The identification and characterisation of novel antimicrobial targets in *Burkholderia pseudomallei*

Submitted by Laura Emma Marshall, to the University of Exeter as a thesis for the degree of
Doctor of Philosophy in Biological Sciences

July 2012

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

The bacterium *Burkholderia pseudomallei* causes the disease melioidosis, a significant public health threat in endemic regions and is a potential biowarfare agent. Treatment of melioidosis is intensive and prolonged and there is no licensed vaccine to protect against it. The aim of this study was to characterise novel targets for antimicrobials to improve treatment of melioidosis.

A holistic down selection process was undertaken in order to identify a range of possible novel and exploitable antimicrobial targets in *Burkholderia pseudomallei*. Four targets: FtsA, FtsZ, MraW and TonB were selected for characterisation by mutagenesis study.

FtsA and FtsZ are early effectors of cell division and are considered potential antimicrobial drug targets in other pathogenic bacteria. Genes for both were shown likely to be essential for viability in *Burkholderia pseudomallei,* following attempted deletion of the genes, thus confirming their potential for drug targeting for treatment of melioidosis.

MraW, a highly conserved methyltransferase, and TonB, the energiser for high affinity iron uptake in Gram negative bacteria, were also selected for characterisation as antimicrobial targets. In-frame deletions of the genes encoding these targets were constructed in *B. pseudomallei* K96243. In order to determine the roles played by MraW and TonB during infection, these mutants were characterised in several models of *Burkholderia pseudomallei* infection.

Deletion of *mraW* rendered the bacteria non-motile and led to attenuation during infection of Balb/C mice. A small growth defect was seen early during infection of macrophages by this mutant, whilst no attenuation was seen on deletion of *mraW* in *Galleria mellonella. Burkholderia pseudomallei ΔtonB* required free iron supplementation for growth. This mutant had an improved ability to invade murine macrophages, though the mutant was attenuated in both *Galleria mellonella* and Balb/C mice.

Attenuation of both mutants in a mammalian model of infection, support the strategy to target either of these proteins as novel targets for inhibition with small molecules during *Burkholderia pseudomallei* infection. However, an improved ability to infect macrophages by *Burkholderia pseudomallei ΔtonB* and non-complementation of this mutant by iron supplementation to *Galleria mellonella* suggests additional roles to iron uptake alone for TonB in *Burkholderia pseudomallei*, such as bacterial iron sensing and signalling.

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