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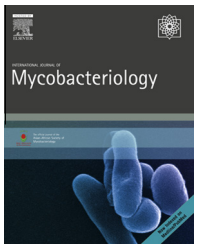

Full Length Article

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.

6. RFF resistance is resistance to RIF detected using LPA, with or without resistance to INH.

Specimen collection and transport

Morning sputum sample was collected from each of the TB cases in 50-mL sterile falcon tubes. All specimens were packed and transported to Jimma University-Mycobacteriology Research Center according to the international standards of WHO recommendation for transport of biological substances and arrived within 3 days of collection for processing within 7 days of its collection.

Sputum smear microscopy

Smears were prepared on the spot of specimen collection or acceptance on clean slides. Standard Ziehl–Neelsen staining procedure was applied [16]. Stained slides were examined for AFB under a 100× oil immersion objective. AFB results were reported for the presence or absence of AFB using the WHO/International Union Against Tuberculosis and Lung Disease scale, with a positive result corresponding to ⩾1 AFB per 100 high-power fields.

Culture and identification

Mycobacterial culture and identification was done in a Biosafety Level-2 laboratory following the standard protocols [17]. All sputum specimens were digested and decontaminated by the standard N-acetyl-L-cysteine and sodium hydroxide method with a final sodium hydroxide concentration of 1%. An equal volume of standard N-acetyl-L-cysteine and sodium hydroxide solution was added to the specimen and incubated for 15 min. After centrifugation, the sediment was resuspended in 1 mL of sterile phosphate buffered saline (pH = 6.8). Finally an aliquot of 0.5-mL sediment was inoculated into a MGIT 960 tube and loaded into a BACTEC MGIT 960 instrument. The laboratory strain, *M. tuberculosis* H_{37}Rv, (American Type Culture Collection 27294), was used as a positive control.

Differentiation of *M. tuberculosis* complex from non-TB mycobacteria (NTM) was done using a SD BIO LINE MPT64 TB Ag test (Standard Diagnostics, Yongin, South Korea). One hundred microliter of sample sediment taken from processed smear positive sputum or 100 μL of mycobacterial growth taken from positive MGIT culture was added into the sample well. The test result was interpreted within 15 min of sample addition.

GenoType MTBDRplus (version 2.0) DST

The GenoType MTBDRplus assay was performed according to the manufacturer's instruction (Hain Lifescience, Nehren, Germany). DNA was extracted from decontaminated smear-positive sample sediment or from MGIT culture positives. Briefly, smear-positive sputum specimens were decontaminated using N-acetyl-L-cysteine-sodium hydroxide [17]. After resuspension, 500-μL decontaminated sample was transferred to a 1.5-mL microcentrifuge tube and centrifuged at 10,000*g* for 15 min. The supernatant was discarded and the pellet was resuspended in 100-μL lysis buffer, incubated for 5 min at 95 °C in a hot air oven. Then 100-μL neutralization buffer was added and centrifuged for 5 min at 10,000*g*. Finally, 5-μL of the DNA supernatant was used for polymerase chain reaction while the remainder was stored at −20 °C. For culture-positive cases, 1 mL of liquid culture was transferred to a microcentrifuge tube and centrifuged for 15 min at 10,000*g*. The supernatant was discarded and the same procedure as in the case of direct sputum proceeded starting from the addition of lysis buffer.

A master mixture for amplification consisted of 35-μL primer nucleotide mixture (provided with kit), 5 μL of 10× polymerase chain reaction buffer with 15 mM $MgCl_2$, 2 μL of 25 mM $MgCl_2$, 0.2 μL (1 U) of HotStarTaq DNA polymerase (Hain Lifescience, Nehren, Germany), 3-μL nuclease free molecular grade water, and 5 μL of DNA supernatant in a final volume of 50 μL. The amplification protocol consisted of 15 min of denaturation at 95 °C, followed by 10 cycles comprising denaturation at 95 °C for 30 s, and 65 °C for 2 min. This was followed by 20 cycles comprising 95 °C for 25 s, 50 °C for 40 s, and 70 °C for 40 s, and a final extension at 70 °C for 8 min. Hybridization was performed with the automatic machine (TwinCubator). After hybridization and washing, strips were removed, fixed on paper, and results were interpreted.

