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ORIGINAL ARTICLE

Comparison of Different Methods for the Diagnosis of Mycobacterium Tuberculosis and Rifampin-Resistance

Chao Lu [1], Hui Jing [2], Zhiming Lu [1, *], Yunfeng Deng [2, *]

[*] *The authors contributed equally to the work*
[1] *Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, PR China*
[2] *Katharine Hsu International Research Center of Human Infectious Diseases, Shandong Public Health Clinical Center, Jinan, Shandong, PR China*

SUMMARY

Background: **Xpert MTB/RIF is recommended by the World Health Organization (WHO) for a rapid and simultaneous detection of Mycobacterium tuberculosis (Mtb) and rifampicin resistance specific to patients who have symptoms and signs.**
Methods: **The aim of this study was to evaluate the diagnostic significance possessed by various assays specific to the detection of Mtb. This study included 345 suspected TB patients who received treatment at the Shandong Public Health Clinical Center during May 2019 and August 2019. Related data included demographics, gender, age, past medical history (PMH), country of birth, country of residence, clinical information and laboratory test outcomes. The smear method was performed three times, the BD960 method was conducted two times and the MTB/RIF and Xpert Ultra (phlegm precipitation) assays were performed once. All methods were completed simultaneously.**
Results: **The Xpert Ultra MTB (phlegm precipitation) method exhibited the highest consistency and sensitivity, followed by the Xpert MTB, Mtb culture, and smear methods, respectively. The Xpert Ultra MTB method also exhibited a significantly higher detection rate relative to the smear method ($X^2 = 13.411$, $p < 0.001$).**
Conclusions: **Xpert MTB/RIF along with Xpert Ultra (phlegm precipitation) exhibited higher sensitivity specific for the diagnosis of TB and rifampicin-resistance. The combined effects of these four methods showed outstanding sensitivity compared with single methods alone.**
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Correspondence:
Zhiming Lu
Department of Clinical Laboratory
Shandong Provincial Hospital Affiliated to
Shandong First Medical University
Jinan 250000, Shandong
PR China
Phone: +86 0531 68772479
Email: luzhiming@sdu.edu.cn

Yunfeng Deng
Katharine Hsu International Research Center of
Human Infectious Diseases
Shandong Public Health Clinical Center
Jinan 250000, Shandong
PR China
Email: yfdeng@126.com

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KEY WORDS

Xpert MTB/RIF, Xpert Ultra, phlegm precipitation, pulmonary, tuberculosis

INTRODUCTION

Mycobacterium tuberculosis (Mtb), the pathogen that causes tuberculosis (TB), contributes to a significant number of deaths related to infectious diseases worldwide [1,2]. To manage TB in the clinic, it is essential to rapidly and accurately detect Mtb as well as drug-resistance [3]. Identifying the source of infection is important to help determine treatment of the patient. Based on the 2018 Global Tuberculosis Report generated by the World Health Organization (WHO) [4], approximately 10 million people worldwide were diagnosed with TB

in 2017. The reported number is less than the estimated number mainly due to missed cases. An accurate assessment of the impact of this disease is crucial for the planning of adequate control and prevention strategies [5]. In the last few years, researchers have developed immunodiagnostic methods to detect Mtb [6,7]. The laboratory assays currently used to detect active TB diagnosis include a mycobacterial culture, acid-fast bacilli (AFB) microscopy, and interferon gamma releasing assays (IGRAs) [8]. The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), endorsed by the WHO in 2010, is a nucleic acid application test to rapidly and automatically detect the susceptibility exhibited by Rifampicin (RIF) [9,10]. This study adopted four methods to detect Mtb in sputum samples. These tests include sputum smear acid-fast staining microscopy (hereafter referred to as "smear method"), BACTEC MGIT 960 ("MGIT 960 method") liquid culture, GeneXpert MTB/RIF ("GeneXpert method"), and GeneXpert Sputum precipitation assay [11]. The tests were compared and evaluated for independent diagnosis effects as well as combined effects. The combined treatment exhibited better diagnosis effects [12]. It is necessary to discover and treat TB in the early stage to prevent transmission. Therefore, this study assessed the diagnostic significance of the different methods in the detection of Mtb.

MATERIALS AND METHODS

Patients

A total of 345 suspected TB patients receiving treatment at the Shandong Public Health Clinical Center in May 2019 and August 2019 were enrolled in this study. There were 219 males and 126 females with an age range from 12 - 92 years (the median age was 48). Among these patients, 255 patients were diagnosed with TB, accounting for 73.91% of all patients. Sputum was collected from each patient and the smear method was performed three times, the BD960 method was conducted two times, and the MTB/RIF and Xpert Ultra (phlegm precipitation) assays were conducted once. All these methods were completed at the same time. Polymerase chain reaction (PCR) assisted in the identification of solid or liquid cultures, whereas microarray analysis assisted in detecting non-tuberculous mycobacterium (NTM).

