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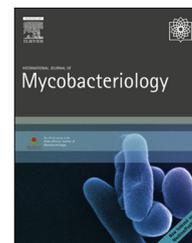


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Whole genome sequencing as the ultimate tool to diagnose tuberculosis

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ABSTRACT

In the past two decades, DNA techniques have been increasingly used in the laboratory diagnosis of tuberculosis (TB). The (sub) species of the *Mycobacterium tuberculosis* complex are usually identified using reverse line blot techniques. The resistance is predicted by the detection of mutations in genes associated with resistance. Nevertheless, all cases are still subjected to cumbersome phenotypic resistance testing. The production of a strain-characteristic DNA fingerprint, to investigate the epidemiology of TB, is done by the 24-locus variable number tandem repeat (VNTR) typing. However, most of the molecular techniques in the diagnosis of TB can eventually be replaced by whole genome sequencing (WGS). Many international TB reference laboratories are currently working on the introduction of WGS; however, standardization in the international context is lacking. The European Centre for Infectious Disease Prevention and Control in Stockholm, Sweden organizes a yearly round of quality control on VNTR typing and in 2015 for the first time also WGS. In this first proficiency study, only three out of eight international TB laboratories produced WGS results in line with those of the reference laboratory. The whole process of DNA isolation, purification, quantification, sequencing, and analysis/interpretation of data is still under development. In this presentation, many aspects will be covered that influence the quality and interpretation of WGS results. The turn-around-time, analysis, and utility of WGS will be discussed. Moreover, the experiences in the use of WGS in the molecular epidemiology of TB in The Netherlands are detailed. It can be concluded that many difficulties still have to be conquered. The state of the art is that bacteria still have to be cultured to have sufficient quality and quantity of DNA for successful WGS. The quality of sequencing has improved significantly over the past 7 years, and the detection of mutations has, therefore, become more reliable. The resistance mutations detected in WGS are in line with the ones visualized in reverse line blot techniques. The turnover in the genome of *M. tuberculosis* is very low, ~0.3–0.5 mutations per genome per year. However, there is a wide variation in the occurrence of mutations per strain and genotype. Still, the resolution of WGS in epidemiological typing is higher than that in VNTR typing; previously suggested epidemiological links by VNTR typing are sometimes refuted on the basis of WGS. Although WGS offers the highest resolution in typing, in a country like The Netherlands, there are many strains with a limited genetic distance up to 100 mutations, without an apparent epidemiological link between the respective cases. These lookalikes are

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presumably even more prevalent in settings where predominant genotypes of *M. tuberculosis* are circulating. In summary, WGS seems to yield a more reliable prediction of resistance by the (lack of) detection of mutations in all 25 genes ever associated with resistance. This may within a short while prevent the need for many phenotypic resistance tests. Although more robust algorithms need to be developed, the recognition of the (sub) species in the *M. tuberculosis* complex seems possible. The first detailed studies on the population structure of *M. tuberculosis* strains in The Netherlands provide more resolution in typing but also an interesting observation that a part of the strains are genetically so conserved that they are separated by less than 100 mutations. This demands a more extended and accurate validation and understanding of the utility of WGS in the epidemiology of TB.

Conflicts of interest

The authors have no conflicts of interest to declare.