EV0542
ePoster Viewing
Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

Rapid identification of bloodstream infection using MinION metagenomics sequencing

Solomon Mwaigwisya*[1], Leary Tim[2], Nick Loman[3], Paul Dark[4], John Wain[1], Justin O'grady[1]

[1]*University of East Anglia, Norwich Medical School, Norwich, United Kingdom*

[2]*Norfolk and Norwich University Hospital, Critical Care Complex, Norwich, United Kingdom*

[3]*Univerity of Birmingham, Institute of Microbiology and Infection, Institute of Microbiology and Infection, Birmingham, United Kingdom*

[4]*Manchester Academic Health Sciences Centre,, Infection, Injury and Inflammation Research Group, Biomedical Facility, Clinical Sciences, Manchester, United Kingdom*

Background: Sepsis is an important cause of morbidity and mortality. Mortality rate rises significantly with delays in initiation of appropriate antimicrobial therapy, therefore rapid and accurate diagnosis is crucial. Traditional culture based methods for diagnosing sepsis/blood stream infection (BSI) have long turnaround times and poor clinical sensitivity. Current molecular methods, all based on PCR, are rapid and accurate but are not comprehensive, seeking only pre-set targets. We investigated the use of MinION metagenomics sequencing and WIMP™ automated bioinformatics analysis for the rapid diagnosis of BSI.

Material/methods: We have used a pathogen DNA enrichment method capable of removing the vast majority of human DNA in a 1 ml blood sample without significant loss of bacterial DNA. This was achieved by immunomagnetic separation of leucocytes using anti-CD45 labelled paramagnetic beads followed by differential lysis of the remaining leukocytes and DNase digestion of the human DNA. DNA was then extracted from the remaining pathogens (if present), whole-genome amplified (Qiagen REPLI-g) and sequenced. Sequences were identified using WIMP, real-time MinION sequence identification and classification software for bacteria, viruses and antimicrobial resistance markers supplied by Oxford Nanopore Technologies. A total of 15 blood samples have been collected and analysed by culture, 16s rDNA PCR and NGS all in parallel. Seven samples were collected and analysed prospectively and 8 retrospectively. Prospective samples were collected from patients in the Norfolk and Norwich University Hospital intensive care unit with suspected sepsis. Biobanked blood samples for the retrospective study were obtained from the University of Manchester as part **of** sepsis diagnostics evaluation study.

Results: Of the 7 prospective samples, one was positive for Group A *Streptococcus* (*S. pyogenes*) by culture, 16S PCR/Sanger sequencing and the metagenomics sequencing based method. This sample was negative by culture, however a sample taken from the patient earlier that day was positive, also

for *S. pyogenes*. The remaining samples were negative by culture and molecular methods. Six of the 8 retrospectively collected samples were concordant with culture and two were discordant. The two discordant samples both contained Gram-negative species according to culture (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) but were negative by 16S PCR analysis. Possible reasons for discordant results include loss of bacteria due to freeze - thaw or low bacterial load. WIMP identified the pathogen/s within 15 minutes of beginning a MinION run and within 8 hours of receiving the sample. Detection of antibiotic resistance genes was possible with longer sequencing run times and was dependent on genome coverage (related to efficiency of human DNA removal and bacterial load in the blood sample). MinION sequencing results were confirmed by Illumina sequencing.

Conclusions: We have demonstrated the great potential of MinION metagenomics sequencing for the rapid, comprehensive diagnosis of BSI.