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RESEARCH ARTICLE

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Assessing the utility of the Xpert *Mycobacterium tuberculosis*/rifampin assay for analysis of bronchoalveolar lavage fluid in patients with suspected pulmonary tuberculosis

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Abstract

Background: There is limited research assessing the utility of the Xpert *Mycobacterium tuberculosis*/rifampin (MTB/RIF) assay for the analysis of bronchoalveolar lavage fluid (BALF) in Chinese patients with suspected pulmonary tuberculosis (PTB). Thus, our objective was to determine the diagnostic accuracy of the Xpert MTB/RIF assay and evaluate its utility for the determination of rifampicin resistance.

Methods: We retrospectively analyzed BALF from 214 patients with suspected PTB between January 2018 and March 2019. Using mycobacterial culture or final clinical diagnosis as the reference standard, the diagnostic accuracy of the smear microscopy (SM), tuberculosis bacillus DNA (TB-DNA), Xpert MTB/RIF assay, and the determination of rifampicin resistance based on the Xpert MTB/RIF assay were compared.

Results: As compared to mycobacterial culture, the sensitivity of the Xpert MTB/RIF assay, SM, and TB-DNA were 85.5% (74.2%–93.1%), 38.7% (26.6%–51.9%), and 67.7% (54.7%–79.1%), respectively. As compared to the final diagnosis, the specificity of the Xpert MTB/RIF assay, SM, and TB-DNA were 100.0% (95.9%–100.0%), 94.3% (87.1%–98.1%), and 98.9% (93.8%–100.0%), respectively. The sensitivity and specificity of the rifampicin resistance detection using the Xpert MTB/RIF assay were 100% and 98.0%, respectively, with liquid culture as the reference.

Conclusions: This study demonstrates that the analysis of BALF with the Xpert MTB/RIF assay provides a rapid and accurate tool for the early diagnosis of PTB. The accuracy of diagnosis was superior compared with the SM and TB-DNA. Moreover, Xpert is a quick and accurate method for the diagnosis of rifampicin-resistant tuberculosis and can also provide more effective guidance for the treatment of PTB or multidrug-resistant tuberculosis (MDR-TB).

KEYWORDS
bronchoalveolar lavage fluid, diagnosis, tuberculosis, Xpert MTB/RIF assay

Yong Pan and Xueqin Xu contributed equally to this work.

1 | INTRODUCTION

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*. In 2017, there were an estimated 10 million new tuberculosis cases worldwide; China accounted for 60% of all new tuberculosis cases.[1]

Currently, the diagnosis of tuberculosis primarily relies on the smear microscopy (SM) and mycobacterial culture; however, both have limited sensitivity.[2,3] Mycobacterial culture is still the gold standard for the diagnosis of tuberculosis, but it takes a long time (2–6 weeks) to obtain a result. As a result, the diagnosis of tuberculosis is delayed and drug resistance can occur, leading to the spread of tuberculosis. Therefore, the identification of a rapid and accurate diagnostic method is of great importance for the prevention and control of tuberculosis. With the development of molecular technology, many methods for the rapid diagnosis of tuberculosis have emerged in recent years. The Xpert *Mycobacterium tuberculosis*/rifampin (MTB/RIF) assay is one of them. The Xpert MTB/RIF assay is based on the real-time polymerase chain reaction technology; it simultaneously detects MTB and RIF resistance within 2 h. It is recommended by the World Health Organization (WHO) for the rapid diagnosis of MTB and the detection of RIF resistance.[1] However, it has not been routinely tested in Chinese laboratories. To date, there are many reports on the practical value of the Xpert MTB/RIF assay for the analysis of sputum in tuberculosis; however, it is difficult to ensure the quality of sputum specimens sent for examination, especially for patients with tuberculosis who are unable to self-discharge sputum or those without sputum. Unacceptable quality of the sputum samples received by laboratories is a common issue and has become a significant cause of low diagnostic yield.[4,5] An alternative to sputum is the bronchoalveolar lavage fluid (BALF). However, with the extensive application of the Xpert MTB/RIF detection in the diagnosis and treatment of early pulmonary tuberculosis (PTB), it is important to evaluate the diagnostic efficiency of the Xpert MTB/RIF assay on BALF and compare the accuracy of detecting rifampicin resistance with the Xpert MTB/RIF assay versus liquid culture.

