DNA EXTRACTION OF MICROBIAL DNA DIRECTLY FROM INFECTED TISSUE

An optimized protocol for use in nanopore sequencing

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Abstract:

- Traditional methods make discovering bacteria that cause tissue infections difficult, in the case of low-growing bacteria or difficult to cultivate microbes.
- We use shotgun metagenomic sequencing on bacterial DNA extracted directly from the infected tissue may reduce time to diagnosis and targeted treatment.
- Infected tissue, is made up of human DNA (hDNA), which makes bacterial identification difficult, so we present a modified version of the Ultra-Deep Microbiome Prep kit for DNA extraction, which removes additional human DNA.
- Tissue biopsies from 3 patients with orthopedic implant-related infections containing varying degrees of Staphylococcus aureus were included. Microbe and antibiotic resistance genes were identified using DNA shotgun metagenomic sequencing and Oxford Nanopore Technologies' (ONT) MinION network, as well as ONT's EPI2ME WIMP and ARMA bioinformatics workflows.
- The modified DNA extraction protocol reduced human DNA by a tenth of a percent while keeping S. aureus DNA. The presented protocol has the ability to classify the infection-causing pathogen in infected tissue within 7 hours of biopsy.
- Positive discovery of antibiotic resistance genes was not effective due to a lack of S. aureus reads.

Introduction:

As next-generation sequencing (NGS) gains acceptance as the gold standard in bacteriology, the market for efficient DNA extraction procedures increases. The ability to remove microbial DNA directly from human samples and then sequence it with shotgun metagenomic sequencing will speed up diagnosis. The development of a DNA extraction protocol capable of depleting hDNA while preserving microbial DNA is important for improving NGS microbe identification.

The Ultra-Deep Microbiome Prep kit (Molzym, Bremen, Germany) is a DNA extraction kit that extracts enriched microbial DNA from a range of sample forms, including biopsies. It can recognize and identify all types of bacteria and fungi, including those that aren't culturable. The kit has been used for shotgun metagenomic sequencing of bronchi-alveolar lavage fluid using Illumina sequencing platform. Additional examples are found in the characterization of human breast tissue biopsies, and the profiling of oral bacteria on pathologically changed heart valves using 16 S rRNA sequencing on an Ion PGM Sequencer and Sanger sequencing. However, used NGS sequencing platforms for metagenomic sequencing, such as Ion Torrent and Illumina, require extensive pre-sequencing sample preparation and require the sequencing run to be completed before analysis can begin (exception of a recently published tool) (LiveKraken).These obstacles may be overcome by using nanopore sequencing technology where pre-sequencing preparation is short (15 min–2 h) and data analysis can be done in near real-time using the web- analysis platform including for microbe identification, and ARMA for antibiotic resistance genes based on the Comprehensive Antibiotic Resistance Database .

These technological advancements may help patients with orthopedic implant-associated infections. The implications of these infections are severe and relying on empirical antibiotic therapy may cause inefficient treatment, increasing risk of mortality.

The rapid detection of these infectious agents is critical for medical care. Traditional OIAI microbiological diagnostics necessitate the cultivation of 5 biopsies from each patient for at least 5 days on several media. Another 24 hours would be required to test phenotypic antibiotic resistance.

The aim of this proof was to demonstrate the feasibility of using a modified version of the Ultra-Deep Microbiome Prep kit for DNA extraction and subsequent shotgun metagenomic sequencing with ONT's nanopore sequencing and bioinformatics platform for near real-time detection of microbes and antibiotic resistance genes directly from infected tissue.

Related work:

From Jan 2017 to December 2018, diagnostic soft tissue biopsies were taken from patients at Akershus University Hospital who had OIAI. They were collected from the areas directly adjacent to the infected implant.

Every biopsy was split into two parts, one was cultivated using standard microbiological diagnostics and the other used for sequencing, was initially frozen at 80 °C. Antibiotic sensitivity testing was carried out according to the European Committee on Antimicrobial Susceptibility Testing guidelines, with EUCAST breakpoints used to classify the isolate as susceptible (S), intermediate (I), or resistant (R). There were a total of 33 patients in the study. Based on the semi-quantification of Staphylococcus aureus development during routine diagnostic cultivation, biopsies from three patients (13 unique biopsies) were chosen. In this proof of concept analysis, patients with S. aureus infection were chosen because it is one of the most frequent causes of OIAI12.

This study was accepted by the Regional Committee for Medical and Health Research Ethics and the local Data Protection Officer (17/024) at Akershus University Hospital. The patients gave their written. All research was performed in accordance with relevant guidelines.