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Evaluation of three rapid assays for *Mycobacterium tuberculosis* complex detection in a comprehensive hospital from West China

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ABSTRACT

Objectives: To assess the capacity of rapid and accurate confirmation of the *Mycobacterium tuberculosis* complex (MTBC) in a Chinese clinical laboratory.

Design and methods: This prospective study investigated three rapid assays, the Amplified Mycobacterium Tuberculosis Direct (MTD) test, real-time PCR, and acid-fast bacilli (AFB) smear, for direct detection of MTBC in a large consecutive series of different clinical specimens. Performance parameters were estimated and compared overall and for separate specimen categories using a combined reference gold standard.

Results: The overall sensitivities were similar for MTD and real-time PCR (62.26% vs. 58.49%), significantly higher than those of AFB smear (31.13%). Among three assays, MTD had a satisfactory sensitivity in respiratory specimen (73.33%) and a nearly perfect detection for smear-positive samples (96.97%). Real-time PCR showed a high positive rate (58.97%) in regard to nonrespiratory specimen. A combination of molecular assays with conventional methods reached marked additive diagnostic values (sensitivity up to 76.42%), higher than each method individually. All detection systems showed excellent specificities (>96.00%).

Conclusions: The present study indicated that our lab had a moderate diagnostic performance for tuberculosis. Quality guarantee for specimen pretreatment, as well as combination analysis, will enable these assays to better incorporate into the routine laboratory workflow in China.

Introduction

Tuberculosis (TB) remains a serious public health problem worldwide [1]. WHO estimates that in 2012, approximately 8.6 million new cases and 1.3 million deaths occurred from TB globally, and 2.9 million cases were still undiagnosed and continued to spread the disease [1]. Rapid and accurate diagnosis for tuberculosis remains challenging because of the low yield of acid-fast bacilli (AFB) smear microscopy and the time-consuming constraint of mycobacterial culture. As sensitive supplementary tools, molecular assays have speeded up diagnosis of this deadly disease. Real-time PCR has proved good diagnostic proficiency in identifying mycobacterial DNA, as a standard molecular technique widely used [2–8]. Another molecular test, the Amplified Mycobacterium Tuberculosis Direct (MTD) test (Gen-Probe, Inc., San Diego, CA), targeting mycobacterial 16S rRNA, has been approved by the US Food and Drug Administration for detecting respiratory sputum, showing sufficient efficiency [5,9–15]. However, molecular methods also have recognized limitations such as carry-over contamination, sophisticated instrument and high cost, and their sensitivities have not lived up to clinical expectation. Strengthening the laboratory performance for better diagnosis, treatment and prevention of tuberculosis, is still an important topic worthy to be investigated further.

China ranks second among the 22 high-burden countries, with numerous TB patients (1.3 million) and the highest annual number of multi-drug resistant tuberculosis (MDR-TB) cases (110,000) worldwide [16]. According to the fifth national tuberculosis epidemiological sampling survey in 2010, the incidence of active pulmonary tuberculosis in West China was 695 cases per 100,000 population, much higher than that of the rest parts of China [17]. The prevalence of MDR-TB showed the rate of 28.3% in sputum smear-positive patients, which was also higher than average for all the developed regions in China [18]. Rapid and accurate technology diagnosing *Mycobacterium tuberculosis* complex (MTBC) is of great importance for TB control in China. Some studies have evaluated the performance of MTD and/or real-time PCR [5,19–21], but most of them were conducted in developed countries and there are still limited data assessing the comparison of these rapid assays so far. Their roles in the TB diagnosis in developing countries like China remain poorly defined. The aim of this study was a comparison of the diagnostic performance of three rapid assays (MTD, real-time PCR, and AFB smear) for the TB diagnosis from a large consecutive series

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of highly suspected tuberculosis patients. It's also a preliminary evaluation of rapid diagnostic capacity for tuberculosis in a comprehensive hospital in West China.

Materials and methods

Study design and patient population

This prospective study was conducted in West China Hospital of Sichuan University, West China, from April 2012 to December 2013. After screening 672 consecutively new patients with clinical and/or radiological suspicion of tuberculosis, 632 patients satisfied all the inclusion criteria and were preliminarily included. Only those patients who could provide detailed clinical history, radiological results and/or histological reports, along with an adequate and eligible specimen were enrolled. Patients who were retreated, initiated anti-tubercular treatment (ATT) > 7 days prior to specimen collection, and lost to follow-up were excluded [22]. The final criterion for the diagnosis of tuberculosis was defined as using a combination of the patient's history, symptoms, radiological and laboratory results, the response to ATT, and a six-month follow-up observation [12,23]. The final diagnoses assigned by at least two respiratory physicians were considered as the reference gold standard. Written Informed consents were obtained from all included individuals and this study was approved by the Ethical Committee of West China Hospital, Sichuan University.

