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Multidrug-resistant and heteroresistant *Mycobacterium tuberculosis* and associated gene mutations in Ethiopia

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SUMMARY

Background: The prevalence of multidrug-resistant tuberculosis (TB) among new and retreatment cases in 2011 in Ethiopia was 2.7% and 17.9%, respectively. However, data on heteroresistance and gene mutation profiles of *Mycobacterium tuberculosis* were not documented.
Methods: A cross-sectional study was conducted on 413 TB-positive clinical specimens submitted between 2012 and 2014 to Bahir Dar Regional Laboratory Center for confirmation of multidrug resistance. Resistance determining genes were analyzed using a line probe assay.
Results: Of 413 *M. tuberculosis* isolates, 150 (36.3%) were multidrug-resistant, 19 (4.6%) were resistant only to rifampicin, and 26 (6.3%) were resistant to isoniazid. Of 169 rifampicin-resistant and 176 isoniazid-resistant isolates, only eight (4.7%) showed rifampicin heteroresistance and only two (1.13%) showed isoniazid heteroresistance. Failing of the *rpoB* WT8 gene with corresponding hybridization of *rpoB* MUT3 (S531L substitution) accounted for 85 (50.3%) rifampicin-resistant mutations. Among 176 isoniazid-resistant isolates, 155 (88.1%) strains had the Ser315Thr1 substitution.
Conclusions: The prevalence of multidrug-resistant *M. tuberculosis* was high in the study area. Ser531Leu and Ser315Thr1 substitutions were the highest gene mutations for rifampicin and isoniazid, respectively.

1. Introduction

Multidrug-resistant tuberculosis (MDR-TB) is caused by strains of *Mycobacterium tuberculosis* that are resistant to isoniazid (INH) and rifampicin (RMP).[1,2] Despite the availability of highly efficacious treatment for decades, tuberculosis (TB) remains a major global health problem.[1,3–5] Globally, 3.5% of MDR-TB has been reported in new TB cases and 20.5% in previously treated TB cases.[6,7] In Ethiopia in 2011, the prevalence of MDR-TB among new and retreatment cases was reported to be 2.7% and 17.9%, respectively.[8]

An erratic drug supply, suboptimal physician prescription, poor patient adherence,[9] initial high bacterial population, and monotherapy have been associated with the emergence of resistance.[10] Some patients with TB harbor mixed populations of drug-susceptible and resistant organisms, a phenomenon that is referred to as heteroresistance. Therefore, heteroresistant strains are precursors for full resistance.[11]

The genetic background of *M. tuberculosis* related to INH resistance is complex. However, mutations in several genes, including *katG* (catalase peroxidase coding genes),[12] *ahpC*, *inhA*, *kasA*, and *ndh*, have all been associated with INH resistance.[10,13,14] Between 50% and 95% of INH-resistant strains contain mutations in codon 315 of the *katG* gene.[9] Furthermore, 20% and 35% of INH-resistant strains contain mutations in the *inhA* regulatory region.[13,14] The most common *inhA* mutation occurs in its promoter region (C15T) and this is frequently associated with monoresistance.[13,14] Strains bearing mutations in the coding region of *inhA* show low-level resistance.[10,15]

Mutations in the RNA polymerase β subunit (*rpoB*) gene have been found in about 96% of RMP-resistant *M. tuberculosis*

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isolates.[14–16] Mutations in codons 531 and 526 are the most frequently reported mutations.[14–16]

Information on the current prevalence of MDR-TB, heteroresistance, and drug resistance mutations has not been documented in Amhara National Regional State (ANRS), Ethiopia. This study was conducted to determine the prevalence of MDR-TB, heteroresistance, and gene mutations to RMP and INH among presumptive MDR-TB cases in ANRS, Ethiopia.

2. Materials and methods

2.1. Study design and sampling technique

A cross-sectional study was conducted between May 2012 and February 2014. During the study period, 856 presumptive MDR-TB cases (sputum and extrapulmonary (peritoneal fluid, tissue, lymph node aspirate, and pus specimens)) were referred to the Bahir Dar Regional Health Research Laboratory Center (BRHRLC). However, only 413 (48.2%) of these clinical samples were TB-positive. This study included the 413 *M. tuberculosis* isolates for gene mutation analysis.

