ASSOP – Investigating the genetic factor(s) responsible for daptomycin resistance in *Staphylococcus aureus* reported in the Australian *Staphylococcus aureus* Sepsis Outcome Program (ASSOP)

Globally *Staphylococcus aureus* is one of the most frequent causes of hospital-onset and community-onset blood stream infections. Although prolonged antimicrobial therapy and prompt source control are used to treat *S. aureus* bacteraemia (SAB), mortality ranges from as low as 2.5% to as high as 40%. In 2013 the Australian Group on Antimicrobial Resistance (AGAR) commenced the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP). Consisting of up to 40 institutions located across Australia, all SAB isolates and metadata are referred to the ASSOP reference laboratory located at Murdoch University.

Daptomycin, a cyclic lipopeptide antibiotic, is considered a last line treatment against multi-resistant Gram-positive bacteria, such as methicillin-resistant *S. aureus* (MRSA) with reduced susceptibility to vancomycin. Owing to its low prevalence of resistance, daptomycin is considered a valuable antimicrobial agent in the treatment of invasive *S. aureus* infections. However, the development of daptomycin resistance during therapy seems to be an important problem affecting its clinical efficacy. The exact mechanisms of daptomycin resistance remains to be elucidated, but current knowledge suggests they are complex, diverse and multifactorial, arising from mutational changes. Although *mprF*, *rpoB* and *rpoC* mutations are believed to be associated with daptomycin resistance, mutations in several other genes have also been proposed.

Since 2013, 83 *S. aureus* have been referred to the ASSOP reference laboratory as daptomycin-resistant (using the Vitek 2 or BD Phoenix automated systems): 33 MRSA and 50 methicillin-sensitive *S. aureus* (MSSA). As per the agreement with the Australian Department of Health short read sequencing on the MRSA was performed at the reference laboratory using Illumina sequencing.

Funding permitting, we propose to:

1. Determine the minimum inhibitory concentration (MIC) on *S. aureus* initially classified as daptomycin-resistant by the AGAR participating laboratories
    - The MIC will be determined by broth microdilution according to CLSI criteria
2. Evaluate the performance of the daptomycin Etest susceptibility strip with broth microdilution
3. Identify single nucleotide polymorphisms (SNPs) in *S. aureus* associated with daptomycin resistance on isolates determined daptomycin resistant by the AGAR participating laboratory
    - SNPs will be identified by short read sequencing using Illumina sequencing
4. Elucidate the role of identified SNPs in daptomycin-resistant *S. aureus*
    - Primers targeting SNPs will be designed and recombinant plasmids harbouring the mutated genes will be constructed. Daptomycin susceptible *S. aureus* isolates will be transformed with the recombinant plasmids and mutant strains will be confirmed by whole genome sequencing. Daptomycin MIC using broth microdilution will be performed to confirm the role of the mutant gene conferring daptomycin resistance
5. Perform long read sequencing on representative isolates harbouring SNPs associated with daptomycin resistance. Sequenced isolates will be used as reference strains for future research
    - Reference strains will be sequenced using the Oxford Nanopore

All laboratory work including the bioinformatics will be performed at the ASSOP Reference Laboratory, Murdoch University

Budget: $46,780 (Includes consumables and labour costs)

Objective 1: Broth microdilution @ $50/isolate, up to 83 isolates = $4,150

Objective 2: Etest: @ $10/isolate up to 83 isolates = $830

Objective 3: Illumina sequencing and bioinformatics: @ $125/isolate, up to 50 MSSA isolates = $6,250 (Note: the 33 MRSA have previously been sequenced)

Objective 4: Cloning in *E. coli*, Site-directed mutagenesis in *S. aureus*, and Illumina sequencing: $30,000

Objective 5: Oxford nanopore sequencing and bioinformatics: @ $550 per isolates, up to 10 isolates = $5,550

Timeline: 12 months

Ongoing Additional Funding: Not required