Each strip of Genotype MTBDRplus assay has 27 reaction zones (bands), including six controls (conjugate, amplification, *M. tuberculosis* complex, *rpoB*, *katG*, and *inhA* controls), eight *rpoB* wild-type (WT1–WT8), and four mutant (MUT) probes (*rpoB* MUT D516V, *rpoB* MUT H526Y, *rpoB* MUT H526D, and *rpoB* MUT S531L), one *katG* WT and two MUT probes (*katG* MUT S315T1 and *katG* MUT S315T2), and two *inhA* WT and four MUT probes (*inhA* MUT1 C15T, *inhA* MUT2 A16G, *inhA* MUT3A T8C, and *inhA* MUT3B T8A).

An internal quality control program with positive and negative controls was implemented during the study. An interpretable Genotype MTBDRplus assay was defined as a test strip with all control markers positive, including results of the markers for positive control (H37Rv strain), negative control for DNA extraction, and for mix preparation. If a WT band was missing or if a MUT band was present, this was taken as an indication of a resistant strain.

Statistical analysis

Data were double entered and analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as frequency (percentage). The rate of mutations in *rpoB*, *katG*, and *inhA* genes in the categories of patients (new, relapse, failure, or defaulter) were estimated. Chi-square test was applied to assess factors associated with drug resistance. A p value < .05 was taken as statistically significant.

Results

A total of 122 smear- and/or-culture positive cases from October 2013 to September 2014 were included in this study. *M. tuberculosis* was isolated in 96.7% (118/122) of patients and NTM in four patients. Of 118 *M. tuberculosis* isolates subjected for LPA test, six had invalid results. Patients with NTM and invalid LPA results were excluded, leaving 112 TB patients

for the final analysis. The majority, 56.2% (63/112), of patients were men. The age of the study participants ranged from 15 years to 75 years with a median age of 28.5 (±13.5 standard deviation) years. Based on their TB-treatment history, 36.6% (41/112) of patients were classified as new, 28.6% (32/112) failure, 26.8% (30/112) relapse, and 8% (9/112) defaulter.

Out of 112 *M. tuberculosis* isolates, 60.7% (68/112) were susceptible to both RIF and INH, 2.7% (3/112) were RIF monoresistant, and 8.9% (10/112) were INH monoresistant. Resistance to RIF and/or INH was noted in 39.3% (44/112) of patients. MDR-TB (resistance to both RIF and INH) was found in 27.7% (31/112) of the cases. MDR-TB was most frequently seen among failure cases (50%), followed by defaulters (33.3%), and relapse cases (23.3%; Table 1).

Men accounted for the majority, 71% (22/31), of MDR-TB patients. More than half, 51.6% (16/31), of MDR-TB patients were found in the age range of 15–25 years. Neither sex nor age of the patients was significantly associated with MDR-TB ($p > .05$). Unlike INH-resistant strains ($p = .98$), RIF-resistant strains were most frequently seen in male patients ($p = .043$). Mutations conferring resistance to RIF ($p = .02$), INH ($p = .01$), and MDR-TB ($p = .004$) commonly occurred in treatment failure cases compared with other treatment categories (Table 2).

Mutation patterns in RIF- and INH-resistant strains

Frequency of gene mutations associated with resistance to RIF (*rpoB*) and INH (*katG* and *inhA*) in relation to TB-treatment history is shown in Table 3. Mutations conferring resistance to RIF and INH were detected in 30.4% (34/112) and 36.6% (41/112) of *M. tuberculosis* isolates respectively. Among 34 RIF-resistant isolates, 82.4% (28/34) had a mutation at position S531L and 2.9% (1/34) at position H526D (Table 3). In five of 34 RIF-resistant isolates, only WT probes (4 *rpoB* WT8 and 1 *rpoB* WT7) were missing with no gain in mutant probes (Table 4). These later isolates were depicted as unknown. But in 82.4% (28/34) of RIF-resistant isolates, *rpoB* gene mutations detected at WT probes were also detected at MUT probes (27 *rpoB* WT8/*rpoB* MUT3 and 1 *rpoB* WT7/*rpoB* MUT2). The majority, 83.9% (26/31), of MDR-TB strains and 66.6% (2/3) of RIF-monoresistant strains had a mutation in *rpoB* (codon 531) gene with an amino acid change of Ser531-Leu. The difference of *rpoB* gene mutation in MDR-TB strains compared with RIF-monoresistant strains was not statistically significant ($p = .06$; Table 4).

