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Full Length Article

Evaluation of the GenoType MTBDR*plus* assay for detection of rifampicin- and isoniazid-resistant *Mycobacterium tuberculosis* isolates in central Ethiopia ☆

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ABSTRACT

Objective/Background: Multidrug-resistant tuberculosis (MDR-TB) is growing globally and becoming a major challenge for national TB control programs. Therefore, rapid identification of MDR strains of *Mycobacterium tuberculosis* and monitoring their transmission could contribute significantly to the control of TB. The GenoType MTBDR*plus* assay has been recommended by the World Health Organization to identify rifampicin (RIF)- and isoniazid (INH)-resistant *M. tuberculosis* isolates. This study was carried out to evaluate the performance of the GenoType MTBDR*plus* assay for the detection of RIF- and INH-resistant *M. tuberculosis* isolates in central Ethiopia. *Methods:* A total of 279 *M. tuberculosis* strains isolated from active TB cases in central Ethiopia were evaluated for their drug sensitivity by the conventional drug-susceptibility test (DST) and compared with data derived from the GenoType MTBDR*plus* assay. The DST served as the gold standard for evaluating the GenoType MTBDR*plus* assay. *Results:* The sensitivity and specificity of the GenoType MTBDR*plus* assay for the detection of RIF-resistant *M. tuberculosis* isolates were 80.0% and 99.6%, respectively. Its sensitivity and specificity for the detection of INH-resistant *M. tuberculosis* isolates were 82.7% and 99.6%, respectively, whereas they were 75.0% and 100%, respectively, for the detection of MDR *M. tuberculosis* strains. The concordances of the GenoType MTBDR*plus* assay and the conventional DST for the detection of RIF and INH susceptibility were 80% (8/10) and 86.2% (25/29), respectively. Furthermore, the concordance of the two tests for the detection of MDR *M. tuberculosis* strains was 75%. Specific mutations were detected in 55.6% (5/9) of the RIF-resistant isolates, with the highest mutation rate (33.3%) for the *rpoB* gene (Codon S531L). For INH-resistant isolates, the highest mutation

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rate (88.8%) related to a *katG* mutation (Codon S315T1). *Conclusion:* The findings of this study revealed that the GenoType MTBDR*plus* assay has high sensitivity and specificity for the detection of RIF and INH resistance. These preliminary data support the notion that the assay should be considered as an alternative to the DST for the characterization of MDR in *M. tuberculosis* isolates and the control of TB.

Introduction

Tuberculosis (TB) is one of the major global health problems, with high prevalence in developing countries. A major concern is multidrug-resistant TB (MDR-TB), which is defined as the resistance to isoniazid (INH) and rifampicin (RIF), two therapeutic compounds for first-line TB treatment. The emergence of strains resistant to major anti-TB drugs has increased the need for identifying rapid and simple methods to detect such resistances and their molecular basis in the *Mycobacterium tuberculosis* genome. Using such tests promises to improve the physician's decision to appropriately treat the disease in patients on an individual basis and allows better monitoring of the emergence of MDR-TB strains in distinct geographical regions, ultimately contributing to the prevention of the spread of resistant strains. Drug-susceptibility testing by conventional methods using solid media such as Löwenstein–Jensen is time consuming because *M. tuberculosis* grows slowly in culture requiring several weeks to identify the pathogen and test its drug-resistance profile. Even with more automated fluid culture methods, the former method takes an average of 14 days. Two additional weeks are required to obtain information about the strain's drug susceptibility [1]. Molecular methods for drug-resistance testing based on the identification of mutations in genes associated with drug resistance, such as the GenoType MTBDR*plus* assay, offer an effective, alternative method to determine drug-resistance strains [2]. The GenoType MTBDR*plus* assay is a molecular-line probe assay containing probes specific for the *M. tuberculosis* complex, wild type as well as probes for common RIF- and INH-resistance-conferring mutations. The assays are based on reverse hybridization of amplicons immobilized on membranes. The GenoType MTBDR*plus* assay detects mutations in the *rpoB*, *katG*, and *inhA* genes, and delivers results with a rapid turnaround time of 48–72 h. Nearly all RIF-resistant strains contain mutations in the *rpoB* gene, which encodes the RNA polymerase subunit β [3]. Mutations in the *katG* and *inhA* genes are related to the high-level and low-level INH resistance, respectively [3]. More than 95% of the RIF-resistant strains harbor a mutation within an 81-bp region of the *rpoB* gene from Codons 507 to 533 and this is region is called the RIF resistance-determining region [4–6]. The highest level of RIF resistance of the *rpoB* gene occurs in Codons 531 and 526. The *rpoB* gene mutations occur in Codons 511, 516, 518, 522, and 533 and cause low-level resistance to RIF. Resistance mutations are rarely identified in other regions of the *rpoB* gene [4].

Mutations causing INH resistance are located in several genes. Several studies have demonstrated that 34.6–94.3% of INH resistance is most frequently associated with a mutation in Codon 315 of the *M. tuberculosis* catalase peroxidase (*katG*) gene [7,8]. The *inhA* gene has 2.9–21.5% of its mutations in the promoter region [9], and an additional 2–11.5% in the *ahpC–oxyR* intergenic region [10,11].

Ethiopia is one of the 27 high MDR-TB-burden countries in the world. According to the 2015 World Health Organization report, 1.6% of new TB patients and 12.0% of previously treated patients had MDR-TB [12]. Annually, 2000–2500 MDR-TB cases are estimated to occur among all reported pulmonary TB cases. However, for example, in the year 2012, only 212 (10.1%) TB cases were detected [13], indicating that the many of the expected MDR-TB cases remain undiagnosed and continue to spread in various communities. Therefore, improved monitoring of TB drug resistance—with respect to the time required for detection of resistance and the sensitivity and specificity of detection of MDR—is important, and may benefit from molecular tests such as the GenoType MTBDR*plus* assay. The objective of this study was to evaluate the performance of the GenoType MTBDR*plus* test in detecting INH- and RIF-resistant *M. tuberculosis* isolates in central Ethiopia.

Materials and methods

M. Tuberculosis isolates

A total of 279 *M. tuberculosis* specimens isolated from smear-positive TB patients who visited St. Lukas, Atat, and Fitche hospitals in central Ethiopia between October 2012 and September 2013 were used for this study. The study was ethically approved by the Ethical Review Board of College of the Natural Sciences of the Addis Ababa University, Ethiopia (Ref. No. CNSDO/379/07/15).

Conventional drug-susceptibility testing using Löwenstein–Jensen media

The isolates were evaluated for their drug sensitivity using the conventional proportion method and the sensitivity of each isolate against the first-line drugs (INH, streptomycin, RIF, and ethambutol) was evaluated by the indirect proportion

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