2 | METHODS

A total of 214 patients hospitalized with suspected PTB at Wenzhou Central Hospital in China, between January 2018 and March 2019, were retrospectively reviewed. Clinical suspicion of PTB was based on the clinical features (eg, cough, hemoptysis, fever, night sweats, weight loss, and other clinical features of tuberculosis) or chest-computed tomography (CT) images suggestive of PTB (eg, nodules and shadows). Confirmed PTB was defined as a positive culture of *Mycobacterium tuberculosis*. Clinical diagnosis of tuberculosis was defined as (1) both clinical symptoms and radiological findings compatible with active PTB and (2) improvement in response to antituberculosis treatment within 2 months or caseating granulomatous inflammation present on the histological examination. The diagnostic criteria for nontuberculous mycobacterial lung disease were based on the integration of clinical, radiological, and microbiological findings.[6] The samples that were liquid culture-positive and SM-positive were tested by rapid immunochromatography. If the rapid immunochromatographic test was positive, tuberculous mycobacteria was considered; if negative, the sample was submitted to the DNA microarray chip analysis. Identification of nontuberculous mycobacteria species was carried out by the DNA microarray chip analysis. This study was approved by the ethics committee of Wenzhou Central Hospital. The need to obtain informed consent was waived due to the retrospective nature of the study. Patients who had previously received antituberculosis drugs were excluded from the analysis.

2.1 | Xpert MTB/RIF assay

A volume of 1 ml of BALF was transferred into the Xpert MTB/RIF (Cepheid, USA) cartridge, and a 1:1 volume of sample reagent buffer was added. Then, the sample was thoroughly mixed, and the Xpert MTB/RIF cartridge was left to stand at room temperature for 15 min. The Xpert MTB/RIF assay was then performed according to the manufacturer's instructions.

2.2 | SM, tuberculosis bacillus DNA

Acid-fast bacilli staining (Korea Standard Instruments Co. Ltd.) was used for SM. TB-DNA was analyzed using the fluorescent polymerase chain reaction (PCR) method (Amplly, Xiamen, China). The samples were liquefied and centrifuged to obtain a precipitate. Then, the nucleic acid extract and sediment were added to the nucleic acid extraction tube. PCR was carried out under the following conditions: predenaturation at 95°C for 5 min, followed by 95°C for 15 s, 58°C for 50 s, 40 cycles. This procedure was carried out in accordance with the manufacturer's instructions.

2.3 | Mycobacteria growth indicator tube (MGIT) drug-susceptibility testing

A mycobacteria growth indicator tube (MGIT) 960 system (BD Diagnositics) was used for the liquid culture. All strains inoculated in the MGITs were incubated in the MGIT 960 instrument. The culture was used for susceptibility testing within one to five days of the instrument returning a positive signal. MGIT suspension culture broth was used undiluted on the first and second day following the positive report; the suspensions were diluted 1:5 with sterile saline on the third to fifth day. The culture was subcultured in a new MGIT if it was more than five days since the instrument returned a positive result. Then, susceptibility testing using the MGIT 960 system was performed (growth control, streptomycin

at 1.0 μg/ml, isoniazid at 0.1 μg/ml, rifampicin at 1.0 μg/ml, ethambutol at 5 μg/ml). The results were reported using the predefined algorithms.

