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Research Article

Concordance of Sputum and Feces Samples for Detecting Mycobacterium Tuberculosis using Xpert® MTB/RIF Ultra

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ABSTRACT

Introduction: Tuberculosis (TB) remains a disease with high morbidity and mortality worldwide, and Indonesia ranks among the countries with the highest TB prevalence. There is a need to develop improved detection tools and explore alternative sample sources beyond sputum. Feces samples are one such non-sputum alternative. Xpert® MTB/RIF Ultra is a novel diagnostic tool used in Indonesia. This study aims to assess the concordance of both sputum and feces samples in detecting Mycobacterium tuberculosis (MTB) using Xpert® MTB/RIF Ultra. **Methods:** An analytical observational study with a cross-sectional design was conducted on TB subjects at Dr. Soetomo Regional Public Hospital (RSUD Dr. Soetomo), Surabaya, and several community health centers (puskesmas) in Surabaya. Sputum and feces samples were collected from the same subjects. These subjects underwent Feces acid-fast bacilli (AFB) tests, sputum and Feces Xpert® MTB/RIF Ultra tests, and sputum culture tests (considered the gold standard), as well as rifampicin resistance tests for positive cultures. Sensitivity and positive predictive value (PPV) tests were conducted using Medcalc software, and the concordance test employed the Kappa value. **Results:** The study involved 71 research subjects. The sensitivity of Feces AFB tests, sputum, and Feces Xpert® MTB/RIF Ultra tests was 7.3%, 97.6%, and 97.6%, respectively. The Cohen's Kappa consistency test for Feces AFB tests and sputum culture produced a Kappa value of 0.063 ($p > 0.05$). The Cohen's Kappa consistency test on sputum and Feces Xpert® MTB/RIF Ultra tests yielded a Kappa value of 0.409 ($p < 0.05$). The Cohen's Kappa consistency test on sputum and Feces Xpert® MTB/RIF Ultra tests compared with the rifampicin resistance tests resulted in Kappa values of 0.902 and 0.951 ($p < 0.05$). The CT value of Feces Xpert® MTB/RIF Ultra tests was higher than that of sputum Xpert® MTB/RIF Ultra tests. **Conclusion:** A concordance exists between the results of sputum and Feces Xpert® MTB/RIF Ultra tests, but no concordance is observed between the results of Feces AFB tests and sputum culture tests. The higher CT value of Feces Xpert® MTB/RIF Ultra tests compared to sputum Xpert® MTB/RIF Ultra tests indicates a lower bacterial load in feces. Feces can be considered a viable alternative sample to sputum for MTB detection using Xpert® MTB/RIF Ultra.

Keywords: Xpert® MTB/RIF Ultra, Mycobacterium tuberculosis (MTB), Feces AFB test, Feces Xpert® MTB/RIF Ultra test, rifampicin resistance test.

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INTRODUCTION

Tuberculosis (TB) is an infectious disease and a leading global cause of death.[1,2] In 2020, there were 5.8 million new TB cases, and Indonesia ranks as the second-highest country in TB prevalence worldwide. It is estimated that there are 845,000 new TB cases annually, resulting in a death rate of 98,000 cases, equivalent to approximately 11 deaths per hour.[2] One of the World Health Organization's (WHO) strategies is to detect Mycobacterium tuberculosis (MTB) at the earliest possible stage, aiming to bring an end to the global TB epidemic.[1] Accurate and prompt diagnosis is a fundamental priority in TB control programs.[3] Rapid diagnosis enables clinicians to promptly initiate treatment, reducing TB transmission, expediting the patient's recovery, and minimizing the emergence of multidrug-resistant (MDR) or extensively drug-resistant (XDR) strains.[4] Prevention of MDR also necessitates the involvement of various stakeholders, including the patient's family.[5]

TB diagnosis commonly involves the examination of sputum specimens, with the identification of MTB bacteria through microscopic examination, molecular Polymerase Chain Reaction (PCR), or culture. While culture remains the gold standard for TB diagnosis, it is time-consuming.[6] PCR, specifically with Xpert® MTB/RIF, is endorsed by WHO globally, including in Indonesia, as a rapid molecular test (TCM) for diagnosing TB and drug resistance in under 2 hours.[7,8] Xpert® MTB/RIF Ultra, the latest TCM used in Indonesia, has a lower Limit of Detection (LOD) compared to Xpert® MTB/RIF.[9]

