



Category: Metagenomics

Towards Point-of-Care Diagnosis of Pulmonary Tuberculosis and Drug Susceptibility Testing by Whole Genome Sequencing of DNA Isolated from Sputum

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Abstract

Preliminary screening of pulmonary tuberculosis (TB) in India still relies on sputum microscopy, which has low sensitivity leading to high rate of false negatives. Moreover, conventional phenotypic drug susceptibility testing (DST) is conducted over a period of weeks leading to delays in correct treatment. Next generation sequencing technologies (Illumina and Oxford Nanopore) have made it possible to sequence miniscule amount of DNA and generate enough data within a day for detecting specific microbes and their DST profile. Sputum samples from two pulmonary TB patients were processed by decontamination and DNA was isolated from the decontaminated sputum sediments. The isolated DNA was used for sequencing by Illumina and by MinION (Oxford Nanopore Technologies). The sequence data was used to diagnose TB and to determine the DST profiles for the first- and second-line drugs by *Mykrobe Predictor*. Validation was conducted by sequencing DNA (by Illumina) isolated from pure growth culture from both the samples individually. DNA sequencing data (for both, Illumina and MinION) from one of the sputum samples indicated the presence of *Mycobacterium tuberculosis* (*M. tb*) resistant to streptomycin, isoniazid, rifampicin and ethambutol and its lineage was predicted to be Beijing East Asia. The second sample indicated the presence of *M. tb* sensitive to the first- and second-line drugs by MinION and showed minor resistance call only to rifampicin by Illumina. Lineage of the second sample was predicted to be East Africa Indian Ocean, whereas Illumina data indicated it to be Delhi Central Asia. The two samples were correctly diagnosed for the presence of *M. tb* in the sputum DNA. Their DST profiles and lineage were also successfully determined from both the sequencing platforms (with minor discrepancies) paving the way towards diagnosis and DST of TB from DNA isolated from sputum samples at point-of-care. Nanopore sequencing currently requires skilled personnel for DNA processing and is a costly method – these drawbacks need to be addressed for successful implementation of Nanopore as a point-of-care diagnostic method in future.

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