2.4 | Rapid immunochromatographic test

The samples that were liquid culture-positive and SM-positive were submitted to rapid immunochromatography (SD MPT64TB Ag kit) (Alere, Shanghai, China). This test is highly specific for the *Mycobacterium tuberculosis* complex, including *M. tuberculosis* (MTB), *Mycobacteriuma fricanum*, *M. bovis*, and some substrains of *M. bovis* bacilli Calmette-Guerin (BCG).[7,8] The culture, 0.1 ml, was placed on the sample kit area, and the analysis of colloidal gold was performed. The result was read within 15–60 min at room temperature. This procedure was performed according to the manufacturer's instructions.

2.5 | DNA microarray chip

The bacterial strains were identified by the DNA microarray chip analysis (CapitalBio Technology).

2.5.1 | PCR amplification

The amplification reaction system was 20 μl, consisting of 18 μl for the amplification reaction solution and 2 μl for the DNA template. PCR was carried out under the following conditions: initial activation at 94°C for 10 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 40 s, followed by 10 cycles at 94°C for 30 s and 72°C for 60 s, and then remaining at 72°C for 7 min.

2.5.2 | Hybridization and determination of the results

A total of 13.5 μl of hybridization mixture (a ratio of 9 μl of hybridization buffer and 6 μl of PCR products) was added to the chip array through the wells. Hybridization was carried out at 50°C for 2 h. The results were determined using a LuxScanTM 10KB microarray chip scanner.

2.6 | Statistical analysis

SPSS 23.0 (IBM) and Medcalc for Windows, version 11.4.2.0 (MedCalc Software, Ostend, Belgium), were used for statistical analyses. Continuous variables are expressed as mean (SD). Sensitivity, specificity, accuracy, and predictive values were calculated with 95% confidence intervals (95% CI). McNemar's test was used for the comparison of sensitivities. A two-tailed $p < 0.05$ was considered statistically significant.

TABLE 1 Demographic and clinical characteristics of 214 patients with suspected pulmonary tuberculosis

Characteristic	*N* (%)
Median age, years	45.0 ± 18.4
Gender	
Male	137 (64.0)
Female	77 (36.0)
Symptoms	
Smoking	49 (22.9)
Cough	162 (75.7)
Expectoration	143 (66.8)
Hemoptysis	58 (27.1)
Chest pain	32 (15.0)
Chest distress	31 (14.5)
Fever	39 (18.2)
Night sweat	13 (6.1)
Asthenia	24 (11.2)
Loss of weight	18 (8.4)
Radiological features	
Nodules	83 (48.5)
Patchy shadow	134 (78.3)
Cavitation	50 (29.2)
Mediastinal lymphadenopathy	70 (40.9)
Lack of imaging data	43 (20.0)
Final diagnosis	
Pulmonary tuberculosis	127 (59.35)
Previous pulmonary tuberculosis	16 (7.48)
Nontuberculous mycobacterial lung disease	9 (4.21)
Bacterial pneumonia	42 (19.63)
Pulmonary fungal infection	7 (3.27)
Lung cancer	6 (2.80)
Enterophthisis	2 (0.93)
COPD	2 (0.93)
Silicosis	3 (1.40)

Abbreviation: COPD, chronic obstructive pulmonary diseases. Age is expressed as the median (SD).

3 | RESULTS

3.1 | Patient characteristics

We identified 214 patients with suspected PTB who underwent bronchoscopy during the study period. A total of 62 patients had culture-confirmed PTB. Based on the clinical data, including clinical symptoms, chest CT images, and response to treatment, 127 patients were clinically diagnosed with tuberculosis. The demographic and clinical-radiological characteristics of the patients with suspected PTB are shown in Table 1. The principal clinical symptoms were cough (75.7%), expectoration (66.8%), and hemoptysis (27.1%). Among the 214 patients, 137 (64.0%) were male and 77

(36.0%) were female. The main radiological features were patchy shadows (78.3%) and nodules (48.5%). Among the nine patients with nontuberculous mycobacterial infection, seven had *M. intracellulare* and two had mixed infection of *M. intracellulare* and *M. avium*, as identified by the DNA microarray chip analysis (data not shown).

