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Full Length Article

Drug resistance-conferring mutations in *Mycobacterium tuberculosis* from pulmonary tuberculosis patients in Southwest Ethiopia

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ABSTRACT

Objective/background: The nature and frequency of mutations in rifampicin (RIF) and isoniazid (INH) resistant *Mycobacterium tuberculosis* isolates vary considerably according to geographic locations. However, information regarding specific mutational patterns in Ethiopia remains limited.

Methods: A cross-sectional prospective study was carried out among confirmed pulmonary tuberculosis cases in Southwest Ethiopia. Mutations associated with RIF and INH resistances were studied using GenoType MTBDRplus line probe assay in 112 *M. tuberculosis* isolates. Culture (MGIT960) and identification tests were performed at the Mycobacteriology Research Center of Jimma University, Jimma, Ethiopia.

Results: Mutations conferring resistance to INH, RIF, and multidrug resistance were detected in 36.6% (41/112), 30.4% (34/112), and 27.7% (31/112) of *M. tuberculosis* isolates respectively. Among 34 RIF-resistant isolates, 82.4% (28/34) had *rpoB* gene mutations at S531L, 2.9% (1/34) at H526D, and 14.7% (5/34) had mutations only at wild type probes. Of 41 INH-resistant strains, 87.8% (36/41) had mutations in the *katG* gene at Ser315Thr1 and 9.8% (4/41) had mutations in the *inhA* gene at C15T. Mutations in *inhA* promoter region were strongly associated with INH monoresistance.

Conclusion: A high rate of drug resistance was commonly observed among failure cases. The most frequent gene mutations associated with the resistance to INH and RIF were observed in the codon 315 of the *katG* gene and codon 531 of the *rpoB* gene, respectively. Further studies on mutations in different geographic regions using DNA sequencing techniques are warranted to improve the kit by including more specific mutation probes in the kit.

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Introduction

Multidrug-resistant tuberculosis (MDR-TB) has become a major public health problem and presents a barrier to TB control [1]. In Ethiopia, MDR-TB is becoming a challenge because of poor adherence to treatment and use of inappropriate treatment regimens [2]. Moreover, culture and drug susceptibility testing (DST) for *Mycobacterium tuberculosis* are not routinely performed. Only a few laboratories in Ethiopia are equipped with facilities to perform DST. In 2010, only 10% of MDR-TB cases were detected [3]. This indicates that a majority of the expected MDR-TB cases in Ethiopia remain undiagnosed and continue to transmit the disease in the community.

The World Health Organization (WHO) has proposed a wide-scale implementation of rapid molecular methods to screen patients at risk of MDR-TB. Rapid tests can provide results within days and thus enable rapid and appropriate treatment, decrease morbidity and mortality, and interrupt transmission [4]. Among these, line probe assay (LPA) has been developed for the rapid detection of *M. tuberculosis* complex and its resistance to rifampicin (RIF) and isoniazid (INH). The assay detects mutations in the *rpoB* gene for RIF resistance, the *katG* gene for high-level INH resistance, and the *inhA* gene for low-level INH resistance from smear-positive or culture-positive sputum sample [5].

Genetic diversities of drug resistant isolates might be attributable to some host factors besides strain evolution in different geographic regions [6]. The principal patient-related factor that is associated with the occurrence of MDR-TB is poor adherence to TB treatment [7]. In particular, those patients that have a previous TB treatment history such as treatment failures, defaulters, or relapse cases are at greater risk of developing MDR-TB. A study in Northwest Ethiopia [8] reported that history of previous TB treatment was significantly associated with gene mutations conferring resistance to INH and RIF.

RIF and INH are the principal first-line drugs used in combination for TB treatment [9]. More than 95% of RIF-resistant *M. tuberculosis* strains harbor a mutation in the 81-bp region of *rpoB*, known as the RIF resistance-determining region [10,11]. INH resistance can occur due to mutations in several genes, such as *katG*, *inhA*, *kasA*, *oxyR*, and *ahpC*. However, 70–80% of INH resistance is associated with mutations in codon 315 of the *katG* gene [12,13]. Studies have shown that >90% of RIF-resistant *M. tuberculosis* strains are also resistant to INH, making RIF resistance a good surrogate marker for MDR-TB [5,9,14].

The nature and frequency of mutations in the *rpoB* gene in RIF-resistant *M. tuberculosis* strains and *katG* and *inhA* genes in INH-resistant *M. tuberculosis* strains vary considerably with geographical locations or ethnic groups [14]. So far in Ethiopia, there was very limited information on the frequency of gene mutations associated with resistance to RIF, INH, and MDR strains in relation to patients' TB history (new, relapse, failure, or return after default). Since mutations that cause RIF and INH resistance in Ethiopia were not well studied, it is difficult to choose the most efficient and cost-effective molecular method to detect such mutations in order to guide therapy. The primary aim of this study was to determine the magnitude and mutation profile of RIF- and INH-resistant *M. tuberculosis* strains with GenoType MTBDRplus in Southwest Ethiopia.

Materials and methods

Study design and setting

This cross-sectional study was carried out at the Mycobacteriology Research Center of Jimma University in Jimma, Jimma, Ethiopia. Jimma University-Mycobacteriology Research Center is the only laboratory equipped with culture and DST in the Southwest part of Ethiopia. It was established as part of interuniversity collaborative research project between Jimma University and a consortium of Flemish Universities from Belgium in November 2010. The laboratory activities are mainly focused on basic research and training in the field of mycobacteriology. It is also involved in the provision of service to patients as part of a national mycobacteriology laboratory network and referral center for DST in Southwest Ethiopia.

Study participants

Pulmonary-TB cases referred from health facilities in Jimma and the surrounding area for DST were enrolled. Individuals were eligible if they were 15 years or older and provided a sputum specimen that was positive for acid-fast bacilli (AFB) on smear microscopy and/or TB was confirmed subsequently by growth of the *M. tuberculosis* in liquid culture (Mycobacteria Growth Indicator Tube [MGIT] 960). At the time of patient presentation, study participants were classified according to the WHO definitions (new, relapse, treatment failure, or default) [15]. The study was approved by the Ethical Review Committee of Jimma University. Written informed consent was obtained from all participants. All confirmed MDR-TB patients were referred to Shenin Gibe Hospital (a nearby hospital, 5 km) for MDR-TB treatment.

Definitions

New cases: patients that have never been treated for TB or have taken anti-TB drugs for <1 month.

Previously treated cases: patients that have received ⩾1 month of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment as follows:

1. Relapse patients have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB.
2. Treatment failure patients are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment.
3. Defaulter (treatment after loss to follow-up) patients have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment.
4. Monoresistance is resistance to one first-line anti-TB drug only (RIF or INH).
5. MDR is resistance to both INH and RIF.