Molecular Pathogenesis of *Clostridium difficile*

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Clostridium difficile has emerged as one of the most important causes of healthcare-associated infections in the western world. Despite its infamy, the molecular basis of pathogenesis remains little understood. Two large cytotoxins (A & B) are the only definitive virulence factors, although the relative role of Toxin A has been questioned. The situation has been exacerbated by the emergence and spread of hyper-virulent strains, responsible for more severe disease. Presently, explanations for hyper-virulence remain conjecture. They include, increased toxin production as a consequence of defects in TcdC repressor, more prolific sporogenesis and enhanced gut epithelial cell adherence.

The elucidation of the molecular basis of pathogenesis has been impeded by ineffective gene systems for forward and reverse genetic studies. We have developed a battery of tools with which both directed (ClosTron) and random (transposon *mariner*) insertional mutants can be made, as well as allelic exchange technologies which allow gene replacement, gene addition and in-frame deletions. Mutants carrying a variety of altered alleles have been created and the physiological consequences assessed.

Through the generation of appropriate multiple mutants, and the use of an *in vivo* infection model, our studies have re-established the role of Toxin A in disease. Furthermore, we have determined a correlation between Quorum Sensing and increased toxin production which may prove to be more telling than variations in the sequence of TcdC. In parallel, we have identified, for the first time, several factors essential to the process of spore germination, and amassed data that suggests the link between hyper-virulence and spore prolificacy is debatable. Whilst our data support the view that hyper-virulent strains adhere more strongly to epithelial cells, the assumption that flagella have a role to play in adhesion has been called into question.