Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
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LAIDLAW SCHOLARS

LAIDLAW UNDERGRADUATE LEADERSHIP AND RESEARCH PROGRAMME

APPLICATION – 2023 COHORT

Name	Domhnall Roe
Programme	Medicine
Year of Study	2nd year
Research Project Title	Novel approaches in antimicrobial therapy: An investigation into the efficacy of WaaG inhibitors against Uropathogenic Escherichia Coli (UPEC)
Supervisor(s)	Primary Supervisor: Julie Renwick, Department of Clinical Microbiology, School of Medicine Co-supervisor: Naoise McGarry, Department of Clinical Microbiology, School of Medicine

Research Project Proposal

Introduction

Effective treatment of urinary tract infections (UTIs) is crucial in modern healthcare, with 40-50% of the adult population of women experiencing a UTI in their lifetime, and failure to properly treat UTIs having the potential to progress to sepsis. The main causative agent of UTIs is *Uropathogenic Escherichia coli* (UPEC), accounting for 80% of the 150 million cases seen worldwide annually.

Two important factors in the virulence of UPEC are its capsule and the O antigen, as they protect the bacteria from immune attack and facilitate its survival in the body. The O antigen is a component of lipopolysaccharide (LPS) which is an important part of bacteria. The O antigen has been identified as a major determinant for survival in the blood, playing an important role in urosepsis. A keen interest of mine is the identification of new compounds that target these factors to treat infection.

WaaG is an enzyme required to make LPS. A novel finding by McGarry and Smith in 2022 found a significant reduction in capsule association with UPEC cells when WaaG was non-functional due to a failure to form full-length LPS. The research also showed that these bacteria deficient in the enzyme were almost completely sensitive to blood compared with the prototype strain showing 100% resistance. Furthermore, Muheim et al identified 4-(2-amino-1,3-thiazol-4-yl) phenol as an

inhibitor of the WaaG enzyme produced by the CFT073 strain of UPEC in vitro. My aim is to bring this finding further and look at the effect of this inhibitor on live bacteria.

The hypothesis of my research is that the use of a WaaG inhibitor will not only increase the sensitivity of the live UPEC strain CFT073 to common antibiotics but will also reduce capsule expression, rendering the bacteria less virulent and less capable of causing sepsis. Thus, I expect my research to reinforce the importance of O antigen and capsule as major determinants of serum resistance in UPEC, as well as provide a novel compound that could be developed to treat UTIs.

Project Methodology & Timeline

As previously stated, I will be investigating the effect of a WaaG inhibitor on the sensitivity of a specific UPEC strain to common antibiotics, as well as re-establishing the significant role of O antigen and capsule in serum resistance.

Research Location:
Central Pathology Lab, Saint James' campus.

Week 1:
Following safety induction and training in the relevant laboratory techniques (culturing, streaking plates etc.), I will identify the minimum concentration of WaaG inhibitor required to inhibit bacterial growth using a UPEC CFT073 urosepsis isolate as the prototype. I will also establish the minimum inhibitory concentration of WaaG inhibitor for a non disease-causing UPEC strain and a mutant strain in which WaaG is absent for comparison. Towards the end of week 1, I will perform viability assays to confirm that the inhibitor does not stop the bacteria from growing.

Week 2:
I will perform serum killing assays which involves exposing the UPEC isolate and inhibitor to normal serum (blood) and comparing this to exposure of the bacteria to heat-inactivated serum. The aim of this step is to show how the inhibitor affects bacterial survival in blood. This procedure will be repeated with the WaaG mutant for comparison.

Week 3:
I will perform Western blot analysis of the capsule to qualitatively assess if capsule expression is affected by WaaG inhibition. Later in week 3, I will perform quantitative analysis of capsule expression in the presence and absence of inhibitor using Enzyme-Linked ImmunoSorbent Assay (ELISA).

Weeks 4 and 5:
I will assess the impact of the WaaG inhibitor on the susceptibility of cells in the presence/absence of inhibitor be exposing them to varying concentrations of several antibiotics. I will repeat this procedure with the control and negative control bacteria in the presence/absence of WaaG inhibitor. This will give me vital information on how the inhibitor affects the antibiotic sensitivity of UPEC. I will also perform reverse transcriptase-PCR to show that the changes in capsule expression occur after the WaaG enzyme has been made by the bacteria, not before.

Week 6:
If time permits, I will perform immunofluorescence microscopy to assess changes in cell shape and capsule pattern in the presence of WaaG inhibitor. This step is not essential to achieve the desired outputs, therefore I can use this week as a backup if any steps run over time.
As the above methodology has been previously optimized by my co-supervisor Naoise McGarry, I do not anticipate major delays in any step involved. The strains, reagents and equipments required are readily available in the laboratory also. Finally, I will analyze the data obtained and write up the results.

Intended Outcomes

The key goal of my research is to show that the WaaG inhibitor previously identified is effective against live UPEC bacteria. Past research has shown the importance of capsule and O antigen in the ability of UPEC to cause disease and sepsis. Since WaaG is needed to allow O antigen to become a part of LPS, and capsule association is linked to this process, an inhibitor of this enzyme would profoundly impact the ability of UPEC to cause disease. With antibiotic resistance becoming an increasingly threatening problem across the globe, novel developments in antimicrobial therapy will be essential in treating bacterial infections. If my research is successful, this inhibitor could potentially be used therapeutically on its own or in combination with existing therapies to control bacterial infections. There is scope for collaboration with pharmacologists, as more specific WaaG inhibitors could be designed based upon the chemical structure of the existing inhibitor, opening up a host of new weapons in our arsenal against bacteria.