Collecting sputum samples can be challenging, especially for the elderly, children, and uncooperative patients. Invasive induction methods, such as bronchoscopy, can be used[10], but these are impractical in peripheral health facilities and may cause patient discomfort, leading to reluctance to provide sputum samples. Feces sample collection, being more convenient and less invasive, is explored in this study to assess its concordance alongside sputum samples when using Xpert® MTB/RIF Ultra. This exploration aims to offer alternative sample options for TB diagnosis.

MATERIALS AND METHODS

Research Sample

This analytical observational study employed a cross-sectional design and involved 71 sputum and Feces samples collected from individuals with pulmonary tuberculosis (TB) treated at RSUD Dr. Soetomo,

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Surabaya, and several community health centers (puskesmas) in Surabaya from November 2022 to February 2023. Inclusion criteria encompassed individuals of all ages and genders capable of spontaneous phlegm production, those undergoing clinical and/or bacteriological tuberculosis examination, and those willing to participate by providing informed consent. Exclusion criteria included extrapulmonary TB patients, TB patients under treatment for more than one month, and samples with insufficient volume. Ethical approval for this research was obtained from the ethics committee of RSUD Dr. Soetomo.

Examinations

The study involved four types of examinations: Feces acid-fast bacilli (AFB) tests, sputum and Feces Xpert® MTB/RIF Ultra tests, sputum culture tests, and rifampicin resistance tests, conducted in case of positive cultures. The microscopic examination of AFB in the Feces samples utilized Ziehl Nellshen staining, followed by observation under a microscope. The sputum culture employed Lowenstein Jensen (LJ) media, which was observed weekly up to the eighth week. The sputum Xpert® MTB/RIF Ultra tests followed the manufacturer's protocol. The Feces Xpert® MTB/RIF Ultra tests required Feces sample preparation, involving the mixing of 1g of feces with 10 mL of phosphate-buffered saline (PBS) for 30 seconds until homogeneous, followed by a 15-minute incubation. Further processing was done by extracting 2 mL of the supernatant following the equipment manufacturer's instructions.

Data Analysis

The sensitivity values for the Feces AFB tests and the sputum and Feces Xpert® MTB/RIF Ultra tests were analyzed using Medcalc software. Concordance analysis was conducted using Cohen's Kappa consistency test in SPSS software.

RESULTS

Subject Characteristics and Research Results

The majority of the subjects fell within the 36-65 age group (71.8%). Several subjects presented with more than one comorbidity or complaint. The most common complaint was cough (77.3%), and the most common comorbidity was diabetes mellitus (DM) (76.7%). The results of the sputum Xpert® MTB/RIF Ultra tests indicated that the highest MTB intensity category was the high category, with 25 subjects (35.2%). In contrast, the results of the Feces Xpert® MTB/RIF Ultra tests showed that the highest MTB intensity category was the low category, comprising 32 subjects (45.1%). The sputum culture tests revealed that 30 subjects tested negative, with 4 of them showing Non-Tuberculosis Mycobacterium (NTM) results. The sputum Xpert® MTB Ultra tests detected traces in 3 subjects, and the Feces Xpert® MTB Ultra tests showed traces in 4 subjects. These trace values, adjusted for the clinical characteristics of the subjects, were categorized into detected or not detected. The majority of subjects with traces were included in the detected group. However, one subject with traces in the Feces Xpert® MTB Ultra tests was classified in the not detected group due to having a history of TB within the last five years. All subjects with indeterminate RIF resistance were classified in the not-detected RIF resistance group (refer to Table 1).

Table 1: General Characteristics of the Research Samples.

