Evaluation of GenoType MTBDRplus assay for rapid detection of isoniazid- and rifampicin-resistance in Mycobacterium tuberculosis isolates from diabetes mellitus patients

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Background: To evaluate the performance of GenoType MTBDRplus assay for rapid detection of isoniazid (INH)- and rifampicin (RIF)-resistance in Mycobacterium tuberculosis (M. TB) isolates from diabetes mellitus (DM) patients, a retrospective study was conducted.

Methods: Between May, 2012 and Sep, 2016, 227 tuberculosis (TB) patients with DM were enrolled in the study. Culture and phenotypic drug susceptibility test were performed in all cases. The GenoType MTBDRplus assay were done on sputum or culture specimens and carried out according to manufacturer's instructions. The sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio for detecting INH- and RIF-resistance by GenoType MTBDRplus assay were calculated using the phenotypic drug susceptibility test as the gold standard.

Results: The total sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio of GenoType MTBDRplus assay: (I) for RIF-resistance detection, were 96.7% (83.3%, 99.4%), 97.5% (94.2%, 98.9%), 38.1 (16.0, 90.7), 0.034 (0.005, 0.235) and 1,113.6 (125.6, 9,873.5), respectively; (II) for INH-resistance detection, were 82.1% (64.4%, 92.1%), 91.9% (87.3%, 95.0%), 10.2 (6.2, 16.8), 0.194 (0.088, 0.43) and 52.3 (17.5, 156.2), respectively; (III) for multiple drug resistance (MDR) detection, were 92.9% (68.5%, 98.7%), 96.2% (92.8%, 98.0%), 24.7 (12.3, 49.5), 0.074 (0.011, 0.491) and 333.1 (38.7, 2,868.7), respectively.

Conclusions: GenoType MTBDRplus assay is highly sensitive and specific for rapid diagnosis of MDR-TB in DM patients. The application of this assay could be considered for some settings. Further studies were needed to assess the role and value of GenoType MTBDRplus assay in treatment for MDR-TB with DM patients.

Keywords: GenoType MTBDRplus assay; sensitivity; specificity; diabetes mellitus (DM)

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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 multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RIF), is an increasing threat to TB control as the treatment is difficult, expensive, and a major health care cost burden.
for developing countries (2). According to World Health Organization (WHO), in the year 2014, an estimated 3.3% of new cases and 20% of previously treated TB cases have MDR-TB (3).

Diabetes mellitus (DM) is a non-communicable chronic disease whose incidence is increasing globally (4). Several studies have elucidated an association between DM and TB. The prevalence of DM among TB patients around the world varies according to different regions that range from 12% to 44% and tended to increase in the past decade (5). DM increases the risk of developing active TB by a factor of 2–3 as compared with the general population (6). Except accelerating TB disease, DM is also an increasingly recognized comorbidity that can complicate its treatment and increases the risk of a poor TB outcome (6-9). Among MDR-TB patients, DM is a relatively common comorbidity in patients undergoing treatment for MDR-TB, it was found to be independently associated with an increased risk of both treatment failure and death (10). Moreover, DM increases the risk of developing serious adverse events in the therapy against drug-resistant TB, such as nephrotoxicity and hypothyroidism (9).

Current studies support that transmission of drug-resistant TB strains drives the drug-resistant TB epidemic, including MDR and extensively drug-resistant TB (11,12). Early detection of MDR-TB is essential for starting an effective treatment regimen and reducing its transmission in the population. However, for drug susceptibility testing (DST), conventional methods relying on solid or liquid culture are time-consuming and labor-intensive. GenoType MTBDR plus assay (Hain Lifescience GmbH, Nehren, Germany) which is designed for the rapid detection of Mycobacterium tuberculosis (M.TB) complex and INH- and RIF-resistance was endorsed by WHO (13). 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 (14). In a meta-analysis, GenoType MTBDR plus showed excellent pooled sensitivity and specificity for detection of resistance to INH (91%, 99%), RIF (96%, 98%), and MDR-TB (91%, 99%) (15). The MTBDR plus assay demonstrated excellent performance and offers great promise in improving MDR-TB care and prevention.

The aim of this study was to evaluate the performance of GenoType MTBDR plus assay for rapid detection of INH- and RIF-resistance in M.TB isolates from DM patients.

### Methods

### Subjects

This study was approved by the Human Research Ethics Committees of Shandong Provincial Chest Hospital. The Ethics Approval ID number is: SPCHEC 2016-11-01. Because of the retrospective nature, written consent was waived.

Between May, 2012 and Sep, 2016, 227 TB patients with DM were enrolled in the study. Culture and phenotypic DST were performed on all cases. The absolute concentration method (INH: 1 μg/mL, RIF: 50 μg/mL) on Löwenstein-Jensen medium was used to screen M.TB isolates (16). Their clinicopathological characteristics were reviewed and analyzed.

All TB patients were confirmed by mycobacterial culture (Löwenstein-Jensen medium). DM was diagnosed according to the WHO criteria, i.e., a fasting blood glucose ≥ 7 mmol/L or HbA1c ≥6.5%. Also, DM was diagnosed if patients had a history of known DM or were receiving anti-diabetic agents.