Specimen process

One corresponding sample per patient, the similar amount of specimen was collected as our routine laboratory flowchart did. The minimum volumes of specimens required were: 5 mL for morning sputum, 15 mL for morning urine, and 3 mL for body fluids; 2 mL for cerebrospinal fluids (CSF), and 1 cm by 1 cm for biopsy specimens. The specimens were processed by a standard NALC–NaOH digestion–decontamination method [22] and subjected in parallel to MTD, real-time PCR and AFB smear for testing MTBC. A portion of each specimen was used to prepare a smear, the remaining stored at −80 °C for the later MTD and real-time PCR detections. Laboratory investigators were blinded to all clinical data.

AFB smears

Specimens were air dried, heat fixed, and stained according to standard Ziehl–Neelsen acid-fast staining procedures. The stained slides were examined under oil immersion (1,000× lens objective). The AFB smear result was defined as the International Union Against Tuberculosis and Lung Disease scale [24]: Scanty (1–9/100 fields), 1+ (10–99/100 fields), 2+ (1–10/100 fields) and 3+ (>10/field). A patient with scanty or higher grade was considered smear positive.

Real-time PCR

TB-DNA was extracted using automated NucliSens EasyMag (BioMe'rieux, Lyon, French). PCR was performed using 'Care TB®' real-time PCR commercial kit (Qiagen China [Shenzhen] Co Ltd, Shenzhen, China) in the LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Germany). A volume of 40 μL PCR reaction mixture contained 37.8 μL master mix, 0.2 μL Taq DNA polymerase, 0.06 μL uracil-N-glycosylase enzyme and 2 μL template DNA. The reaction program was initial anti-pollution at 37 °C for 5 min, then denaturation at 94 °C for 1 min, then amplification for 40 cycles by denaturing at 95 °C for 5 s, annealing at 60 °C for 30 s, and cooling to 40 °C for 1 min. *Mycobacterium tuberculosis* (MTB) H37Rv ATCC 2794 strain and a master mix with all reagents but no DNA template were used as the positive and negative control. Cycle threshold (Ct) ≤ 37 was considered PCR-positive, Ct ≥ 40, 0 or invalid value reported negative. Specimen with Ct in the suspicious range (37 < Ct < 40) needed repeated test. A repeat result of 37 < Ct < 40 was considered positive.

MTD

The MTD test (Gen-Probe Inc., San Diego, CA) was performed and interpreted according to the manufacturer's instructions. Briefly, a 450 μL aliquot of sediment was added to a tube containing glass beads and sample buffer and sonicated for 15 min in a water bath sonicator at room temperature. A 25 μL lysate was transferred into a tube containing 40 μL of amplification reagent and 200 μL of oil. Tubes were incubated at 95 °C for 15 min, and then cooled to 42 °C for 5 min. Enzyme reagent (25 μL) was added, and incubated at 42 °C for 30 min. Hybridization reagent (100 μL) was added, and incubated at 60 °C for 15 min. Selection reagent (300 μL) was added, and incubated at 60 °C for 15 min. Tubes were cooled and placed in a Leader 50 luminometer (Gen-Probe) to determine the number of relative light units (RLU). Positive and negative controls offered by the commercial kit were included in every run. MTD results were acceptable if the negative and positive controls were <20,000 and >1,000,000 RLU, respectively. Those specimens with values of ≤30,000 RLU were considered negative and ≥500,000 RLU were considered positive. The test should be repeated when a value was equivocal (30,000 to 499,999 RLU), and the repeated result >30,000 RLU was considered positive.

Protocol for inhibitor detection

MTD-negative samples could be analyzed the inhibitory substances as by Sloutskys et al. described [23]. Briefly, 450 μL of diluent buffer was added to 50 μL of the original specimen sediment. Of this 1:10 diluted sample, 450 μL was transferred to the amplification tube and tested along with the positive and negative control in accordance with the MTD process. Specimen with a value ≥500,000 RLU was positive for MTBC and contains substances inhibiting amplification.

Statistical analysis

Continuous variables were described with means ± standard deviation (SD). Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) were estimated at a 95% confidence interval (95% CI); the κ test was used to evaluate the consistency between two methods, and κ > 0.75 was considered very good consistency. Chi-square or Fisher's exact test was used to analyze differences between proportions. Statistical analyses were performed with SPSS software (version 17.0; SPSS; Chicago, IL). A p value of 0.05 was considered statistically significant.

Results

Clinical patients determined

A total of 610 patients were finally included and analyzed. Fig. 1 shows the flow chart of the patient. The average age was 41.57 ± 18.40 years, and the male-to-female ratio was 1.68 (382/228) (Table 1), and other demographics of the study population also were shown in Table 1.

Clinical evaluation of three rapid assays

Of 610 highly suspected patients, 318 (52.13%) were finally diagnosed as tuberculosis cases, and 292 (47.87%) were non-tuberculosis, using the composed diagnosis as the references gold standard. Sensitivities, specificities, and predictive values of three diagnostic tools for all specimens are presented in Table 2. The overall sensitivities and specificities were 62.26% and 98.29% for MTD, 58.49% and 98.97% for real-time PCR, and 31.13% and 98.97% for AFB smear, respectively. Both the