Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among Mycobacterium tuberculosis isolates from Ethiopia

Belay Tessema1,2,3*, Joerg Beer2, Frank Emmrich3,4, Ulrich Sack3,4 and Arne C Rodloff2

Abstract

Background: The emergence of drug resistance is one of the most important threats to tuberculosis control programs. This study was aimed to analyze the frequency of gene mutations associated with resistance to isoniazid (INH), rifampicin (RMP) and ethambutol (EMB) among Mycobacterium tuberculosis isolates from Northwest Ethiopia, and to assess the performance of the GenoType® MTBDRplus and GenoType® MTBDRsl assays as compared to the BacT/ALERT 3D system.

Methods: Two hundred sixty Mycobacterium tuberculosis isolates from smear positive tuberculosis patients diagnosed between March 2009 and July 2009 were included in this study. Drug susceptibility tests were performed in the Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University Hospital of Leipzig, Germany.

Results: Of 260 isolates, mutations conferring resistance to INH, RMP, or EMB were detected in 35, 15, and 8 isolates, respectively, while multidrug resistance (MDR) was present in 13 of the isolates. Of 35 INH resistant strains, 33 had mutations in the katG gene at Ser315Thr and two strains had mutation in the inhA gene at C15T. Among 15 RMP resistant isolates, 11 had rpoB gene mutation at Ser531Leu, one at His526Asp, and three strains had mutations only at the wild type probes. Of 8 EMB resistant strains, two had mutations in the embB gene at Met306Ile, one at Met306Val, and five strains had mutations only at the wild type probes. The GenoType® MTBDRplus assay had a sensitivity of 92% and specificity of 99% for INH resistance, and 100% sensitivity and specificity to detect RMP resistance and MDR. The GenoType® MTBDRsl assay had a sensitivity of 42% and specificity of 100% for EMB resistance.

Conclusion: The dominance of single gene mutations associated with the resistance to INH and RMP was observed in the codon 315 of the katG gene and codon 531 of the rpoB gene, respectively. The GenoType® MTBDRplus assay is a sensitive and specific tool for diagnosis of resistance to INH, RMP and MDR. However, the GenoType® MTBDRsl assay shows limitations in detecting resistance to EMB.

Keywords: Mycobacterium tuberculosis, Drug resistance, Gene mutation

Background

According to the World Health Organization (WHO) report, the proportion of multidrug resistant tuberculosis (MDR-TB), resistant to at least isoniazid and rifampicin among new and previously treated TB cases globally ranges from 0% to 28.3% and from 0% to 61.6%, respectively [1]. In Ethiopia, the countrywide anti-TB drug resistance survey conducted in 2005 showed that the prevalence of MDR-TB was 1.6% and 11.8% among new and previously treated TB cases, respectively [2]. Moreover, 5825 MDR-TB cases were estimated to have occurred in 2006 in Ethiopia [3]. MDR-TB treatment involves prolonged use of second-line anti-TB drugs that are less effective, less tolerated, more toxic, and more expensive than first-line anti-TB drugs [4]. In most high-burden TB countries, MDR-TB is only
diagnosed after prolonged treatment with first-line TB drugs and clinical recognition that treatment has failed. Treatment of drug-resistant TB with standard first-line drugs, instead of a regimen designed according to the resistance pattern has several potential adverse consequences: patients remain on inadequate treatment longer, increasing the risk of treatment failure or death; selection of drug resistant strains and patients remain infectious, promoting transmission to close contacts [5].

In Ethiopia, the treatment regimens for category I and category II (retreatment regimen) tuberculosis cases are 2 (RMP-INH-EMB-PZA)/4(RMP-INH) and 2 STM (RMP-INH-EMB-PZA)/1(RMP-INH-EMB-PZA)/5(EMB3 (RMP-INH))3, respectively [6]. The standard treatment regimen for MDR-TB is 6(EMB-PZA-KM (AMK)-LFXETO-CS)/12(EMB-PZA-LFX-ETO-CS). For proper treatment and control of tuberculosis, WHO is recommending countries to expand their capacity for culture based drug-susceptibility testing (DST) and consider new, molecular-based assays for diagnosing drug resistance [7,8]. Since M. tuberculosis usually grows slowly, the identification and drug-resistance testing usually require several weeks. The gold-standard of TB diagnosis by culture takes weeks to become positive, and even with the up-to-date automated fluid culture methods it takes an average of 14 days. Another 14 days for additional testing are required to get the information on drug susceptibility [9-11]. Molecular methods of drug resistance testing, based on the identification of mutations in genes associated with drug resistance, like GenoType MTBDRplus assay, offer an effective tool for determining drug resistance because of their high sensitivity, specificity and speed [12].

