*Publishable Summary*

*P. aeruginosa* is a ubiquitous environmental pathogen and is one of the three major causes of opportunistic human infections. *P. aeruginosa* is found in an estimated 10-20% of all hospital acquired infections, most likely due to its high resistance to antibiotics and disinfectants that eliminate other environmental bacteria. It is implicated in respiratory infections, urinary tract infections, gastrointestinal infections, keratitis, otitis media, and bacteraemia. Respiratory infections can either be acute or chronic. Acute infections are usually acquired at hospital due to *P. aeruginosa* contamination on hospital equipment such as respirators and are normally cleared following antibiotic treatment. In contrast, chronic *P. aeruginosa* lung infections are found in patients suffering from cystic fibrosis or bronchiectasis and once *P. aeruginosa* colonisation is established, it is almost impossible to remove by current methods.

In recent years a quickly expanding family of proteins in Gram-negative bacteria have been identified, termed autotransporters (AT). AT proteins have been identified in many Gram-negative pathogens and have diverse functions ranging from cell associated adhesins to secreted toxins. Our research aims to investigate the contribution of autotransporter proteins of *P. aeruginosa* to virulence and colonisation. This will also involve investigating the role of autotransporter proteins in biofilm formation of *P. aeruginosa*. Finally we aim to explore the interaction between surface factors of *P. aeruginosa* with the human immune system during chronic lung infection.

To achieve these aims many different assays and techniques were used. These included a variety of phenotypic assays measuring motility, adhesion, enzymatic and protease activities. In addition novel assays looking at *Pseudomonas* binding to salad leaves and tomato cuticle was used. Pathogenicity of the strains was also determined using the nematode *C. elegans* model. Dr Wells also used his previous expertise to set-up and optimise a continuous flow biofilm chamber. Finally, to investigate the interaction between the patient immune system and the *Pseudomonas* strains, a variety of methods were used including serum bactericidal assays, flow cytometry, immunofluorescence microscopy and ELIZAs.

The most exciting results obtained so far have been by studying the interactions between the patient immune system and infecting bacteria. We studied eleven patients with chronic *Pseudomonas aeruginosa* lung infections for the ability for their serum to kill their infecting strains. Three patients were found to have serum that was unable to kill their infecting strains. This impairment was found to be due to a blocking in their serum, which could only be overcome with very high titres (up to 94%) of healthy control serum. Using flow cytometry and a variety of columns we identified the blocking factor as high titres of *P. aeruginosa* specific IgG2. The inhibitory IgG2 was found to be specific for the O-antigen region of lipopolysaccharide of the infecting *P. aeruginosa.* Importantly, patients with impaired immunity were linked to increased severity of disease. Thus, lipopolysaccharide O-antigen can induce inhibiting IgG2 antibodies in chronic *Pseudomonas* infection which may be a mediating factor for severity of disease. This work has been submitted to be published in a high ranking journal.

This work has the potential to be used as a prognostic and diagnostic test for chronic *P. aeruginosa* infections and thus we have submitted a patent based on this research. Most critically however, currently the O-antigen of *P. aeruginosa* LPS is thought to be an optimal target for protective antibodies and has been developed as a vaccine target in multiple trials. So far three LPS based vaccines have reached Phase II or III trials. Two LPS based vaccines, actually led to worse clinical status in the vaccinated group. The third vaccine, Aerugen® is primarily based on the O-antigen of *P. aeruginosa* LPS. Although shown to be somewhat effective in animal models and small human trials, no efficacy was seen in a Phase III clinical trial and the trial suspended. Our data may partially explain why the vaccines were not effective and suggests that the O-antigen is not the ideal target for vaccine development, and may actually lead to detrimental effects.

In addition to these results, we also have identified a role of the autotransporter esterase, EstA in digestion of tomato cuticle. This autotransporter has an orthologue in *Salmonella* (ApeE) and we found that expression of ApeE or EstA resulted in digestion of the tomato cuticle localized to the bacterial position 20 h post inoculation. In addition expression of the autotransporter enabled the bacteria to utilize TWEEN®80 as an alternative carbon source most likely by hydrolysis of oleic acid. Thus, the AT proteins ApeE and EstA enables strains to digest tomato cuticle and utilize an additional carbon source, which may be key for long-term growth and survival on produce.

Finally, the most important aim in this fellowship was the transfer of Dr Wells’ knowledge to European researchers. Thus, in addition to his work on *Pseudomonas*, Dr Wells set up, optimized and trained researchers in the use of a continuous flow biofilms chamber. He also visited another laboratory to learn the *C. elegans* nematode pathogenicity model and train others in its use. The successful transfer of knowledge can be seen in Dr Wells’ published work on the *Salmonella* autotransporter SadA, which was found to promote biofilm formation and provide limited protection against infection. In addition, Dr Wells was critical in investigating the role lipopolysaccharide plays in biofilm formation and *C. elegans* pathogenicity in *Escherichia coli.*