### GenoType MTBDRplus assay

The GenoType MTBDR plus assay was done on sputum or culture specimens and carried out according to manufacturer’s instructions. Briefly, three steps are required: (I) DNA extraction from specimens; (II) amplification of target region by PCR; (III) hybridization of PCR product to the specific oligonucleotide probes, immobilized on the strip. Drug resistance was read as the absence of wild-type band and/or presence of mutation band.

### Statistical analysis

Statistical analysis was carried out using SPSS 17.0 software. Data were expressed as mean ± standard deviation (SD), all calculations were estimated at a 95% confidence interval (95% CI). The result of the phenotypic DST assay was used as the gold standard to calculate the sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio for detecting INH- and RIF-resistance by GenoType MTBDR plus assay.

### Results

A total of 227 specimens (40 culture and 187 sputum samples) collected from DM patients were enrolled in the study. Their mean age was 52.2 (13.2, SD) years. Of these
patients, 86.8% [197] were male, all (203/203) tested for HIV status were HIV-negative, 58 patients were retreatment cases, 187 patients have isolated pulmonary TB, 40 pulmonary + extra-pulmonary TB (25 pleural, 5 lymph node, 4 meningitis, 7 others). Amongst the 227 onset isolates, 182 (80.2%) were sensitive, 30 (13.2%) mono-resistant (14 INH, 16 RIF), 14 (6.2%) MDR. The clinical characteristics of the participants were presented in Table 1.

The total sensitivity, specificity, false positive ratio, false negative ratio and diagnostic odds ratio of GenoType MTBDRplus assay: (I) for RIF-resistance detection, were 96.7% (83.3%, 99.4%), 97.5% (94.2%, 98.9%), 38.1 (16.0, 90.7), 0.034 (0.005, 0.235) and 1113.6 (125.6, 9873.5), respectively; (II) for INH-resistance detection, were 82.1% (64.4%, 92.1%), 91.9% (87.3%, 95.0%), 10.2 (6.2, 16.8), 0.194 (0.088, 0.43) and 52.3 (17.5, 156.2), respectively; (III) for MDR detection, were 92.9% (68.5%, 98.7%), 96.2% (92.8%, 98.0%), 24.7(12.3, 49.5), 0.074 (0.011, 0.491) and 333.1 (38.7, 2,868.7), respectively. The diagnostic performance of GenoType MTBDRplus compared to DST in solid medium, for RIF- and INH-resistance detection in M.TB isolates from DM patients, is summarized in Table 2.

### Discussion

Although from 1990 to 2010, the prevalence of TB decreased significantly, China ranked the second largest country in terms of the number of TB patients (17). A cohort study showed that the prevalence of latent TB infection ranged from 13% to 20% (18). Since TB with a high prevalence in many developing countries, it enables the coexistence with chronic diseases, which increased in the last decades (19,20).

In China, the prevalences of total diabetes and pre-diabetes were 9.7% and 15.5%, respectively (21). Therefore, it was concluded that there are much TB patients with TB-DM comorbidity. DM and TB affect vulnerable groups, such as older adults and people with other morbidities.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Resistance</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>FPR</th>
<th>FNR</th>
<th>DOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>INH</td>
<td>0.50 (0.15–0.85)</td>
<td>0.89 (0.74–0.96)</td>
<td>4.4 (1.1–16.8)</td>
<td>0.565 (0.210–1.515)</td>
<td>7.6 (0.8–71.3)</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>0.86 (0.49–0.97)</td>
<td>0.67 (0.85–1.00)</td>
<td>28.3 (4.0–199.5)</td>
<td>0.147 (0.024–0.905)</td>
<td>192.0 (10.5–3,509.5)</td>
</tr>
<tr>
<td></td>
<td>MDR</td>
<td>1.00 (0.34–1.00)</td>
<td>1.00 (0.91–1.00)</td>
<td>–</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Sputum</td>
<td>INH</td>
<td>0.88 (0.69–0.96)</td>
<td>0.93 (0.88–0.96)</td>
<td>11.9 (6.8–20.9)</td>
<td>0.135 (0.047–0.389)</td>
<td>88.1 (23.0–338.1)</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>1.00 (0.86–1.00)</td>
<td>0.98 (0.94–0.99)</td>
<td>41.0 (15.6–107.9)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>MDR</td>
<td>0.92 (0.65–0.99)</td>
<td>0.95 (0.91–0.97)</td>
<td>17.8 (9.2–34.4)</td>
<td>0.088 (0.013–0.574)</td>
<td>202.9 (23.5–1,749.1)</td>
</tr>
<tr>
<td>Total</td>
<td>INH</td>
<td>0.82 (0.64–0.92)</td>
<td>0.92 (0.87–0.95)</td>
<td>10.2 (6.2–16.8)</td>
<td>0.194 (0.088–0.43)</td>
<td>52.3 (17.5–156.2)</td>
</tr>
<tr>
<td></td>
<td>RIF</td>
<td>0.97 (0.83–0.99)</td>
<td>0.98 (0.94–0.99)</td>
<td>38.1 (16.0–90.7)</td>
<td>0.034 (0.005–0.235)</td>
<td>1,113.6 (125.6–9,873.5)</td>
</tr>
<tr>
<td></td>
<td>MDR</td>
<td>0.93 (0.69–0.99)</td>
<td>0.96 (0.93–0.98)</td>
<td>24.7(12.3–49.5)</td>
<td>0.074 (0.011–0.491)</td>
<td>333.1 (38.7–2,868.7)</td>
</tr>
</tbody>
</table>