Molecular methods that have been developed to detect drug resistance include the GenoType MTBDRplus for detection of INH and RMP resistance and the GenoType MTBDRsl for detection of resistance against EMB, flooroquinolones, and aminoglycosides/cyclic peptides (Hain Lifescience, Nehren, Germany). These assays are DNA strip assays that use PCR and hybridization. Mutations in katG gene and inhA gene were related to the high-level and low-level INH resistance, respectively [13]. Nearly all RMP resistant strains contain mutation of the rpoB gene, coding RNA polymerase subunit β and mutation in the embB gene was associated with EMB resistance [14,15].

In Ethiopia, culture and drug susceptibility testing (DST) for M. tuberculosis are not performed routinely in clinical microbiology laboratories. Laboratory diagnosis of TB remains in a stage of acid-fast staining. Currently, five regional laboratories are being rebuilt and equipped to perform culture and drug susceptibility testing using methods including GenoType MTBDRplus assay. The GenoType MTBDRplus and GenoType MTBDRsl assays have been studied in several laboratories of other countries, however, there is a wide variation in circulating M. tuberculosis strains worldwide [16,17], and false negative results may occur due to unique genetic mutations [18-24], affecting the performance of molecular assays for drug susceptibility testing. Therefore, analysis of gene mutations associated with resistance to anti-tuberculosis drugs and assessment of the performance of molecular methods for drug resistance testing in different settings are needed to ensure acceptable performance of the assays. So far, there was no report on the frequency of gene mutations associated with resistance to INH, RMP and EMB and the applicability of these molecular assays for M. tuberculosis isolates from Ethiopia. In this study, we analyzed the frequency of gene mutations associated with resistance to INH, RMP and EMB among M. tuberculosis isolates from Northwest Ethiopia, and assessed the performance of the GenoType MTBDRplus for detection of resistance to INH, RMP and MDR and GenoType MTBDRsl assay for detection of EMB resistance compared to the automated, culture-based, BacT/ALERT 3D system drug susceptibility testing.

Methods

Study design, area and study period

Two hundred sixty M. tuberculosis isolates from smear positive tuberculosis patients diagnosed between March 2009 and July 2009 at Gondar Hospital, Gondar Health Center, Metemma Hospital, Bahir Dar Hospital and Debre Markos Hospital in Northwest Ethiopia were included in this study. Diagnosis of smear-positive tuberculosis was based on the national guideline for microscopic examination of tuberculosis (6): direct smears were prepared from three sputum specimens and stained by Ziehl-Neelsen staining technique for microscopic examination. Drug susceptibility tests using GenoType MTBDRplus, GenoType MTBDRsl and BacT/ALERT 3D system were performed at the mycobacteriology laboratory in the Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University Hospital of Leipzig, Germany. Informed consent was obtained from the study subjects. Institutional ethical clearance was obtained from the research and publication committee of Gondar University, Ethiopia. Details of sputum storage, transportation, isolation and identification of the isolates have described previously [25].

GenoType MTBDRplus and GenoType MTBDRsl drug susceptibility testing

GenoType MTBDRplus assay for detection of INH and RMP resistance, and GenoType MTBDRsl assay for detection of ethambutol resistance were performed
according to the manufacturer’s instructions (Hain Life-science GmbH, Nehren, Germany). Briefly, DNA was extracted from cultures by heating the bacteria in a heating block for 20 minutes at 95°C followed by sonication in ultrasonic water bath for 15 minutes. Amplification was performed using 2.5 μl (1 unit) Taq DNA polymerase (ROCHE, Mannheim, Germany). For the amplification profile the instructions of the manufacturer were followed. Hybridization of the single-stranded, biotin-labeled amplicons to membrane-bound probes on the strip followed by addition of conjugate, and substrate to detect visible band patterns on the strip was performed manually using a shaking water bath, Memmert-SV1422 (Memmert GmbH & CO.KG, Schwabach, Germany) at 45°C. Then strips were allowed to dry and interpreted according to the manufacturer’s recommendation.

