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A feasibility study of the Xpert MTB/RIF test at the peripheral level laboratory in China

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SUMMARY

Objective: To evaluate the performance of Xpert MTB/RIF (MTB/RIF) in the county-level tuberculosis (TB) laboratory in China.
Methods: From April 2011 to January 2012, patients with suspected multidrug-resistant tuberculosis (MDR-TB) and non-MDR-TB were enrolled consecutively from four county-level TB laboratories. The detection of *Mycobacterium tuberculosis* (MTB) by MTB/RIF was compared to detection by Löwenstein–Jensen culture. The detection of rifampin resistance was compared to detection by conventional drug-susceptibility testing. The impact of multiple specimens on the performance of MTB/RIF was also evaluated.
Results: A total of 2142 suspected non-MDR-TB cases and 312 suspected MDR-TB cases were enrolled. For MTB detection in suspected non-MDR-TB cases, the sensitivity and specificity of MTB/RIF were 94.4% and 90.2%, respectively. The sensitivity in smear-negative patients was 88.8%. For the detection of rifampin resistance in suspected non-MDR-TB cases, the sensitivity and specificity of MTB/RIF were 87.1% and 97.9%, respectively. For the detection of rifampin resistance in suspected MDR-TB cases, the sensitivity and specificity of MTB/RIF were 87.1% and 91.0%, respectively. Using multiple sputum specimens had no significant influence on the performance of MTB/RIF for MTB detection.
Conclusions: The introduction of MTB/RIF could increase the accuracy of detection of MTB and rifampin resistance in peripheral-level TB laboratories in China. One single specimen is adequate for TB diagnosis by MTB/RIF.

1. Introduction

Tuberculosis (TB) remains a significant public health problem globally. Each year, millions of people suffer from TB, and TB ranks as the second leading cause of death among infectious diseases worldwide.[1] The emergence of multidrug-resistant TB (MDR-TB) poses another challenge in the programmatic management of TB.[1] China has the second highest TB burden and the greatest number of MDR-TB cases in the world.[2] According to the National Anti-Tuberculosis Drug Resistance Survey in 2007,[3] approximately 12 000 new MDR-TB cases emerge annually in China, accounting for 24% of MDR-TB cases worldwide.[2] Although MDR-TB represents only 8% of incident TB cases in China, controlling MDR-TB remains a great challenge due to the difficulties in diagnosis and treatment. A lack of laboratory capacity and delays in obtaining test results in resource-limited settings are also barriers to MDR-TB control.[4,5]

The Xpert MTB/RIF assay (referred to as MTB/RIF in this article) is an in vitro diagnostic technology of semi-nested, real-time fluorescent quantitative PCR that targets the 81-bp rifampin

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resistance-determining region of the *rpo*B gene. MTB/RIF detects *Mycobacterium tuberculosis* (MTB) and rifampin resistance simultaneously.[6–10] The MTB/RIF assay has shown excellent performance in multicenter studies undertaken in reference laboratories and in district and sub-district health facilities in resource-poor countries.[11,12] Furthermore, the MTB/RIF assay has been endorsed by the World Health Organization (WHO) as a promising new rapid diagnostic technology that has the potential for large-scale roll-out.[13] However, evaluation of MTB/RIF has never been conducted in China.

We conducted a multicenter feasibility study across four sites in China with the aim of assessing the performance of the MTB/RIF assay in the detection of MTB and rifampin resistance.

2. Methods

2.1. Study population

From April 2011 to January 2012, suspected non-MDR-TB patients (persons with a cough and expectoration, or hemoptysis for more than 2 weeks) and suspected MDR-TB patients (TB patients with a history of relapse, return after default, initial treatment failure, or retreatment failure) were enrolled consecutively at four county-level TB laboratories in both southern and northern China (Xiangtan and Yueyang counties in Hunan Province, and Beilin and Lanxi counties in Heilongjiang Province).

2.2. Sample processing

Three sputum samples (spot, night, and morning sputum) were collected from each patient. The specimens were examined by acid-fast bacillus (AFB) smear microscopy, solid Löwenstein–Jensen culture, and MTB/RIF test simultaneously.

