Original Article
Comparison of genotype MTBDRplus results between TB patients with different levels of drug resistance: a retrospective study in China

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Abstract: Background and aim: To evaluate and compare the performance of GenoType MTBDRplus for the detection of isoniazid (INH) and rifampicin (RIF) resistance at two different levels of drug-resistant tuberculosis (TB) in China, a retrospective study was conducted. Methods: Between May 2012 and September 2016, 253 INH- or RIF-resistant TB patients were enrolled in the study. Phenotypic drug susceptibility test (DST) was performed using the absolute concentration method. The MTBDRplus assay was done on culture specimens. Comparisons of the positivity were made using Fisher’s exact test. Results: Positivity of MTBDRplus for detecting resistance in low-level INH-resistant TB was 84.0% (78.1%, 88.6%) and according to resistance to high-level INH, the groups were divided as follows: 1) resistant subgroup (+), the positivity was 63.6% (43.0%, 80.3%); 2) sensitive subgroup (-), the positivity was 86.8% (80.8%, 91.1%). For RIF, positivity of MTBDRplus in low-level RIF-resistant TB was 91.4% (86.6%, 94.7%). According to resistance to high-level INH (+ and -), positivity was 97.2% (93.0%, 98.9%) and 73.3% (59.0%, 84.0%), respectively. The statistical analysis indicated significant differences in positivity between subgroups with different levels of drug-resistant TB isolates (INH or RIF-resistant, all P<0.05). Conclusions: Although MTBDRplus assay accurately predicts drug resistance in most TB isolates in China, conventional DST remains necessary to confirm MDR-TB. It was demonstrated that MTBDRplus assay has significant differences in positivity between low- and high-level drug-resistant groups. The exact mechanism behind the phenomenon is still unclear, and warrants further study, since high-dose drugs may have a role in MDR-TB therapy.

Keywords: GenoType MTBDRplus assay, isoniazid, rifampicin, discordance

Introduction

Despite the availability of effective chemotherapy, tuberculosis (TB) remains one of the world’s deadliest diseases. In 2012, TB killed 1.3 million people [1]. Drug resistance develops as a result of inadequate treatment creating a selection pressure on spontaneously occurring mutants and thus complicates management of TB. Multidrug-resistant tuberculosis (MDR-TB) represents one of the most important threats in the control of TB worldwide. MDR-TB is defined as resistance to two of the most potent first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF) with or without resistance to other drugs [2]. According to a WHO estimate, there were approximately 300,000 new cases of MDR-TB and around 190,000 fatalities from TB worldwide only in 2014 [3].

Solid and liquid culture methods for drug susceptibility test (DST) of Mycobacterium tuberculosis (M.TB) are time consuming requiring weeks to months in providing the results [4, 5]. Genotypic (molecular) methods for DSTs that target MDR-TB make it possible for the patients to be detected earlier, thus improving outcomes. GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany) which is used for the rapid detection of M.TB complex and resistance to INH and/or RIF was endorsed by WHO [6]. The molecular line probe assay detects mutations associated with the rpoB gene for RIF resistance, katG genes and inhA regulatory region gene for INH resistance [7].
Three previously published meta-analyses found that MTBDRplus assay had good accuracy for rapid detection of drug resistance to INH and/or RIF of \textit{M. TB} [8-10].

However, although greater than 95\% of RIF resistance is associated with mutations in an 81 base pair section of the \textit{rpoB} gene, INH resistance appears more complex and has been associated with multiple genes [11], like \textit{katG}, \textit{inhA}, \textit{oxyR-aphC}, \textit{kasA}, and \textit{ndh} [12-14]. In addition, the two mutations, \textit{katG}315 and \textit{inhA}-15, combined with ten of the most commonly occurring mutations in the \textit{inhA} promoter and the \textit{ahpC-oxyR} intergenic region, explain 84\% of global phenotypic INH resistance [11]. Therefore, the accuracy of MTBDRplus is associated with prevalence of antibiotic resistance genes and its mutant locus. Mutation loci on these genes (such \textit{rpoB}, \textit{katG}) are associated with a wide range of INH or RIF minimum inhibitory concentrations [15-17]. Cessation of INH and RIF are generally recommended in the confirmation of MDR-TB. However, high doses might have a role [18, 19]. This implied that: 1) it is necessary to perform DST at different levels; 2) these mutations may have effect on the choice of anti-MDR-TB therapy; 3) The composition of mutations related with antibiotic resistance should be different between TB isolates with different levels of drug resistance.

