PATHOGEN GENOMICS FOR ANTIBIOTIC DISCOVERY & NEW RESISTANCE GENES

More than ever we need a new arsenal to fight the microscopic war against bacteria – especially as antibiotic resistance rates continue to sky-rocket. The World Health Organisation has recognised antibiotic resistance as one of the “greatest threats to global health, food security, and development today”. Thus, it is critical to study antibiotic resistance in great molecular detail and monitor new resistance genes, as well as to look for new antibiotic targets to develop therapeutically. Our research group is firmly rooted in using cutting edge genomics techniques to understand antibiotic resistance mechanisms, discover new antibiotic targets and uncover detailed mechanisms of action (MOA) for novel antimicrobials and current empirical antibiotic therapies.

CUTTING EDGE GENOMICS TECHNIQUES TO STUDY ANTIBIOTIC RESISTANCE

We have pioneered the Transposon directed insertion-site sequencing (TraDIS) method, which combines large-scale random mutagenesis and whole genome sequencing, to assay the fitness of every gene in the bacterial genome simultaneously, under any selection (1,2). We have applied this method to tens of G-ve and G+ve bacterial species including hospital ESCAPE pathogens, environmental and gut strains across many assays, especially antibiotic resistance (3), but also in bacteriophage selection (4), motility (5), animal infection models (6), and sporulation (7). We currently have projects open using TraDIS (learning both the lab and bioinformatics side) to explore gene function in bacterial genomes, and identify new antibiotic resistance genes as well as novel therapeutic tags.

In 2016, we developed a new TraDIS-based method at MQ, that uses physical separation of mutants for the first time ever. This method, named “TraDISort”, combines TraDIS and cell sorting to differentiate mutant populations and identify novel drug pumps (9,10). One potential research project is to utilise TraDISort to find novel antibiotic resistance genes using fluorescently-labelled antibiotics.

UNDERSTANDING THE GENETICS OF ANTIBIOTIC SYNERGY

Our research using genomic techniques also focuses on gaining a detailed molecular understanding of antibiotic synergy. Antibiotic combination therapy presents a rare opportunity to revive failing options within our existing arsenal of antibiotics and is a potent tool to combat multi-drug resistant bacterial infections. Shockingly, however, we have little to no understanding of the basic molecular mechanisms underlying antibiotic synergy. Preliminary studies performed previously in our lab using TraDIS show that treatment with 2 synergistic antibiotics separately and together yielded a unique gene set during the synergistic reaction. Laboratory follow-ups of these synergy-specific genes identified the first ever antibiotic synergistic resistance gene, which only gives resistance to both antibiotics together, but not to either of the individual
antibiotics separately. This preliminary work, together with a handful of published studies indicate that unique mechanisms of action occur during synergistic killing compared with those of the original antibiotics. Understanding these synergy-specific mechanisms of killing and identifying any secondary drug targets opens up the possibility of improving combination therapy to minimise adverse side-effects. Further, these unknown secondary targets represent an untapped reservoir of primary drug discovery of safe and effective antibiotics in the future. Elucidating these complex interactions has only become possible recently with the advent of high-throughput methods, like TraDIS. We have a current ARC grant on this area of research and many projects within this space.

UNCOVERING HETERO-RESISTANT POPULATIONS OF PATHOGENS

Bacterial cells do not live separately, but in communal populations. Heteroresistance is when genetic changes occur in subpopulations of bacteria to acquire resistance to antimicrobials. Using deep whole genome sequencing of bacterial communities during a resistance-induction (where we train bacterial cultures to become antibiotic resistant), directed evolution experiment, we have previously found a new colistin resistance gene in Klebsiella pneumoniae (10). Here, SNPs (mutations), were present in only ~30% of the population and would have been overlooked using standard SNP calling methods. We are finding that by sequencing these resistant subsections of the populations in clinical samples, we can find more new antibiotic resistance genes and further understand how bacterial communities work together to maintain low level resistance SNPs in their populations.

Selected Publications

1. Barquist L, Boinett CJ, Cain AK. Approaches to querying bacterial genomes with transposon-insertion sequencing. RNA Biology, 2013 (Review)
4. L Cowley, A Low, D Pickard, ... D Gally, J Parkhill, C Jenkins, and AK Cain. Transposon insertion sequencing elucidates novel gene involvement in phages T4 and T7 susceptibility and resistance in Escherichia coli O157, Mbio, 2018
5. LM Nolan, CB Whitchurch, L Barquist, .. IG Charles, A Filloux, J Parkhill and AK Cain. A global genomic approach uncovers novel components for twitching motility-mediated biofilm expansion in Pseudomonas aeruginosa, Mbio, 2018
7. Dembek M, Barquist L, Boinett CJ, Cain AK et al., High-throughput analysis of gene essentiality and sporulation in Clostridium difficile, Mbio, 2015
8. IT. Paulsen, AK Cain, KA. Hassan, Physical enrichment of transposon mutants from saturation mutant libraries using the TraDISort approach, Mobile Genetic Elements, 2017
9. KA Hassan, AK Cain, TT Huang, ... Parkhill J & Paulsen IT, Fluorescence-Based Flow Sorting in Parallel with Transposon Insertion Site Sequencing Identifies Multidrug Efflux Systems in Acinetobacter baumannii mBio 2016
10. AK Cain, CJ Boinett, L Barquist, J Dordel, M Fookes, M Mayho, M Ellington, D Goulding, DJ Pickard, J Parkhill & NR Thomson, Investigating cellular and genetic responses after exposure to the last-line antibiotic colistin Klebsiella pneumoniae, Scientific Reports, 2018