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Original Article

Evaluation of Xpert MTB/RIF in detection of pulmonary and extrapulmonary tuberculosis cases in China

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Abstract: Background: To evaluate the performance of Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis (Mtb) and rifampin resistance in pulmonary and extrapulmonary tuberculosis (TB) patients in China. Material/Methods: We prospectively enrolled 506 TB patients to evaluate sensitivity and specificity of Xpert MTB/RIF by comparing with Mtb culture, fluorescent staining smear microscopy and clinical diagnosis. Results: Overall, 402 of 506 (79.4%) included patients were clinically diagnosed with TB. Sensitivities of Xpert MTB/RIF, Mtb culture, and AFB microscopy, and combined three methods among clinically-diagnosed pulmonary TB patients were 67.7% (130/192), 65.1% (125/192), 61.5% (118/192), and 69.8% (134/192) respectively; and 19.0% (40/210), 23.8% (50/210), 5.2% (11/210), and 34.3% (72/210) respectively among clinically-diagnosed extrapulmonary TB patients. Specificities of Xpert MTB/RIF assay for pulmonary and extrapulmonary TB were 100%. Compared with MGIT drug susceptibility test, the sensitivity and specificity of Xpert MTB/RIF for diagnosis of rifampin-resistant TB was 77.3% and 96.0% respectively. Conclusion: The Xpert MTB/RIF assay has a comparable sensitivity with Mtb culture for pulmonary TB, but less sensitive than Mtb culture for extrapulmonary TB. Combined three methods achieved a better sensitivity than any individual test.

Keywords: Xpert MTB/RIF, pulmonary, extrapulmonary, tuberculosis, China

Introduction

Tuberculosis (TB), especially drug resistant TB, remains one of the major public health concerns worldwide with a mortality ranging from 1.6 to 2.2 million per year [1]. Rapid and accurate diagnosis of active TB disease can significantly prevent TB transmission in populations. Laboratory assays currently in use for active TB diagnosis include the acid-fast bacilli (AFB) microscopy, mycobacterial culture, and the interferon gamma releasing assays (IGRAs), all of which still suffer from either low sensitivity, specificity, long turnaround time, and/or high costs [2]. Xpert MTB/RIF assay, a method based on DNA amplification, was recently introduced to tackle TB diagnostic limitations, with increased sensitivity and specificity for rapid TB diagnosis. The Xpert MTB/RIF assay was recommended by World Health Organization (WHO) in 2011 to facilitate the rapid detection of active TB and rifampin resistance with more than 95% sensitivity for smear-positive cases and 55% for smear-negative cases [3]. However, data on the utility of Xpert MTB/RIF in extra-pulmonary specimens are still limited. China has the second highest incidence of TB and the highest drug-resistant TB in the world [4]. Performance of the Xpert MTB/RIF assay in the Chinese population is still under investigation. The objectives of this study were to evaluate the performance of the Xpert MTB/RIF assay for the direct detection of *M. tuberculosis* (Mtb) and rifampin resistance in pulmonary and extrapulmonary TB patients in China.

Materials and methods

This study was conducted in Shandong Provincial Chest Hospital, which is the only pro-

Table 1. Comparison of GeneXpert MTB/RIF test with MTB culture and fluorescent staining smear tests with sputum and extrapulmonary specimens

Specimens	GeneXpert MTB/RIF			MTB Culture				Fluorescent staining Smear test	
	Indeterminate	Negative	Positive	Culture Negative	MTB Positive	NTM Positive	Contaminated	Negative	Positive
Sputum (n = 229)	1			0	0	1	0	0	1
		98		82	4	9	3	86	12
			130	6	121	0	3	15	115
CSF (n = 101)		91		83	8	0	0	90	1
			10	6	4	0	0	10	0
Pleural Fluid (n = 124)		108		87	21	0	0	108	0
			16	4	12	0	0	13	3
Tissue (n = 22)		11		11	0	0	0	11	0
			11	10	1	0	0	5	6
Urine (n = 21)		19		19	0	0	0	18	1
			2	0	2	0	0	2	0
Ascitic fluid (n = 9)		8	0	7	1	0	0	8	0
			1	0	1	0	0	1	0
Total (n = 506)	1			0	0	1	0	0	1
		335		289	34	9	3	321	14
			170	26	141	0	3	46	124

Table 2. Sensitivities of GeneXpert MTB/RIF test, MTB culture, and fluorescent staining smear in diagnosing pulmonary and extrapulmonary TB

