

PROGRAMME 3: Antimicrobial resistance and novel antimicrobial targets of CF pathogens: mechanisms of biocide resistance and action

Cardiff School of Biosciences, Cardiff University

Ms Helen Rose (PhD student) & Dr Eshwar Mahenthalingam (Principal Investigator)

Background

The major CF pathogens *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex (Bcc) and Methicillin Resistant *Staphylococcus aureus* (MRSA) are highly resistant to both antibiotics and disinfectants (specifically referred to as biocides in this application). Biocides such as chlorhexidine and triclosan form the primary agents used to prevent microbial infection in both clinical settings and the home (2). Yet in comparison to antibiotics, little is known about the molecular basis of biocide action and what factors they target in bacteria (2). If the mechanisms of biocide action can be uncovered and studied in molecular detail, they may provide novel therapeutic targets, enhancing our search for new ways to fight CF infection.

For many of the multi-resistant CF pathogens, especially the Bcc and emerging epidemic *P. aeruginosa* strains, resistance or susceptibility to biocides has not been systematically studied. Thus it is unclear whether current clinical practices for biocidal disinfection are adequate to kill these 'superbugs' in all situations. Up-to-date studies on these agents and disinfection procedures may help prevent further spread of these CF pathogens.

Preliminary data

In Cardiff, we have begun preliminary studies on the genetic basis of resistance to the biocides chlorhexidine and triclosan in *B. cenocepacia*. Transposon mutagenesis was used to isolate mutants susceptible to each biocide and the genetic identity of each mutant was then identified. Both known and novel antimicrobial target genes were identified from this screen. Triclosan and chlorhexidine mutants were susceptible to other biocides and to the antibiotics trimethoprim, chloramphenicol, ciprofloxacin and gentamicin, suggesting a potential link between biocide and antibiotic resistance.

Hypothesis and reasoning behind proposal

"Antimicrobial resistance in multidrug-resistant CF pathogens is multifactorial and the widespread use of biocides is contributing to the emergence of antibiotic resistant strains".

To prove or disprove this controversial hypothesis we will use a systematic genetic and genomic experimental strategy to investigate biocide resistance at the molecular level. Our overall aim will be to identify mechanisms of resistance and novel drug targets associated with biocides.

Methods of research

Determine the biocide resistance of an up-to-date panel of multidrug resistant CF 'superbugs'.

The first step in our studies will be to perform a comprehensive and up-to-date survey of biocide and non-antibiotic resistance within representative multi-drug resistant (MDR) CF pathogens such as *P. aeruginosa*, Bcc, and MRSA. Characterisation of biocides will be expanded beyond triclosan and chlorhexidine to include all major classes of biocide that have inherent solubility allowing MIC determination and genetic screening. The following biocide classes will be examined: (i) Quaternary Ammonium Compounds (QAC): cetylpyridinium chloride (CPC), cetrimide and benzalkonium chloride; (ii) Phenolics, phenol and cresol; (iii) anilides such as Triclocarban and finally (iv) Parabens, esters of para(4)-hydroxybenzoic acid. All these compounds are in widespread use (2) and hence, they should facilitate a broad range of resistance mechanisms to be characterised. The Minimal Inhibitory Concentration (MIC) of each biocide against the genome sequenced *B. cenocepacia* J2315 (1) and the test panel of MDR bacteria will be determined. Biocide MICs will also be correlated to the concentrations and applications used in clinical situations to determine if current procedures are adequate to kill all MDR CF pathogens.

Perform transposon mutagenesis screens of *B. cenocepacia* to identify novel biocide and non-antibiotic resistance genes and pathways

B. cenocepacia will be used as the model MDR CF pathogen with which to identify antimicrobial resistance genes, as it is highly resistant and has the largest sequenced genome of the CF pathogens, potentially encoding the greatest variety of resistance mechanisms and novel target genes (1). Transposon mutagenesis will be used to identify genes associated with biocide resistance.

Identify transposon-mutated genes from *B. cenocepacia* genome sequence and correlate antimicrobial resistant for each

Sequencing of transposon mutants will identify the genetic basis for each biocide mutant. Biocide susceptible mutants will then be screened for susceptibility to other biocides and antibiotics to determine all antimicrobial resistance/susceptibility traits associated with each genetic region identified. In addition to mutagenesis, we will use a recently developed *B. cenocepacia* J2315 DNA microarray to analyse the global expression that occurs in response to biocide resistance. Overall, the systematic genomic approach will build a novel picture of genes behind the high antimicrobial resistance of MDR CF pathogens such as *B. cenocepacia*.

References

1. **Mahenthiralingam, E., T. A. Urban, and J. B. Goldberg.** 2005. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol **3**:144-56.
2. **McDonnell, G., and A. D. Russell.** 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev **12**:147-79.