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Perspective

Determination of Multidrug-Resistant Tuberculosis Using Genotype Method

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DESCRIPTION

Several countries are suffering from the high prevalence of Multidrug-Resistant Tuberculosis (MDR-TB). For first and second-line drugs, the public health system provides free diagnosis and drug-susceptibility testing using phenotypic (proportions in 7H10) and molecular genotype methods. Molecular drug resistance testing has been shown to significantly reduce the time for diagnosis, particularly for isoniazid and rifampicin, two anti-tuberculosis regimen pillars, and thus contribute to better clinical outcomes. However, phenotypic methods, such as MGIT 960 and proportions in agar plaque, remain the reference standard at National Reference Laboratory of Mycobacteria.

The Genotype method is based for identifying mutations in the rpoB gene's Rifampicin-resistance determining region (RRDR), an 81 base-pair segment of the gene. To diagnose isoniazid resistance, mutations in the katG and inhA genes are identified. However, this assay only detects the most common mutations, not every possible mutation that confers resistance to these drugs. There could be mutations that these tests miss ("non-canonical" mutations), in that case the test would be reported as susceptible. A resistant pattern would be detected if phenotypic methods were used. There have been reports of novel mutations being proposed to explain discrepancies between molecular and phenotypic methods, which could explain the susceptible results. Whole Genome Sequencing (WGS) is a technology that can identify new sequences that could explain certain resistance traits, and it is currently being used to investigate the genome of *Mycobacterium tuberculosis* strains with discordant results.

The below provided laboratory procedure is followed to detect the presence of drug resistance in *Mycobacterium tuberculosis* strains:

- Strains kept at 80°C in the laboratory's bio bank were reactivated in Middel brook 7H9 broth for 7 days. The 0.5 mL aliquot was then transferred to Löwenstein-Jensen media for further growth.
- To confirm the results, we repeated the Genotype® MTBDRplus, Hain LifeScience, Nehren, Germany v2.0 test and the proportions in the agar-plaque test, GenoType MTBDRplus v2.0 was carried out in accordance with the manufacturer's instructions. The GenoLyse v1.0 kit was used for DNA extraction, and the Genotype MTBDRplus v2.0 probes were used for DNA amplification and hybridization to identify mutations in the rpoB, katG, and inhA genes.
- The colonies were transferred from the Löwenstein-Jensen media to Middlebrook 7H9 for 7 days and then to Middlebrook 7H10 for 21 days for the proportions in the agar-plaque test. The plaques contained rifampicin showed minimum inhibitory concentration at 1.0 g/mL and isoniazid at 0.2 g/mL and 1.0 g/mL. If the critical proportion was greater than 1%, the strain was considered resistant; if it was less than 1%, it was considered sensible. Only strains with persistent discordance after repeating the tests were considered for WGS analysis.
- According to the manufacturer's instructions, genomic DNA was extracted from these strains using the "GenJet Genomic DNA purification" kit. The concentration of double-stranded DNA was then determined by fluorescence using the Qubit 2.0 fluorometer kit (Invitrogen, Carlsbad, CA, USA). Whole genome sequencing was carried out using paired-end Nextera XT kits of two 250 and two 300 pb with the NGS illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The MiSeq Reagent Kit v3 (600 Cycles) was used exactly as directed.

However, not all existing mutations cause resistance, so existing evidence about their association with drug resistance must be considered. The World Health Organization has issued interpretation recommendations for detection. Knowledge on this topic is constantly evolving, and it is critical to generate additional data on these novel mutations. Using whole genome sequencing, we set out to find mutations that could explain discrepancies in the evaluation of susceptibility to rifampicin and isoniazid between molecular and phenotypic methods. This information could help to develop more comprehensive diagnostic devices for assessing drug resistance in the future.

CONCLUSION

We were able to report several mutations identified through WGS in *Mycobacterium tuberculosis* strains with discordant results

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between phenotypic and genotypic diagnostic tests for evaluating drug resistance to rifampicin and isoniazid. Some of these, specifically rpoB V170F and I491F for rifampicin and katG R463L, kasA G269S, and Rv1592c I322V for isoniazid, may be considered in the future for the development of local genotypic diagnostic tests for drug resistance diagnosis.