In this retrospective study, we aimed to evaluate and directly compare the performance of MTBDRplus for the detection of INH and RIF resistance at two different levels of drug-resistant TB in China.

**Materials and methods**

This study was approved by the Human Research Ethics Committees of Shandong Provincial Chest Hospital (SPCH). Because of the retrospective nature, written consent was waived.

**Subjects**

Between May, 2012 and September, 2016, 253 INH- or RIF-resistant TB patients were enrolled in the study. Culture and phenotypic DST were performed on all cases. Cases were divided according to whether high-level resistance to INH (or RIF) was present. Their clinico-pathological characteristics were reviewed and analyzed.

**Methods**

The absolute concentration method (INH: 1 and 10 μg/mL, RIF: 50 and 250 μg/mL) on Löwenstein-Jensen medium was used to screen \textit{M. TB} isolates [20]. The MTBDRplus assay was done on culture specimens. There were three steps: i) DNA extraction from processed sputum specimen, ii) amplification of target region by PCR, and iii) Hybridization of PCR product to the specific oligo-nucleotide probes, immobilized on the strip. All three steps were carried out simultaneously.
Table 1. Positivity of GenoType MTBDRplus assay in patients with different levels resistance to isoniazid or rifampicine

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Number</th>
<th>Positivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (High-level resistance)</td>
<td>+ 22</td>
<td>63.6% (43.0%, 80.3%)</td>
</tr>
<tr>
<td></td>
<td>- 166</td>
<td>86.8% (80.8%, 91.1%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>188 84.0% (78.1%, 88.6%)</td>
</tr>
<tr>
<td>Rifampicin (High-level resistance)</td>
<td>+ 142</td>
<td>97.2% (93.0%, 98.9%)</td>
</tr>
<tr>
<td></td>
<td>- 45</td>
<td>73.3% (59.0%, 84.0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>187 91.4% (86.6%, 94.7%)</td>
</tr>
</tbody>
</table>

Drug resistance was expressed as the absence of wild-type band, presence of mutation band or both.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 16.0. Continuous data are summarized as mean with standard deviation, all calculations were estimated at a 95% confidence interval (95% CI). Binary data are presented as percentages. Comparisons of the percentages were made using a two-tailed Fisher’s exact test. P<0.05 was taken as statistically significant.

Results

From May, 2012 to September, 2016, the MTBDRplus assay was performed on 1633 samples. In total, 253 low-level INH- or RIF-resistant TB patients were enrolled in the study. The average age was 40.1 ± 16.9 years (range 5 to 61 years), with 70.4% (178/253) male. All (223/223) were HIV-negative. The flow chart used to evaluate the performance of MTBDRplus is shown in Figure 1.

As shown in Table 1, positivity of MTBDRplus for detecting antibiotic resistance in low-level INH-resistant TB was 84.0% (78.1%, 88.6%); According to resistance to high-level INH, the group was divided into the: 1) resistant subgroup (+), the positivity was 63.6% (43.0%, 80.3%); 2) sensitive subgroup (-), the positivity was 86.8% (80.8%, 91.1%).

For RIF, the positivity of MTBDRplus for detecting antibiotic resistance in low-level RIF-resistant TB was 91.4% (86.6%, 94.7%); According to resistance to high-level INH, the group was divided into the: 1) resistant subgroup (+), the positivity was 97.2% (93.0%, 98.9%); 2) sensitive subgroup (-), the positivity was 73.3% (59.0%, 84.0%).

Fisher’s exact test was used to compare the positivity of MTBDRplus assay for detecting INH or RIF resistance. The results (Table 2) indicated:

1) significant difference between subgroups with different types (+ and -) of high-level resistance to INH (or RIF) (all P<0.05); 2) significant difference between groups with high-level resistance to INH and RIF (P<0.05).