3.2 | Diagnostic parameters with culture as the reference standard

For the 62 cases of culture-positive PTB, the sensitivity of the Xpert MTB/RIF assay (85.5%; 95% CI 74.2–93.1%) was significantly higher than that of SM (38.7%; 26.6%–51.9%) with culture as the reference standard, $p < 0.05$. Among the 152 culture-negative patients, 22 were positive according to the Xpert MTB/RIF assay (22 patients were finally clinically diagnosed with tuberculosis) and 13 were positive according to SM (eight were clinically diagnosed with tuberculosis, and the remaining five patients were diagnosed with nontuberculous mycobacterial disease). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TB-DNA were 67.7% (95% CI 54.7%–79.1%), 85.5% (95% CI 78.9%–90.7%), 65.6% (95% CI 55.6%–74.5%), and 86.7% (95% CI 81.8%–90.4%), respectively (Table 2). Compared with the Xpert MTB/RIF assay, the diagnostic performance of TB-DNA (eg, sensitivity, PPV, and NPV) was poor.

3.3 | Diagnostic parameters with clinical diagnosis as the reference standard

The sensitivity of the Xpert MTB/RIF assay (59.1%; 95% CI 50.0%–67.7%) was significantly higher than that of the culture (48.8%; 95% CI 39.9%–57.8%), SM (25.2%; 95% CI 17.9%–33.7%), and TB-DNA (49.6%; 95% CI 40.6%–58.6%) with clinical diagnosis as the reference standard (Table 3). When we combined the Xpert MTB/RIF assay, TB-DNA and SM results, the diagnostic accuracy was no better than the Xpert MTB/RIF assay alone, $p = 0.2$ (data not shown).

3.4 | Gain in early pulmonary tuberculosis diagnosis

Compared with SM and TB-DNA, the Xpert MTB/RIF assay exhibited gains of 29/62 (46.8%) and 11/62 (17.7%), respectively, for the early diagnosis of culture-confirmed PTB and gains of 43/127 (33.9%) and 12/127 (9.4%), respectively, for the final diagnosis of PTB (Table 4).

3.5 | Assessment of resistance to rifampicin in *Mycobacterium tuberculosis* infection using the Xpert MTB/RIF assay and conventional drug susceptibility assay

Of the 214 patients, 62 were liquid culture-positive, and among these, nine were negative on the Xpert MTB/RIF assay; a total of 53 cases were included in the final analysis. The diagnostic efficacy of the Xpert MTB/RIF assay for the detection of rifampicin resistance is shown in Table 5. The sensitivity and specificity of the Xpert MTB/RIF assay for the detection of rifampicin resistance were 100% and 98.0%, respectively. The RIF critical concentrations of isoniazid/rifampicin/streptomycin/ethambutol (H/R/S/E) used in the MGIT culture system were 0.1 g/ml, 1.0 μg/ml, 1.0 μg/ml, and 5 μg/ml, respectively (Table 6). In the current study, three patients were rifampicin-resistant as determined by the Xpert MTB/RIF assay. One was susceptible to H/R/S/E, and two had multidrug-resistant tuberculosis (MDR-TB), one of which was resistant to H/R, and the other was resistant to H/R/S, as determined by a conventional drug susceptibility assay (Table 7). Three patients were rifampicin-resistant as determined by a conventional drug susceptibility assay, and two cases were resistant to rifampicin as determined by the Xpert MTB/RIF assay. The remaining case was negative according to the Xpert MTB/RIF assay.