M.TB, mycobacterium tuberculosis; DM, diabetes mellitus; INH, isoniazid; RIF, rifampicin; 95 CI, confidence interval; FPR, false positive ratio; FNR, false negative ratio; DOR, diagnostic odds ratio; MDR, multidrug resistance.

Table 1: The clinical characteristics of the participants

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Number</th>
<th>Sex, male (%)</th>
<th>Age (years)</th>
<th>HIV negative status (%)</th>
<th>INH</th>
<th>RIF</th>
<th>MDR</th>
<th>PTB</th>
<th>PTB + EPTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>40</td>
<td>35 (87.5)</td>
<td>52.0±14.1</td>
<td>0 (34/34)</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Sputum</td>
<td>187</td>
<td>162 (86.6)</td>
<td>52.2±13.0</td>
<td>0 (169/169)</td>
<td>24</td>
<td>23</td>
<td>12</td>
<td>152</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>227</td>
<td>197 (86.8)</td>
<td>52.2±13.2</td>
<td>0 (203/203)</td>
<td>28</td>
<td>30</td>
<td>14</td>
<td>187</td>
<td>40</td>
</tr>
</tbody>
</table>

INH, isoniazid; RIF, rifampicin; MDR, multidrug resistance; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.
(hypertension, respiratory diseases, mental disorders, cancer) (22). Diabetes contributes to worsen outcomes and increased severity, when active TB disease develops (23). Thus, there is an urgent need to implement strategies for TB prevention and control among the millions of DM patients exposed to M.TB.

In our study, the GenoType MTBDRplus assay demonstrated excellent performance in detecting MDR-TB in DM patients, with a sensitivity of 92.9% (68.5%, 98.7%) and a specificity of 96.2% (92.8%, 98.0%). In various other studies, it was shown that the GenoType MTBDRplus assay achieved a high sensitivity and specificity for MDR-TB (15). The results in this study differ slightly, with a sensitivity and specificity for RIF of 96.7% (83.3%, 99.4%) and 97.5% (94.2%, 98.9%) for INH of 82.1% (64.4%, 92.1%) and 91.9% (87.3%, 95.0%), respectively. The sensitivity and specificity for the detection of INH-resistance are slightly lower than that published in the meta-analysis [sensitivity: 91% (88%, 94%), specificity: 99% (98%, 99%)] (15).

In general, there still remain several problems difficult to be solved, for example, few mutant probes, heteroresistance and requirement of high bacillary load. The 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. Until now, alterations in multiple genes (24), like katG, inhA, oxyR-aphC, kasA and ndh (25-27), have been reported to be associated with INH-resistance. However, the MTBDRplus assay for the detection of INH-resistance is designed to detect only one mutation in katG and three in inhA. Moreover, the limited numbers of probes in GenoType MTBDRplus restricted its detection of all mutation loci, which might also have decreased its sensitivity. Hetero-resistance is the phenomenon of simultaneous occurrence of drug resistant and drug sensitive TB isolates in the same sample (28,29). The phenomenon has been reported in clinical practice in China (30). It may also contribute to the discordant results between the molecular assay and phenotypic DST. Bacillary load is also an important factor influencing the performance of the test. A study conducted by Seifert M. et al. showed that smear gradation appeared to influence test sensitivity and specificity, indicating a significant association between bacillary load and test performance (31).

Our study also has several limitations. First, because of its retrospective nature, it may produce selection bias. However, consecutive subjects were enrolled, which should reduce the likelihood of a selection bias. Although the MTBDRplus assay demonstrated excellent performance, its impact on TB control strategies for DM patients haven’t been addressed yet. In the next studies, we would conduct studies evaluating the role of GenoType MTBDRplus assay in reducing adverse events in MDR-TB treatment for MDR-TB with DM patients.

Overall, the molecular assay is rapid, reliable and easy to interpret, although the test requires more technical expertise. The results of diagnosis thus have been found to be significantly shorter than the conventional DST method. It is concluded from the present study that GenoType MTBDRplus assay is highly sensitive and specific for rapid diagnosis of MDR-TB in DM patients. The application of this assay could be considered for some settings.

Acknowledgements
None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by institutional/regional/national ethics committee/ethics board of Human Research Ethics Committees of Shandong Provincial Chest Hospital (No. SPCHEC 2016-11-01). Because of the retrospective nature, written consent was waived.

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