To detect high level INH resistance, the GenoType® MTBDRplus has incorporated one wild type (WT-315) and two mutation-type probes specific for mutation Ser315Thr1 and Ser315Thr2 of the katG gene. For detection of low-level INH resistance this assay has two wild-type probes (WT-15/-16 and WT-8) and four mutation-type probes, covering mutations of C15T, A16G, T8C and T8A in the inhA gene. To detect rifampicin resistance, the Genotype MTBDRplus has incorporated eight wild-type probes for the rpoB gene, covering codons in the rpoB gene from 505 to 533, and four other probes specific for mutations Asp516Val, His526Tyr, His526Asp and Ser531Leu. For detection of ethambutol resistance, the GenoType® MTBDRsl employs one wild-type probe (WT-306) and two mutation probes specific for mutations Met306Ile and Met306Val in the embB gene.

**BacT/ALERT 3D system drug susceptibility testing**

Drug susceptibility testing for isoniazid, rifampicin and ethambutol was performed by BacT/ALERT 3D system (BioMerieux, S.A, France) according to the methods published previously [26,27]. The final drug concentration in the test bottles was 1 μg/ml for INH and RMP, and 2 μg/ml for EMB. Two control bottles, one with 1% control (0.5 ml of the 1:100 diluted test organisms suspension) and one original control bottle without drug were used for interpretation of the test results. *M. tuberculosis* isolate was determined to be resistant to an antibiotic when the drug-containing bottle had a time to detection (TTD) that was less than or equal to the TTD of the 1% control.

**Statistical analysis**

All laboratory data were entered, cleared and analyzed using SPSS version 13 statistical package software (SPSS Inc., Chicago, IL). The standard chi-square tests (χ²) were used to assess statistical relationships between predisposing factors and drug-resistant TB. Sensitivity, specificity, positive predictive value and negative predictive value of the molecular methods were analyzed using crosstabulation after arranging the results of the molecular methods in the rows and gold standard, BacT/ALERT 3D system in columns. P values of less than 0.05 were considered statistically significant.

**Results**

Of the 260 patients included in this study, the majority of patients, 59% were males. The median age of the study subjects was 28.0 years (range, 7-75 years). History of previous treatment for tuberculosis was significantly associated with gene mutations conferring resistance to INH (P = 0.001), RMP (P = 0.002) and MDR (P = 0.044). HIV co-infection, gender and age of the study subjects had no significant association with gene mutations conferring resistance to INH, RMP and EMB. A summary of patient demographic characteristics and associated drug susceptibility pattern according to the molecular methods is shown in Table 1.

**Mutations associated with INH, RMP and EMB resistance**

Mutations conferring resistance to isoniazid, rifampicin and ethambutol were detected in 14%, 6% and 3% of the isolates, respectively. Five percent of the isolates showed mutation in both rpoB gene and katG gene or inhA promoter region indicating that they were multidrug resistant. There was no isolate that showed mutations at both katG and inhA genes. Mutations associated with isoniazid resistance were more often encountered as compared to those seen in rifampicin and ethambutol. Of 35 INH resistant strains, 94% had mutation in the katG (codon 315) gene with amino acid change of Ser315Thr1, indicating high level resistance, while 6% of the strains had mutation in the inhA gene, C15T, indicating low level resistance. All katG gene mutations detected at wild type probes were also present at mutant probes, as was the case with the inhA gene mutations (Table 2). Additionally, three strains showing resistance to isoniazid and two strains sensitive to isoniazid by the BacT/ALERT 3D system did not display concordant results by GenoType® MTBDRplus even on repeat assays (Table 3).

The rifampicin resistant isolates displayed different mutations: 73% of the isolates had mutation at position Ser531Leu, one isolate had mutation at His526Asp, while in three of the isolates mutation was detected only at the wild type probes. Of the isolates with mutation that detected only at wild type probes, one isolate had mutation at rpoB WT2 and WT3, one isolate at rpoB WT4 and one isolate at rpoB WT6. According to the kit manufacturer’s recommendation, the three isolates with
mutation that detected only at wild type probes were considered resistant (Table 2).