TABLE 2 Performance of SM, TB-DNA, and Xpert MTB/RIF using culture as the reference for the detection of pulmonary tuberculosis

Tests	Sensitivity% (95% CI) *n*	Specificity% (95% CI) *n*	Accuracy% (95% CI)	PPV % (95% CI)	NPV % (95% CI)
SM	38.7 (26.6–51.9) 24/62	91.4 (85.8–95.4) 139/152	76.2 (69.9–81.7)	64.9 (50.2–77.2)	78.5 (74.9–81.8)
TB-DNA	67.7 (54.7–79.1) 42/62	85.5 (78.9–90.7) 130/152	80.4 (74.4–85.5)	65.6 (55.6–74.5)	86.7 (81.8–90.4)
Xpert MTB/RIF	85.5 (74.2–93.1) 53/62	85.5 (78.9–90.7) 130/152	85.5 (80.1–89.9)	70.7 (61.8–78.2)	93.5 (88.7–96.4)

Abbrevitions: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; SM, smear microscopy; TB-DNA, tuberculosis bacillus DNA.

TABLE 3 Performance of SM, TB-DNA, Xpert MTB/RIF, and culture using clinical diagnosis as the reference for the detection of pulmonary tuberculosis

Tests	Sensitivity% (95% CI) *n*	Specificity% (95% CI) *n*	Accuracy% (95% CI)	PPV % (95% CI)	NPV % (95% CI)
SM	25.2 (17.9–33. 7) 32/127	94.3 (87.1–98.1) 82/87	53.3 (46.4–60.1)	86.5 (72.2–94.0)	46.3 (43.5–49.2)
TB-DNA	49.6 (40.6–58.6) 63/127	98.9 (93.8–100.0) 86/87	69.6 (63.0–75.7)	98.4 (89.9–99. 8)	57.3 (53.0–61.5)
Xpert MTB/RIF	59.1 (50.0–67.7) 75/127	100.0 (95.9–100.0) 87/87	75.7 (69.4–81.3)	100.0 -	62.6 (57.6–67.3)
Culture	48.8 (39.9–57.8) 62/127	100.0 (95.9–100.0) 87/87	69.6 (63.0–75.7)	100.0 -	57.2 (53.0–61.3)

Abbreviations: SM, smear microscopy; TB-DNA, tuberculosis bacillus DNA; PPV, positive predictive value; NPV, negative predictive value. CI, confidence interval.

TABLE 4 Gain in early pulmonary tuberculosis diagnosis ($n = 214$)

	Culture-confirmed ($n = 62$)	Final diagnosis ($n = 127$)
Xpert MTB/RIF-positive	53	75
SM-positive	24	32
TB-DNA	42	63
Gain in early PTB diagnosis*	29	43
Gain in early PTB diagnosis #	11	12

Abbreviations: #, comparison of Xpert MTB/RIF and TB-DNA; *, comparison of Xpert MTB/RIF and SM; PTB, pulmonary tuberculosis; SM, smear microscopy.

TABLE 5 Diagnostic accuracy of Xpert MTB/RIF for the detection of RMP resistance using the conventional drug susceptibility assay as the reference

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Xpert MTB/RIF	*n* (%)	*n* (%)	*n* (%)	*n* (%)
RMP resistance detection	2 (100)	50 (98.0)	2 (66.7)	50 (100)

Abbreviation: RMP, rifampicin.

TABLE 6 Analysis of *Mycobacterium tuberculosis* resistance to rifampicin and isoniazid by the Xpert MTB/RIF and conventional drug susceptibility assays and the RIF critical concentration of H/R/S/E

Samples		Xpert MTB/RIF	Conventional drug susceptibility assay@rifampicin / isoniazid / streptomycin / ethambutol			
Resistance						
Total	Liquid culture	Xpert MTB/RIF	Rifampicin	Rifampicin@ (1.0 μg/ml)	Isoniazid@ (0.1 g/ml)	Streptomycin@ (1.0 μg/ml)	Ethambutol@ (5 μg/ml)
214	62	75	3 (4%)	3 (4.8%)	6 (9.7%)	6 (9.7%)	0 (0)