Resistance to INH is associated with a mutation at two genes; *katG* and *inhA*. Of 41 INH-resistant isolates, 90.2% (37/41) had a mutation in the *katG* gene, while 9.8% (4/41) in

Table 1 – Rifampin (RIF) and Isoniazid (INH) resistance pattern in relation to tuberculosis treatment history (new, failure, relapse, and default; *n* = 112).

Resistance pattern	New (*n* = 41)	Failure (*n* = 32)	Relapse (*n* = 30)	Defaulter (*n* = 9)
Susceptible to RIF & INH	32 (78)	13 (40.6)	19 (63.3)	4 (44.4)
Resistance to RIF & INH[a]	5 (12.2)	16 (50)	7 (23.3)	3 (33.3)
RIF monoresistance	2 (4.9)	0	1 (3.3)	0
INH monoresistance	2 (4.9)	3 (9.4)	3 (10)	2 (22.2)

Note: Data are presented as *n* (%).
a Resistance to rifampin and isoniazid is defined as multidrug resistant tuberculosis.

Table 2 – Patient characteristics and their association with resistance to rifampin (RIF) and isoniazid (INH) based on GenoType MTBDRplus line probe assay (*n* = 112).

Patient characteristics	RIF and INH resistance pattern					
	RIF	*p*	INH	*p*	MDR	*p*
Sex						
Male (*n* = 63)	24 (38)	.043	23 (36.5)	.98	22 (34.9)	.052
Female (*n* = 49)	10 (20.4)		18 (36.7)		9 (18.4)	
Age (y)						
15–25 (*n* = 42)	16 (38)		20 (47.6)		16 (38)	
26–35 (*n* = 34)	10 (29.4)	.24	10 (29.4)	.20	8 (23.5)	.21
36–45 (*n* = 16)	6 (37.5)		7 (43.8)		5 (31.2)	
46–55 (*n* = 12)	1 (8.3)		3 (25)		1 (8.3)	
>55 (*n* = 8)	1 (12.5)		1 (12.5)		1 (12.5)	
TB Tx history						
New (*n* = 41)	7 (17)		7 (17)		5 (12.2)	
Failure (*n* = 32)	16 (50)	.02	19 (59.4)	.01	16 (50)	.004
Relapse (*n* = 30)	8 (26.7)		10 (33.3)		7 (23.3)	
Default (*n* = 9)	3 (33.3)		5 (55.6)		3 (33.3)	

Note: Data are presented as *n* (%). MDR = multidrug resistance; Tx = treatment.

Table 3 – Frequency of gene mutations associated with resistance to rifampicin (*rpoB*) and isoniazid (*katG* or *inhA*) in relation to tuberculosis (TB) treatment history.

Gene	Band	Gene region/mutation		TB treatment history			
			Total (n = 34)	New (n = 7)	Failure (n = 16)	Relapse (n = 8)	Default (n = 3)
rpoB							
	WT1	506–509	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT2	510–513	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT3	513–517	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT4	516–519	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT5	518–522	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT6	521–525	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	WT7	526–529	2 (5.9)	0 (0)	2 (12.5)	0 (0)	0 (0)
	WT8	530–533	31 (91.2)	7 (100)	14 (87.5)	7 (87.5)	3 (100)
	MUT1	D516V	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MUT2A	H526Y	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MUT2B	H526D	1 (2.9)	0 (0)	1 (6.3)	0 (0)	0 (0)
	MUT3	S531L	28 (82.3)	5 (71.4)	13 (81.3)	8 (100)	2 (66.7)
Gene	Band	Gene region/mutation	Total (n = 41)	New (n = 7)	Failure (n = 19)	Relapse (n = 10)	Default (n = 5)
katG							
	WT	315	31 (75.6)	7 (100)	14 (73.7)	8 (80)	2 (40)
	MUT1	S315T1	36 (87.8)	6 (85.7)	17 (89.5)	10 (100)	3 (60)
	MUT2	S315T2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
inhA							
	WT1	−15/−16	4 (9.8)	0 (0)	2 (10.5)	0 (0)	2 (40)
	WT2	−8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MUT1	C15T	4 (9.8)	0 (0)	2 (10.5)	0 (0)	2 (40)
	MUT2	A16G	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MUT3A	T8C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MUT3B	T8A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: Data are presented as n (%). MUT = mutant; WT = wild type.

