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Four-year evaluation of tuberculosis diagnostics and drug resistance patterns: A retrospective analysis of smear microscopy, MTB/RIF PCR, and culture-confirmed *Mycobacterium tuberculosis* isolates

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MAIN POINTS

- MTB/RIF PCR demonstrated markedly higher sensitivity than direct smear microscopy in diagnosing culture-confirmed tuberculosis.
- Direct smear microscopy showed excellent specificity but limited sensitivity, particularly in smear-negative tuberculosis cases.
- Tuberculosis positivity rates declined over the four-year study period; however, resistance to isoniazid remained the most frequently detected first-line drug resistance.
- Despite low multidrug-resistant tuberculosis rates, routine molecular diagnostics and drug susceptibility testing remain essential for effective tuberculosis management.

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ABSTRACT

Aim: Accurate and timely diagnosis of tuberculosis remains a major clinical challenge. This study aimed to evaluate the diagnostic performance of direct smear microscopy and MTB/RIF PCR compared with culture, and to assess first-line drug resistance patterns among *Mycobacterium tuberculosis* complex (MTBC) isolates.

Materials and Methods: This retrospective study analyzed non-duplicate mycobacterial culture records obtained between 2020 and 2023 at a tertiary care center. Culture-confirmed MTBC cases served as the reference standard. The diagnostic performance of direct smear microscopy and MTB/RIF PCR was evaluated in patients who underwent both the index test and the culture. Drug susceptibility testing was performed for first-line antituberculosis agents, including isoniazid, rifampicin, ethambutol, and streptomycin. The study was reported in accordance with the STROBE and STARD guidelines.

Results: A total of 8,863 mycobacterial cultures were evaluated, yielding 165 MTBC-positive isolates. MTB positivity rates decreased from 2.18% in 2020 to 1.31% in 2023. When data from all years were combined, direct smear microscopy demonstrated a sensitivity of 34.5% and a specificity of 99.8%, whereas MTB/RIF PCR showed a sensitivity of 91.5% and a specificity of 98.5%, all for culture-confirmed tuberculosis. Drug susceptibility testing was available for 160 isolates. Resistance to isoniazid, rifampicin, ethambutol, and streptomycin was detected in 14.4%, 1.9%, 6.3%, and 8.8% of isolates, respectively. Multidrug-resistant tuberculosis was identified in 1.25% of cases.

Conclusion: Direct smear microscopy exhibited limited sensitivity despite high specificity, whereas MTB/RIF PCR demonstrated excellent diagnostic performance and a high negative predictive value. Although overall drug resistance rates were relatively low, the presence of isoniazid resistance underscores the importance of routine susceptibility testing in the management of tuberculosis.

Keywords: *Mycobacterium tuberculosis* complex, Smear microscopy, MTB/RIF PCR, Drug resistance, Diagnostic performance

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INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality worldwide, despite major advances in diagnostic technologies and treatment strategies [1]. Rapid and accurate identification of *Mycobacterium tuberculosis* complex (MTBC) is essential to initiate timely, appropriate therapy, prevent transmission, and limit the emergence of drug-resistant strains. Although mycobacterial culture is considered the reference standard for TB diagnosis, its prolonged turnaround time limits its usefulness in urgent clinical decision-making.

Direct smear microscopy has traditionally served as a cornerstone of TB diagnosis, particularly in resource-limited settings, due to its simplicity, low cost, and high specificity. However, its sensitivity is highly variable and depends on factors such as bacillary load, specimen quality, and laboratory expertise [2,3]. In recent years, molecular diagnostic assays, including MTB/RIF PCR, have substantially improved TB

diagnostics by enabling rapid detection of MTBC and rifampicin resistance, thereby facilitating earlier initiation of targeted treatment [4,5]. Nevertheless, the diagnostic performance of these molecular methods may vary in real-world settings depending on patient populations, laboratory workflows, and specimen characteristics [6–9].