Related data involved demographics, gender, age, PMH, country of birth, country of residence, clinical information as well as laboratory test outcomes. Patient files were retrieved through the medical record system. Active TB was diagnosed taking into account symptoms, microbiologic evidence, and radiologic results. *Mycobacterium tuberculosis* species were identified using P-nitro benzoic acid (PNB) and 2-thiophenecarboxylic acid hydrazide (TCH) resistance tests. The drug rifampicin was applied to drug sensitivity testing (DST) specific to Mtb strains. This study obtained approval from the SPCH and obtained informed consent from adult patients and parents of those under 18 years of age. Non-TB mycobacterium patients were excluded from analysis.

Protocols

Procedures followed clinical and laboratory practices of the ISO15189 certified TB reference laboratory in China. All specimens received smear tests using the fluorescent staining method (Baiao Laibo, Beijing, China). Smear microscopy tests were performed together with laboratory testing procedures for TB [8]. Any one of the three smear samples from the same patient were determined as a positive smear. The MGIT 960 method strictly complied with BACTEC MGIT960 system operation instructions. The BACTEC™ MGIT™ 960 Mycobacterial Detection System (Becton, Dickinson and Company, USA) assisted in performing Mtb cultures and DST. A total of 0.5 mL of sputum sample was added into a round bottom centrifuge tube (50 mL). 1 - 2 mL of pretreatment solution (NALC-NaOH) was added to the sputum sample, followed by 15 minutes of shaking for digestion. A total of 40 mL of phosphate buffer solution (PBS) was added to the solution for neutralization, followed by 18 minutes of centrifugation at 3,000 x g. Lastly, the supernatant was discarded. Negative culture results were set to 42 days. As long as the instrument showed a positive, the culture medium would undergo acid-fast staining microscopy to determine bacterial purity. GeneXpert (Cepheid, Sunnyvale, CA, USA) strictly complied with operation instructions. A total of 1 mL of sputum sample was added into 2 mL of sample treatment solution, which was then fully mixed. Following a 15-minute incubation, 2 mL of sample was added into the reaction kit and was placed into the tester to receive automatic detection. Results were directly visualized using the detection system. The GeneXpert Sputum precipitation assay was performed as follows: the sputum sample in a screw-cap tube was added to digestive solution and was mixed for 30 seconds. Results were directly observed using the detection system.

Statistical Analysis

True negative, true positive, false negative, and false positive data were extracted from analysis. Additionally, negative-predictive (probability of true-negative results occurring) and positive-predictive (probability of true-positive results occurring) values were calculated. The sensitivity and specificity formulas used in calculations were as follows: sensitivity = [true positive/(true positive + false negative) x 100%], and specificity = [true negative/(true negative + false positive) x 100%]. Positive predictive value = true positives/(true positive + false positives)), and negative predictive value = (true negative/(true negative + false negatives)) were calculated per session. We defined the accordance rate as [(true positive + true negative)/total number of samples] x 100%. Statistical analyses were performed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA). The X^2 test was used to compare

Table 1. Diagnostic efficacy of single method.

Methods	Clinical diagnosis		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Consistency rate (%)
	Tuberculosis	Non-tuberculosis					
Smear method							
Positive	146	2	57.25	96.67	98.65	34.73	64.76
Negative	109	58					
Culture method							
Positive	166	2	65.01	96.67	98.81	39.46	71.11
Negative	89	58					
GeneXpert							
Positive	177	3	69.41	95.00	98.33	42.22	74.29
Negative	78	57					
Precipitation assay							
Positive	95	3	80.51	90.91	96.94	56.60	82.78
Negative	23	30					

Notes: Consistency - (true positive cases + true negative cases)/total sample number.

Table 2. Diagnostic efficacy of four combined methods.

Methods	Clinical diagnosis		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Consistency rate (%)
	Tuberculosis	Non-tuberculosis					
Two combined methods							
Positive	183	4	71.76	93.33	97.86	43.75	75.87
Negative	72	56					
Three combined methods							
Positive	195	6	76.47	90.00	97.01	47.37	79.05
Negative	60	54					
Four combined methods							
Positive	200	3	84.75	90.91	97.09	62.5	86.09
Negative	55	57					

Notes: Two combined methods: Smear method + culture method, Three combined methods: Smear method + culture method + GeneXpert method, Four combined methods: Smear method + culture method + GeneXpert method + Xpert Ultra MTB method, Consistency - (true positive cases + true negative cases)/total sample number.

counting data. A $p < 0.05$ was identified as being statistically significant.

RESULTS

Comparison of diagnostic efficiency

Among all TB patients, 255 active patients were under clinical diagnosis before being discharged from the hospital. As for the sputum specimens collected from PTB patients, 146 (57.25%) were positive for Mtb as deter-

Table 3. Sensitivity, specificity of the GeneXpert MTB/RIF and precipitation assay with the culture method and clinical diagnosis as reference.

	The culture method as reference	The clinical diagnosis as reference
GeneXpert MTB/RIF		
Sensitivity (%)	**91.07**	**69.41**
Specificity (%)	**81.63**	**95.00**
Sputum precipitation		
Sensitivity (%)	**97.59**	**80.51**
Specificity (%)	**72.86**	**90.91**

Table 4. Comparison between GeneXpert MTB/RIF or Xpert Ultra MTB/RIF assay and conventional MTB drug susceptibility test (DST).

	Conventional DST	
	RIF^R	RIF^S
GeneXpert MTB/RIF		
RIF^R	**37**	**8**
RIF^S	**0**	**108**
Precipitation assay		
RIF^R	**26**	**5**
RIF^S	**0**	**50**

mined by a fluorescent staining smear test, while 166 (65.09%) were positive as shown by Mtb culture. A total of 177 (69.41%) were positive based on the Xpert MTB Assay, and 95 (80.51%) were positive based on the GeneXpert Sputum precipitation assay. Clinical diagnosis was considered as a reference standard for judging the diagnostic significance possessed by these four methods (Table 1). The GeneXpert Sputum precipitation assay presented the highest consistency and sensitivity, followed by the Xpert MTB, Mtb culture, and smear methods. The GeneXpert method presented a significantly higher detection rate relative to the smear method ($X^2 = 8.114$, $p = 0.004$). The GeneXpert Sputum precipitation assay also exhibited a significantly higher detection rate relative to the smear, Mtb culture, and GeneXpert methods ($X^2 = 19.077$, $p < 0.001$; $X^2 = 9.118$, $p = 0.002$; $X^2 = 19.077$, $p = 0.025$, respectively). Clinical diagnosis consistency ranged between 64.76% - 82.78% for the four methods.

Combined diagnostic efficacy

The diagnostic sensitivity exhibited by a combined method reached as high as 84.75%, larger than what was observed for each single test (Table 2).

Detection status

The detection status of the Mtb culture, GeneXpert, as well as GeneXpert Sputum precipitation assay were performed for patients with smear negative suspected TB. Compared with the culture reference, the clinical reference was more specific but less sensitive. The Xpert MTB/RIF assay and the GeneXpert Sputum precipitation assay identified 45 and 31 RIF resistant cases, respectively. Relative to traditional DST test, the sensitivity and specificity in determining RIF resistance were 93.4% and 85.6%, respectively, for the GeneXpert Sputum precipitation assay (Table 3). Compared with the conventional DST test, the Xpert MTB/RIF assay presented 100% (37/37) sensitivity and 93.1% (108/116) specificity for the identification of RIF resistance. The precipitation assay presented 100% (26/26) sensitivity and 90.9% (50/55) specificity for the identification of RIF resistance (Table 4).

DISCUSSION

TB diagnosis using direct smear microscopy and culture forms the backbone of TB diagnostics worldwide [13]. Etiological examination of TB includes Mtb nucleic acid detection as well as liquid culture. Using sputum samples collected from suspected TB patients, this study used four etiological testing methods, including the smear, mycobacterial culture, GeneXpert, and GeneXpert Sputum precipitation methods. As previously demonstrated, the smear method was used due to its simple, rapid, low cost, and high sensitivity, remaining the cornerstone methods for Mtb diagnosis worldwide [14]. Nevertheless, our results demonstrated that the smear method possessed a significantly lower positive detection rate compared with the GeneXpert and the GeneXpert Sputum precipitation methods. In line with published literature [15], Xpert on sputum presented stronger sensitivity relative to microscopy. Mycobacterial culture was treated as the "gold standard" specific for TB diagnosis, TB control, epidemiological investigation, clinical diagnosis, as well as genotyping [16]. However, Mtb grows slowly, making it difficult for physicians to diagnose disease using this method [17]. To this effect, the increased sensitivity observed using the smear concentration method ideally compares with PCR.

The WHO recommended the adoption of the GeneXpert MTB/RIF molecular diagnosis technique [18]. This technique, adopting the semi-nested quantitative PCR amplification process, can be automatically completed in a closed kit and detect Mtb in no more than two hours [19]. Meanwhile, it is capable of detecting mutations in rifampicin Mtb resistance genes, acting as a reference to predict rifampicin resistance. Researchers developed a novel GeneXpert Sputum precipitation assay to overcome shortcomings possessed by the traditional Xpert MTB/RIF assay, which presents enhanced sensitivity when detecting TB and RIF resistance [20]. In

this study, the GeneXpert Sputum precipitation assay presented the highest consistency and sensitivity, followed by Xpert MTB/RIF, Mtb culture, and fluorescent staining smear microscopy, respectively. Obviously, the GeneXpert Sputum precipitation assay showed the highest sensitivity among the four methods, confirming results by John Osei Sekyere et al. [21]. This method is characterized by its simple, rapid, and lower biosafety requirements as well as being capable of detecting drug resistance to rifampicin and is therefore recommended to be widely applied in laboratories at the basic level. This study paid attention to the diagnostic significance possessed by the GeneXpert Sputum precipitation assay regarding the detection of TB and resistance, thereby determining its efficiency in rapidly diagnosing TB in pulmonary specimens. The GeneXpert Sputum precipitation assay and GeneXpert method showed obviously higher sensitivity relative to the traditional smear method.

Currently, the majority of hospitals use the traditional acid-fast staining microscope for TB treatment. It is still difficult to diagnose smear negative suspected TB patients [22]. As concluded in this study, the Mtb culture, GeneXpert, and GeneXpert Sputum precipitation assay show different positive detection rates of smear negative TB patients. The detection rate exhibited in our results was different compared with results shown by Jason P. Rice et al. [23]. Based on the cause analysis, sputum specimen quality may be a possible influencing factor. Analyses in the study did not include contaminated samples. It was difficult to control the sputum specimen quality. However, the diagnosis of smear-negative PTB patients is perceived as difficult. Due to the lack of bacteriological indicators, the diagnosis of smear-negative PTB was more complicated than smear-positive PTB. This is especially the case for smear-negative TB patients to choose crucial tools for detecting active TB.

To master identifying the etiological basis of TB diagnosis, as well as meeting the TB prevention and treatment requirements, the combined test integrating various detection methods was studied. Considering the actual situation of TB detection laboratories in Jinan, the combined tests that involved four methods were conducted and the diagnostic values were compared. As demonstrated, combined methods were more sensitive than any independent method. Combing Mtb culture with the fluorescent staining smear microscopy method increased the pathogen-positive rate up to 71.76%. Combining the four methods contributed to a pathogen-positive rate up to 84.75%. Despite the slightly complex experimental procedure and instrument for liquid culture, as well as the bacteria collection during pretreatment process, there are relatively high requirements for laboratory personnel and for experimental conditions. Nevertheless, considering the improved etiology positive rate due to BD 960, as well as the advantages of shorter culture time and the follow-up molecular/phenotypic drug sensitivity tests specific to positive culture, it is encouraged to use well-equipped TB testing laboratories in districts or counties to actively conduct liquid cultures.

Unlike most previous studies [24,25], this study adopted the clinical diagnosis of active TB as well as the culture as the evaluation standard for the performance of the Xpert MTB/RIF assay. Clinical reference is more specific and less sensitive compared with culture reference. Relative to previous studies that focused on countries with low TB incidence, the Xpert MTB/RIF assay exhibited a much lower sensitivity when identifying RIF resistance. The GeneXpert Sputum precipitation assay exhibited a similar accuracy in determining rifampicin resistance as Xpert MTB/RIF. According to this study, the GeneXpert Sputum precipitation assay was the most specific. In the case that BACTEC MGIT 960 system liquid media was used as the gold standard, the GeneXpert Sputum precipitation assay was more specific than smear. In the case that CRS was used as a reference, it had better specificity than AFB smear as well as MGIT 960 [26]. In many studies, the GeneXpert, centrifugal concentrated smear and MGIT 960 methods were used to replace traditional smears and cultures. Testing methods exert a significant impact in various laboratories [27] and may not be easily replaced. To better serve patients, the four methods should be integrated to obtain the largest efficiency.

Altogether, the GeneXpert Sputum precipitation assay rapidly and specifically tests active TB diagnosis and exhibits a sensitivity to PTB similar to that of Mtb culture. Combining the four methods results in stronger sensitivity compared to any of the four methods used alone.

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Declaration of Interest:
All authors declare that no conflict of interest exists.

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