Principal Supervisor: Prof. Marco R Oggioni

Co-supervisor: Dr. Richard Badge

**PhD project title:** Microbes; qualified genomic and metabolic indicators of man-made selective pressure. A new molecular approach to tackle AMR.

**University of Registration:** University of Leicester

**Project outline**

1. Project outline describing the scientific rationale of the project

The UK Prime Minister announced recently a Review on Antimicrobial Resistance (AMR), calling for ideas to bring this growing threat under control. The Department of Health, Department for Environment, PHE and NHS England are all contributing to the implementation of the UK 5 year AMR strategy and AMR has become a priority for the Research Councils. This PhD project is designed to bring an innovative and alternative approach to analysing the selective pressures driving AMR in the environment.

Mobile genetic elements are the vehicles that allow rapid horizontal spread of AMR genes within and between bacterial populations. Among these elements, insertion sequences (IS) are often the fastest to recruit novel genes and mobilise them. Often these mobilised genes are initially metabolic genes in one organism, but may confer resistance when transferred to another organism. While much effort is placed in tracing known resistance genes, there is scarce work on their metabolic origin. We hope in this project to take a non-medically biased approach to discover global rules governing the generation and spread of mobile elements. We have experience of IS-mobilised duplicated metabolic genes conferring resistance to antibiotics and biocides in staphylococci. The observation that hetero-diploidy (a feature easily detectable in genome sequences) of a metabolic gene of the core-genome (i.e. a gene with a name and known function) could be evidence for selective pressure, suggests the possibility of screening the thousands of published microbial genomes for similar evidence of selective pressure exerted by almost any compound to which bacteria have been exposed. Consequently the selective agent and hence our investigation will extend from the more obvious antimicrobials to include, more importantly, other man-made compounds that may act as a selective pressure in the environment. These compounds could include any type of drug, but also compounds like waste contaminants and pollutants.

The overarching scientific aim of this PhD project is to gain proof of concept i.e. that a strategy of scanning databases for duplicated core-genome metabolic genes is able to reveal new targets and resistance mechanisms. Genes are expected to show up multiple times in lists obtained by *in silico* screening of thousands of genomes. These numerical differences could represent a quantification of selective pressure on single genes and thus indirectly a quantification of selective capacity of the relative compounds. The quantification of such selective pressure could correlate to the relative risk or efficacy of such compounds. While definition of a strategy of risk quantification is a long-term goal, this project is intended to be the proof of the concept. The project work will start with staphylococci due to the extensive expertise of the supervisors on this species. To reach this aim we have the following objectives.

- Identification of duplicated hetero-diploid core genome metabolic genes in the NCBI staphylococcal database.
- Proof of concept that a newly identified hetero-diploid core-genome metabolic gene confers phenotypic resistance to a compound by phenotypic profiling and cloning.
• Production of a global analysis of hetero-diploid genes in public microbial databases in order to produce lists of duplicated genes for a series of organisms.

This interdisciplinary PhD project has the challenging aim to be able, in just three years, to propose a novel paradigm based upon a “globally valid” strategy for the use of bacteria as qualified indicators of man-made selective pressure, by leveraging public genome databases – as a modern “Ames²-test”.

The variety of cutting edge technologies involved, ranging from phenotypes to genomics, make this an interdisciplinary project on the edge between microbial sciences and bioinformatics. As a result, it is an ideal training opportunity for MIBTP students, and should provide the student with an excellent background increasing its competitiveness and employability.

Relevant BBSRC Strategic Research Priority: Combatting antimicrobial resistance

Techniques that will be undertaken during the project.

Wet bench work will include training in biosafety level 2 work, bacterial growth, DNA manipulation, microscopy, molecular manipulation of bacteria including cloning and generation of genetically modified microorganisms and work on next generation sequencing technologies to be performed in-house including gene expression analysis by RNaseq. Phenotypic tests will be on a case by case basis and will include phenotype microarray with BIOLOG plates for chemical sensitivity. Multiple bacterial species will be analysed during the study and

The bioinformatic part of the project will involve learning appropriate programming skills and developing expertise to run and optimise software running on the local high performance computer clusters ALICE and SPECTRE, within the Linux environment. Databases will be searched both online and locally using programmatic approaches. The search for duplicated metabolic genes will be performed using BLAST [using back a reference pan genome approach with the need to optimise parameters] -not sure what this means. Detection of duplicated metabolic genes will be performed by testing for variation in sequence coverage, revealed by the analysis of raw sequencing data. Scripts for hit extraction and vicinity checking of IS elements, extraction of MLST data will have to be developed by the student, with the support of Supervisors. Separate databases and search tools will be built for whole genomes and plasmids, to encompass chromosomal and non-chromosomal elements. The analysis of these large datasets will need dedicated skills development, that could be supplemented by the student attending the appropriate modules of the MSc Bioinformatics.

Contact: Prof Marco Oggioni, University of Leicester