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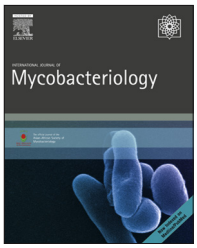

Use of WGS in *Mycobacterium tuberculosis* routine diagnosis

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ABSTRACT

Objective/background: Whole Genome Sequencing (WGS) is becoming affordable with overall costs comparable to other tests currently in use to perform the diagnosis of drug-resistant tuberculosis (TB) and cluster analysis. The WGS approach allows an "all-in-one" approach providing results on expected sensitivity of the strains, genetic background, epidemiological data, and indication of risk of laboratory cross-contamination.
Methods: Although ideal, WGS from the direct diagnostic specimen is not yet standardized, and to date the two most promising approaches are WGS from early positive liquid culture and targeted sequencing from diagnostic specimens using Next-Generation Technology. Both have advantages and disadvantages. Sequencing from early MGIT requires positive cultures, whereas targeted sequencing can be performed from a specimen positive for *Mycobacterium tuberculosis* with a consistent gain in time to information. The aim of this study is to evaluate the feasibility and cost of using WGS with a centralized approach to speed up diagnosis of TB in a low-incidence country.
Methods: From March 2016 to September 2016, we collected and processed by WGS 89 early positive routine MGIT960 tubes. Time to diagnosis and accuracy of this technique were compared with those of standard testing performed in a regular laboratory. A 2-mL aliquot of early positive MGIT was processed, starting with heat inactivation. DNA was then isolated by using the Maxwell 16 Cell DNA Purification Kit and Maxwell 16 MDx for automated extraction. Paired-end libraries of read-length 75–151 bp were prepared using the Nextera XT DNA Sample Preparation kit, and sequenced on Illumina Miseq/Miniseq platform (based on the 1st available run). Total variant calling was performed according to the pipeline of the Phyresse web-tool. The DNA isolation step required 30 min for inactivation plus 30 min for extraction. The concentration obtained ranged from 0.1 to 1 ng/μL, suitable for library preparation. Samples were sequenced with a turnaround time of 24–48 h. The percentage of reads mapped to the H37Rv reference genome was 83% on average. The mean read coverage was 65×. The main challenge was the presence of nonmycobacterial DNA contamination in a variable amount. Lineage detection was possible for all cases, and mutations associated with drug resistance to antitubercular drugs were examined. We observed high diagnostic accuracy for species identification and detection of full drug resistance profile compared to standard DST testing performed in MGIT.

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Results: Two events of recent transmissions including respectively three and two patients were identified, and two laboratory cross-contaminations were investigated and confirmed based on the analysis. Time to availability of report was about 72 h from MGIT positivity compared to up to 6–9 weeks for XDR-TB diagnosis with standard testing. In addition to speed, the main advantages were the availability of a full prediction of resistance determinants for rifampicin-resistant cases, and the fast detection of potential cross-contaminations and clusters to guide epidemiological investigation and cross-border tracing. Cost analysis showed that the cost per strain was approximately €150 inclusive of staff cost, reagents, and machine cost.

Conclusion: WGS is a rapid, cost-effective technique that promises to integrate and replace the other tests in routine laboratories for an accurate diagnosis of DR-TB, although it is suitable nowadays for cultured samples only.

Conflicts of interest

All authors declare no conflicts of interest.