Rapid pathogen and antimicrobial resistance characterisation

The majority of existing molecular assays target specific genomic regions of suspected pathogens or classes of pathogen (e.g. 16S-based assays target bacteria and archaea only); however, this requirement for *a priori* knowledge of the pathogen, combined with the limited genomic data retrieved, severely limit the application of such assays. From clinical research to animal and plant diagnostics, many researchers are now exploiting the advantages of real-time nanopore metagenomic sequencing to detect and fully characterise pathogens, allowing the rapid identification of co-infections[1], antimicrobial resistance[2,3], and evolutionary adaptations[1].

At the Quadram Institute Bioscience (QIB) in Norwich, UK, researchers have developed a nanopore sequencing workflow offering enhanced, real-time detection of the pathogens responsible for lower respiratory infections (LRIs), which cause over three million deaths worldwide each year[2]. A novel saponin-based host DNA depletion methodology removed up to 99.99% of host nucleic acids prior to sequencing. The nanopore sequencing results showed 96.6% concordance to traditional culture techniques; however, importantly, the entire process — from sample acquisition to pathogen and antimicrobial resistance (AMR) profiling — was achieved within just six hours (**Figure 1**). This is significantly faster than the 48–72 hours required for culture-based techniques[2].

'Metagenomic sequencing-based approaches have the potential to overcome the shortcomings of both culture and PCR, by combining speed with comprehensive coverage of all microorganisms present'[2]

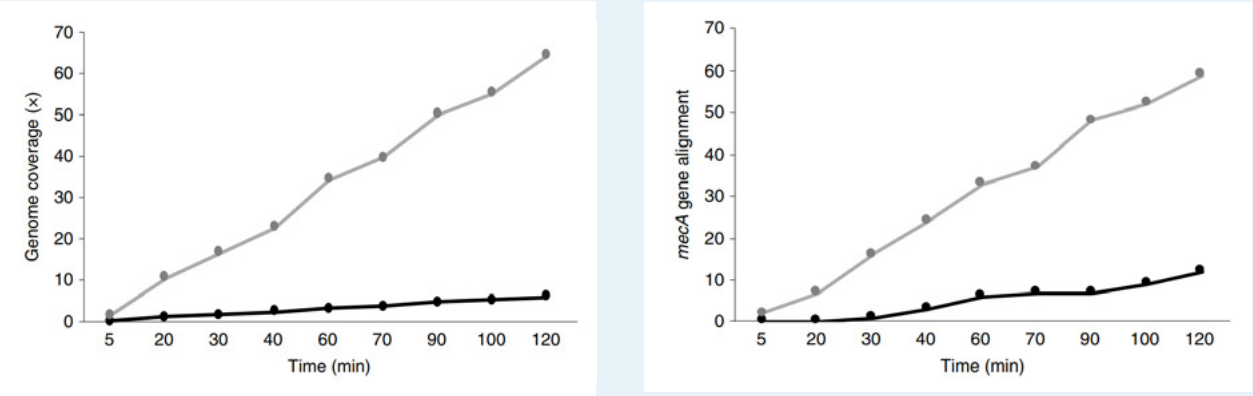

Figure 1
Nanopore sequencing was performed on an LRI sample containing methicillin-resistant *Staphylococcus aureus* (MRSA) both with ('Depleted') and without ('Control') host depletion. Within five minutes of sequencing, the host depleted sample had a) 1.6x genome coverage and b) two *mecA* gene alignments. This compared with a) 0.2x coverage and b) zero *mecA* alignments for the undepleted control. Figure from Charalampous *et al.*[2] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

Recently Břinda *et al.* demonstrated that real-time nanopore sequencing could enable AMR profiling of clinical research samples within just 10 minutes of sequencing and 4 hours of sample acquisition[3]. Their novel technique infers AMR phenotype by identifying the detected pathogen's closest relatives in a database of microbial genomes.

According to the authors '*This flexible approach has wide application for pathogen surveillance and may be used to greatly accelerate appropriate empirical antibiotic treatment*'[3].*

*Oxford Nanopore Technologies products are currently for research use only.

Find out more about metagenomic sequencing at www.nanoporetech.com/applications

References

1. Kafetzopoulou, L. E. et al. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. Science 363(6422):74-77 (2019).
2. Charalampous, T. et al. Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection. Nat. Biotechnol. 37 (7), 783-792 (2019).
3. Břinda, K. et al. Rapid inference of antibiotic resistance and susceptibility by genomic neighbour typing Nat. Microbiol. DOI: 10.1038/s41564-019-0656-6 [Online ahead of print] (2020).