Table 4 – Mutation pattern of rifampicin (RIF; *rpoB*) and isoniazid (*katG* and *inhA*) resistant *Mycobacterium tuberculosis* strains by GenoType MTBDRplus assay.

Gene	Mutation pattern (wild type/mutant)	Amino acid change	RIF resistant (n = 34)	MDR-TB (n = 31)	RIF-MR (n = 3)	p
rpoB						
	rpoB WT8/*rpoB* MUT3	S531L	27 (79.4)	26 (83.9)	1 (33.3)	.06[a]
	rpoB WT8/ND	Unknown	4 (11.8)	3 (9.7)	1 (33.3)	
	rpoB WT7/*rpoB* MUT2B	H526D	1 (2.9)	1 (3.2)	0 (0)	
	rpoB WT7/ND	Unknown	1 (2.9)	1 (3.2)	0 (0)	
	rpoB MUT3	S531L	1 (2.9)	0 (0)	1 (33.3)	
	Mutation pattern (wild type/mutant)	Amino acid change	INH resistant (n = 41)	MDR-TB (n = 31)	INH-MR (n = 10)	p
katG						
	KatG WT/*katG* MUT1	S315T1	30 (73.2)	24 (77.4)	6 (60)	.002[b]
	KatG WT/ND	Unknown	1 (2.4)	1 (3.2)	0 (0)	
	katG MUT1	S315T1	6 (14.6)	6 (19.4)	0 (0)	
inhA						
	inhA WT1/inhA MUT1	C15T	4 (9.8)	0 (0)	4 (40)	

Note: Data are presented as n (%).
a RIF-monoresistant versus MDR-TB.
b INH-monoresistant versus MDR-TB. INH-MR = isoniazid monoresistant; MUT = mutant; ND = not detected; RIF-MR = rifampicin monoresistant; WT = wild type.

the *inhA* gene (Table 3). A mutation in the *katG* gene at codon Ser315Thr1 was documented most frequently and seen in 87.8% (36/41) of INH-resistant isolates. Six *katG* gene mutations detected at MUT probes (*katG* MUT1) were not present in wild probes but all *inhA* gene mutations detected at WT probes were also present at MUT probes. Only one had

a missing WT (*katG* WT) with no gain in MUT probes (Table 4). There was no combined *katG* and *inhA* gene mutations found among INH-resistant isolates.

All MDR-TB strains and 60% (6/10) of INH-monoresistant strains had mutations at the *KatG* gene. However, 40% (4/10) of INH-monoresistant strains and none of the MDR-TB strains had a mutation at the *inhA* gene. This difference of mutations in MDR-TB strains compared with INH-monoresistant strains was statistically significant ($p = .002$; Table 4). Mutations at the *KatG* gene were significantly associated with MDR-TB compared with *inhA* gene mutations.

Discussion

Drug resistance in *M. tuberculosis* appears to result from the stepwise acquisition of new mutations in the genes for different drug targets [18]. Resistance to drugs is mainly due to treatment that is inadequate, often because of an irregular drug supply, inappropriate regimens, or poor compliance [19,20]. Genetic characterization and identification of mutations that cause resistance will allow the selection of most efficient molecular methods to detect such mutations in order to optimize an effective antibiotic treatment. In the present study, we determined the frequency of gene mutations associated with RIF and INH resistance in *M. tuberculosis* strains among pulmonary TB patients.