2.2. Specimen processing

The clinical samples were processed using the *N*-acetyl-L-cysteine NaOH (NALC-NaOH) method. The processed samples were suspended in 1.0 ml sterile phosphate buffer (pH 7.0) and then 100 μl of resuspended pellet was inoculated onto two Lowenstein–Jensen (LJ) medium slants. Smears for microscopic examination were stained using the Ziehl–Neelsen (ZN) method.

DNA was extracted from samples that were smear- and/or culture-positive using GenoLyse chemical methods. From the extracted DNA, 5 μl was used directly for PCR amplification. Master mix preparation, DNA addition, amplification, hybridization, and interpretation were performed as recommended by the manufacturer (Hain Lifescience GmbH, Nehren, Germany).[13,17] Resistance determining genes were analyzed using a line probe assay (LPA).

2.3. LPA interpretation

Susceptibility to anti-TB drugs was defined as hybridization (presence of a band) to all the wild-type (WT) probes and no hybridization (absence of a band) to the mutant probes. The absence of hybridization of any WT and/or hybridization of any mutant gene indicates resistance to the respective drugs. Hybridization of WT and mutant genes indicates heteroresistance or a mixed infection (Figure 1).

2.4. Statistical analysis

All data were entered, cleared, and analyzed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp. Armonk, NY, USA). Descriptive statistics were used to visualize differences within the data. Binary logistic regression was used to assess possible factors associated with MDR-TB and heteroresistance. Gene mutations were analyzed manually.

2.5. Data quality assurance

DNA extraction positive (H37Rv) and negative controls and master mix controls were used. Fifty isolates were characterized at the national TB laboratory using the BACTEC MGIT (Mycobacteria Growth Indicator Tube) 960 TB system (BD Diagnostics, USA). Lot-to lot quality assurance systems were in place to verify the quality of the commercial kit. All procedures were done using standard operating procedures.

2.6. Ethical considerations

Ethical clearance was obtained from the Amhara Regional Health Bureau Research Ethics Review Committee and official permission was obtained from BRHRLC.

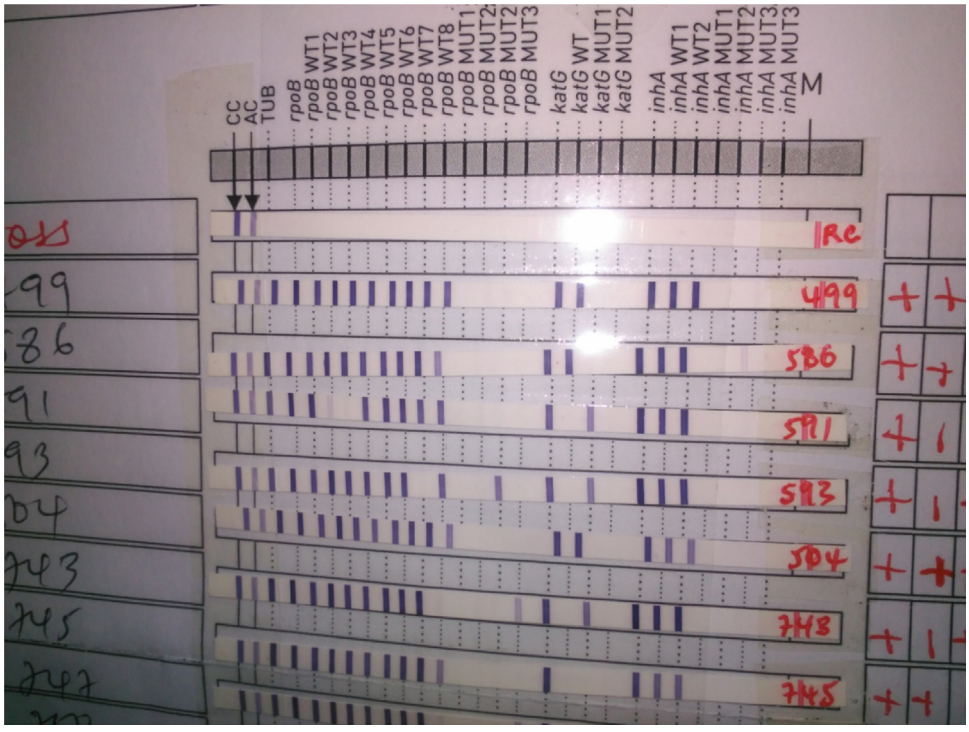

Figure 1. Line probe assay strips showing controls and rifampicin (RMP) and isoniazid (INH) resistance-associated banding patterns; Ethiopia, 2015.