4 | DISCUSSION

In this study, we evaluated the utility of the Xpert MTB/RIF assay for the analysis of BALF in patients with suspected PTB; mycobacterial culture or final diagnosis based on clinical criteria was used as the reference standard. The accuracy of the Xpert MTB/RIF assay on BALF was found to be higher than the accuracy of SM and TB-DNA, respectively; all $p < 0.05$. In this study, all MDR-TB cases were identified by the Xpert MTB/RIF assay. Thus, the Xpert MTB/RIF assay may serve as an important initial diagnostic test for patients suspected of having MDR-TB.[9]

At present, acid-fast bacilli staining and culture are routine tests in hospitals.[10-12] However, the low sensitivity of SM limits its use for the diagnosis of tuberculosis. Furthermore, SM cannot distinguish

TABLE 7 Analysis of *Mycobacterium tuberculosis* resistance to rifampicin by the Xpert MTB/RIF and conventional drug susceptibility assays

	Liquid culture		
Xpert MTB/RIF	**RMP-resistant**	**RMP-susceptible**	**Total**
RMP-resistant	2	1	3
RMP-susceptible	0	50	50
Total	2	51	53

between *Mycobacterium tuberculosis* and nontuberculosis mycobacteria. Mycobacterial culture is the gold standard for the diagnosis of tuberculosis; however, the results take a long time to obtain, and thus, it is not able to provide rapid early clinical diagnosis. As such, the development of a highly accurate and early detection method is imperative for the diagnosis of patients with suspected PTB. With the development of bronchoscopy, the Xpert MTB/RIF assay has been routinely carried out on BALF in our hospital (a comprehensive teaching hospital and tuberculosis-focused hospital in China) for the diagnosis of PTB.

When culture was used as the reference standard, the Xpert MTB/RIF assay showed high sensitivity (85.5%; 95% CI 74.2%–93.1%), which is consistent with previous studies.[13-17] In contrast, the sensitivities of SM and TB-DNA were low, 38.7% (95% CI 26.6%–51.9%) and 67.7% (95% CI 54.7%–79.1%), respectively. In addition, compared with SM and TB-DNA, the Xpert MTB/RIF assay exhibited gains of 29/62 (46.8%) and 11/62 (17.7%), respectively, for the early diagnosis of suspected PTB.[15,16] This can be explained by the analytical limit of detection of the Xpert MTB/RIF assay. Specifically, the analytical limit of detection of the Xpert MTB/RIF assay is 131 colony-forming units (cfu)/ml, while that of SM is 10,000 cfu/ml.[18]

In our study, nine cases were found to be Xpert MTB/RIF assay-negative but culture-positive. Ultimately, all nine cases were clinically diagnosed with PTB. These false negatives may be due to PCR inhibitors (eg, bloody BALF) or insufficient nucleic acid material in some specimens.[19] In addition, a total of 22 patients were found to be Xpert MTB/RIF-positive but culture-negative. When clinical diagnosis was used as the reference standard, these 22 patients were eventually diagnosed with PTB. This occurred because the Xpert MTB/RIF assay is a fast, automated PCR method that amplifies any DNA, whether from live or dead bacilli.[20] However, positive culture requires live bacilli. Furthermore, Pagliotto et al. reported that beta-lactams contain early antitubercular activity. In our study, some patients had used nontuberculosis antibiotics (eg, beta-lactams) before antituberculosis treatment; this might have resulted in negative cultures. We hypothesize that these factors led to a positive Xpert MTB/RIF result but negative culture result. Culture analysis of BALF can miss a number of cases in patients with suspected PTB. Thus, it is still necessary to obtain a clinical diagnosis based on the various detection results and clinical manifestations combined with the analysis of culture.[21]

When we used clinical diagnosis as the reference standard, the accuracy of the Xpert MTB/RIF assay (75.7%; 95% CI 69.4%–81.3%) was higher than SM (53.3%; 95% CI 46.4%–60.1%), TB-DNA (69.6%; 95% CI 63.0%–75.7%), and culture (69.6%; 95% CI 63.0%–75.7%); all $p < 0.05$. A combination of the above parameters (Xpert MTB/RIF assay, SM, and TB-DNA) had a diagnostic sensitivity that was not significantly different from the sensitivity of the Xpert MTB/RIF assay alone, $p = 0.2$. This result may imply that the Xpert MTB/RIF assay is superior to the traditional diagnostic indicators (SM and TB-DNA).

