Original Article

Comparison of Two Molecular Assays For Detecting Smear Negative Pulmonary Tuberculosis

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Abstract

Objective To compare the performance of MTBDRplus V2 and Xpert MTB/RIF for detecting smear negative pulmonary tuberculosis (PTB).

Methods Clinical PTB suspects were enrolled consecutively in Anhui Chest Hospital and Xi'an Chest Hospital from January to December in 2014. The sputum samples of smear negative PTB suspects were collected and decontaminated. The sediment was used to conduct MTBDRplus V2, Xpert MTB/RIF and drug susceptibility test (DST). All the samples with discrepant drug susceptibility result between molecular methods and phenotypic method were confirmed by DNA sequencing.

Results A total of 1973 cases were enrolled in this study. The detection rates of *Mycobacterium tuberculosis* complex (MTBC) by MTBDRplus V2 and Xpert MTB/RIF were 27.67% and 27.98%, respectively. When setting MGIT culture result as a gold standard, the sensitivity and specificity of MTBDRplus V2 were 86.74% and 93.84%, and the sensitivity and specificity of Xpert MTB/RIF were 86.55% and 93.43%, respectively. For the detection of the resistance to rifampin, the sensitivity and specificity of MTBDRplus V2 were 94.34% and 96.62%, and the sensitivity and specificity of Xpert MTB/RIF were 88.68% and 95.96%, respectively. For the detection of the resistance to isoniazid, the sensitivity and specificity of MTBDRplus V2 were 77.38% and 98.02%, respectively.

Conclusion MTBDRplus V2 and Xpert MTB/RIF can be used to detect MTBC in smear negative samples with satisfactory performance.

Key words: Smear negative pulmonary tuberculosis, Diagnosis; Drug resistance

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INTRODUCTION

Tuberculosis (TB) remains a major global public health problem, affecting millions of people each year, and TB is the second leading cause of death among infectious diseases worldwide[1]. China ranks third in the countries with heavy TB burden in the world. In recent years, the proportion of smear positive TB cases declined, while the prevalence of active PTB showed no significant decrease according to the fifth national TB epidemiology survey in China in 2010[2]. Smear negative PTB patients, especially drug resistant patients, cannot receive timely and effective diagnosis and treatment due to the lack of sensitive laboratory test.

Because of its rapid detections for MTBC and the resistance to rifampin, Xpert MTB/RIF (Cepheid, USA) is widely used in the world[3-4]. Multicenter studies have demonstrated that Xpert MTB/RIF can be used for the detection of MTBC with high sensitivity and specificity[5-6], and it was recommended by World Health Organization (WHO) for the diagnosis of TB in 2010[7]. MTBDRplus V1 (Hain, Germany) can be used to detect MTBC and the resistance to rifampin and

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isoniazid from smear positive sputum with high accuracy[8-9] in 1 work day, and it was recommended by WHO for screening of multidrug resistance tuberculosis (MDR TB) in countries with heavy TB burden[10]. MTBDRplus V2 has significantly improved sensitivity of detection for MTBC compared with MTBDRplus V1, which can be directly applied to test the sputum samples collected from TB suspects[11]. However, there is limited data of MTBDRplus V2 in the clinical practice.

In this study, the performance of MTBDRplus V2 and Xpert MTB/RIF were compared among smear negative PTB suspects to provide scientific evidence for the diagnosis of smear negative PTB.

MATERIALS AND METHODS

Clinical Sample

PTB suspects were enrolled at outpatient departments in Anhui Chest Hospital and Xi'an Chest Hospital from January to December in 2014. One sputum sample was collected from each patient for different laboratory tests. After smear tests at laboratory, a total of 1993 smear negative sputum samples were collected for other laboratory tests.

Sample Processing

A 2 mL sputum sample of each suspect was processed by using N-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method[12]. The supernate was discarded following centrifugation, and the sediments were resuspended in 2 mL of phosphate buffer solution. Three aliquots were prepared to perform MTBDRplus V2, Xpert MTB/RIF and MGIT 960 culture.

MTBDRplus V2 test

The assay was performed according to manufacturer's protocol (Hain, germany)[13]. The test has three steps: DNA extraction, PCR amplification and hybridization.

Xpert MTB/RIF test

The test was conducted according to manufacturer's protocol (Cepheid USA). 0.5 mL aliquot was mixed with sample reagent buffer at a ratio of 3:1, followed by incubation at room temperature for 15 min. Two mL sample was transferred to Xpert MTB/RIF cartridge and the cartridge was loaded into the instrument.

BACTEC MGIT 960 Culture and Drug Susceptibility Test

A 0.5 mL aliquot sample was inoculated in Bactec-MGIT 960 tube. After the culture flashed positive, the susceptibility test to rifampin and isoniazid was performed according to the manufacturer's protocol[14].