Mutations associated with ethambutol resistance were less frequent compared to those seen in isoniazid and rifampicin resistance. Of the 8 EMB resistant strains according to the molecular method, two strains had mutations in the \textit{embB} (codon 306) gene with amino acid change of Met306Ile and one strain had mutation in the \textit{embB} gene with amino acid change of Met306Val, whereas five strains had mutation that detected only at the wild type probes (\textit{embB} WT) but not at the mutant probes (Table 2). Moreover, 58\% of the isolates showing resistance to ethambutol by the BacT/ALERT 3D system did not display a concordant result by GenoType\textsuperscript{®} MTBDRsl assay even on repeat assays (Table 3).

| Characteristics | Number of patients | Anti-TB drug resistance |
|-----------------|--------------------|-------------------------|
|                 | INH N (%) | P-value | RMP N (%) | P-value | MDR N (%) | P-value | EMB N (%) | P-value |
| Gender          |           |         |           |         |           |         |           |         |
| Male            | 153       | 19 (12.4)| 0.556     | 9 (5.9) | 0.925     | 7 (4.6) | 0.707     | 5 (3.3) | 0.831   |
| Female          | 107       | 16 (15.0)|           | 6 (5.6) |           | 6 (5.6) |           | 3 (2.8) |         |
| Age (years)     |           |         |           |         |           |         |           |         |
| < 40            | 214       | 29 (13.6)| 0.927     | 11 (5.1)| 0.348     | 9 (4.2)| 0.205     | 7 (3.3)| 0.696   |
| ≥ 40            | 46        | 6 (13.0) |           | 4 (8.7) |           | 4 (8.7)|           | 1 (2.2)|         |
| TB history      |           |         |           |         |           |         |           |         |
| New             | 214       | 22 (10.3)| 0.001     | 8 (3.7)| 0.002     | 8 (3.7)| 0.044     | 6 (2.8)| 0.582   |
| Previously treated | 46   | 13 (28.3)|           | 7 (15.2)|           | 5 (10.9)|           | 2 (4.3)|         |
| HIV status      |           |         |           |         |           |         |           |         |
| Negative        | 194       | 29 (14.9)| 0.228     | 9 (4.6)| 0.180     | 9 (4.6)| 0.647     | 7 (3.6)| 0.395   |
| Positive        | 66        | 6 (9.1)|           | 7 (10.6)|           | 4 (6.1)|           | 1 (1.5)|         |
| Total           | 260       | 35 (13.5)|           | 15 (5.8)|           | 13 (5.0)|           | 8 (3.1)|         |

\(N\) number, \textit{INH} isoniazid, \textit{RMP} rifampicin, \textit{MDR} multidrug resistance, \textit{EMB} ethambutol

In this study had no mutations conferring resistance to fluoroquinolones and aminoglycosides. This might be due to low use/access to these drugs in Northwest Ethiopia.

Performance of GenoType\textsuperscript{®} MTBDRplus and GenoType\textsuperscript{®} MTBDRsl assays

Compared with the automated, culture-based, BacT/ALERT 3D system drug susceptibility testing, the GenoType\textsuperscript{®} MTBDRplus assay had a sensitivity of 92\% and specificity of 99\% for detection of INH resistance, a sensitivity of 100\% and specificity of 100\% for RMP resistance, and a sensitivity of 100\% and specificity of 100\% for multidrug resistance. The GenoType\textsuperscript{®} MTBDRsl assay had a sensitivity of 42\% and specificity of 100\% for detection of EMB resistance (Table 3).

### Table 1 Characteristics of study subjects and their association with resistance to isoniazid, rifampicin and ethambutol based on GenoType\textsuperscript{®} MTBDRplus and GenoType\textsuperscript{®} MTBDRsl assays