Direct smear microscopy of each sputum specimen was performed using Ziehl–Neelsen staining in accordance with the China NTP-Sputum Smear standard procedures and Quality Assurance Manual.[14] Sputum specimens were processed for solid culture and inoculated onto Löwenstein–Jensen medium following WHO guidelines.[15] One milliliter of raw sputum was collected by pipette and tested using the MTB/RIF assay according to the manufacturer's protocol.[8]

All culture-positive strains were transported to provincial TB reference laboratories for conventional drug susceptibility testing (DST) of rifampin and isoniazid as per the WHO guidelines,[15] using 40 μg/ml rifampin and 0.2 μg/ml isoniazid.

The China National TB Reference Laboratory (NTRL) collected all strains to perform 16S–23S rDNA internal transcribed spacer (ITS) sequencing for species identification.[16]

Strains with discordant results between conventional DST and the MTB/RIF assay were subjected to PCR amplification and DNA sequencing of the 81-bp *rpo*B core region with forward (CTTGCACGAGGGTCAGACCA) and reverse (ATCTCGTCGCTAACCACGCC) primers.[17] The sequencing results were entered into the Basic Local Alignment Search Tool (BLAST), an international data bank (http://www.ncbi.nlm.nih.gov/BLAST), for comparison with the reference strain H37Rv.

2.3. Ethical considerations

The study was approved by the Ethics Committee of the Chinese Center for Disease Control and Prevention. Because the study was conducted on routine samples only and did not involve any changes in intervention, the requirement to obtain individual informed consent was waived by the review board.

2.4. Statistical analysis

Statistical analysis was performed using Microsoft Excel and SPSS v. 15.0 (SPSS Inc., Chicago, IL, USA). A Chi-square test was used for the statistical analysis. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Study population

From April 2011 to January 2012, a total of 2454 suspected TB cases (2142 suspected non-MDR-TB and 312 suspected MDR-TB) were enrolled consecutively into this study; 1741 subjects were male and 713 were female.

3.2. Performance analysis of the MTB/RIF assay for MTB detection in suspected non-MDR-TB cases

In 2142 suspected non-MDR-TB cases, the rates of MTB detection by smear microscopy, solid culture, and MTB/RIF assay were 13.3% (284/2142), 26.9% (577/2142), and 31.9% (683/2142), respectively. The detection rate of MTB/RIF was significantly higher than that of smear microscopy (Chi-square 214.68, $p = 1.3 \times 10^{-48}$) and that of solid culture (Chi-square 12.31, $p = 0.000451$).

Of the 2142 suspected non-MDR-TB cases enrolled in the study, 48 were excluded from analysis of the performance of the MTB/RIF assay for MTB detection (Figure 1). For the remaining 2094 suspected non-MDR-TB cases, the sensitivity and specificity of the MTB/RIF assay for MTB detection were analyzed using solid culture as the reference standard (Table 1). The diagnostic results of 1911 patients (91.3%) by MTB/RIF were consistent with those obtained by solid culture. A total of 519 out of 550 culture-positive patients were detected positive by the MTB/RIF assay resulting in a sensitivity of 94.4%, while 1392 out of 1544 culture-negative patients were detected negative by the MTB/RIF assay resulting in a specificity of 90.2%; the positive predictive value (PPV) and negative predictive value (NPV) of the MTB/RIF assay for MTB detection were 77.4% and 97.8%, respectively.

3.3. Performance analysis of the MTB/RIF assay for the detection of rifampin resistance in suspected non-MDR-TB cases

Among the 550 culture-positive suspected non-MDR-TB cases, one failed the MTB/RIF assay and 31 tested negative for MTB by MTB/RIF. Therefore, 518 suspected non-MDR-TB cases were used for evaluation of MTB/RIF for the detection of rifampin resistance (Figure 1).

For the 518 culture-positive suspected non-MDR-TB cases, the sensitivity and specificity of the MTB/RIF test for the detection of rifampin resistance were analyzed using conventional DST as the reference standard. Twenty-seven out of 31 rifampin-resistant isolates identified by DST were confirmed resistant by the MTB/RIF assay, resulting in a sensitivity of 87.1%. Out of 487 isolates that were rifampin-sensitive TB by DST, 477 were confirmed sensitive by the MTB/RIF assay, resulting in a specificity of 97.9%. The PPV and NPV of the MTB/RIF test for the detection of rifampin resistance in suspected non-MDR-TB cases were 71.1% and 99.4%, respectively (Table 2).

3.4. Performance analysis of the MTB/RIF assay for the detection of rifampin resistance in suspected MDR-TB cases

Of the 312 suspected MDR-TB cases enrolled in this study, four were excluded for the analysis of the performance of the MTB/RIF assay for the detection of rifampin resistance due to culture