CRISPR/Cas-Mediated Pathogenesis Disruption to Sustainably Combat Multidrug-Resistant Pathogens

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Antimicrobial resistance (AMR) is an emerging health problem that threatens global health and prosperity. According to the World Health Organization, with limited therapeutic options, AMR currently kills over 700,000 people annually and is estimated to kill 10 million by 2050. Furthermore, antibiotics eliminate microbiomes crucial to proper host metabolic function and are increasingly becoming associated with diseases such as obesity, diabetes, cancer and cardiovascular disease. This project explores a novel CRISPR/Cas-based approach to targeting and disabling bacterial virulence as a pathogen-specific alternative to antibiotics. The CRISPR-Cas9 nuclease is used to cleave and degrade Shigella's virulence plasmid, and the newly characterized INTEGRATE transposon-insertion CRISPR system is used to disable the SPI-1 pathogenicity island of Salmonella through transposon insertion. The latter approach also allows for the insertion of customizable DNA cargo into specific pathogenic loci, which is applied to selectively eliminate Salmonella biofilms through a rationally engineered DJK-5 anti-biofilm peptide. Both systems are tested in an in vivo Galleria Mellonella invertebrate infection model, and significantly improve survival when delivered through the P1 phagemid delivery vector. Ultimately, both plasmid and chromosomal virulence systems are targeted and eliminated using a novel, promising two-CRISPR approach. This ensures that host microbiomes necessary for normal physiological function are not disrupted, and dramatically reduces potential bacterial resistance - all while providing an effective, targeted weapon against emerging drug-resistant pathogens.

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