| Characteristics | Number of patients | Anti-TB drug resistance |
|-----------------|--------------------|-------------------------|
|                 | INH N (%) | P-value | RMP N (%) | P-value | MDR N (%) | P-value | EMB N (%) | P-value |
| Gender          |           |         |           |         |           |         |           |         |
| Male            | 153       | 19 (12.4)| 0.556     | 9 (5.9) | 0.925     | 7 (4.6) | 0.707     | 5 (3.3) | 0.831   |
| Female          | 107       | 16 (15.0)|           | 6 (5.6) |           | 6 (5.6) |           | 3 (2.8) |         |
| Age (years)     |           |         |           |         |           |         |           |         |
| < 40            | 214       | 29 (13.6)| 0.927     | 11 (5.1)| 0.348     | 9 (4.2)| 0.205     | 7 (3.3)| 0.696   |
| ≥ 40            | 46        | 6 (13.0) |           | 4 (8.7) |           | 4 (8.7)|           | 1 (2.2)|         |
| TB history      |           |         |           |         |           |         |           |         |
| New             | 214       | 22 (10.3)| 0.001     | 8 (3.7)| 0.002     | 8 (3.7)| 0.044     | 6 (2.8)| 0.582   |
| Previously treated | 46   | 13 (28.3)|           | 7 (15.2)|           | 5 (10.9)|           | 2 (4.3)|         |
| HIV status      |           |         |           |         |           |         |           |         |
| Negative        | 194       | 29 (14.9)| 0.228     | 9 (4.6)| 0.180     | 9 (4.6)| 0.647     | 7 (3.6)| 0.395   |
| Positive        | 66        | 6 (9.1)|           | 7 (10.6)|           | 4 (6.1)|           | 1 (1.5)|         |
| Total           | 260       | 35 (13.5)|           | 15 (5.8)|           | 13 (5.0)|           | 8 (3.1)|         |

\(n\) number of isolates, \textit{WT} wild-type, \textit{MUT} mutant, \textit{ND} no mutation detected at mutant probe, \textit{NA} mutant probe is not available
Table 3 Performance of GenoType® MTBDRplus assay for detection of resistance to INH, RMP and MDR and GenoType® MTBDRsl assay for detection of EMB resistance in comparison to BacT/ALERT 3D system

| Molecular methods | DST results | Bact/ALERT 3D DST results | Sensitivity % | Specificity % | PPV % | NPV % |
|-------------------|-------------|---------------------------|---------------|---------------|-------|-------|
|                   |             | Susceptible | Resistant | Susceptible | Resistant |              |             |             |             |       |       |
| INH               | Susceptible | 222         | 3         | 91.7         | 99.1       | 94.3     | 98.7          |
|                   | Resistant   | 2           | 33        |              |            |          |               |
| RMP               | Susceptible | 245         | 0         | 100          | 100        | 100      | 100            |
|                   | Resistant   | 0           | 15        |              |            |          |                |
| INH +RMP (MDR)    | Susceptible | 247         | 0         | 100          | 100        | 100      | 100            |
|                   | Resistant   | 0           | 13        |              |            |          |                |
| EMB               | Susceptible | 241         | 11        | 42           | 100        | 100      | 95.6           |
|                   | Resistant   | 0           | 8         |              |            |          |                |

DST drug susceptibility testing, INH isoniazid, RMP rifampicin, MDR multidrug resistance, EMB ethambutol, PPV positive predictive value, NPV negative predictive value

Discussion

Almost all TB laboratories in Ethiopia have only been equipped with the acid-fast staining and lack resources for culture, identification and drug susceptibility testing of mycobacteria, which present a huge hindrance for tuberculosis control in the country. Therefore, there is an urgent need for laboratories to find a rapid and efficient method for TB diagnosis as a complement to the smear microscopy, and meanwhile to establish MDR-TB diagnostic route for rapid detection of drug-resistant TB. The GenoType® MTBDRplus and GenoType® MTBDRsl assays are rapid and technically simple to perform and do not require sophisticated equipment when compared with the conventional culture-based techniques. These assays have been studied in other countries. However, false negative results reported due to unique genetic mutations associated with resistance to anti-tuberculosis drugs in different countries [18-24]. Therefore, in this study, we investigated the frequency of gene mutations associated with resistance to INH, RMP and EMB and evaluated the performance of these molecular assays for detection of resistance to INH, RMP and EMB on M. tuberculosis isolates from Northwest Ethiopia.

In this study, the GenoType® MTBDRplus assay had a sensitivity of 92% and specificity of 99% for INH resistance, and 100% sensitivity and specificity for RMP resistance and for multidrug resistance. Other reports have shown that the performance of the GenoType® MTBDRplus assay in sensitivity and specificity almost comes up to that of conventional culture-based susceptibility testing: Causse et al. [28] reported a sensitivity of 95% for INH and 100% for RMP, a Meta analysis report by Bawanga et al. [29] showed that GenoType® MTBDRplus assay has a sensitivity and specificity of 96% and 100% for INH and 99% and 99% for RMP, respectively. In the present study, 8% of phenotypically defined isoniazid-resistant strains had no mutations in codon 315 of the katG gene and in the regulatory region of the inhA gene, demonstrating that other mechanisms or mutations in other codons of the katG gene may be responsible for the development of INH resistance in M. tuberculosis strains. Interestingly, all phenotypically defined rifampicin-resistant strains and multidrug-resistant strains had mutations conferring resistance to rifampicin, and both isoniazid and rifampicin resistance (MDR). Suggesting that the set of the DNA probes used in the GenoType® MTBDRplus assay covers most of the mutations prevalent in Northwest Ethiopia. However, previously reported associations between the gene mutations and Beijing strains [30,31] suggest that the assay may be potentially useful in the area with a high prevalence of Beijing family (Eastern Europe, China and South-East Asia).