Sample Characteristics	n	%
Age (years)		
Mean ± Std. Deviation (min-max)	45.96 ± 15.45 (18-79)	
17–25 years	11	15.5
26–35 years	4	5.6
36–45 years	17	23.9
46–55 years	19	26.8
56–65 years	15	21.1
> 65 years	5	7.0
Sex		
Male	45	63.4
Female	26	36.6
Treatment Duration (days)		
Mean ± Std. Deviation (min-max)	6.56 ± 6.05 (0-28)	
0–7 days	45	63.4
8–14 days	21	29.6
15–21 days	4	5.6
22–30 days	1	1.4
Complaint		
Had complaints	65	91.5
Cough*	51	77.3
Shortness of breath*	30	45.5
Weight loss*	12	18.2
Decreased appetite*	8	12.1
Night sweats*	7	10.6
Pain*	7	10.6
Weak*	6	9.1
Others*	24	36.4
Had no complaints	6	8.5
Comorbidities		
Had comorbidities	30	42.3
DM*	23	76.7
Hypertension*	3	10
Hepatitis B*	4	13.3
Others*	7	26.7
Had no comorbidities	41	57.7
History of TB disease		
Yes	6	8.4
No	65	91.6
Feces AFB test results		
Negative	68	95.8
Positive	3	4.2
Sputum Xpert® MTB Ultra test results		
Not Detected	11	15.5
Detected		
Trace, no history of TB	2	2.8
Trace, HIV	1	1.4
Very Low	2	2.8
Low	18	25.4
Medium	12	16.9
High	25	35.2
Sputum Xpert® RIF Ultra test results		
Not Detected	40	56.3
Indeterminate, had no history of TB	3	2.8
Indeterminate, HIV	1	1.4
Detected	28	39.4
Feces Xpert® MTB Ultra test results		
Not Detected	12	16.9
Trace, had a history of TB < 5 years	1	1.4
Detected		
Trace, no history of TB	2	2.8
Trace, had a history of TB > 5 years	1	1.4
Very Low	17	23.9
Low	32	45.1
Medium	5	7
High	1	1.4
Feces Xpert® RIF Ultra test results		
Not Detected	40	56.3
Indeterminate, had no history of TB	2	2.8
Indeterminate, had a history of TB > 5 years	1	1.4
Indeterminate, had a history of TB < 5 years	1	1.4
Detected	27	38
Sputum Culture test results		
Negative	30	42.3
Positive	41	57.7
Rifampicin (40 µg/mL) resistance test		
Sensitive	21	51.2
Resistant	20	48.8

Note: * = percentage calculated from those who have complaints/comorbidities.

Table 2: Results of the Feces AFB Tests, Sputum Xpert® MTB/RIF Ultra Tests, and Feces Xpert® MTB/RIF Ultra Tests Compared with the Sputum Culture Tests.

Examinations	Sputum culture		Sensitivity (95% CI)	PPV (95% CI)
	Positive	Negative		
Feces AFB				
Positive	3	0	7.3 % (1.5-19,9)	100% (29.2-100)
Negative	38	30		
Sputum Xpert® MTB/RIF Ultra				
Detected	40	17	97.6% (87.1-99.9)	66.7% (60.7-72.1)
Not detected	1	13		
Feces Xpert® MTB/RIF Ultra				
Detected	40	15	97.6% (87.1-99.9)	72.73% (65-79.3)
Not detected	1	15		

Table 3: Results of the Sputum Xpert® MTB/RIF Ultra Tests and Feces Xpert® MTB/RIF Ultra Tests Compared with the Rifampicin Resistance Tests.

Examinations	Rifampicin Resistance		Concordance	
	Positive	Negative	Kappa value	p-value
Sputum Xpert® RIF Ultra test results			0.902	< 0.001
Detected	19	1		
Not detected	20	20		
Feces Xpert® MTB/RIF Ultra			0.951	< 0.001
Detected	20	1		
Not detected	0	20		

Table 4: Results of the Sputum and Feces Xpert® MTB/RIF Ultra Tests.

Sputum Xpert® MTB/RIF Ultra	Feces Xpert® MTB/RIF Ultra		Total
	Detected	Not detected	
Detected	54	6	60
Not detected	4	7	11
Total	58	13	71
Kappa value	= 0.409		
p-value	= < 0.001		

Results of the AFB tests and Xpert® MTB/RIF Ultra tests compared with the results of the sputum culture tests as the gold standard

The results of the AFB tests were compared with those of the sputum culture tests, and the concordance of the Feces AFB tests with the sputum culture tests resulted in a kappa value of 0.063 (p = 0.130). The RIF resistance results from the sputum and Feces Xpert® MTB/RIF Ultra tests were also compared with the results of the rifampicin resistance tests (Table 3).

