AESOP – Molecular Characterisation of the *van* operon in vancomycin variable *Enterococcus faecium* isolated in the Australian *Enterococcus* Sepsis Outcome Program (AESOP)

Enterococcus faecium, a resident of the normal human gastrointestinal flora, has emerged as an important nosocomial pathogen responsible for bloodstream infections. Acquired resistance to ampicillin, high-level gentamicin, vancomycin and linezolid has increased worldwide amongst hospital-associated *E. faecium* narrowing treatment options. In 2013 the Australian Group on Antimicrobial Resistance (AGAR) commenced the Australian *Enterococcus* Sepsis Outcome Programme (AESOP). Consisting of up to 40 institutions located across Australia, all enterococcal bacteraemia isolates and metadata are referred to the AESOP reference laboratory located at Murdoch University.

Phenotypically vancomycin susceptible *E. faecium* harbouring the *vanA* gene complex are known as vancomycin variable *E. faecium* (VVEfm). VVEfm have been reported in Canada, South Korea, Norway, and recently in Australia. Molecular analyses have shown VVEfm typically lack the *vanS* (sensor kinase) and *vanR* (regulator) genes despite harbouring the *vanHAX* gene cassette. Because of their susceptibility to vancomycin, VVEfm are not detected by culture-dependent susceptibility methods. The prevalence of VVEfm among *vanA*-positive enterococci has mostly been reported in single institution reports and only rarely on a multi-institutional or regional level. In contrast, *vanB*-positive VVEfm have not been reported.

Since 2013, using short read sequencing, the AGAR AESOP has identified 102 VVEfm: 47 harbouring the *vanA* gene and 55 the *vanB* gene. The genetic factors responsible for *vanA* and *vanB* VVEfm in Australia are not known.

Funding permitting, we propose to:

1. Determine the vancomycin minimum inhibitory concentration (MIC) on VVEfm identified in AESOP 2013 to 2020
    - The MIC will be determined by broth microdilution according to CLSI criteria
2. Determine the genetic factors responsible for v*anA* VVEfm
    - Bioinformatic analysis of *vanA* VVEfm genomes previously sequenced by Illumina Sequencing
3. Determine the genetic factors responsible for *vanB* VVEfm
    - Bioinformatic analysis of *vanB* VVEfm genomes previously sequenced by Illumina Sequencing
4. Confirm the molecular mechanism responsible for VVEfm
    - By introducing the *van* operon mutations identified into a vancomycin-resistant *E. faecium* (VREfm) isolate, we will be able to confirm the genetic factors responsible for the inactivation of the *van* operon.
5. Determine how VVEfm exposed to increasing concentrations of vancomycin can revert to a VREfm phenotype
    - Although inactive, the *van* operon harboured by VVEfm isolates can mutate in the presence of vancomycin, resulting in resistance to the drug. We will investigate the molecular mechanisms which can cause activation or over-expression of the *vanA* and *vanB* operon in VVEfm, in the presence of vancomycin.
6. Perform long read sequencing on a representative set of VVEfm isolates. Sequenced isolates will be used as reference strains for future research
    - Reference strains will be sequenced using the Oxford Nanopore

- Long read sequencing is required to capture the comple *van* operon and will provide a better resolution for the identification of polymorphisms responsible for the activation of the *vanA* and *vanB* operons of VVEfm.

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

Budget: $45,650 (Includes consumables and labour costs)

Objective 1: Broth microdilution @ $50/isolate, up to 102 isolates = $5,100

Objective 2: Analysis performed in-kind

Objective 3: Analysis performed in-kind

Objective 4: Molecular cloning in *E. coli*, Transformation of recombinant plasmids into *E. faecium*, MIC determination by broth microdilution, and Illumina sequencing of mutants. $25,000

Objective 5: Forced evolution experiments in the presence of vancomycin, MIC determination by broth microdilution, and Illumina sequencing of mutants = $10,000

Objective 6: Oxford nanopore sequencing and bioinformatics:@ $550 per isolates, up to 10 isolates = $5,550

Timeline: 12 months

Ongoing Additional Funding: Not required