Rapid pathogen detection in ambiguous infections using real-time nanopore sequencing technology

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Infectious diseases

- Infectious diseases are among the main causes of mortality and morbidity worldwide
- Timely diagnosis and targeted antimicrobial treatment are essential for successful treatment of infections

Current pathogen detection methodology

- Current diagnosis of clinically significant infections relies on a variety of laboratory-based tests including
  - Microscopy, culturing, immunoassays, nucleic-acid amplifications, etc.

Current pathogen detection methodology

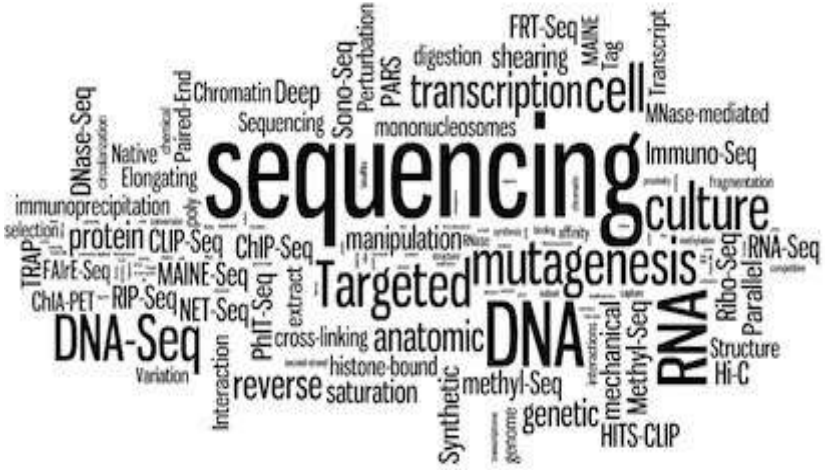
- However, the conventional microbiological methods are not always confirmatory for the accurate diagnosis in some clinical scenarios
  - Pneumonia (15-25% unknown cause)
  - Sepsis (20% unkown cause)
  - Menengitis / Encephalitis (60-80% unknown cause)
- It might take days/weeks to characterize the infectious agents

Current pathogen detection methodology

Lack of timely pathogen detection or no detection at all negatively affects the mortality rates!

Next Generation Sequencing?

- NGS could be a novel diagnostic tool for the detection of pathogens
- However;
  - High-throughput is not feasible for single samples
  - Sequencing duration is typically long
  - Not plausible for the out-of-lab application (in field)



illumina

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life technologies

PACIFIC BIOSCIENCES

Helicos BioSciences Corporation

ion torrent

Oxford NANOPORE Technologies

Next Generation Sequencing- MinION

- MinION (Oxford Nanopore) is a real-time-whole-genome sequencer
  - Which enables custom use
- Is a portable 'USB stick'
  - Which enables out-of lab use

Objectives of the study

- This study aimed real-time nanopore sequencing based rapid detection of infection agents for clinically significant yet laboratory-confirmation-negative cases
- **This study was designed as a proof-of-concept**

Methods

- Two hospitalized patients were included
- **The first patient**
  - had several weeks of abdominal pain, fever, weight loss
  - positive peritoneal thickening
  - peritoneal biopsy was negative for the carcinoma
  - cultures (peritoneal& bronchoalveolar lavage fluid) were negative (ENZ stain-negative, PCR-negative, Bactec-negative)
  - Purified protein derivative (PPD) score was 18
- This patient was clinically diagnosed with **peritoneal tuberculosis**

Methods

- **For the second patient** who had been diagnosed with *Brucellosis 9 months ago*, clinical findings were indicating relapse of ***Brucellosis***
  - Blood cultures were negative
  - Serological determinants for brucellosis were under the threshold (1/20 and 1/40)

- Peritoneal fluid (patient 1) and the blood samples from these patients were collected
- Modified producers were optimised for the Microbial DNA isolation (Qiagen) and depletion of host DNA (MolYsis)
- Fragmentation were done using Covaris-G-Tubes

Methods

- Sequence-ready libraries were generated using the MinION-low input expansion kit and MinION-genomic DNA sequencing kit
- A 72-h sequencing protocol were initiated using the MinION control software
- Read event data were base-called by the software Metrichor using workflow v2.39.3
- Generated sequences were searched in the microbial databases using developed software pipeline with a time-period of 10 minutes as the sequencing continues

Results

- On average, ~9 Mbp DNA was read in 10 minutes intervals
- In 20 minutes, the first *Mycobacterium tuberculosis* classification was done
- In 30 minutes, the first *Brucella melitensis* was detected
- Confident detection thresholds were satisfied for both species in 50 minutes time-point
- Altogether, **the total detection time was calculated to be 6 hours**

Discussion

- Cultures might fail to reveal a pathogen in patients with signs and symptoms consistent with an infectious disease
- We employed the novel real-time nanopore sequencing in detection of infectious agents in two cases
  - where traditional microbiological methods failed to reveal the pathogens!

Discussion

- This approach can not only detect conventionally unrevealed pathogens, but it can achieve this in a tight time-frame

- In addition to this pathogen detection at "point-of-care" can be avaliable
  - if the wet-lab procedure is optimized to be used in field

Future work!

- ✧Currently we are working on improving the procedures
  - ✧Fast sample preparation and library preparation

- ✧Developing the next version of the rapid pathogen detection software using artificial intelligence (deep-learning)
  - ✧The pathogen detection includes species identification, antimicrobial resistance profiling and virulence factor recognition

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