TITLE: APPLICATION OF A NEXT-GENERATION NANOPORE-BASED RAPID DNA SEQUENCING PROTOCOL FOR USE IN SURVEILLANCE AND INVESTIGATION OF OUTBREAKS CAUSED BY ANTIBIOTIC-RESISTANT BACTERIA

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ABSTRACT:
The global increase in antimicrobial resistance has been identified by the World Health Organization (WHO) as one of the three main threats to human health. Surveillance and investigation of outbreaks related to antimicrobial resistant bacteria is essential to limit the transmission of these pathogens. In healthcare-related settings, rapid outbreak investigations are crucial to implement adequate control strategies, preventing patients from being affected by serious infections and saving healthcare costs. Next generation sequencing (NGS) can provide unprecedented resolution in the discrimination of closely related bacterial strains, which is a key feature in outbreak investigation. However, the NGS implementation is generally far from ideal for routine diagnostics due to time-consuming procedures, expensive equipment, and the need for specialized personnel. To achieve the high resolution of NGS and overcome some of the limitations, we developed and validated a fast and straightforward protocol using DNA whole genome sequencing based on Oxford Nanopore technology (ONT) to investigate outbreaks related to antibiotic resistant bacteria. The protocol was developed using 42 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates identified from former well-characterized outbreaks. The suggested protocol includes: 1. a 20h sequencing run; 2. identification of the sequence type (ST); 3. *de novo* genome assembly; 4. polishing of the draft genomes; and 5. phylogenetic analysis based on single nucleotide polymorphisms (SNPs). Overall, the developed protocol was able to at least discard an outbreak in 27h (mean) after the bacterial identification and less than 33h to confirm it. All these estimated times were calculated considering the average time for six MRSA isolates per sequencing run. The validation of the protocol was performed using Illumina technology (MiSeq). To test the ONT-based protocol, a real-time outbreak investigation of six clinical *S. aureus* isolates was conducted. Additionally, the protocol was also tested for *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates related to Norwegian outbreaks. The suggested protocol enables to identify outbreaks in early stages (<33h) using a portable and low-cost device along with a streamlined downstream analysis, therefore having the potential to be incorporated in routine surveillance analysis workflows.

Keywords: outbreak, antimicrobial-resistant bacteria, nanopore sequencing, whole genome sequencing

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