacterial cell death but rather in antibiotic sensitization due to plasmid loss (9,11).

CRISPR/CAS9 COMBINED WITH ANTIBIOTICS:

Antibiotics will always be a necessity, however due to their misuse, their efficacy is being drastically decreased through resistance. Studies suggest that using CRISPR antimicrobials can be employed with even greater success in a combination therapy with conventional antibiotics, bringing a multivalent approach to antimicrobial resistance. An insect model infected with E. coli was first treated with CRISPR/Cas9 targeting an antibiotic resistance gene (blaTEM-1). After administering CRISPR and an antibiotic (ceftriaxone), a 70% survival rate was seen, compared to the 30% survival rate with antibiotic alone. (14)

CONCLUDING REMARKS:

Antibiotic resistance is a naturally occurring mechanism that can be delayed but not totally eliminated since resistance is an unavoidable result of medication selection pressure. Antibiotics are the foundation of modern medicine and they have made a significant contribution to the advancement of animal quality of life and food production in the last half-century. The propagation of antimicrobial resistance must be halted to ensure antimicrobials that are both effective and cost-effective and to maintain animal health, welfare, and food security. CRISPR/Cas9 is a viable solution to overcoming antimicrobial resistance, however it will require a combination of novel CRISPR therapeutics and global cooperation to further regulate the misuse of antibiotics, reduce their usage and begin to reverse this issue.

REFERENCES:

1. Årdal C, Baraldi E, Ciabuschi F, Outterson K, Rex J, Piddock L, et al. To the G20: incentivising antibacterial research and development. Lancet Infect Dis. 2017;17(8):799–801. pmid:28689703.
2. Ventola C. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277–283. pmid:25859123; PubMed Central PMCID: PMC4378521.
3. Czepiel J, Dróżdż M, Pituch H, Kuijper E, Perucki W, Mielimonka A, et al. Clostridium difficile infection: review. Eur J Clin Microbiol Infect Dis. 2019;38(7):1211–1221. pmid:30945014; PubMed Central PMCID: PMC6570665.
4. Chilambi G, Nordstrom H, Evans D, Ferrolino J, Hayden R, Marón G, et al. Evolution of vancomycin-resistant Enterococcus faecium during colonization and infection in immunocompromised pediatric patients. Proc Natl Acad Sci U S A. 2020;117(21):11703–11714. pmid:32393645; PubMed Central PMCID: PMC7261057.
5. Beisel C, Gomaa A, Barrangou R. A CRISPR design for next-generation antimicrobials. Genome Biol. 2014;15(11):516. pmid:25417800; PubMed Central PMCID: PMC4282009.
6. Barrangou R, Doudna J. Applications of CRISPR technologies in research and beyond. Nat Biotechnol. 2016;34(9):933–941. pmid:27606440.
7. Barrangou R, Ousterout D. Repurposing CRISPR-Cas systems as DNA-based smart antimicrobials. Cell Gene Ther Insights. 2017;3(1):63–72.
8. Greene A. CRISPR-Based Antibacterials: Transforming Bacterial Defense into Offense. Trends Biotechnol. 2018;36(2):127–130. pmid:29157535.
9. Citorik R, Mimee M, Lu T. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat Biotechnol. 2014;32(11):1141–1145. pmid:25240928; PubMed Central PMCID: PMC4237163.
10. Selle K, Fletcher J, Tuson H, Schmitt D, Mcmillan L, Vridhambal G, et al. In Vivo Targeting of Clostridioides difficile Using Phage-Delivered CRISPR-Cas3 Antimicrobials. MBio. 2020;11(2):e00019–20. pmid:32156803; PubMed Central PMCID: PMC7064742.
11. Bikard D, Euler C, Jiang W, Nussenzweig P, Goldberg G, Duportet X, et al. Exploiting CRISPR-cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol. 2014;32(11):1146–1150. pmid:25282355; PubMed Central PMCID: PMC4317352.
12. Rodrigues M, McBride S, Hullahalli K, Palmer K, Duerkop B. Conjugative Delivery of CRISPR-Cas9 for the Selective Depletion of Antibiotic-Resistant Enterococci. Antimicrob Agents Chemother. 2019;63(11):e01454–19. pmid:31527030; PubMed Central PMCID: PMC6811441.
13. Edgar R, Qimron U. The Escherichia coli CRISPR system protects from λ lysogenization, lysogens, and prophage induction. J Bacteriol. 2010;192(23):6291–6294. pmid:20889749; PubMed Central PMCID: PMC2981215.
14. Tagliaferri T, Guimarães N, Pereira M, Vilela L, Horz H, Dos Santos S, et al. 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. 2020;11:578. pmid:32425894; PubMed Central PMCID: PMC7203346.
15. Drug-resistant infections a threat to our economic future; 2017. Available from: www.worldbank.org. Accessed June 7th, 2022.
16. United Nations meeting on antimicrobial resistance. Bull World Health Organ. 2016;94(9):638–639. doi: 10.2471/BLT.16.020916
17. Rhouma M, Beaudry F, Thériault W, Letellier A. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front Microbiol. 2016;7:1789. doi: 10.3389/fmicb.2016.01789
18. Antibiotic resistance threats in the United States; 2013. https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed June 7th, 2022.
19. Aydin S, Ince B, Ince O: Assessment of anaerobic bacterial diversity and its effects on anaerobic system stability and the occurrence of antibiotic resistance genes. Bioresour Technol 2016, 207:332-338.
20. Moreno AM, Palmer N, Alemán F, Chen G, Pla A, Jiang N, et al. Immune-orthogonal orthologues of AAV capsids and of Cas9 circumvent the immune response to the administration of gene therapy. Nat Biomed Eng. (2019) 3:806–16. doi: 10.1038/s41551-019-0431-2