Discussion

While the number of cases of TB has been steadily declining over the past decade, the prevalence of drug-resistant disease threatens to reverse these declines [3]. Recognition of drug resistance and the timely initiation of effective therapy are essential for treating and preventing transmission of MDR-TB. Lots of studies have evaluated the diagnostic performance of the MTBDRplus assay. However, there are little data comparing the performance of MTBDRplus for the detection of INH and RIF resistance in TB isolates with two different levels of drug resistance. Our study demonstrates that MTBDRplus assay for detection of INH and RIF resistance has significantly higher sensitivity in low-level drug-resistant group than that in high-level resistant group. Moreover, the sensitivity of MTBDRplus assay for detection of INH resistance was lower than that of RIF resistance.

In this study, the total sensitivity for INH and RIF resistance was 91.4% (86.6%, 94.7%) and 84.0% (78.1%, 88.6%), respectively. The sensitivities were lower than that was reported in a meta-analysis (INH: 91% (88%, 94%); RIF: 96% (95%, 97%)) conducted in 2016. This may be partially attributed to limitations of the molecular methods for detection of first line drug resistance.
Brossier F et al., the assay can detect 89% of the INH-resistant strains with a high level of resistance, but only 17% of the strains are characterized by a low level of INH resistance. The results are inconsistent with findings of our study, although this may be contributed to the different concentrations of INH used in the DSTs (0.1, 0.2 and ≥1 vs. 1 and 10 μg/mL).

Although the molecular basis of these discordant results between different-levels resistant TB has not been fully elucidated, we can speculate as to the mechanisms behind test discordance. First, for example, alterations in multiple genes [11], like $kat_G$, $inh_A$, oxyR-aphC, $kas_A$, and ndh [12-14], are associated with INH resistance. As is known, the MTBDRplus assay for the detection of INH resistance is designed to detect only one mutation in $kat_G$ and three in $inh_A$. The limited numbers of probes in MTBDRplus restricted its detection of all mutation loci, which might also have decreased its sensitivity. Moreover, the types of mutations and the types of resistant genes are associated with different-levels resistance [24]. So other mutations may play role in the discordant results. An alternative explanation for the discordant results may be the presence of mixed populations of bacteria, consisting of both susceptible and resistant strains, and hetero-resistance [25, 26].

Molecular methods for detecting resistance in $M. TB$ have some limitations. These assays have significantly increased MDR-TB detection. However, no test has yet replaced phenotypic DST as the gold standard for MDR-TB diagnosis. The MTBDRplus assay is designed to detect the more frequent mutations related to INH and RIF resistance, not to detect the whole mutations. Therefore, this would decrease the sensitivity in detection of drug resistance, especially of INH resistance. Although common mutations confer drug resistance are well known for some drugs, in some cases the mutations are silent and are not always related to the resistance acquisition. Unfortunately, we cannot reveal exact mechanisms behind the observed discordance. Future studies will conduct research to detail the full mechanism.

In conclusion, although the MTBDRplus assay accurately predicts drug resistance in most of TB isolates in China, conventional DST remains necessary to confirm MDR-TB. It was demonstrated that MTBDRplus assay for detection of INH and RIF resistance has significantly higher sensitivity in low-level resistant group than that in high-level group. This implied that there is difference in the MTBDRplus results between TB isolates with low- and high-level drug resistance. The exact mechanism behind the phenomenon is still unclear, and warrants further study, since high-dose drugs may have a role in MDR-TB therapy [18, 19].

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Disclosure of conflict of interest

None.

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Table 2. Comparison of GenoType MTBDRplus assay results between patients with different levels resistance to isoniazid or rifampicine

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Isoniazid (High-level resistance)</th>
<th>Rifampicine (High-level resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td>P&lt;0.05</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

In this table, the resistance to isoniazid and rifampicine is compared between patients with high-level resistance and those with low-level resistance. The table shows the statistical significance of the differences in the assay results between the two groups.
Genotype MTBDRplus in TB with different drug resistances

References


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