Concordance between the Sputum Xpert® MTB/RIF Ultra Tests and the Feces Xpert® MTB/RIF Ultra Tests

The sputum and Feces Xpert® MTB/RIF Ultra tests resulted in 76.1% of detected subjects, with moderate agreement (κ 0.409) (Table 4).

Comparison between the Differences in the Targeted Genes in the Sputum and Feces Xpert® MTB/RIF Ultra Tests

A separate test was conducted to determine differences in the targeted genes between the Sputum and Feces Xpert® MTB/RIF Ultra tests. According to the non-parametric Wilcoxon Sign-Rank test, significant differences were found in the IS1081-6110, rpoB1, rpoB2, rpoB3, and rpoB4 genes.

For each targeted gene in detecting MTB, the median total CT value in the Feces Xpert® MTB/RIF Ultra tests was higher than that in the sputum Xpert® MTB/RIF Ultra tests. There was one subject with undetected MTB whose rpoB2 was detected, but the IS1081 & 6110 were not detected, thus not affecting the overall conclusion. There was one subject with detected MTB at a very low intensity whose rpoB3 was detected with a value of 0, indicating an undetected rpoB3 result. However, this did not impact the final determination of MTB detection because the IS1081 & 6110 and other rpoB genes were detected. The CT values in the high category were lower than the CT values in other intensity categories, except for rpoB3, where two samples were detected as having MTB at a very low intensity, and one of them had a value of 0, potentially affecting the mean and median values. Only one subject was observed with detected MTB at a high intensity in the feces sample, making it impossible to determine the minimum and maximum values for the results in the high category in the feces sample. The Area Under the Curve (AUC) values for each targeted gene showed good results, with values close to 1 ($p < 0.05$) (Table 5).

DISCUSSION

Subject Characteristics and Research Results

This study revealed a higher incidence of TB cases in men. Although TB can affect individuals of all ages and genders, our findings align with the global trend observed in 2020, where a higher prevalence of TB cases was reported in men.[1] Consistent with prior research, the study also identified a predominant occurrence of TB in older men.[11] Various factors contribute to the increased susceptibility of men to TB, including habits such as smoking and alcohol consumption, both of which exhibit immunosuppressive effects. Additionally, social roles and community dynamics may expose men more frequently to infections, including TB.[12-14]

The age distribution in our study predominantly fell within the range of 36-65 years. Age-related immunosenescence plays a role in shaping the manifestation of TB in older patients. Older individuals with TB tend to exhibit fewer clinical and radiological symptoms, leading to delayed diagnoses and, subsequently, delayed treatment initiation compared to their younger counterparts.[15,16] Notably, other studies have suggested that individuals aged 10 to 18 years are most strongly correlated with positive GeneXpert® MTB results.[17]

DM emerged as the most prevalent comorbidity in our study. DM-TB patients with elevated and uncontrolled blood sugar levels face compromised immune systems, escalating the risk of TB treatment failure, severe complications, and a heightened mortality risk compared to those without comorbidities.[18] Effective management of DM in these patients is imperative. Considering adjunctive DM therapy, metformin is deemed beneficial for DM-TB co-infected patients. It demonstrates the capacity to elevate pro-inflammatory and anti-inflammatory cytokines, respond to Th-1 and Th-2 immunity, particularly enhancing IFNγ, and mitigate insulin-related IL-10 regulation.[19] Beyond DM, hepatitis emerged as a common comorbidity in our research. While the side effects of TB treatment may exacerbate hepatitis, it is crucial to note that these side effects should not serve as a reason to discontinue therapy.[20]

In our study, cases with trace and indeterminate RIF resistance results underwent a thorough examination of the patient's clinical history to establish conclusive outcomes. This approach aligns with WHO guidelines, which consider factors such as age and comorbidities, including HIV, extrapulmonary TB, and previous TB treatment history.[1]

Table 5: Interpretation of CT Values in the Xpert® MTB/RIF Ultra Tests.