Previous studies have shown that 40-95% of isoniazid resistance are defined as the high-level drug-resistance due to the katG gene mutations. Of which, 75-90% are recognized as mutations in the 315th codon of the katG gene, which mainly result in Ser315Thr1 and Ser315Thr2 mutation [13,15,32]. In the present study, 94% of INH resistances, close to the high limit of reported range, were attributed to katG mutations which confer high level resistance to INH. Of which, 100% were identified as Ser315Thr1 mutation. Studies have also shown that 8% to 43% of INH resistance are defined as the low-level drug resistance mainly caused by the mutations in the promoter region of inhA gene [33]. In this study, we have observed that the low-level drug-resistance proportion was 6%, close to the low limit of the reported range.

In the previous studies [14,15,34], about 95% of resistance to RMP are associated with the rpoB gene mutations which are found to cluster mainly in the region of codon 507-533. In this study, the distribution of gene
mutation among RMP resistant isolates was 73% at position Ser531Leu and 7% at His526Asp. In 20% of the resistant isolates, mutation was detected only at the wild type probes, which is different from the previously reported gene mutation distribution in China, 37% at Ser531Leu, 3% at His526Asp and in 60% of the isolates, mutation was detected only at the wild type probes [35], reflecting the difference in the distribution of gene mutations associated with RMP resistance in different geographical locations. The high frequency (20%) of RMP resistant isolates with no mutation at the mutant probes, probably indicating the presence of less common mutations at rpoB gene that can not be detected by the current version of the GenoType® MTBDRplus assay.

In this study, the distribution of gene mutation among 8 isolates showing resistance to EMB by GenoType® MTBDRsrl assay was 25% at Met306lle, 13% at Met306-Val and 63% of the strains had mutation only at the wild type probes. Furthermore, 58% of the isolates showing resistance to ethambutol by the BacT/ALERT 3D system did not display a similar result by this molecular assay even on repeat assays. Consequently, in the present study, GenoType® MTBDRsrl assay had sensitivity of 42% and specificity of 100% for ethambutol resistance. Similarly, other previous studies have shown that this assay has low sensitivity, only about 50% for detection of EMB resistance [36-38]. The present study together with previous reports, highlight the fact that the molecular basis of EMB resistance in M. tuberculosis is still insufficiently understood to allow detection of EMB resistance by molecular methods.

Conclusions
In our study, the dominance of single gene mutations associated with the resistance to isoniazid and rifampicin in the codon 315 of the katG gene and codon 531 of the rpoB gene was observed. The GenoType® MTBDRplus assay is a sensitive and specific tool for diagnosis of resistance to INH, RMP and MDR. The short turnaround times and the potential for rapid screening of large numbers of isolates make it suitable as a first-line screening assay for TB drug resistance. Its application and popularization will help better solve the long-standing problem of laboratory diagnosis of drug resistance in Ethiopia. In the majority of phenotypically ethambutol resistant isolates, gene mutation associated with the resistance to ethambutol was not detected by this assay. This indicates that the present version of the GenoType® MTBDRsrl assay shows limitations in detecting resistance to ethambutol. Further studies are required to understand the mechanism of resistance to ethambutol and to evaluate GenoType® MTBDRplus assay for the diagnosis of INH and RMP resistance from direct sputum specimens of tuberculosis patients in Ethiopia.

Acknowledgements
This study was carried out with the financial support obtained from Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany; Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University Hospital of Leipzig, Germany; German Academic Exchange Service (DAAD), Germany and University of Gondar, Ethiopia. We acknowledge the data collectors and the study participants from all study areas in Northwest Ethiopia. We also give our appreciation to Elisabeth Krawczyk for her kind assistance during drug resistance testing.