Sequencing

All the culture positive strains were collected for DNA sequencing to identify TB related gene (*16S rRNA*) and drug resistance related gene mutation for rifampin (*rpoB*) and isoniazid (*katG* and *inhA*) at national TB reference laboratory (Table 1). The sequencing results were entered into the Basic Local Alignment Search Tool (BLAST), an international database (http://www.ncbi.nlm.nih.gov/BLAST), for the alignment with reference strain H37Rv. The mutations of *rpoB, katG*, and *inhA* gene were compared with H37Rv.

Data Analysis

SPSS 22.0 was used for data analysis. χ^2 test was used for comparison of detection rate of different methods.

Table 1. Primer used for Sequencing to Identify MTBC and Detection of Drug Resistance Related Genes

Gene	Primer Pairs (5'-3')	Amplification Length (bp)
16S rRNA	F: GGCCTAACCCTCGGGAGGGAG	440
	R: CCCGAGGCATATCGCAGCCTC	
rpoB	F: ACCGACGACATCGACCACTT	430
	R: GTACGGCGTTTCGATGAACC	
katG	F: AATCGATGGGCTTCAAGACG	500
	R: CTCGTAGCCGTACAGGATCTCG	
inhA	F: CCTCGCTGCCCAGAAAGGGA	248
	R: ATCCCCCGGTTTCCTCCGGT	

RESULTS

Performance of Different Methods in Detection of MTBC

Twenty cases were excluded due to culture contamination and error result of Xpert MTB/RIF test. The detection rates of MTBC by MTBDRplus V2, Xpert MTB/RIF and MGIT 960 were 27.67% (546/1973), 27.98% (552/1973), and 26.76% (528/1973). No significant difference was observed (P>0.05) (Table 2).

Out of 528 MTBC isolates identified by DNA sequencing, 458 was positive in MTBDRplus V2, the sensitivity was 86.74%, and 457 were positive in Xpert MTB/RIF, the sensitivity was 86.55%. The specificities of MTBDRplus V2 and Xpert MTB/RIF were 93.84% and 93.43%, respectively (Table 3).

Performance of MTBDRplus V2 and Xpert MTB/RIF in Detection of Resistance to Rifampin

The sensitivity and specificity MTBDRplus V2 and Xpert MTB/RIF in detection of resistance to rifampin were analyzed by using MGIT DST as standard. Among 53 resistant cases identified by MGIT DST, MTBDRplus V2 detected 50 resistant cases, the sensitivity was 94.34%, but Xpert MTB/RIF detected only 47 resistant cases, the sensitivity was 88.68%. The overall specificities of MTBDRplus V2 and Xpert MTB/RIF for rifampin susceptibility were 96.62% and 95.96%, respectively (Table 4).

The sequencing results demonstrated that the results of 12 strains were consistent with MTBDRplus V2 among 16 strains which had discrepant results between genotypic and phenotypic drug susceptibility result for rifampin. The result showed an accordance rate of 75%. However, only 11 out of

Table 2. Comparison of MTBDRplus V2, Xpert MTB/RIF and MGIT 960 Culture in Detection of MTBC

Sites	MTBDRplus V2 (%)	Xpert MTB/RIF (%)	MGIT 960[a] (%)	*P* Value[*]
Anhui	30.02 (290/966)	30.02 (290/966)	28.57 (276/966)	
Xi'an	25.42 (256/1007)	26.02 (262/1007)	25.02 (252/1007)	
Total	27.67 (546/1973)	27.98 (552/1973)	26.76 (528/1973)	0.974

Note. [a]MTBC identified by sequencing. [*]χ^2=0.052, P=0.974.

Table 3. Performance of MTBDRplus V2 and Xpert MTB/RIF in Detection of MTBC

Methods	Sites	Sensitivity (%)	Specificity (%)
MTBDRplus V2	Anhui		
	Correct No./Total No.(%)	241/276 (87.32)	641/690 (92.90)
	95% CI	83.4-91.2	91.0-94.8
	Xi'an		
	Correct No./Total No.(%)	217/252 (86.74)	715/755 (94.70)
	95% CI	81.8-90.4	93.1-96.3
	Total		
	Correct No./Total No.(%)	458/528 (86.74)	1356/1445 (93.84)
	95% CI	83.8-89.6	92.6-95.1
Xpert MTB/RIF	Anhui		
	Correct No./Total No.(%)	238/276 (86.23)	638/690 (92.46)
	95% CI	82.2-90.3	90.5-94.4
	Xi'an		
	Correct No./Total No.(%)	219/252 (86.90)	712/755 (94.30)
	95% CI	82.7-91.1	92.7-96.0
	Total		
	Correct No./Total No.(%)	457/528 (86.55)	1350/1445 (93.43)
	95% CI	83.6-89.5	92.1-94.7

22 strains that had discrepant results in the detection of resistance to rifampin between Xpert MTB/RIF and MGIT were identified by sequencing.