Xpert® MTB/RIF Ultra	Sputum Xpert® MTB/RIF Ultra									Feces Xpert® MTB/RIF Ultra								
	AUC	p-value	Median total	Not detected	Trace	Detected				AUC	p-value	Median total	Not detected	Trace	Detected			
						Very low	Low	Medium	High						Very low	Low	Medium	High
IS1081-6110																		
Median	1.000	< 0.001	16.2	0	24.9	19.4	17.9	16.3	16.1	0.924	< 0.001	17.1	0	22.9	18.7	17.1	16.3	16.1
Mean				0	25.1	19.4	18.1	16.4	16.1				0	23.6	18.8	17.6	16.3	16.1
Min-max				0	24.8-25.6	17.9-20.8	16.4-21.3	16.1-17.4	15.8-16.3				0	19.4-29	17.2-19.1	16.2-18.8	16.2-16.4	16.1
rpoB1																		
Median				0	0	35.6	24.7	20.1	18.2				0	0	31.1	25	20.1	19.1
Mean	0.975	< 0.001	18.8	0	0	35.6	24.4	20	18.4	0.974	< 0.001	24.6	0	0	31.5	24.4	20.3	19.1
Min-max				0	0	32.2-38.9	20.9-28.2	19.3-20.6	17.3-23.8				0	0	24.6-34.6	21-28	19.7-21	19.1
rpoB2																		
Median				0	0	34.2	25	20.2	18				0	0	30.4	24.5	20.2	19.5
Mean	0.888	< 0.001	18.9	3.1	0	34.2	24.7	20.3	18.2	0.931	< 0.001	24.4	2.2	0	31	24.6	20.1	19.5
Min-max				0-34.6	0	31.8-36.6	20.7-29.8	19.3-22.3	17.2-22.6				0-26.7	0	28.3-2.1	21.3-27	19.5-20.7	19.5
rpoB3																		
Median				0	0	17.7	27.4	22.6	20.4				0	0	34.1	27.1	23.8	22.6
Mean	0.967	< 0.001	21.7	0	0	17.7	27.2	22.7	20.9	0.966	< 0.001	26.7	0	0	32.7	26.5	23.8	22.6
Min-max				0	0	0-35.4	22.5-31.5	21.5-24.4	18.7-26.7				0	0	0-36.8	23.4-29.3	22.2-25.3	22.6
rpoB4																		
Median				0	0	33.6	27	22.9	20.7				0	0	32.7	27.8	19.5	18.3
Mean	0.975	< 0.001	21.6	0	0	33.6	27.5	22.2	20	0.974	< 0.001	27.3	0	0	33.4	27.8	21	18.3
Min-max				0	0	32.2-34.9	23.6-31.1	19-24.9	16.8-22.9				0	0	28.9-37.2	22.1-30.8	19.2-24.6	18.3

These criteria are consistent with the recommendations provided by the Ministry of Health of the Republic of Indonesia (Kemenkes RI), which outlines the management flow for patients exhibiting MTB trace results on Xpert® MTB/RIF Ultra.[21] Trace results invariably yield indeterminate RIF resistance, as they indicate outcomes corresponding to the lowest bacterial load for MTB detection.[22]

In our study, four subjects were identified with Non-Tuberculous Mycobacteria (NTM) detected in the culture tests. One NTM subject went undetected in the sputum and Feces Xpert® MTB/RIF Ultra tests, while two were identified solely in the sputum Xpert® MTB/RIF Ultra tests. Deby's research underscores a significant association between NTM and the severity of pulmonary TB. TB is one of the risk factors for NTM.[23] Although Xpert® MTB/RIF Ultra has demonstrated 100% sensitivity in testing 30 NTM species, there are more than 180 NTM species, some of which can cause diseases in humans.[9,24] The signs and symptoms of NTM are often unclear and non-specific, resembling those seen in TB patients and other diseases. To diagnose NTM, further examinations are essential, applying the criteria set by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA).[24] The researchers posit that the NTM species identified may not belong to the 30 NTM species initially tested, or contamination might have occurred. NTM species with lower virulence may result from colonization or infection, emphasizing the need for effective communication between pulmonary doctors and laboratory professionals to accurately determine whether NTM serves as the causative pathogen, represents colonization, or is merely a contaminant.[25]

Sensitivity Values of the AFB and Xpert® MTB/RIF Ultra Tests Compared with the Sputum Culture Tests as the Gold Standard

AFB exhibited a very low sensitivity value but demonstrated a PPV of 100%. In contrast, another study reported a higher sensitivity value for Feces AFB at 53.9%, coupled with a specificity of 100%.[26] The specificity value indicates that Feces AFB has a 100% ability to generate negative results in patients with negative MTB culture test results, aligning with our findings where Feces AFB was negative in all cases with negative culture test results.