Author details
1Department of Medical Laboratory Technology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia. 2Institute of Clinical Immunology, University Hospital of Leipzig, Leipzig, Germany. 3Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University Hospital of Leipzig, Leipzig, Germany. 4Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany.

Competing interests
The authors declare that they have no competing interests.

Received: 13 October 2011 Accepted: 10 February 2012 Published: 10 February 2012

References
1. World Health Organization: Multidrug and extensively drug-resistant TB (M/ XDR-TB): 2010 global report on surveillance and response. WHO/HTM/TB/2010.3 Geneva: WHO; 2010.
2. World Health Organization: Anti-tuberculosis drug resistance in the world. Fourth Global Report. WHO/HTM/TB/2008.394 Geneva: WHO; 2008.
3. World Health Organisation: Global tuberculosis control. Surveillance, planning and financing. WHO report WHO/HTM/TB/2008.393 Geneva: WHO; 2008.
4. World Health Organization: Guidelines for the programmatic management of drug-resistant tuberculosis. WHO/HTM/TB/2006.361 Geneva: WHO; 2006.
5. Dorman SE, Chaisson RE: From magic bullets back to the Magic Mountain: the rise of extensively drug-resistant tuberculosis. Nature Med 2007, 13:295-298.
6. Ministry of Health of Ethiopia. Tuberculosis, Leprosy and TB/HIV Prevention and Control Programme Manual Addis Ababa. MOH, 2008.
7. World Health Organisation: Strategic and technical advisory group for tuberculosis (STAG-TB) report on conclusions and recommendations. Seventh meeting Geneva: WHO, 2007.
8. World Health Organization: Policy Statement: Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB) Geneva: WHO, 2008.
9. Ahmad M, Mokaddas E: Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis. Respir Med. 2009, 103:1777-179.
10. Ani AE: Advances in the laboratory diagnosis of Mycobacterium tuberculosis. Ann Afr Med 2008, 7:57-61.
11. Grandjean L, Moore DA: Tuberculosis in the developing world: recent advances in diagnosis with special consideration of extensively drug-resistant tuberculosis. Curr Opin Infect Dis 2008, 21:454-461.
12. Ioannidis P, Papaventzi D, Karabata S, Nikolaidou S, Panagi M, Raftopoulou E, Konstantinidou E, Marioni J, Kanavaki S, Cepheid GeneXpert MTB/RIF assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of...
tuberculosis and smear-negative microscopy results. J Clin Microbiol 2011, 49:3068-3070.

13. Viteche C, Jacobs WR Jr. The mechanism of isoniazid killing: clarity through the scope of genetics. Am J Respir Crit Care Med 2007, 175:35-50.

14. Cole ST. Rifampicin resistance in mycobacteria. Res Microbiol 1996, 147:48-52.

15. Riccardi G, Pasca MR, Buroni S. Mycobacterium tuberculosis: drug resistance and future perspectives. Future Microbiol 2009, 4:597-614.

16. Malik AN, Godfrey-Faussett P. Effects of genetic variability of Mycobacterium tuberculosis strains on the presentation of disease. Lancet Infect Dis 2003, 3:174-183.

17. Nicol WP, Wilkinson RJ. The clinical consequences of strain diversity in Mycobacterium tuberculosis. Trans R Soc Trop Med Hyg 2008, 102:955-965.

18. Barnard M, Albert H, Coetzee G, O’Donnell AJ, Barnard O, Mabaso ME. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med 2008, 177:787-792.

19. Brossier F, Veziris N, Tuftf-Pernot C, Jarlier V, Sougakoff W. Performance of the Genotype MTBDR line probe assay for detection of resistance to rifampin and isoniazid in strains of Mycobacterium tuberculosis with low- and high-level resistance. J Clin Microbiol 2000, 44:3659-3664.

20. Evans J, Stead MC, Nicol MP, Segal H. New rapid genotypic assays to identify drug-resistant Mycobacterium tuberculosis in South Africa. J Antimicrob Chemother 2009, 63:111-116.

21. Hillemann D, Weizenegger M, Kubica T, Richter E, Niemann S. Use of Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2008, 46:3699-3703.

22. Ling D, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J 2008, 32:1165-1174.

23. Makanjani N, Mathia J, Marjamaki M, Viljanen MK, Soini H. Comparison of two commercially available DNA line probe assays for detection of multidrug-resistant Mycobacterium tuberculosis. J Clin Microbiol 2006, 44:350-352.

