A novel approach for reducing multiple-drug resistance in foodborne bacteria: application of CRISPR technology

Lead Supervisor: Prof Simon C Andrews, School of Biological Sciences, University of Reading
Email: s.c.andrews@reading.ac.uk

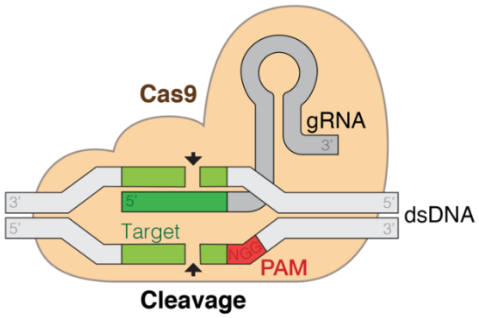
Co-Supervisors:
Prof Roberto M. La Ragione, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey
Dr Arnoud H. M. van Vliet, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey
Dr Caroline Rymer FRSB FHEA, School of Agriculture, Policy and Development, University of Reading
Dr Holger Kneuper, Folium Science, Bristol
Prof Martin J Woodward, Folium Science, Bristol

Project Description: Antibiotic resistance is a major threat to both global health and food security with increasing numbers of bacterial infections, (including food-borne diseases, that are becoming more difficult to treat as new resistance mechanisms emerge and spread and our portfolio of antibiotic options becomes less effective. Recent estimates indicate death dues to antibiotic treatment failure could exceed those caused by cancer by 2050 and we are now on the threshold of entering a 'post-antibiotic era' where infectious disease is once again the major cause of death in the developed world. There is a pressing need to develop new strategies to counter the threat of antibiotic resistance.

Our approach is to utilise the power of CRISPR-Cas technology to remove antibiotic resistance from the food production chain and thus eliminate one of the major sources of human health risk. Animal production contributes to human health issues due to the presence of multidrug resistant (MDR) bacteria and there is a drive to remove antibiotics from the food chain including EU directives on antibiotic reduction in animal production and the "No Antibiotics Ever" campaign in the USA. The objective of this significant and socially responsible study is to re-enable the efficacy of our antibiotics by developing a novel strategy for removal of resistance genes. This a highly innovative approach has high potential impact to provide front line and last resort antibiotics an extended lifespan through treatments that remove the resistance genes in animals and thus reduce their presence in humans.

The testable hypothesis is that ***the CRISPR-Cas systems can be exploited to reduce the burden of AMR in bacterial populations such that:***

a) the risk of resistance passing into the human food chain is dramatically reduced and
b) *the treatment permits use of the antibiotic, thus expanding the potential utility of current antibiotics*.



The CRISPR-Cas system consists of a sequence-specific nuclease (Cas9) and a guide RNA (gRNA) that provides sequence specificity to its DNA cleavage activity (see **Fig**). CRISPR-Cas9 is a versatile and highly specific next-generation antimicrobial that allows targeting of specific pathogenic bacteria while leaving the remaining microbiome intact. The preliminary data generated by the commercial partner, Folium Science, has demonstrated the application of CRISPR technology to reduce the burden of *Salmonella* in poultry (Cogan et al., in press). Conjugative delivery of CRISPR-Cas9 and target-specific gRNA by a mobilisable plasmid from a donor bacterium (a 'probiotic') to *Salmonella* caused degradation of the DNA of the targeted bacterium and subsequent bacterial cell death. Folium Science is providing this know-how and access to its patent portfolio to enable our consortium to tackle one of the most pressing needs currently - countering MDR bacteria in animal production.

Our recent studies demonstrated very high prevalence of MDR bacteria in commercial poultry. Avian Pathogenic *E. coli* (APEC) have been linked with Urinary Tract Infection (UTI) in humans and resistance means treatment failure. The overall goal is to devise a CRISPR-Cas system that effectively reduces the incidence of selected test antibiotic resistance genes (e.g. CTX-M 15) for use in poultry in the first instance.

The four objectives are:

(1) *In silico* analysis of WGS data from commercial poultry microbiomes to identify antibiotic resistance genes, their prevalence and vectors. Targets (guides with relevant PAM motif for Cas activity) will then be designed.

(2) Synthetic target sequences will be cloned by Golden Gate synthesis approaches onto Folium vectors constitutively expressing Cas9 or Cas cascade enzymes. The constructs will be mobilised into test strains and loss of the targeted resistance monitored both phenotypically and genotypically.

(3) *In vitro* gut models will be utilised to determine the dynamics of delivery of the CRISPR-Cas system to the poultry gut microbial community and to assess reduction of the target resistance gene and its associated vector.

(4) The CRISPR-Cas probiotic strain developed above will then be used in commercial broiler chicks. Microbial molecular profiling by NGS will be undertaken to examine dissemination of the CRISPR-Cas system, loss of the targeted AMR gene and any population shifts in the gut microbiome.

References:

- https://www.agritech-east.co.uk/folium-sciences-new-guided-biotic-triggers-salmonella-self-digestion
- https://www.forbes.com/sites/donaldmarvin/2019/06/11/folium-science-uses-crispr-to-remove-antibiotics-in-livestock/#518ba4d7359a
- Lutful Kabir, SM. (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health, 7(1), 89–114. http://doi.org/10.3390/ijerph7010089
- Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, McDermott P, Hung-Fan M, Riley LW. A Population-Based Surveillance Study of Shared Genotypes of *Escherichia coli* Isolates from Retail Meat and Suspected Cases of Urinary Tract Infections. mSphere. 2018 Aug 15;3(4). pii: e00179-18. doi: 10.1128/mSphere.00179-18