Performance of MTBDRplus V2 in Detection of Resistance to Isoniazid

Compared with MGIT DST, MTBDRplus V2 showed an overall sensitivity of 77.38% in the detection of the resistance to isoniazid. It correctly showed the susceptibility to isoniazid in 347 of 354 cases, with an overall specificity of 98.02% (Table 5).

For 26 strains with discrepant results between MTBDRplus V2 and MGIT DST for the detection of resistance to isoniazid, the accordance rate was about 88.46% (23/26) between sequencing result and MTBDRplus V2 result.

DISCUSSION

In this study, MTBDRplus V2 and Xpert MTB/RIF were firstly compared for detecting smear negative PTB suspects in China. Our results showed that both MTBDRplus V2 and Xpert MTB/RIF can efficiently provide bacterial evidence from sputum samples for almost one third of smear negative PTB suspects. Although the detection rates of MTBC by these two methods were little higher than that by MGIT culture, the turnaround time was significantly shortened from 15 days to 1 day. In addition, no difference was observed in performance between MTBDRplus V2 and Xpert MTB/RIF for the detection of MTBC.

Xpert MTB/RIF is a rapid and fully automatic assay. The evaluation at county level revealed that the performance for the detection of MTBC was

Table 4. Performance of MTBDRplus V2 and Xpert MTB/RIF in Detection of Resistance to Rifampin

Methods	Sites	Sensitivity (%)	Specificity (%)
MTBDRplus V2	Anhui		
	Correct No./Total No.(%)	28/30 (93.33)	202/208 (97.12)
	95% CI	84.4-100.0	94.8-99.4
	Xi'an		
	Correct No./Total No.(%)	22/23 (95.65)	170/177 (96.05)
	95% CI	87.3-100.0	93.2-98.9
	Total		
	Correct No./Total No.(%)	50/53 (94.34)	372/385 (96.62)
	95% CI	88.1-100.0	94.8-98.4
Xpert MTB/RIF	Anhui		
	Correct No./Total No.(%)	25/29 (86.21)	196/201 (97.51)
	95% CI	73.7-98.8	95.4-99.1
	Xi'an		
	Correct No./Total No.(%)	22/24 (86.21)	184/195 (94.36)
	95% CI	80.6-100.0	91.1-97.6
	Total		
	Correct No./Total No.(%)	47/53 (88.68)	380/396 (95.96)
	95% CI	80.1-97.2	94.0-97.9

Table 5. Performance of MTBDRplus V2 in Detection of Resistance to Isoniazed

Sites	Sensitivity (%)	Specificity (%)
Anhui		
Correct No./Total No.(%)	29/33 (87.88)	198/205 (96.59)
95% CI	76.7-99.0	94.1-99.1
Xi'an		
Correct No./Total No.(%)	36/51 (70.59)	149/149 (100.00)
95% CI	58.1-83.1	100.0-100.0
Total		
Correct No./Total No.(%)	65/84 (77.38)	347/354 (98.02)
95% CI	68.4-86.3	96.6-95.5

excellent [15]. However, after its wide use worldwide, false-negative and false-positive results in detecting resistance to rifampin were reported[16-17]. In our study, the sensitivity of Xpert MTB/RIF for the detection of resistance to rifampin was 88.68% and the specificity was 95.96% when compared with phenotypic DST. The sequencing of *rpoB* gene showed that only one half (8/16) of strains which were sensitive to rifampin by MGIT but were resistant by Xpert were identified as rifampin sensitive. Further analysis indicated that almost 90% (7/8) of the strains were reported to have low or very low bacteria load level for MTBC detection by Xpert MTB/RIF. Further improvement are needed when it is used for sputum samples with low bacteria load.

MTBDRplus V2 can detect the resistance to isoniazid and rifampin at the same time of detection of MTBC, which overcome the intrinsic shortcoming of Xpert for the diagnosis of MDR-TB. Our study indicates that the performance of MTBDRplus V2 is better than Xpert MTB/RIF for the detecting resistance to rifampin. The sensitivity for detection of resistance to rifampin was up to 94.34%, consistent with an international report[18]. Although the accordance rate between sequencing and MTBDRplus V2 for discrepant samples was lower than the research from India[19], it was similar to several other reports[20-21], which might be due to the high differentiation of MTBC circulating in China.

In addition, the sensitivity in the detection of resistance to isoniazid was not high for clinical use. The reason of low performance might be due to the inclusion of only *katG* gene and *inhA* gene in MTBDRplus V2 assay which conferred about 70% isoniazid-resistant isolates[22]. However, an important gene, *oxyR-ahpC,* which has been proven as an indicator of the resistance to isoniazid according to previous literatures[23-24], was not included in the test panel of MTBDRplus V2. Our results indicate that the detection of additional gene should be added to improve performance of MTBDRplus V2 for the detection of the resistance to isoniazid.

CONCLUSION

The present study indicates that both MTBDRplus V2 and Xpert MTB/RIF can be used as a rapid and reliable method for the detection of TB and its drug susceptibility in clinical smear negative sputum sample.

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