Similar to other developing countries such as India, Bangladesh, and South Africa, there is a high rate of MDR-TB in Ethiopia [2,3]. This is proving to be an emerging threat to TB control because very few laboratories in Ethiopia are equipped with DST facilities. The overall MDR-TB rate of 27.7% observed in this study is higher than 11.8% estimated in the WHO 2011 report [3] and 18% documented in a drug resistance survey in Ethiopia [21] but lower than 46% reported in Addis Ababa, Ethiopia by Abate et al. [22]. In our study, the treatment failure category predicted a high rate of drug resistance, with 59.4% of patients in this category exhibiting resistance to INH and 50% resistance to RIF and INH. This is because adding one drug in the failing regimen could change susceptible strains and lead to MDR. The "treatment failure" category could be used to identify patients who may benefit from alternative regimens instead of the current standard retreatment regimen.

The genetic basis of antibiotic resistance in *M. tuberculosis* isolates has been widely studied [8,23,24]. This is the first report of mutation patterns associated with drug resistance in *M. tuberculosis* isolates from Southwest Ethiopia. Mutations conferring resistance to RIF and/or INH were detected in 39.3% of *M. tuberculosis* isolates. Several studies have shown that >95% of RIF-resistant strains harbor a mutation within the 81-bp region of the *rpoB* gene [10,11]. In this study, the most common mutation among RIF-resistant isolates was at position Ser531Leu, seen in 82.4% of the cases. Similarly, previous studies indicated [8,25] this was the most frequently reported mutation in RIF-resistance isolates in Ethiopia. However, in five (14.7%) of our RIF-resistant isolates, only a WT band (found in drug-susceptible strains) was missing, but a corresponding MUT band (found in drug-resistant strains) was not present. It is likely that this banding pattern is the result of mutations associated with drug resistance. However, there is a slight possibility that the pattern represents a silent mutation, one that does not result in an amino acid change or may indicate the presence of less common mutations at the *rpoB* gene that cannot be detected by the current Version 2 of the GenoType MTBDRplus assay.

In GenoType MTBDRplus assay, INH resistance is detected by probes of two genes; *katG* and *inhA*. Results reported from many areas of the world and Ethiopia [8,12,13] have shown that *katG* mutations vary geographically, but 40–95% of INH resistance was due to *katG* gene mutations of which 75–90% of resistant isolates involved base changes at codon 315 of the *katG* gene. In agreement with these results, we found that >85% of INH-resistant strains from Jimma and surrounding areas have a mutation at codon 315 of the *katG* gene. Previous studies have also shown that 8–43% of INH resistance were mainly caused by the mutations in the promoter region of the *inhA* gene [13,26]. In our study, 10% of INH-resistant strains were associated with mutations in the promoter region of the *inhA* gene (mutation in codon C15T). All *inhA* gene mutations were found only in INH-monoresistant strains. However, mutations at the *katG* gene were most frequently associated with *rpoB* gene mutations, making *katG* mutation a better predictor of MDR-TB compared with *inhA* gene mutations.

It is interesting to note that monoresistance to INH is relatively common while monoresistance to RIF is rare. In fact, nearly 90% of RIF-resistant strains are also INH resistant, making RIF resistance a good surrogate marker for MDR-TB [5,9]. In this study, three RIF-resistant isolates were not MDR-TB (RIF monoresistance). This finding is slightly higher than previous studies that reported a very low RIF-monoresistance rate by phenotypic DST in Ethiopia [22,27]. This could be explained by the presence of some unidentified mutations in other genomic regions (like *kasA*, *oxyR*, and *ahpC*) of INH-resistant *M. tuberculosis* isolates that were not targeted by the assay (GenoType MTBDRplus) used in the present study. This emphasizes the importance of collecting more information on the local prevalence of drug resistance (RIF monoresistance) patterns before implementing molecular assays such as GeneXpert MTB/RIF test.

Conclusions

There was high rate of MDR-TB among previously treated patients, particularly in the treatment failure category, in Southwest Ethiopia. The most dominant gene mutations associated with resistance to INH and RIF were observed in codon 315 of the *katG* gene and codon 531 of the *rpoB* gene in Ethiopia. Mutations in the *inhA* promoter region were strongly associated with INH monoresistance. Since there are clear geographical differences in the presence and proportion of resistance-related mutations, it is crucial to study more drug-resistant clinical isolates from different regions of the country to improve the kit by including more specific mutation probes.

Conflicts of interest

None.

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