Diagnostic Performance of the GenoType MTBDRplus and MTBDRsl Assays to Identify Tuberculosis Drug Resistance in Eastern China

Qiao Liu¹, Guo-Li Li², Cheng Chen¹, Jian-Ming Wang², Leonardo Martinez³, Wei Lu¹, Li-Mei Zhu¹

¹Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, Nanjing, Jiangsu 210009, China
²Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu 211166, China
³Department of Epidemiology and Biostatistics, University of Georgia School of Public Health, Athens 21401, Georgia, USA
⁴Center for Global Health, University of Georgia School of Public Health, Athens 21401, Georgia, USA

Abstract

Background: The WHO recently has recommended the GenoType MTBDRplus version 1.0 and MTBDRsl version 1.0 assays for widespread use in countries endemic with drug-resistant tuberculosis. Despite this, these assays have rarely been evaluated in China, where the burden of drug-resistant tuberculosis is among the highest globally.

Methods: Mycobacterium tuberculosis clinical isolates were obtained between January 2008 and December 2008. Isolates were tested for drug resistance against rifampicin (RFP) and isoniazid (INH) using the GenoType MTBDRplus assay and drug resistance against ethambutol (EMB), ofloxacin (OFX), and kanamycin (KM) using the Genotype MTBDRsl assay. These results were compared with conventional drug-susceptibility testing (DST).

Results: Readable results were obtained from 235 strains by GenoType MTBDRplus assay. Compared to DST, the sensitivity of GenoType MTBDRplus assay to detect RFP, INH, and multidrug resistance was 97.7%, 69.9%, and 69.8%, respectively, whereas the specificity for detecting RFP, INH, and multidrug resistance was 66.7%, 69.2%, and 76.8%, respectively. The sensitivity and specificity of the GenoType MTBDRsl assay were 90.9% and 95.2% for OFX, 77.8% and 99.5% for KM, 63.7% and 86.4% for EMB, respectively. Mutations in codon S531L of the rpoB gene and codon S315T of KatG gene were dominated in multidrug-resistant tuberculosis (MDR-TB) strains.

Conclusions: In combination with DST, application of the GenoType MTBDRplus and MTBDRsl assays may be a useful supplementary tool to allow a rapid and safe diagnosis of multidrug resistance and extensively drug-resistant tuberculosis.

Key words: GenoType MTBDRplus Assay; GenoType MTBDRsl Assay; Multidrug-resistant Tuberculosis; Rapid Diagnosis

Introduction

The emergence of drug-resistant tuberculosis is a major public health concern and threatens global progress toward reaching the World Health Organization’s (WHO) post-2015 new End TB Strategy goal of tuberculosis elimination.[¹] China has the third highest burden of new tuberculosis. Globally, 3.9% of new cases and 21% of previously treated cases have multidrug-resistant tuberculosis (MDR-TB) and more than half of these patients are located in India, China, and the Russian Federation.[¹,²] In a nationwide survey across China in 2007, the prevalence of MDR-TB was 10.2%. Estimates of MDR-TB prevalence were 5.7% and 25.6% among new and previously treated cases, respectively. Approximately 8% of MDR-TB patients had extensively drug-resistant (XDR) tuberculosis.[³]

Although laboratories in many of these countries can perform sputum smear microscopy, a shortage of laboratories capable of performing accurate, rapid culture and drug-susceptibility testing.

Address for correspondence: Dr. Li-Mei Zhu, Department of Chronic Communicable Disease, Center for Disease Control and Prevention of Jiangsu Province, Nanjing, Jiangsu 210009, China
E-Mail: jsjkmck@163.com

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testing (DST) still exists. Due to this, the time to obtain a bacteriological culture-based diagnosis may range from weeks to months for many patients. Furthermore, many cases with low bacillary loads are misdiagnosed, underdiagnosed, or poorly treated. Among the nearly half a million estimated cases of MDR-TB that occurred globally in 2014, about one in four were detected. Comparatively, China detects only 11% of estimated MDR-TB cases.

To enlarge the capacity for the detection of drug resistance, the WHO recommends the use of a line-probe assay, the GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany), which can identify the Mycobacterium tuberculosis (MTB) complex as well as resistance to rifampicin (RFP) and isoniazid (INH) drugs. The assay detects mutations in the rpoB gene for RFP resistance, katG gene for INH resistance, and inhA regulatory region gene for low-level INH resistance. Subsequently, a new DNA strip assay, GenoType MTBDRsl version 1.0 (Hain Lifescience GmbH, Nehren, Germany), was developed to detect resistance to ethambutol (EMB), fluoroquinolones, and injectable aminoglycosides/cyclic peptides allowing diagnosis of XDR-TB among MDR-TB patients.

Several evaluation studies of GenoType MTBDRplus and MTBDRsl assays have been conducted in different countries, including in China where the burden of drug-resistant tuberculosis has reached epidemic levels and programmatic detection is poor. The objective of the present study was to evaluate the diagnostic performance of the GenoType MTBDRplus and MTBDRsl assays in a high-burden Chinese population using a culture-based phenotypic DST as a gold standard.

**Methods**

**Ethics approval and consent to participate**

This study was reviewed and approved by the Ethics Committee of Jiangsu Province Centre for Disease Control and Prevention. The study was conducted in accordance with approved guidelines, and written informed consent was obtained from all eligible TB patients.

**Study population and isolates**

The study design has been described previously. Briefly, MTB isolates were collected from Jiangsu province in 2008. In all, 235 isolates were evaluated, including 192 MDR-TB, 25 RFP monoresistant, four INH monoresistant, and 14 fully susceptible isolates.

An extensive investigation of treatment history of chemotherapy was undertaken by trained field workers and nurses using a structured questionnaire. Other demographic information collected from participants included age, gender, smoking status, drinking status, occupation, and family contact with tuberculosis.

**Isolate identification and drug-susceptibility testing**

Sputum samples were cultured and isolated on Lowenstein-Jensen (LJ) culture media. Culture-positive isolates were used for isolate identification and DST. Identification of MTB was completed using p-nitrobenzoic acid (PNB) method. Growth in LJ medium impregnated containing PNB indicated that the bacilli were not an MTB complex. Species other than MTB were excluded from all final analyses.

LJ medium impregnated one antituberculosis drug was used for DST and the critical drug concentrations were 0.2 μg/ml for INH, 40 μg/ml for RFP, 2 μg/ml for EMB, 30 μg/ml for kanamycin (KM), and 2 μg/ml for ofloxacin (OFX). Growth on the control medium was compared with growth on a drug-containing medium to determine susceptibility. DST results were categorized as resistant or susceptible. For internal quality assurance of DST, a standard H37Rv isolate was included with each new batch of LJ medium.

**Genomic DNA preparation**

Mycobacterial genomic DNA was extracted from mycobacterial colonies growing on LJ medium by resuspending one loop of mycobacterial colonies in 200 μl TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA) and was incubated at 85°C for 30 min to obtain genomic DNA. After centrifugation of the suspension, the supernatant fluid containing DNA was removed and stored at −20°C until further use. Laboratory isolate H37Rv was used as a control for all microbiological and genetic procedures.

**Molecular methods**

GenoType MTBDRplus and GenoType MTBDRsl assays were performed according to the manufacturer’s instructions. Genotypic assays were evaluated blindly by two medical technologists independently. In addition, the presence of wild-type sequence along with the corresponding mutant probe indicated the sample carrying heteroresistance isolate.

**Statistical analysis**

Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with 95% confidence intervals (CI) of the GenoType MTBDRplus and MTBDRsl assay results were calculated. A value of \( P < 0.05 \) was considered statistically significant. SPSS (version 13.0, SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses.

**Results**

A total of 235 patients were included in this study. The majority of patients (168/235, 71.5%) were male. The participants’ median age was 49.6 years (interquartile range, 35.8–60.0 years). Of the 235 participants, 88 (37.4%) were new cases and 147 (62.6%) were previously treated cases. There was a higher rate of MDR-TB among patients with a prior history of TB treatment compared to persons never treated (65.6% vs. 48.8%, \( P = 0.04 \) [Table 1].

When DST was performed on participants, 81.7% (192/235) were MDR-TB, 92.3% (217/235) displayed any RFP resistance, and 10.6% (25/235) demonstrated monoresistant specimens to RFP. Furthermore, 83.4% (196/235) displayed any form of INH resistance, 1.7% (4/235) were INH monoresistant.
50.6% (119/235) were any EMB resistant, and 22.1% (52/235) were EMB monoresistant. Among the 235 clinical isolates with a positive culture for MTB, 97.9% (230/235) displayed results to OFX and KM. 34.8% (80/230) isolates showed any OFX resistance, and 9.79% (23/230) isolates were OFX monoresistant. 7.8% (18/230) were any KM resistant and 2 (0.85) were KM monoresistant. Only 6.25% (12/192) of the MDR isolates were XDR.

**Genetic mutations**

In the GenoType MTBDRplus assay, RFP resistance was detected using probes from the \( \text{rpoB} \) gene. Among 74 RFP monoresistant isolates, 62.2% (46/74) had \( \text{rpoB} \) MUT3, 8.1% (6/74) had \( \text{rpoB} \) MUT1, and 4.1% (3/74) had \( \text{rpoB} \) MUT2A. All RFP monoresistant isolates had \( \text{rpoB} \) WT1 band present, 73 (98.6%) had WT2, WT5, and WT6 bands, 91.9% (68/74) had WT3 and WT4 band, 83.8% (64/74) had WT7 band, and 27.0% (20/74) had WT8 band. 54.9% (79/144) of MDR-TB isolates had \( \text{rpoB} \) MUT3, 11.8% (17/144) had \( \text{rpoB} \) MUT2A, 9.7% (14/144) had \( \text{rpoB} \) MUT2B, and 3.5% (5/144) had \( \text{rpoB} \) MUT1.

In the GenoType MTBDRplus assay, INH resistance was detected using probes of the \( \text{katG} \) and \( \text{inhA} \) genes. The most prevalent mutation of INH monoresistant was MUT1 (1/5) of INH monoresistant isolates and in 18.1% (26/144) of MDR-TB isolates [Table 2].

Among the 235 clinical isolates with a positive culture for MTB, 223 (94.9%) had a completely interpretable MTBDRsl assay. The distributions of genetic mutations of drug-resistant MTB isolates with an interpretable MTBDRsl assay are shown in Table 3. The predominant mutations of the GenoType MTBDRsl assay identified as conferring OFX resistance was MUT1 (44/77, 57.1%) followed by MUT3C (25/77, 32.5%). In addition, a similar proportion of isolates demonstrated a lack of binding to the \( \text{gyrA} \) WT3 (34/77, 44.2%) probe. All KM drug-resistant MTB isolates had an MUT1 mutation (15/15, 100%) and 53.3% (8/15) did not bind to the WT1 probe. EMB resistance was detected in 87 isolates of which the MUT1B gene was the most prevalent (50.6%, 44/87) followed by MUT3C (25/77, 32.5%).

The distribution of gene mutations in the 31 OFX-monoresistant isolates identified by the GenoType MTBDRsl assay is shown in Table 3. The most prevalent mutation of OFX monoresistant was MUT1 (64.5%, 20/31). MUT1 (2/2) was the most prevalent mutation of KM-monoresistant isolates and for EMB-monoresistant isolates was missing WT1 (95.4%, 42/44) followed by MUT1B (56.8%, 25/44). All XDR-TB isolates had \( \text{rrs} \) MUT1 mutation while 5 were missing \( \text{gyrA} \) WT3 mutation and 4, 3, 2, and 2 had the \( \text{gyrA} \) WT2 mutation.

### Table 1: Characteristics in patients with differing drug-susceptibility patterns

| Characteristic | Number of patients | Drug-susceptibility pattern | \( \chi^2 \) | \( P \) |
|---------------|--------------------|-----------------------------|-------------|-----|
|              |                    | MDR (%)                     | Non-MDR (%) |     |
| Age (years)  |                    |                             |             |     |
| ≤40          | 80                 | 71 (37.0)                   | 9 (20.9)    | 5.152 | 0.076 |
| 41–57        | 80                 | 65 (33.9)                   | 15 (34.9)   |     |      |
| ≥58          | 75                 | 56 (29.2)                   | 19 (44.2)   |     |      |
| Gender       |                    |                             |             |     |
| Male         | 168                | 134 (69.8)                  | 34 (79.1)   | 1.484 | 0.223 |
| Female       | 67                 | 58 (30.2)                   | 9 (20.9)    |     |      |
| Treatment history |            |                             |             |     |
| New cases    | 88                 | 66 (34.4)                   | 22 (51.2)   | 4.227 | 0.040 |
| Previously treated cases | 147       | 126 (65.6)                  | 21 (48.8)   |     |      |
| Occupation   |                    |                             |             |     |
| Farmer       | 139                | 111 (57.8)                  | 28 (65.1)   | 0.776 | 0.378 |
| Nonfarmer    | 96                 | 81 (42.2)                   | 15 (34.9)   |     |      |
| Alcohol use  |                    |                             |             |     |
| No           | 116                | 96 (50.0)                   | 20 (46.5)   | 0.577 | 0.750 |
| Occasionally | 79                 | 65 (33.9)                   | 14 (32.6)   |     |      |
| Often        | 40                 | 31 (16.1)                   | 9 (20.9)    |     |      |
| Smoking status |                |                             |             |     |
| No           | 103                | 83 (43.2)                   | 20 (46.5)   | 0.289 | 0.866 |
| Previous smoker |            |                             |             |     |
| Yes          | 96                 | 80 (41.7)                   | 16 (37.2)   |     |      |
| No           | 36                 | 29 (15.1)                   | 7 (16.3)    |     |      |
| Family contact |              |                             |             |     |
| Yes          | 14                 | 13 (6.8)                    | 1 (2.3)     | 0.476* |      |
| No           | 221                | 179 (93.2)                  | 42 (97.7)   |     |      |

MDR: Multidrug resistance. *Fisher’s Exact Test.
MUT3C, gryA MUT1, embB MUT1A, and embB MUT1B mutations, respectively.

Performance of GenoType MTBDRplus and GenoType MTBDRsl assays
Compared with the DST, the GenoType MTBDRplus assay had a sensitivity and specificity of 97.7% and 66.7% for detection of RFP resistance, 69.9% and 69.2% for INH resistance, and 69.8% and 76.8% for MDR-TB resistance, respectively. The GenoType MTBDRsl assay had a sensitivity and specificity of 90.9% and 95.2% for detection of OFX resistance, 77.8% and 99.5% for detection of KM resistance, 63.7% and 86.4% for detection of EMB resistance, and 46.2% and 100.0% for detection of XDR-TB resistance, respectively. The PPV ranged from 82.8% (EMB) to 100.0% (XDR-TB);
the NPV was lowest for INH (31.4%) and highest for XDR-TB (96.3%) [Table 4].

Discussion

In this study, we determined the diagnostic accuracy of the GenoType MTBDRplus and MTBDRsl assays to detect resistance to antituberculosis drugs in a setting with endemic tuberculosis drug resistance. With respect to culture isolates, the sensitivities of the MTBDRplus assay for the detection of RFP resistance were recently reported to be in the range of 95–99%.[17,18] This is in concordance with the high sensitivity of the MTBDRplus assay measured in our study (97.7%). In our study, the specificity for RFP (66.7%) and INH (69.2%) and the sensitivity for INH (69.9%) were much lower than other studies.[19,20] The sensitivity (69.8%) and specificity (76.8%) for the detection of MDR-TB in the present study were also lower than previous reports.[18]

More specifically, 95% of these RFP resistance-causing mutations are located within an 81 bp hotspot region of rpoB spanning codons 507–533, known as the RFP resistant determining region.[21] Mutations in codons 516, 526, and 531 of rpoB are most commonly associated with high-level RFP resistance.[20,22] Our results showed that the S531L mutation in rpoB was most frequent (125/218, 57.3%), followed by mutations in codon 526 (34/218, 15.6%). In 144 (66.1%) isolates resistant to RFP isolates, a missing WT8 band was observed. This correlates well with a recent study;[18] however, the observed distribution varies by geographic location.

Some authors cited the low sensitivity to detect INH resistance as a main limitation of the GenoType MTBDRplus assay.[23,24] Mutations that cause INH resistance are located in several genes and regions. Between 50% and 95% of INH-resistant isolates have been found to contain mutations in codon 315 of the katG gene[25,26] and an additional 10–15% of INH-resistant isolates had mutations in the ahpC-oxyR intergenic region.[26,27] In the study, a mutation at codon 315 of the gene katG was present in 66.4% of INH-resistant isolates.

Although the most common mutations predictive of drug resistance are well known for some antituberculosis drugs, these mutations are sometimes silent and are not always related to the acquisition of resistance. In addition, the exact ratio of resistant to susceptible bacilli that results in phenotypic resistance is unclear. This means that in practice, a molecular assay result can differ from the one obtained by a susceptibility proportion method.[20]

| Molecular methods | Phenotypic DST result |
|-------------------|-----------------------|
|                   | Resistant (n) | Susceptible (n) | Invalid (n) | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) |
| RFP               |              |                |            |                          |                        |                  |                  |
| Resistant         | 212          | 6              | 0          | 97.7 (94.7–99.2)         | 66.7 (41.0–86.7)       | 97.2 (94.1–99.0) | 70.6 (44.0–89.7) |
| Susceptible       | 5            | 12             | 0          |                          |                        |                  |                  |
| INH               |              |                |            |                          |                        |                  |                  |
| Resistant         | 137          | 12             | 0          | 69.9 (63.0–76.2)         | 69.2 (52.4–83.0)       | 91.9 (86.4–95.8) | 31.4 (21.8–42.3) |
| Susceptible       | 59           | 27             | 0          |                          |                        |                  |                  |
| MDR               |              |                |            |                          |                        |                  |                  |
| Resistant         | 134          | 10             | 0          | 69.8 (62.8–76.2)         | 76.8 (61.4–88.2)       | 93.1 (87.6–96.6) | 36.3 (26.4–47.0) |
| Susceptible       | 58           | 33             | 0          |                          |                        |                  |                  |
| OFX               |              |                |            |                          |                        |                  |                  |
| Resistant         | 70           | 7              | 0          | 90.9 (82.2–96.3)         | 95.2 (90.4–98.1)       | 90.9 (82.2–96.3) | 95.2 (90.4–98.1) |
| Susceptible       | 7            | 139            | 0          |                          |                        |                  |                  |
| Invalid           | 3            | 4              | 5          |                          |                        |                  |                  |
| KM                |              |                |            |                          |                        |                  |                  |
| Resistant         | 14           | 1              | 0          | 77.8 (52.4–93.6)         | 99.5 (97.3–100.0)      | 93.3 (68.1–99.8) | 98.1 (95.1–99.5) |
| Susceptible       | 4            | 204            | 0          |                          |                        |                  |                  |
| Invalid           | 0            | 7              | 5          |                          |                        |                  |                  |
| EMB               |              |                |            |                          |                        |                  |                  |
| Resistant         | 72           | 15             | 0          | 63.7 (54.1–72.6)         | 86.4 (78.5–92.2)       | 82.8 (73.2–90.0) | 69.9 (61.4–77.4) |
| Susceptible       | 41           | 95             | 0          |                          |                        |                  |                  |
| Invalid           | 6            | 6              | 0          |                          |                        |                  |                  |
| XDR               |              |                |            |                          |                        |                  |                  |
| Resistant         | 6            | 0              | 0          | 46.2 (19.2–74.9)         | 100 (98.0–100.0)       | 100 (54.1–100.0) | 96.3 (92.5–98.5) |
| Susceptible       | 7            | 181            | 29         |                          |                        |                  |                  |
| Invalid           | 0            | 7              | 5          |                          |                        |                  |                  |

RFP: Rifampicin; INH: Isoniazid; MDR: Multidrug resistant; OFX: Ofloxacin; KM: Kanamycin; EMB: Ethambutol; XDR: Extensively drug resistant; CI: Confidence interval; DST: Drug-susceptibility testing; PPV: Positive predictive value; NPV: Negative predictive value.
A previous study displayed that the sensitivity of GenoType MTBDRplus for detection of MTB increased as the smear grade increased, reflecting an association between assay sensitivity and sputum bacillary burden.[20] Several studies have shown that the sensitivities and specificities of drug resistance detection in culture samples are slightly higher than for those conducted in sputum-positive samples.[18,20] Previous study showed that most invalid results were in sputum specimens with a lower bacillary load (1+) or culture-negative samples. More results were interpretable on sputum samples with higher bacillary load while in samples containing less bacillary load the performance of the assays was poorer.[20] The assays are also not useful in sputum specimens with lower bacillary load and paucibacillary extrapulmonary TB specimens. The sensitivity to detect INH resistance increased from 67.3% to 89.4% when most isolates were highly drug resistant.[19] Similarly, in Cavusoglu et al.[20] sensitivity rose from 72.9% to 87.1% when only highly resistant isolates were tested. The low detection rate of INH resistance by the GenoType MTBDRplus method in the present study might be because this study population comprises a relatively high number of low-level INH resistance or that these isolates harbor resistant mutations at other katG gene regions or in other loci.

Heteroresistance has also been reported as an important factor potentially affecting the accuracy and reliability of DST results by line probe assays and impacting double patterns on GenoType MTBDRplus membranes.[20] We assume that heteroresistance is more likely to occur in high TB burden settings and in cultures isolated from chronic patients because these patients are more likely infected with various populations of mycobacteria.[21] Drug-susceptible isolates contaminated with resistant DNA isolates might also induce false-positive results.[22]

Previous studies have shown that the sensitivity of GenoType MTBDRs/l assay to be between 75.6% and 90.6% for detecting fluoroquinolone resistance, 77–100% for detecting KM resistance, and 57–69.2% for detecting EMB resistance.[33,35] In the study, the GenoType MTBDRs/l assay identified 90.9% of OFX-resistant isolates, 77.8% of KM-resistant isolates, and 63.7% of EMB-resistant isolates. We found that GenoType MTBDRs/l assay had excellent accuracy for detecting phenotypic resistance to OFX, modest accuracy for detecting resistance to KM, but poor accuracy for detecting resistance to EMB, showed similar results to previous study.[20] We also found that GenoType MTBDRs/l was specific for the diagnosis of XDR-TB, although there is room for improvement regarding sensitivity.

We observed that the most prevalent mutation was gyrA MUT1/A90V (44/77, 57.1%) followed by the gyrA MUT3/C/D94G (25/77, 32.5%) mutation from OFX-resistant isolates conflicting with previous studies.[13,34] Furthermore, heteroresistant isolates might result from the coexistence of wild type and mutant alleles of the gyrA gene at the preliminary stage of full-drug resistance.[17] High rates of heteroresistance to fluoroquinolone-resistant isolates were found in the study (40.3%), higher than other studies reporting between 4.2% and 21.9%.[33,34,37]

Specifically, the A1401G mutation in the rrs gene is associated with resistance to KM and AM and in this present study the A1401G mutation appeared in all KM isolates with 99.5% specificity and 93.3% PPV using the GenoType MTBDRs/l assay. The nucleotide changes in the region from positions 1400 to 1500 of the rrs gene indicated that the assay performs well in detecting the presence of these mutations. The sensitivity and specificity of the GenoType MTBDRs/l assay was 77.8% and 99.5% for KM, respectively, similar to a previous study.[33] KM resistance may be caused by a mutation in other genes, such as the eis promoter region.[18]

We noticed that the predominant mutation was embB M306V (50.6%), which presented a close analogy to a Taipei study in which embB-M306V accounted for 59.3% EMB mutations[38] and another study which embB-M306V accounted for 60.0%.[36] This suggests that the significance of mutations in this codon is limited.

A recent meta-analysis by Cheng et al.[39] showed a similar sensitivity and specificity with the MTBDRs/l assay for detecting EMB resistance (55% and 71%). This poor performance of the MTBDRs/l assay is likely caused by the inherent difficulties in phenotypic DST for EMB and by the fact that only mutations at position 306 are screened with this assay. Given the poor performance of the MTBDRs/l assay, this assay can be used neither for detecting nor for ruling out EMB resistance accurately and clinicians should await the results of phenotypic DST before deciding on changes in treatment regimens.

The Genotype MTBDRplus version 1.0 assay prompted a 21.6% increase in the direct detection of INH resistance due to the incorporation of the inhA gene conferring low-level INH resistance.[20,40] Genotype MTBDRplus version 1.0 assay has been limited for the use on smear-positive patient material.[41] GenoType MTBDRs/l version 1.0 assay only targets selected mutations involving gyrA (fluoroquinolone) and rrs (second-line injectable drugs [SLID]) gene loci, mutations encoding resistance to fluoroquinolone, and SLID that occur outside these regions would be missed by the assay.[42] GenoType MTBDRs/l version 2.0 assay is redesigned based on version 1.0 assay and accommodates additional mutations for the molecular detection of resistance to fluoroquinolone involving gyrA and gyrB and SLID resistance covering both rrs and eis genes.[43,44]

In conclusion, rapid diagnosis of MDR and XDR-TB is critically important for clinical and epidemiological reasons. These assays can inform clinicians about MTB resistance patterns of tuberculosis patients within 1 day. However, since discordance still exists between conventional and molecular approaches in resistance testing, we suggest including more target genes, such as the gyrB and eis genes, to improve the sensitivity of this assay and allow for more effective programmatic application. We recommend
that the GenoType assay might serve as an early guide for tuberculosis disease therapy until phenotypic DST results can be administered.

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**Conflicts of interest**

There are no conflicts of interest.

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