Previous studies have reported sensitivities and specificities of the Xpert MTB/RIF assay for the detection of rifampicin resistance ranging from 92.9%–100.0% and 98.6%–100. 0%, respectively.[22,23] This is consistent with the results of our study. It has been shown that resistance to rifampicin is often a marker of drug resistance, and almost 90% of the rifampicin-resistant strains are also resistant to isoniazid. However, the resistance of *Mycobacterium tuberculosis* to rifampin (RMP) in nearly 97% of isolates is due to mutations in an 81-bp rifampin resistance-determining region (RRDR) of the *rpoB* gene.[24-26] Given the importance of prompt and accurate identification of rifampicin resistance, in the USA, the use of the Xpert MTB/RIF assay to confirm rifampicin resistance has been recommended in all TB cases.[27] In our study, all MDR-TB cases were identified by the Xpert MTB/RIF assay. Thus, the Xpert MTB/RIF assay may serve as an important initial diagnostic test for patients suspected of having MDR-TB. However, one in three cases returned inconsistent results across the two methods; the Xpert MTB/RIF assay was able to detect rifampicin-resistant tuberculosis, whereas the conventional drug susceptibility assay was unable to. This discordance between the Xpert MTB/RIF assay and conventional drug susceptibility assay for the detection of rifampin-resistant *Mycobacterium tuberculosis* has been reported previously.[28,29] The possible reasons for the inconsistency between the two methods are as follows. First, silent mutations within the RRDR of the *rpoB* gene have been reported.[30,31] The most probable reason for the Xpert assay result returning a false-positive RMP resistance result is because the silent mutations do not change the properties of encoded proteins. As a result, Xpert detects mutation of the *rpoB* gene, and the conventional drug susceptibility assay shows phenotypic susceptibility. The second possible reason for the inconsistency is laboratory error when performing the MGIT phenotypic conventional drug susceptibility assay, as reported by Hofmann-Thiel S. et al.[32,33] Thus, any discordant rifampin resistance results should be confirmed by sequencing of the *rpoB* gene.

The present study has several limitations. First, it was conducted retrospectively at a single center, and the missing data may have caused some biases. Further prospective, multicenter studies should be performed. Second, this study did not perform the Xpert MTB/RIF assay on sputum. Therefore, we cannot compare the diagnostic accuracy of the Xpert MTB/RIF assay on BALF and sputum. Third, bronchoscopy is not feasible in resource-poor settings. This may limit the widespread application of this technique. Fourth, when the rapid immunochromatographic test was positive, the mixed infection of tuberculosis and nontuberculous mycobacteria was not taken into account. Fifth, the case with

discordant rifampin resistance results from the Xpert MTB/RIF assay, and the MGIT 960 culture was not confirmed by sequencing of the *rpoB* gene.

The main strength of this study was the evaluation of rifampicin resistance in BALF samples taken from a Chinese sample. This is important as variations in the specificity and sensitivity of the Xpert MTB/RIF assay reported in the previous studies may originate from the geographical features of the sampling location, differences in sampling methods, the presence of MDR-TB, and mutations on the *rpoB* gene in specific populations.[34]

5 | CONCLUSION

In summary, this study has shown that the Xpert MTB/RIF assay of BALF is an accurate and more rapid tool for the early diagnosis of PTB. This assay can also provide more effective guidance for the treatment of PTB or MDR-TB. The rapid and accurate laboratory diagnosis of MDR-TB is crucial for the effective treatment and can assist with limiting the transmission of MDR-TB.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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