24. Miotto P, Piana F, Penati V, Caducci F, Migliorini GB, Cirillo DM. Use of Genotype MTBDR assay for molecular detection of rifampicin and isoniazid resistance in Mycobacterium tuberculosis clinical strains isolated in Italy. J Clin Microbiol 2006, 44:2485-2491.

25. Tessaema C, Beer J, Emmrich F, Sack U, Rodloff AC. Rate of recovery of Mycobacterium tuberculosis from frozen acid-fast-bacillus smear-positive sputum samples subjected to long term storage in Northwest Ethiopia. J Clin Microbiol 2011, 49:2557-2561.

26. Beer J, Kuchler R, Rodloff AC. Investigations about the possibility for testing the susceptibility of mycobacteria with the MB/BacT culture system. J Lab Med 1997, 21:390-398.

27. Tortoli E, Mattei R, Savarino A, Bartolini L, Beer J. Comparison of Mycobacterium tuberculosis susceptibility testing performed with BACTEC 460 TB (Becton Dickinson) and MB/BacT (Organon Teknika) systems. Diagnostic Microbio Infec Dis 2000, 38:83-86.

28. Caussé M, Ruz P, Gutierrez JB, Zerolo J, Casal M. Evaluation of new GenoType MTBDRplus for detection of resistance in cultures and direct specimens of Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2008, 12:1456-1460.

29. Bwanga F, Hoffer F, Halle M, Joboba ML. Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. BMC Infect Dis 2009, 9:67.

30. Drobniowski F, Balabanova Y, Nikolayevsky V, Ruddy M, Kuznetsov S, Zakharova S, Melentiev A, Fedorov I. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. JAMA 2005, 293:2726-2731.

31. Lipin MY, Stepanshina VN, Shemyakin YG, Shinnick TM. Association of specific mutations in katG, rpoB, rpsL and rrs genes with spoligotypes of multidrug-resistant Mycobacterium tuberculosis isolates in Russia. Clin Microbiol Infect 2007, 13:620-625.

32. Halton ST, Brimacombe M, Bobadilla del Valle M, Cavatore M, Guerrero MI, Barma-Basil M, Billman-Jacobe H, Lavender C, Fyfe J, Garcia-Garcia L, Leon CI, Bose M, Chaves F, Murray M, Eisenach KD, Sifuentes-Osornio J, Cave MD, Ponce de Leon A, Alland D. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother 2006, 50:2640-2649.

33. Zhang Y, Yew WV. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2009, 13:1320-1330.

34. Telleria A, Imboden P, Marchesi F, Lawrie D, Cole S, Colston MJ, Natter L, Schopfer K, Bodmer T. Detection of rifampin-resistance mutations in Mycobacterium tuberculosis. Lancet 1993, 343:647-650.

35. Zhang L, Ye Y, Dua L, Wang T, Song X, Lu X, Ying B, Wang L. Application of Genotype MTBDRplus in rapid detection the Mycobacterium tuberculosis complex as well as its resistance to isoniazid and rifampin in a high volume laboratory in Southern China. Mol Biol Rep 2011, 38:2185-2192.

36. Huang WL, Chi TL, Wu MH, Jou R. Performance assessment of the GenoType MTBDRsl test and DNA sequencing for detection of second-line and ethambutol drug resistance among patients infected with multidrug-resistant Mycobacterium tuberculosis. J Clin Microbiol 2011, 49:2502-2508.

37. Said HM, Kock MM, Ismail NA, Baba K, Omar SV, Osman AG, Hoosein AA, Ehlers MM. Evaluation of the GenoType MTBDRsl assay for susceptibility testing of second-line anti-tuberculosis drugs. Int J Tuberc Lung Dis 2012, 16:104-109.

38. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by GenoType MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2010, 48:1683-1689.

Pre-publication history
The pre-publication history for this paper can be accessed here: http://www.biomedcentral.com/1471-2334/12/37/prepub

doi:10.1186/1471-2334-12-37

Cite this article as: Tessaema et al: Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among Mycobacterium tuberculosis isolates from Ethiopia. BMC Infectious Diseases 2012 12:37.

Submit your next manuscript to BioMed Central and take full advantage of:

• Convenient online submission
• Thorough peer review
• No space constraints or color figure charges
• Immediate publication on acceptance
• Inclusion in PubMed, CAS, Scopus and Google Scholar
• Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit