Research Article

Evaluation of MTBDRplus and MTBDRsl in Detecting Drug-Resistant Tuberculosis in a Chinese Population

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Background. This study aims to evaluate GenoType MTBDRplus and GenoType MTBDRsl for their ability to detect drug-resistant tuberculosis in a Chinese population. Methods. We collected 112 Mycobacterium tuberculosis strains from Jiangsu province, China. The conventional DST and line probe assay were used to detect drug resistance to rifampicin (RFP), isoniazid (INH), ofloxacin (OFX), kanamycin (Km), and ethambutol (EMB). Results. The sensitivity and specificity were 100% and 50% for RFP and 86.11% and 47.06% for INH, respectively. The most common mutations observed in MTBDRplus were rpoB WT8 omission + MUT3 presence, katG WT omission + MUT1 presence, and inhA WT1 omission + MUT1 presence. For drug resistance to OFX, Km, and EMB, the sensitivity of MTBDRsl was 94.74%, 62.50%, and 58.82%, respectively, while the specificity was 92.59%, 98.81%, and 91.67%, respectively. The most common mutations were gyrA WT3 omission + MUT3C presence, rrs MUT1 presence, embB WT omission + MUT1B presence, and embBWT omission + MUT1A presence. Sequencing analysis found several uncommon mutations. Conclusion. In combination with DST, application of the GenoType MTBDRplus and GenoType MTBDRsl assays might be a useful additional tool to allow for the rapid and safe diagnosis of drug resistance to RFP and OFX.

1. Introduction

Antimicrobial resistance (AMR) is the ability of a microorganism to resist an antimicrobial medicine to which it was originally sensitive [1]. Resistant microorganisms are able to withstand attacks by these medicines, resulting in treatment failure, a prolonged disease process, and increased risks of microorganisms spreading. AMR is detrimental to the successful control of infectious diseases, and it increases the economic burden on individuals and societies. Recently, the emergence of drug-resistant tuberculosis (TB) has drawn greater attention to AMR. The discovery of MDR (at least resistance to isoniazid [INH] and rifampicin [RFP]) and XDR (resistance to INH, RFP, and any fluoroquinolones [FLQs] and to one of amikacin [AM], capreomycin [CAP], and kanamycin [Km]) has posed a difficult challenge for TB control [2, 3].

According to the global tuberculosis report in 2013, 450,000 people developed MDR-TB, and 170,000 die of MDR-TB annually, with the highest levels in eastern Europe and central Asia [4]. Current assays for detecting drug-resistant tuberculosis include conventional drug susceptibility testing (DST), molecular-based DST, sequencing of known genetic loci, line-probe assay, and GeneXpert MTB/RIF. Conventional DST remains a common choice in many countries, especially in source-limited and funding-lacking areas, and it is the only gold standard when evaluating new molecular techniques [5]. However, it is constrained by the slow growth characteristics of M. tuberculosis, which can take two to four weeks on solid culture medium [6].
addition, poor standardization of conventional DST persists, including inoculum size, dispersion of bacillary clumps, subculturing bias, testing environment (temperature and pH), and critical concentrations of certain drugs [5].

Because the genetic mutations in the *M. tuberculosis* genome were proved to be associated with the phenotype of drug resistance [7, 8], molecular techniques have made the rapid detection of MDR or XDR based on these mutations possible. The WHO has recommended two molecular methods: line probe assays (LPAs) [9] and Xpert MTB/RIF [10]. Compared with Xpert MTB/RIF, LPAs are able to detect resistance to other drugs in addition to RFP using hybridization assays. Moreover, LPAs can detect heteroresistance, which is defined as the coexistence of susceptible and resistant bacteria in the same specimen [11, 12]. This type of heteroresistance is difficult to identify using conventional DST [13].

GenoType MTBDRplus and GenoType MTBDRsl are two commercial versions of LPAs designed for the rapid detection of five types of anti-tuberculosis drug resistance, depending on the identification of common mutations in the *rpoB*, *katG*, *inhA*, *rrs*, *gyrA*, and *embB* genes. Relying on specific probes immobilized on nitrocellulose strips, GenoType MTBDRplus can detect drug resistance to RFP and INH, while the second version of GenoType MTBDRsl also enables the detection of mutations involved in resistance to injectable drugs, as well as resistance to FLQs. Evaluation studies of MTBDRplus and MTBDRsl have been conducted in different countries [14], but little research has been conducted in China. Hence, the present study aimed to evaluate the performance of GenoType MTBDRplus and GenoType MTBDRsl compared to conventional DST and to describe the patterns of drug resistance in a Chinese population.

2. Methods

2.1. Sample Collection. Sputum samples from newly diagnosed sputum smear-positive tuberculosis patients were collected in Jiangsu province, China, between May 2008 and December 2008. The samples were cultured and isolated on Lowenstein-Jensen (LJ) medium, followed by DST. Sputum smear microscopy testing and sputum culture were performed in the county-level laboratory, while the DST was performed at the provincial laboratory. The DNA of *M. tuberculosis* was extracted from the isolated culture and was used for the rapid detection of drug resistance by GenoType MTBDRplus and GenoType MTBDRsl. The researchers who performed the LPAs were blinded to the results of conventional DST. DNA sequencing was used to confirm genetic mutations and to explore the inconsistent and controversial results between conventional DST and the LPAs. We used the *M. tuberculosis* H37Rv strain as the control during the microbiological and genetic procedures.

2.2. Conventional DST. After strain isolation, DST was performed using the proportional method on LJ solid medium with critical concentrations of 40 μg/mL for RFP, 0.2 μg/mL for INH, 2.0 μg/mL for OFX, 30.0 μg/mL for Km, and 2 μg/mL for EMB. The growth of colonies on the drug-containing plate was compared to the control plate as a proportion. If the bacterial growth on the medium with the specific drug was ≥1% greater than the control, the strain was declared resistant to the specific drug, and it was defined as sensitive when the growth rate was <1% greater than the control sample.

2.3. Genomic DNA Extraction. One loop of mycobacterial colonies on LJ medium was spun down and suspended in 200 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA) in a 1.5 mL Eppendorf tube. Then, the mixture was incubated at 85°C for 30 minutes before it was centrifuged at 8000 rpm for 5 minutes. The supernatant layer containing DNA was collected and stored at −20°C until used.

2.4. GenoType MTBDRplus and GenoType MTBDRsl Testing. The GenoType MTBDRplus and GenoType MTBDRsl testing was performed according to the instructions supplied by the manufacturer (Hain Lifescience GmbH, Nehren, Germany). If any wild-type band was absent, or any mutation band was present, that particular strain was considered drug resistant. In contrast, if all of the wild-type bands were present and none of the mutation bands were present, that particular strain was considered susceptible.

2.5. PCR and DNA Sequencing. Fragments of *Rv0577* and *16S rRNA* genes were amplified to identify the nontuberculous mycobacterial (NTM) strains, which were inconsistently judged by MTBDRplus, MTBDRsl, and conventional DST. To confirm the genetic mutations, the fragments of eight genes (*rpoB*, *katG*, *inhA*, *gyrA*, *gyrB*, *rrs*, *cis*, and *embB*) were amplified and sequenced with the primers listed in Table 1. PCR was conducted as follows: 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and elongation at 72°C for 1 min, followed by a final extension step for 10 min at 72°C. The PCR products were purified and sequenced.

2.6. Data Analysis. The data were entered using Epidata software, version 3.1 (Denmark), and were analyzed using STATA software, version 10.0 (StataCorp, College Station, TX, USA). Conventional DST was considered the gold standard for calculating the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and agreement rate of the LPAs. The sequencing data were processed and analyzed by ProSeq software, version 3.0, and BioEdit software, version 7.1.9.

2.7. Ethical Consideration. The Institutional Review Board (IRB) of Nanjing Medical University approved the study. Written informed consent was obtained from all participants. The investigation was conducted according to the principles expressed in the Declaration of Helsinki.
Table 1: PCR and DNA sequencing primers.

| Locus      | Primer    | Sequence (5’ to 3’)             | Size (bp) | Position | Product (bp) |
|------------|-----------|---------------------------------|-----------|----------|--------------|
| Rv0577     | Rv0577-f  | ATGCCCAAGAGAAGCGAATACAGGCAA     | 27        | 67166    | 67192        |
| Rv0577     | Rv0577-r  | CAATTGCTCGGGTGGCTGGCTCAA        | 24        | 67191    | 671928       |
| 16s rRNA   | 16SrRNA-f | ACGGTTGGAATACAGGCGACTCGGC      | 25        | 142650   | 674         |
| 16s rRNA   | 16SrRNA-r | TCTCGGAGTACAGGCGACTCGGC        | 28        | 147392   | 165         |
| rpoB       | rpoB-f    | CTTTGACGACGGTTGCGGTGGAAGCA     | 20        | 760829   | 760848       |
| rpoB       | rpoB-r    | ATCTCGTCGCTAACCACGCGC          | 20        | 761371   | 761352       |
| inhA       | inhA-f    | TGGCCGAAAGGATGGTCGTATG         | 23        | 214886   | 214905       |
| inhA       | inhA-r    | ATGAGGAATGTCGGTCGGCA           | 20        | 2153340  | 2153521      |
| katG       | katG-f    | ACGGACGCTGCAAACAGCGGC          | 20        | 2154886  | 2154905      |
| katG       | katG-r    | GCCAATCGTACGGCAATTTC           | 20        | 2155340  | 2155321      |
| gyrA       | gyrA-f    | CCCCTGGTCTCGATTGCAAAC          | 20        | 7273     | 7292         |
| gyrA       | gyrA-r    | CTTCGGTTAATCTCATGCC            | 20        | 7695     | 7676         |
| embB       | embB-f    | CGTGACGACGCCGCTGGTATG          | 22        | 4247345  | 4247366      |
| embB       | embB-r    | TGAATGCGGGTAAACGCGC            | 20        | 4247834  | 4247815      |
| rrs         | rrs-r     | GCTGACGCTGCAAACAGCGGC          | 19        | 1473518  | 1473500      |
| rrs         | rrs-f     | GCTGACGCTGCAAACAGCGGC          | 20        | 1473003  | 1473022      |
| eis         | eis-f     | GCCGTAGCTACGGCGAAAGTGG          | 18        | 271491   | 2714928      |
| eis         | eis-f     | GCCGTAGCTACGGCGAAAGTGG          | 22        | 271547   | 2715456      |
| gyrB       | gyrB-f    | AAGCACCAGTTGCGCAACAC           | 20        | 6353     | 6372         |
| gyrB       | gyrB-r    | CTGCCACGTTGAGTTGTCA            | 20        | 6961     | 6942         |

3. Results

3.1. Strain Identification. We isolated 112 specimens circulating in Jiangsu province. Five strains were confirmed as NTM by both conventional DST and LPAs. For eight suspected NTM strains with inconsistent results, PCR was performed to amplify specific fragments of 16s rRNA and Rv0577 genes. They were confirmed as NTM based on agarose gel electrophoresis and were excluded from subsequent analysis (Table 2).

3.2. Conventional DST. The most common drug-resistant patterns were RFP-R + INH-R + OFX-S + Km-S + EMB-S (24.24%) and RFP-R + INH-R + EMB-R + OFX-S + Km-S (14.14%). The proportions of drug-resistant strains for RFP, INH, OFX, Km, and EMB were 91.92%, 82.83%, 41.30%, 8.70%, and 51.52%, respectively. According to the conventional DST, there were five strains resistant to and one strain susceptible to all five drugs (Table 3).

3.3. Performance of GenoType MTBDRplus and GenoType MTBDRsl. The sensitivity, specificity, PLR, NLR, and Kappa values for RFP, INH, OFX, Km, and EMB using LPAs are shown in Table 4. Predominant patterns of mutation are listed in Table 5. Patterns of drug resistance detected by GenoType MTBDRplus and GenoType MTBDRsl in detail are displayed in Supplementary Table 1 (Supplementary Material available online at http://dx.doi.org/10.1155/2016/2064765).

For GenoType MTBDRplus, 95.96% (95/99) and 78.65% (70/89) had consistent phenotypic and genotypic results for RFP and INH, respectively. GenoType MTBDRplus yielded four false-positive results in RFP-susceptible strains. They showed omission of different rpoB wild-type bands (WT3 + WT4, WT6, WT7, and WT8). No false negative results for RFP were observed. Dual mutation (presence of rpoBMUT2A + MUT3) was observed in one strain. Three strains showed heteroresistance for RFP, with the appearance of both wild and mutation bands. GenoType MTBDRplus did not perform well for INH. Nine false-positive strains showed inhAMUT1 mutations, and 10 false negative strains did not show any omission of katGWT or inhAWT. No strain had inhAMUT2, inhAMUT3A, or inhAMUT3B mutations in our study. Notably, three strains (omission of both the katG control band and wild-type band) and seven strains (inhA control band was very weak) were identified as INH-invalid. Only one strain showed a mixed band pattern (inhAMUT1 presence + WT omission) while showing katG_MUT1. No dual mutation was observed in the katG or inhA bands.

GenoType MTBDRsl correctly recognized 36 cases of OFX resistance in 38 strains, five cases of Km resistance in eight strains, and 30 cases of EMB resistance in 51 strains. Heteroresistance was common for OFX, while 15 (37.5%, 15/40) strains were obvious and six (15%, 6/40) were suspected. Thirteen of the strains had dual mutations. Regarding Km, rrsMUT1 was observed in all of the Km-resistant strains and rrsMUT2 was not observed in any strain. Three (50%) strains heteroresistant for Km presented with
Table 2: NTM detected by conventional DST and GenoType MTBDRplus and GenoType MTBDRsl.

| Strain number | Conventional DST | MTBDRplus | MTBDRsl |
|---------------|------------------|-----------|---------|
|               | Strain           | RFP      | INH     | OFX     | Km      | EMB | Strain           | RFP      | INH     | Strain   | OFX     | Km      | EMB |
| 966           | MTB              | R        | R       | R       | S       | R    | NTM              |          |         |          |         |         |     |
| 1246          | NTM              | —        | —       | —       | —       | —    | NTM              | MTB      | R       | MTB      | S       | S       | R   |
| 1378          | NTM              | —        | —       | —       | —       | —    | MTB              | R         | R       | MTB      | S       | S       | S   |
| 1491          | NTM              | —        | —       | —       | —       | —    | MTB              | R         | R       | MTB      | S       | S       | R   |
| 2052          | NTM              | —        | —       | —       | —       | —    | MTB              | S         | S       | MTB      | S       | S       | S   |
| 1538          | MTB              | R        | S       | S       | S       | R    | NTM              |          |         |          |         |         |     |
| 1581          | MTB              | S        | R       | R       | R       | NA   | NTM              |          |         |          |         |         |     |
| 1782          | MTB              | R        | R       | NA      | NA      | R    | NTM              |          |         |          |         |         |     |
| 1545          | NTM              | —        | —       | —       | —       | —    | NTM              |          |         |          |         |         |     |
| 1897          | NTM              | —        | —       | —       | —       | —    | NTM              |          |         |          |         |         |     |
| 1901          | NTM              | —        | —       | —       | —       | —    | NTM              |          |         |          |         |         |     |
| 1902          | NTM              | —        | —       | —       | —       | —    | NTM              |          |         |          |         |         |     |
| 1939          | NTM              | —        | —       | —       | —       | —    | NTM              |          |         |          |         |         |     |

R: resistant; S: sensitive; NA: not available.

Table 3: Drug resistance patterns detected by conventional DST in 99 strains.

| Number (%) of strains | RFP | INH | OFX | KAN | EMB |
|-----------------------|-----|-----|-----|-----|-----|
| 6 (6.06)              | R   | R   | NA  | NA  | R   |
| 1 (1.01)              | R   | R   | NA  | NA  | S   |
| 5 (5.05)              | R   | R   | R   | R   | R   |
| 1 (1.01)              | R   | R   | R   | R   | S   |
| 16 (16.16)            | R   | R   | R   | S   | R   |
| 11 (11.11)            | R   | R   | R   | S   | S   |
| 1 (1.01)              | R   | R   | S   | R   | R   |
| 1 (1.01)              | R   | R   | S   | R   | S   |
| 14 (14.14)            | R   | R   | S   | S   | R   |
| 24 (24.24)            | R   | R   | S   | S   | S   |
| 1 (1.01)              | R   | S   | R   | S   | R   |
| 2 (2.02)              | R   | S   | R   | S   | S   |
| 3 (3.03)              | R   | S   | S   | S   | R   |
| 5 (5.05)              | R   | S   | S   | S   | S   |
| 1 (1.01)              | S   | R   | S   | S   | R   |
| 1 (1.01)              | S   | R   | S   | S   | S   |
| 1 (1.01)              | S   | R   | S   | R   | S   |
| 1 (1.01)              | S   | S   | R   | S   | S   |
| 3 (3.03)              | S   | S   | S   | S   | R   |
| 1 (1.01)              | S   | S   | S   | S   | S   |

R: resistant; S: sensitive; NA: not available.

3.4. Sequencing. Subsequent sequencing was performed in strains meeting the following criteria: (1) omission of both wild-type bands and mutation bands, indicating uncertain mutations; (2) mutation bands, wild-type bands, or gene locus control bands that were weak; (3) inconsistent results between phenotypes and genotypic assays; and (4) unavailable conventional DST results for OFX and Km. Supplementary Table 1 reveals the sequencing results. For the rpoB gene, codon 531 (47/95) was the most common mutation locus, followed by codon 526 (28/95) and codon 516 (11/95). Four false-positive strains identified by GenoType MTBDRplus were confirmed by sequencing to have mutations at codons 533, 526, 522, and 516 which were consistent with the target mutation regions of probes. Interestingly, two strains with the absence of the WT1 band for mutations were expected at 505–509. However, they were both observed by sequencing to have the mutation at codon 572. One RFP-resistant strain with the omission of both WT3 and WT8 was found to have an uncommon mutation at codon 515 (ATC→ACC). Sequencing of the katG and inhA (promoter region) genes confirmed mutations in all strains with the omission of both the wild and mutation bands and strains with weak bands. Of the three strains with omission of both the katG wild and mutation bands, in addition to codon katG 315, one was also found to have a mutation at codon katG317 and the other was at inhA-15 as determined by sequencing. Of four strains with omission of both the inhA wild-type band and mutation band, three strains were found to have a mutation at codon katG315 and one strain was found to have a mutation at inhA-34, rather than the target mutation of inhA-8. Notably, only one strain with omission of the katG locus control band was confirmed to have no mutation in the katG and inhA promoter region, while the other two strains failed in sequencing because of the quality of the samples. Of strains with weak inhA locus control bands, two strains were found to have mutations at inhA-26. Almost all of the strains

the rrsMUT1 band together with the rrsWT band. MUT1B was commonly observed in EMB-resistant strains. Two heteroresistant strains for EMB were detected by MTBDRsl, showing the presence of the bands of embBMUT1A + WT and embBMUT1B + WT.
Table 4: Performance of GenoType MTBDRplus and GenoType MTBDRsl according to the conventional DST.

| Conventional DST (n) | GenoType MTBDRplus | GenoType MTBDRsl |
|----------------------|--------------------|------------------|
|                      | R      | S      | INV | Se (%) | Sp (%) | PLR | NLR | Agr (%) | Kappa | P  |
| RFP                  |        |        |     |        |        |     |     |         |       |     |
| R (91)               | 91     | 0      | 0   | 100    | 50     | 2   | 0   | 95.96   | 0.65  | <0.001 |
| S (8)                | 4      | 4      | 0   |        |        |     |     |         |       |     |
| Total (99)           | 95     | 4      | 0   | 100    | 50     | 2   | 0   | 95.96   | 0.65  | <0.001 |
| INH                  |        |        |     |        |        |     |     |         |       |     |
| R (82)               | 62     | 10     | 10  |        |        |     |     |         |       |     |
| S (17)               | 9      | 8      | 0   |        |        |     |     |         |       |     |
| Total (99)           | 71     | 18     | 10  | 86.11  | 47.06  | 1.63| 0.30| 78.65   | 0.32  | 0.001 |
| OFX                  |        |        |     |        |        |     |     |         |       |     |
| R (38)               | 36     | 2      | 0   |        |        |     |     |         |       |     |
| S (54)               | 4      | 50     | 0   |        |        |     |     |         |       |     |
| NA (7)               | 3      | 4      | 0   | 94.74  | 92.59  | 12.79| 0.06| 93.48   | 0.87  | <0.001 |
| Total (99)           | 43     | 56     | 0   |         |        |     |     |         |       |     |
| Km                   |        |        |     |        |        |     |     |         |       |     |
| R (8)                | 5      | 3      | 0   |        |        |     |     |         |       |     |
| S (84)               | 1      | 83     | 0   |        |        |     |     |         |       |     |
| NA (7)               | 2      | 5      | 0   | 62.50  | 98.81  | 52.50| 0.38| 95.65   | 0.69  | <0.001 |
| Total (99)           | 8      | 86     | 0   |         |        |     |     |         |       |     |
| EMB                  |        |        |     |        |        |     |     |         |       |     |
| R (51)               | 30     | 21     | 0   |        |        |     |     |         |       |     |
| S (48)               | 4      | 44     | 0   |        |        |     |     |         |       |     |
| Total (99)           | 34     | 65     | 0   | 58.82  | 91.67  | 7.06| 0.45| 74.75   | 0.50  | <0.001 |

R: resistant; S: sensitive; NA: not available; Se: sensitivity; Sp: specificity; INV: invalid; PLR: positive likelihood ratio; NLR: negative likelihood ratio; Agr: agreement.

* Seven strains with very weak inhA locus control bands and 3 strains without katG control bands were identified as invalid results.

* Significant test for Kappa.

Table 5: Predominant mutation patterns for RFP, INH, OFX, Km, and EMB.

| Drug | Predominant mutation patterns | Number of strains |
|------|------------------------------|-------------------|
| RFP  | rpoBWT8 omission + MUT3 appearance | 40                |
| INH  | katGWT omission + MUT1 appearance | 54                |
|      | inhA WT1 omission + MUT1 appearance | 12                |
| OFX  | gyrA WT3 omission + MUT3C appearance | 7                 |
| Km   | rrs MUT1 appearance | 8                 |
| EMB  | embB WT omission + MUT1B appearance | 15                |

with inconsistent INH resistance results were confirmed by sequencing to have concordant results with MTBDRplus except for one strain, which was found to be consistent with the conventional DST and to have a mutation at codon katG299. Sequencing confirmed mutations in strains with omission of both the wild and mutation bands or strains with weak bands in gyrA, rrs, and embB. One OFX-resistant strain with omission of both the gyrA WT2 and MUT bands was found to have mutations at codons gyrA90 and gyrA91, which were in the target mutation region (codons 89–93).

4. Discussion

GenoType MTBDRplus and GenoType MTBDRsl have already been in use in several countries, but they remain in the research stage in China, and information about their...
performance is of significance for future applications. In this study, we analyzed the drug resistance of RFP, INH, OFX, Km, and EMB by comparing LPA with phenotypic conventional DST in 99 *M. tuberculosis* strains from Jiangsu Province, China. Subsequently, we sequenced strains with unclear results, unclear mutations, or inconsistent results.

In this study, the specificity for RFP and INH and the sensitivity for INH were much lower than studies conducted in Spain, Italy, South Africa, and Germany [15–19], while the results for OFX, Km, and EMB were similar to those of previous reports [20–27]. GenoType MTBDRplus and GenoType MTBDRsl showed high sensitivity for RFP and OFX, so it could be used in areas with high prevalence of drug resistance to detect potentially drug-resistant patients. Moreover, its high specificity for OFX could exclude OFX-susceptible patients in screening. However, the low sensitivity and specificity for Km and EMB have restricted the application of previous reports [20–27]. GenoType MTBDRplus and GenoType MTBDRsl could be used for early diagnosis and timely therapeutic instruction, while conventional DST can be used for confirmation, which requires several weeks.

The sensitivity for RFP resistance in this study (100%) was similar to that described in Spain (100%) [15] and Italy (100%) [16] but slightly higher than that reported in South Africa (98.95%) [18] and Germany (96.77%) [19]. Nevertheless, the specificity for RFP was much lower than that in the aforementioned studies, ranging from 95.45% to 100%. Four strains with false-positive results were confirmed by sequencing to have mutations in the *rpoB* gene. Two factors might have contributed to these inconsistent results. First, the sample size of RFP-susceptible strains was small, and only eight RFP-susceptible strains were recruited for this study. In other words, the proportion of RFP-resistant strains was much higher than that in previous studies [15–19], thus incurring sample selection bias. Second, conventional DST, as the gold standard, was not always perfect. Conventional DST for RFP was not absolutely as accurate and reliable as we expected because its performance was not as straightforward in the rounds of proficiency testing among the supranational TB reference laboratories (SRL) [28]. It showed highly inconsistent results between these top laboratories in detecting strains with specific mutations, that is, the “disputed” mutations [29]. The MICs of strains with these “disputed” mutations could be less than the conventional critical concentrations [30], leading to a “susceptible” result. The specificity of molecular detection assay might be underestimated because of the limitations of conventional DST [31]. In this study, we observed that the mutation frequency of *rpoB*533I in RFP-resistant strains detected by GenoType MTBDRplus was 42.11%, which was lower than that in studies conducted in Colombia (64%) and Spain (72.2%) [16, 32]. Mutations confirmed by sequencing were almost all located in the target mutation region of GenoType MTBDRplus except for the mutation of *rpoB*537T in two strains with omission of WT1, for which the target mutation region was codons 505–509. There were many mutations in the *rpoB* gene detected by sequencing, but their roles in RFP resistance require more studies to confirm.

