Original Article
Diagnostic performance of GenoType MTBDRplus on culture specimens in smear-negative retreatment tuberculosis patients

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Abstract: Background: The diagnostic value of GenoType MTBDRplus assay hasn’t been validated in smear-negative treated tuberculosis (TB) patients. Therefore, a retrospective study was conducted to evaluate it. Methods: Between Jun, 2013 and Sep, 2016, 35 retreatment TB patients were enrolled in the study. The phenotypic drug susceptibility test (DST) was evaluated using indirect proportion method with L-J medium. The GenoType MTBDR plus assay was done on culture specimens according to the manufacturer’s instructions. The sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDRplus assay in detection of isoniazid (INH)- and rifampicin (RIF)-resistance were calculated using the phenotypic DST assay as the gold standard. Results: The average age was 28.9 ± 11.3 years (range 5 to 61 years), 57.1% (21/35) were male. For detecting INH-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDRplus assay were 94.1% (73.0%, 99.0%), 55.6% (33.7%, 75.4%), 2.12 (1.25, 3.60) and 0.106 (0.02, 0.74), respectively; For detecting RIF-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio were 100% (85.1%, 100.0%), 91.7% (64.6%, 98.5%), 12.00 (1.84, 78.37) and 0, respectively; For detecting MDR-TB, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio were 93.3% (70.2%, 98.8%), 65.0% (43.3%, 81.9%), 2.67 (1.45, 4.92) and 0.10 (0.02, 0.70), respectively. Conclusion: The GenoType MTBDRplus assay has high sensitivity for detection of INH- and RIF-resistance in retreatment TB patients. However, the specificity is moderate, this should be taken into account when interpreting the test results.

Keywords: Retreatment, tuberculosis, sensitivity and specificity, isoniazid, rifampicin

Introduction
Tuberculosis (TB) is one of the most serious infectious diseases, with an annual incidence of 9 million new cases, killing more than 1.5 million people annually [1]. The emergence of multi-drug resistant TB (MDR-TB) is further complicating the situation. According to a WHO estimate, in 2014, there were approximately 300,000 new cases of MDR-TB and around 190,000 fatalities from TB worldwide [1]. MDR-TB, defined as resistance to two of the most potent first-line anti-TB drugs, rifampicin (RIF) and isoniazid (INH), has become a major barrier to achieving successful control of TB, as therapy is costly, complicated, with less effective.

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. In addition, contamination rates with conventional culture and DST are high. GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany) which is used for the rapid detection of M.TB complex and resistance to INH and RIF was endorsed by WHO [2]. 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 [3]. In a meta-analysis, GenoType MTBDRplus showed excellent pooled sensitivity and specificity for detection of resis-
GenoType MTBDR <\em>plus</\em> in smear (-) retreatment TB patients

The average age was 28.9 ± 11.3 years (range 5 to 61 years), 57.1% (21/35) were male. All were HIV-negative. 59.4% (19/32) were TB-PCR positive. 10 patients had smoking habit (19.0 ± 26.5 pack-years) and were all males. The time difference to INH (91%, 99%), RIF (96%, 98%), and MDR-TB (91%, 99%) [4]. GenoType MTBDR <\em>plus</\em> assay demonstrated excellent performance and offers great promise in improving MDR-TB care and prevention.

In retreatment TB cases, having ≤ 2 treatment courses and not completing retreatment were associated with mortality [5]; A high proportion of MDR-TB exists among retreatment TB cases, especially the relapse and treatment failure cases [6]; Retreatment TB patients, high-risk MDR-TB population, had poor utilization of access to bacteriologic-based TB diagnosis [7]. Therefore, it is necessary to develop cheap, safe and maneuverable DST guiding anti-TB therapy for these high-risk MDR-TB patients. Such as Xpert MTB/RIF, GenoType MTBDR <\em>plus</\em> assay. Meanwhile, in China, mixed infections were observed more frequently among TB patients undergoing retreatment than among new cases (P<0.05) [8]. This may contribute to disagreement between GenoType MTBDR <\em>plus</\em> assay and phenotypic DST results. Considering these problems, the diagnostic performance of GenoType MTBDR <\em>plus</\em> assay deserves an accurate and well-designed evaluation in retreatment TB patients.

The goal of this study was to investigate the diagnostic performance of GenoType MTBDR <\em>plus</\em> assay on culture specimens in comparison to the routine diagnostic standard in smear-negative retreatment TB patients. Therefore, this retrospective study was performed to compare the assay results with phenotypic DST at a Chinese provincial laboratory.

**Materials and methods**

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

**Table 1.** Diagnostic performance of GenoType MTBDR <\em>plus</\em> assay in retreatment tuberculosis patients

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
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<tbody>
<tr>
<td>Isoniazid</td>
<td>94.1% (73.0%, 99.0%)</td>
<td>55.6% (33.7%, 75.4%)</td>
<td>2.12 (1.25, 3.60)</td>
<td>0.106 (0.02, 0.74)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>100% (85.1%, 100.0%)</td>
<td>91.7% (64.6%, 98.5%)</td>
<td>12.00 (1.84, 78.37)</td>
<td>0</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>93.3% (70.2%, 98.8%)</td>
<td>65.0% (43.3%, 81.9%)</td>
<td>2.67 (1.45, 4.92)</td>
<td>0.10 (0.02, 0.70)</td>
</tr>
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</table>

95% CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; MDR-TB, multidrug-resistant tuberculosis.
between initial treatment and retreatment was 3.1 ± 5.3 years (range 1 month to 21 years). Seven patients have contact history with a TB patient in the family. 17 patients were INH-resistant, 22 were RIF-resistant, 9 were mono-resistant TB (INH or RIF), 15 were MDR-TB. The subjects included 21 isolated pulmonary TB and 14 pulmonary + extra-pulmonary TB (1 urinary tuberculosis, 2 tuberculous meningitis, 3 tuberculous lymphadenitis and 10 tuberculous pleurisy).