Our study revealed high sensitivity values for both sputum and Feces Xpert® MTB/RIF Ultra tests, consistent with other research reporting overall ultra sensitivity and specificity exceeding 89% and 94%, respectively. This suggests that the Xpert® MTB/RIF Ultra tool can effectively serve in TB screening and treatment.[27, 28] The heightened sensitivity of the device contributes significantly to increased TB detection, providing a more reliable identification of rifampicin resistance.[29] This is particularly noteworthy as MTB can still be detected even in cases with negative culture test results due to the presence of MTB DNA in the examined samples.[30]

The sensitivity value of Feces Xpert® MTB/RIF Ultra tests in our study was 97.6%, a result consistent with another study reporting a sensitivity of 85.4%. This reaffirms that feces can serve as a viable alternative sample for confirming TB.[31]

Concordance between the Results of the Feces AFB Test and Those of the Sputum Culture Tests as the Gold Standard

In our study, no concordance was observed between the Feces AFB and sputum culture tests, indicating that Feces AFB is unsuitable for monitoring TB therapy. Consequently, reliance on serial detection tests is imperative to identify cases that might go undetected with the AFB test.[32] This finding aligns with another study examining Feces AFB and Feces culture, where all microscopically positive stool samples did not yield positive results in Feces LJ culture. In cases of positive microscopic AFB, most Feces samples showed damaged bacilli. This damage could

be attributed to the sputum, which contains bacteria ingested through the digestive tract, the highly acidic environment of which may kill most bacteria, yet the DNA remains intact in the feces.[26]

Concordance between the Sputum Xpert® MTB/RIF Ultra Test and the Feces Xpert® MTB/RIF Ultra Test Results

The AUC values for each targeted gene in sputum and Feces Xpert® MTB/RIF Ultra tests indicate excellent performance, signifying that Xpert® MTB/RIF Ultra can effectively detect the rpoB and IS1081-IS6110 genes as anticipated. The incorporated genes in Xpert® MTB/RIF Ultra include rpoB and IS1081-IS6110. These insertion element probes enhance the detection of complex Mycobacterium tuberculosis (MTB) by targeting a multi-copy insertion element present in the majority of TB strains. The melting point analysis, facilitated by four rpoB probes, can differentiate between wild-type sequences and mutations associated with RIF resistance.[27] A study in India by Gaur et al. similarly achieved high AUC results, with a Receiver Operating Characteristic (ROC) curve exhibiting an AUC of 0.882 (CI, 0.829-0.935) for Feces PCR, underscoring the high accuracy of Feces PCR in MTB detection.[33]

Our study identified a moderate concordance between Feces and sputum Xpert® MTB/RIF Ultra tests ($p < 0.05$). Another study reported a kappa value of 0.83 ($p < 0.001$), concluding that feces could serve as a viable alternative to respiratory specimens and was proven as an innovative non-invasive test, particularly beneficial for patients unable to provide sputum, positioning it as a valuable tool in health facilities to enhance healthcare services.[34]

Comparison between Differences in the CT Values of the Sputum and Feces Xpert® MTB/RIF Ultra Tests

A notable discrepancy exists in the cycle threshold (CT) values between genes, with the median total CT value for the Feces Xpert® MTB/RIF Ultra tests being higher than that for the sputum Xpert® MTB/RIF Ultra tests. This variance may stem from differences in the level or quantity of bacterial DNA present in feces. A parallel study that compared CT values of rpoB in sputum and feces also reported higher CT in feces, attributed to the lower concentrations of MTB DNA. The explanation posits that a significant number of bacilli in sputum may incur damage during passage through the digestive tract[26], yet bacterial DNA in feces remains detectable by Xpert® MTB/RIF Ultra.