Clinical TB diagnosis	GeneXpert MTB/RIF assay	MTB Culture	Smear microscopy	Combining 3 Methods
Pulmonary (n = 192)				
Sputum	67.7% (130/192)	65.1% (125/192)	61.5% (118/192)	69.8% (134/192)
Extrapulmonary (n = 210)				
CSF (n = 81)	12.3% (10/81)	14.8% (12/81)	1.2% (1/81)	23.5% (19/81)
Pleural fluid (n = 95)	16.8% (16/95)	34.7% (33/95)	3.2% (3/95)	38.9% (37/95)
Tissue (n = 18)	61.1% (11/18)	5.6% (1/18)	33.3% (6/18)	61.1% (11/18)
Urine (n = 9)	22.2% (2/9)	22.2% (2/9)	11.1% (1/9)	33.3% (3/9)
Ascitic fluid (n = 7)	14.3% (1/7)	28.6% (2/7)	0% (0/7)	28.6% (2/7)
Sub-total (n = 210)	19.0% (40/210)	23.8% (50/210)	5.2% (11/210)	34.3% (72/210)
Total (n = 402)	42.3% (170/402)	43.5% (175/402)	32.1% (129/402)	51.2% (206/402)

vincial-level hospital specialized in TB (a referral hospital) and other lung infections in Shandong Province. Shandong is the second largest province in China (population size, 96 million and 59% rural) and has approximately 40,000 new TB cases annually. Between July 2013 and June 2014, a total of 506 TB participants were prospectively enrolled in this study, which included 229 pulmonary TB participants (229 sputum specimens) and 277 extrapulmonary TB participants (101 cerebrospinal fluid [CSF], 124 pleural fluid, 22 tissue, 21 urine, and 9 ascitic fluid specimens). The specimens were collected from each TB suspect, all specimens were processed smear microscopy test by the fluorescent staining method, Mtb culture and drug susceptibility test (DST) by using BACTEC™ MGIT™ 960 Mycobacterial Detection System (BD, NJ, USA), and the Xpert MTB/RIF assay. All procedures were conducted at the Shandong Provincial TB Reference Laboratory (the ISO15189 certified TB reference laboratory in mainland China). A P-nitro benzoic acid (PNB) and 2- thiophenecarboxylic acid hydrazide (TCH) resistance test was utilized to identify Mtb species. *Mtb* H37Rv was

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

Specimen type	The culture method as reference		The clinical diagnosis as reference	
	Sensitivity	Specificity	Sensitivity	Specificity
Sputum	96.8% (91.5%-99.0%)	93.8% (86.5%-97.5%)	67.7% (60.0%-73.8%)	100.0% (89.7%-100.0%)
CSF	33.3% (11.3%-64.6%)	93.3% (85.4%-97.2%)	12.3% (6.1%-21.5%)	100.0% (83.2%-100.0%)
Pleural Fluid	36.4% (21.0%-54.9%)	95.6% (88.5%-98.6%)	16.8% (9.9%-25.9%)	100.0% (88.1%-100.0%)
Lesions	100% (5.5%-100.0%)	52.4% (30.3%-73.6%)	61.1% (35.7%-82.7%)	100.0% (39.8%-100.0%)
Urine	100% (19.8%-100.0%)	100% (79.1%-100.0%)	22.2% (2.8%-60.0%)	100.0% (73.5%-100.0%)
Ascitic fluid	50% (2.7%-97.3%)	100% (56.1%-100.0%)	14.3% (0.4%-57.9%)	100.0% (71.5%-100.0%)
Total	80.6% (73.8%-86.0%)	92.0% (88.3%-94.6%)	42.3% (37.0%-46.9%)	100.0% (96.4%-100.0%)

used as the standard strain for quality control. The DST panel included 2 first-line anti-TB drugs: isoniazid, rifampin. The Xpert MTB/RIF assay was conducted according to the manufacture instructions. Clinical diagnosis of TB patients was made by combining clinical manifestations, chest radiography, positivity of laboratory testing (smear microscopy, Mtb culture, and Xpert MTB/RIF assay) and clinical improvement in response to antimycobacterial treatment.

Results

Among 229 sputum specimens collected from pulmonary TB participants, 130 (56.8%) tested positive for Mtb, 98 (42.8%) negative, and 1 (0.4%) indeterminate by the Xpert MTB/RIF assay; while 125 (54.6%) were positive for Mtb, 88 (38.4%) negative, 10 (4.4%) non-tuberculous mycobacteria (NTM), and 6 (2.6%) contaminated tests by Mtb culture; 128 (55.9%) were positive, and 101 (44.1%) negative by fluorescent staining smear test (**Table 1**). The Mtb positivity agreement between the Xpert MTB/RIF assay and Mtb culture, as well as, between Xpert MTB/RIF and fluorescent staining smear test were 94.3% (κ = 0.885; 95% CI, 0.824-0.946) and 75.1% (κ = 0.751; 95% CI, 0.665-0.838) respectively. A total of 192 pulmonary TB participants were clinically diagnosed as active pulmonary TB cases when discharged from the hospital. Compared with the clinical diagnosis, sensitivities of Xpert MTB/RIF, Mtb culture, and fluorescent staining smear microscopy, and combined three methods were 67.7% (130/192), 65.1% (125/192), 61.5% (118/192), and 69.8% (134/192) respectively (**Table 2**).

Among 277 specimens from extrapulmonary TB participants, 40 (14.4%) were identified Mtb positive by Xpert MTB/RIF assay, 50 (18.1%) positive by Mtb culture, 11 (4.0%) positive by fluorescent staining smear microscopy (**Table 1**). The highest Mtb positivity agreement between the Xpert MTB/RIF assay and Mtb culture was found in urine specimens (n = 2; 100%; κ = 1.0), while the lowest Mtb positivity agreement was found in tissue specimens (n = 11; 54.6%, κ = 0.091) (**Table 1**). A total of 210 extrapulmonary TB participants were clinically diagnosed as active TB cases (**Table 2**). Compared with the clinical diagnosis, sensitivities of Xpert MTB/RIF, Mtb culture, and fluorescent staining smear microscopy, and the combined three methods were 19.0% (40/210), 23.8% (50/210), 5.2% (11/210), and 34.3% (72/210) respectively among clinically-diagnosed extrapulmonary TB patients. Specificities of Xpert MTB/RIF assay for pulmonary and extrapulmonary TB diagnosis were 100% (**Table 2**).

A total of 121 Mtb isolates from pulmonary and extra-pulmonary TB patients had RIF resistance results from both Xpert MTB/RIF assay and conventional DST (**Table 3**). The Xpert MTB/RIF assay identified 17 RIF resistant cases. The conventional DST found 22 RIF resistant cases, of which 17 were also isoniazid (INH) resistant, i.e., multi-drug resistant (MDR). The RIF resistance agreements between the Xpert MTB/RIF assay and conventional DST was 92.6% (κ = 0.746; 95% CI, 0.588-0.903). Compared with the conventional DST test, the Xpert MTB/RIF assay presented 77.2% of sensitivity and 96.0% of specificity in identification of RIF resistant, and 76.5% (13/17) sensitivity

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

GeneXpert MTB/RIF	Conventional DST				Total
	RIF^RINH^R	RIF^RINH^S	RIF^SINH^S	RIF^SINH^R	
RIF^R	13	4	3	1	21
RIF^S	4	1	88	7	100
Total	17	5	91	8	121

R: drug resistant; S: drug susceptible.

and 96.2% (100/104) specificity in estimation of MDR-TB (**Table 4**).

Discussion

Xpert MTB/RIF is a TB-specific automated, cartridge-based nucleic amplification assay with fully integrated and automated sample preparation, amplification and detection of Mtb, as well as, rifampin resistance, within two hours. Importantly, the Xpert MTB/RIF assay procedure and sample testing has minimal biosafety concerns with no detectable infectious aerosols. The WHO has recommended widespread use of Xpert MTB/RIF for detection of TB and rifampicin resistance, and as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB [3]. Our study extended the performance evaluation of Xpert MTB/RIF assay with pulmonary and extrapulmonary TB cases in a high TB-burden setting in China.

This study is different from most of previous studies [5-7] that we used clinical diagnosis of active TB (combining laboratory tests, clinical manifestation, chest radiography, and response to anti-TB treatment) as a standard to evaluate Xpert MTB/RIF assay performance. Among pulmonary TB cases, the Xpert MTB/RIF assay presented a comparable or better sensitivity than Mtb culture (67.7% vs. 65.1%), and fluorescent staining smear tests (67.7% vs. 61.5%) with 100% specificity identifying clinical pulmonary TB cases. As for extra-pulmonary TB cases, Mtb culture showed a higher sensitivity (23.8% vs. 19.0%) than the Xpert MTB/RIF assay. Compared with the data from most previous studies in low-incidence countries [8], the Xpert MTB/RIF assay presented a relatively lower sensitivity (77.2%) in identification of RIF resistant. Molecular epidemiologic studies in TB endemic countries have shown that mixed Mtb infections could account for up to 50% of TB cases (9). Patients may have concurrent infections of drug-susceptible and resistant Mtb isolates. In these cases, the sensitivity of Xpert MTB/RIF assay could be decreased to 80%, which is consistent with our results [9].

China has the highest incidence of MDR-TB in the world, accounting for 24% of global MDR-TB burden [4]. Previous studies have shown 80-90% of RIF resistant TB cases may also have INH resistance (i.e., MDR-TB) [4]. In our study cohort, only 77.3% (17/22) of DST proven RIF resistant cases had MDR-TB disease, of which Xpert MTB/RIF assay only identified 76.5% of these MDR-TB (13/17). Therefore, the Xpert MTB/RIF assay result may not be specific enough to be utilized as an indicator of MDR-TB in China, which is consistent with a recent finding [10].

Conclusion

The Xpert MTB/RIF assay is a rapid and specific test for active TB diagnosis, and has a comparable sensitivity with Mtb culture for pulmonary TB, but less sensitive than Mtb culture for extrapulmonary TB. Combined three methods achieved a better sensitivity than any individual test.

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Disclosure of conflict of interest

None.

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