As shown in Table 1, for detecting INH-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDRplus assay were 94.1% (73.0%, 99.0%), 55.6% (33.7%, 75.4%), 2.12 (1.25, 3.60) and 0.106 (0.02, 0.74), respectively; For detecting RIF-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDRplus assay were 100% (85.1%, 100.0%), 91.7% (64.6%, 98.5%), 12.00 (1.84, 78.37) and 0, respectively; For detecting MDR-TB, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDRplus assay were 93.3% (70.2%, 98.8%), 65.0% (43.3%, 81.9%), 2.67 (1.45, 4.92) and 0.10 (0.02, 0.70), respectively.

Discussion

The results of the present study have shown that the GenoType MTBDRplus assay has high sensitivity for the rapid detection of INH- and RIF-resistant M.TB in retreatment TB patients. However, the specificity is moderate; this should be taken into account when interpreting the test results. To our best knowledge, this is the first study from China to investigate diagnostic performance of GenoType MTBDRplus assay in retreatment TB. Although the technique which detects MDR mutations at onset or during therapy would enable rapid identification of MDR and facilitate the modification of regimens for retreatment TB patients, the moderate accuracy also should be taken into account.

Currently, the GenoType MTBDRplus assay has been proven to be suitable for application both with culture isolates and directly with smear-positive specimens. Chen C et al. evaluated the performance of the GenoType MTBDRplus assay in smear-positive TB, it was shown that the sensitivities and specificities for GenoType MTBDRplus in detecting INH- and RIF-resistance were respectively 76.47%, 95.44%, 85.94% and 93.13% [11]. Yadav RN et al. reported the use of the assay on culture specimens, the sensitivity and specificity were 98% and 99% respectively for detection of RIF-resistance; 92% and 99% respectively for detection of INH-resistance; 97% and 100% respectively for detection of MDR-TB [12]. It looks like that culture materials may be more accurate than smear-positive specimens for detecting INH- and RIF-resistance using GenoType MTBDRplus assay. This may be associated with that the sensitivity of MTBDRplus assay is directly related to the specimen’s bacillary load (spumum smear status) [13].

Usually, GenoType MTBDRplus assay had very high specificity (up to 100%). But in the study, there were a moderated specificity in detection of drug resistance. If the absence of wild-type band, presence of mutation band or both were detected by the assay, the culture would be resistant to the matched drug only if TB DNA was extracted from single colonies in routine practices. However, in a Chinese study, mixed infections were observed frequently among TB patients undergoing retreatment than among new cases (P<0.05) [8, 14]. Therefore, mixed infection may contribution to the moderate specificity of GenoType MTBDRplus assay in detection of INH- and RIF-resistance among retreatment TB patients. Meanwhile, in our routine work, the TB DNA for GenoType MTBDRplus assay was extracted from the whole culture, but only one isolate was preceded for pheno-typic DST.

Although smear-negative specimens have been approved for GenoType MTBDRplus assay, its sensitivity remains to be a problem, because the specimen’s bacillary load is associated with the performance [13]. Cultivated samples maybe a better choice compared to these specimens were collected from patients directly. For TB detection, Lin et al. evaluated the diagnostic value of the combination of MGIT 960 system and real time-PCR, the results showed that the combination is useful for the early detection of M.TB [15]. It implied that the combination of GenoType MTBDRplus assay and MGIT 960 system would improve diagnostic value of GenoType MTBDRplus assay, especially saving a lot of time.
This study had several limitations. Firstly, the weakness of the study was its retrospective nature, so the results should be treated with caution. Secondly, the sample size was small. Although thousands of TB patients were examined by the GenoType MTBDR\textit{plus} assay, there were relatively few patients met the eligibility criteria. Thirdly, further work (such as sequencing, MIRU-VNTR genotyping) would be helpful to improve the use of GenoType MTBDR\textit{plus} assay in retreatment TB patients [16, 17]. Unfortunately, these M.TB isolates from clinical specimens weren’t collected and stored routinely. Lastly, it requires further analysis to determine whether the mixed infection affect the specificity of GenoType MTBDR\textit{plus} assay in retreatment TB cases.

**Conclusion**

The GenoType MTBDR\textit{plus} assay has high sensitivity for the rapid detection of INH- and RIF-resistant M.TB in retreatment TB patients. However, the specificity is moderate; this should be taken into account when interpreting the test results. Meanwhile, further research on GenoType MTBDR\textit{plus} assay is needed. Such as the effect of mixed infection, the diagnostic value of combination with MGIT 960 System.

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**Disclosure of conflict of interest**

None.

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**References**