The determination of non-detected results is grounded in the absence of the IS1081 & 6110 genes, even though the Sputum Culture (SPC) results are detected. This aligns with the findings of our study, where some subjects exhibit the detection of rpoB2 despite the absence of IS1081 & 6110. Traces are ascertained when the IS1081 & 6110 genes are detected, but the detected rpoB is < 2 rpoB, indicating insufficient strength to confirm the presence of MTB.[27] This scenario is consistent with the outcomes of our study, which recorded CT values for IS1081 & 6110 but with undetected rpoB.

Concordance between the RIF Resistance Results of the Sputum Xpert® MTB/RIF Ultra Tests and Those of the Rifampicin Resistance Tests

The outcomes of the sputum Xpert® MTB/RIF Ultra tests indicated a higher detection rate of rifampicin-resistant TB than traditional rifampicin resistance tests. This discrepancy could be attributed to the relatively low sensitivity of culture in general, whereas the rifampicin resistance tests were performed only if the MTB culture results were positive. Other studies investigating the diagnostic value of RIF resistance in the Xpert® MTB/RIF Ultra test reported remarkably high sensitivity and specificity results. It even identified a sample, confirmed by Sanger sequencing, that exhibited a mixture of wild-type and S531L mutants detected in the Xpert® MTB/RIF Ultra test but not in the rifampicin resistance and Xpert® MTB/RIF tests. Improvements in the Xpert® MTB/RIF Ultra test, such as the enhancement of the Sloppy Molecular Beacon (SMB) examination of rpoB based on the melting point (ΔTm) of the MTB target amplicons, have enabled the detection of both wild-type and mutated rifampicin resistance.[35]

Another study corroborated the efficacy of the Xpert® MTB/RIF Ultra test in identifying various rifampin resistance-determining region (RRDR) mutations. Among 30 rifampicin resistance tests, 29 showed mutations in the RRDR that could be well-detected using the Xpert® MTB/RIF Ultra test. These findings contribute to the ability to rule out transmission between RR TB patients and differentiate between relapsed and reinfected TB.[30]

Concordance between the RIF Resistance Results of the Feces Xpert® MTB/RIF Ultra Tests and Those of the Rifampicin Resistance Tests

The rifampicin resistance tests and the Feces Xpert® MTB/RIF Ultra tests indicate a significant concordance. Similar findings were observed in previous studies, indicating a high concordance between the RIF resistance results in the rifampicin resistance tests and the Feces Xpert® MTB/RIF Ultra tests. These results suggest that the Xpert® MTB/RIF Ultra tool can serve as a rapid tool for detecting drug-resistant tuberculosis in healthcare services.[31]

In contrast, another study reported a significant proportion of indeterminate RIF resistance results from the Xpert® MTB/RIF Ultra tests, primarily attributed to high trace results. Notably, trace results from the Xpert® MTB/RIF Ultra tests are accompanied by the detection rpoB, making it challenging to interpret rifampicin resistance results.[34] However, the present study yielded fewer indeterminate RIF resistance results, possibly due to differences in Feces sample preparation, enhancing the detection of MTB DNA in feces.

Several limitations should be acknowledged in this study. Firstly, Feces culture tests were not conducted, precluding a direct comparison between the sputum and Feces culture tests and between the Feces Xpert® MTB/RIF Ultra tests and the Feces culture tests. Secondly, the study focused on adults, limiting the generalizability of results to children. Lastly, no follow-up examination was conducted on NTM culture results, hindering the identification of specific NTM types or species detected.

CONCLUSION

This study concludes that there is concordance between the results of the sputum Xpert® MTB/RIF Ultra tests and those of the Feces Xpert® MTB/RIF Ultra tests. However, no concordance was observed between the Feces AFB tests and the sputum culture tests as the gold standard. The higher CT values in feces compared to sputum are attributed to the lower bacterial concentration in feces. Consequently, feces can be considered a viable alternative sample to sputum for MTB detection using Xpert® MTB/RIF Ultra.

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DISCLOSURE

The authors report no conflict of interest in this work.

AUTHOR CONTRIBUTIONS

D.R., P.W., and H.K.: Investigation, resources, data curation, writing—original draft, writing—review & editing, funding acquisition; Y.P., T.K., and A.: Formal analysis, data curation, writing—original draft, writing—review & editing.

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