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

Aycan Gundogdu*[1], Huseyin Kilic[2], Aysegül Ulu Kilic[3], Ufuk Nalbantoglu[4]

[1]*Erciyes University Medical Faculty; Microbiology and Clinical Microbiology*

[2]*Erciyes University Medical Faculty; Medical Microbiology*

[3]*Erciyes University; Infectious Disease*

[4]*Erciyes University; Computer Engineering*

Background: Infectious diseases are the main causes of mortality and morbidity worldwide. Timely diagnosis and targeted antimicrobial treatment are essential for successful treatment of infections. Current diagnosis of clinically significant infections rely on a variety of laboratory-based tests including microscopy, culturing, immunoassays and nucleic-acid amplifications. However, the routine microbiological methods are by themselves, not always confirmatory for the accurate diagnosis in some clinical scenarios with a significant time delay.

This study aimed real-time nanopore sequencing based confirmation of clinically significant yet laboratory-confirmation-negative peritoneal tuberculosis and Brucellosis cases. For this reason we used a portable, 'USB stick', whole-genome sequencer, the MinION (Oxford Nanopore), as part of their early access programme.

Material/methods: Two hospitalized patients who clinically diagnosed with peritoneal tuberculosis and brucellosis were included in this study. The first patient had several weeks of abdominal pain, fever, weight loss, with positive peritoneal thickening. Purified protein derivative (PPD) score was 18, peritoneal biopsy was negative for the carsinoma, and peritoneal&bronchoalveolar lavage fluid cultures were negative (Ziehl-Neelson stain-negative, PCR-negative, Bactec-negative). For the second patient who diagnosed as Brucellosis 9 months ago, clinical findings were in line with as relapse. Blood cultures were negative and serological determinants for brucellosis were under the treshold (1/20 and 1/40).

Peritoneal fluid and the blood samples from these patients were taken after clinical determination of the infections. Bacterial DNA isolation were done using commercial kits and fragmentation were done using Covaris-G-Tubes. Sequence-ready libraries were generated using the MinION-low input expansion kit and MinION-genomic DNA sequencing kit according to the protocol. A 72-h sequencing protocol were initiated using the MinION control software, MinKNOW. Read event data were base-called by the software Metrichor using workflow v2.39.3. Generated fasta files were processed using WIMP taxonomy analysis workflow with a time-period of 10 minutes as the sequencing continues.

Results: In order to assess the rapid-diagnostic potential of real-time sequencing, the generated data was re-analyzed at time intervals of 10 mins. On average, ~9 Mbp DNA was read in 10 minutes intervals, and the first *Mycobacterium tuberculosis* classification was done in 20 minutes, where *Brucella melitensis* was detected in 30 minutes time-point. Confident detection thresholds were satisfied for both species in 50 minutes time-point. Altogether, the total detection time was calculated to be 6 hours.

Conclusions: It is well known that, even with cultivable bacteria, cultures might fail to reveal an organism in patients with signs and symptoms consistent with an infectious disease. We employed the novel real-time nanopore sequencing technology in rapid detection of infection agents in two crucial cases where traditional microbiological methods failed to reveal the pathogens. It is reported that real-time sequencing can not only detect conventionally unrevealed pathogens, but it can achieve this in a tight time-frame.