The main reasons for the low sensitivity and specificity in detecting INH resistance might be similar to those for RFP, which include sample selection bias and the accuracy of conventional DST. In addition, the mechanism of INH resistance has not been entirely clear, and it might have contributed to the low sensitivity for INH. The proportion of INH-resistant strains in our study was much higher than that in previous studies (26.8%–68%) [15–19]. According to the Kim summary, at the concentration of 0.2 µg/mL for INH, the 1% critically resistant proportion could likely distinguish between susceptible and resistant strains, showing a discrimination power of 77.1% [33]. Moreover, even in different laboratories using the same methods, the most reasonable criteria for resistance could be different [33]. Hazbon et al. found that approximately 10–15% of low-level INH-resistant strains did not have mutations in *katG* or *inhA* [34]. Heym et al. also found mutations in the promoter region of ahpC in INH-resistant strains [35]. All of these findings supported that INH resistance might be due to a new mechanism. The limited numbers of probes in GenoType MTBDRplus restricted its detection of all mutation loci, which might also have decreased its sensitivity. Among three strains with omission of the *katG* control band and WT band, sequencing did not discover any mutations in one strain and failed in the other two strains. The sequencing result was consistent with DST but discrepant with the manufacturer’s instructions, which classified this situation as resistance to INH. We should be more cautious about similar situations and repeat the experiments to confirm the results.

The high sensitivity, specificity, and Kappa value of GenoType MTBDRsl for OFX indicated the high consistency between this rapid detection assay and conventional DST. The common mutations identified by GenoType MTBDRsl or sequencing in OFX-resistant strains were gyrAA91V and D94G. Differing from previous studies in which the D94G mutation was much more common than A91V, the frequency of these two mutations was close to each other in our study [20, 26, 36, 37]. Heteroresistance of OFX detected by GenoType MTBDRsl in our study was higher than previous reports [20, 26, 36, 37]. It is notable that six strains with weak mutation bands which were suspected to have heteroresistance were confirmed to have no heteroresistance by sequencing. Moreover, some of the 15 heteroresistant strains were confirmed to have no heteroresistance by sequencing. These sequenced strains were observed to have mutations at codons 94 and 90 except for one strain, which had a mutation at codon 95, which was considered not to take part in fluoroquinolone resistance [8]. We suspected that mutations at codons 94 and 90 may easily lead to a detection result of heteroresistance by GenoType MTBDRsl. Though we found mutations at gyrB51I and gyrB422 in two OFX-susceptible strains, we could not confirm that the mutations were associated with OFX resistance because the mutation locus in *gyrB* gene in OFX-resistant strains varied greatly in previous studies [38–40].

The Km-resistant strains judged by GenoType MTBDRsl were all confirmed to have mutations of *rrs*A140G, but one Km-susceptible strain was also found to have an *rrs*A140I mutation. It was reported that the A140G mutation in the
The molecular basis of EMB resistance in GenoType MTBDRsl was confirmed to have a mutation at eis-10. The low detection ability of EMB resistance indicated that the molecular basis of EMB resistance in GenoType MTBDRsl was insufficient, although embB306 was common in the EMB-resistant strains [43, 44]. In addition to M306I and M306V, some rare mutations—Y319C, G407A, and A410P—were also observed in this study. There might also exist some mutations in the embA or embB gene related to EMB resistance, rather than mutations in eis [45].

In conclusion, GenoType MTBDRplus and GenoType MTBDRsl could be applied for the rapid detection of drug resistance to RFP and OFX. However, the role of GenoType MTBDRplus for INH resistance detection was not confirmed because the current results were different from previous reports. It cannot be widely applied until further validation in China. In addition, because the mechanism of Km and EMB resistance was not completely identified, GenoType MTBDRsl for detecting resistance to Km and EMB is not currently suitable for clinical applications. The correlation between uncommon mutations identified in this study and drug resistance must be confirmed in the future.

Disclosure

The funders play no role in the study design, data collection and analysis, decision to publish, or in preparation of the paper.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

Wei Lu and Yan Feng contributed equally to this work.

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