In addition to diagnostic challenges, drug-resistant TB continues to represent a major global public health concern. Resistance to first-line antituberculosis agents, particularly isoniazid and rifampicin, is associated with unfavorable treatment outcomes, prolonged infectiousness, and increased healthcare costs [10–13]. Continuous local surveillance of resistance patterns is therefore essential to guide empirical treatment strategies and identify emerging trends in multidrug-resistant tuberculosis.

The present study aimed to conduct a comprehensive four-year evaluation of TB diagnostics at a tertiary care center by comparing the diagnostic performance of direct smear microscopy and MTB/RIF PCR, with culture-confirmed MTBC as the reference standard. Additionally, the study sought to assess the prevalence and patterns of resistance to first-line antituberculosis drugs in MTBC isolates, thus providing clinically relevant data for laboratory specialists and treating clinicians.

MATERIALS AND METHODS

Study design and isolates

This retrospective observational study included non-duplicate clinical specimens submitted to the Microbiology Laboratory of Marmara University Pendik Training and Research Hospital, Istanbul, Turkey, for mycobacterial investigation between January 2020 and December 2023. To avoid repeated sampling, follow-up or repeat specimens/records from the same patient were excluded, and only the first eligible specimen per patient was included in the analysis. The study was conducted following approval by the Marmara University Faculty of Medicine Ethics Committee and in accordance with the principles of the Declaration of Helsinki (Protocol No: 09.2025.25-0982, Date: 10.12.2025), and it was reported in accordance with the STROBE and STARD guidelines for observational and diagnostic accuracy studies. Culture-confirmed *Mycobacterium tuberculosis* complex (MTBC) isolates were included in the analysis.

Direct smear microscopy and mycobacterial culture

Clinical specimens were processed using standard decontamination and concentration procedures. Direct smear microscopy was performed using Ziehl–Neelsen staining. Mycobacterial cultures were incubated on Löwenstein–Jensen medium and in an automated liquid culture system (BACTEC MGIT 960; Becton Dickinson, Sparks, MD, USA). Culture results were reported as positive when mycobacterial growth was detected and as negative when no growth was observed after the recommended incubation period.

Identification of Mycobacterium tuberculosis complex

Isolates were identified as MTBC by routine laboratory identification algorithms. Only culture-confirmed MTBC isolates were accepted as the reference standard for diagnostic performance analysis.

MTB/RIF PCR assay

Molecular detection of MTBC was performed using the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) in accordance with the manufacturer's instructions. Results were reported as positive or negative for MTBC. When TRACE/very-low results were reported by the platform, these were interpreted cautiously in routine practice and evaluated together with smear microscopy, culture results, and clinical information; repeat testing and/or a follow-up specimen were recommended when clinically indicated.

Drug susceptibility testing

Drug susceptibility testing was performed for first-line antituberculosis agents, including isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM), using the BACTEC MGIT 960 system, according to the manufacturer's instructions and the standard critical concentrations (INH 0.1 μg/mL, RIF 1.0 μg/mL, EMB 5.0 μg/mL, SM 1.0 μg/mL). Of the 165 culture-confirmed MTBC isolates, susceptibility results were available for 160. Five isolates were excluded from resistance analysis because of insufficient growth or technical limitations.

Statistical analysis

Culture-confirmed MTBC was accepted as the reference standard. The diagnostic performance of direct smear microscopy and MTB/RIF PCR was evaluated separately by comparing each test with culture results in patients who had both the index test and culture performed. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas, and 95% confidence intervals (CIs) were calculated using the exact (Clopper–Pearson) method. Comparisons of sensitivity and specificity between smear microscopy and MTB/RIF

Table 1. Annual distribution of culture-confirmed *Mycobacterium tuberculosis* complex isolates (2020--2023).

Year	Total cultures (n)	MTBC positive (n)	MTBC positivity (%)
2020	1,847	40	2.18
2021	1,996	56	2.81
2022	2,187	32	1.46
2023	2,833	37	1.31
Total	8,863	165	1.86

MTBC: *Mycobacterium tuberculosis* complex.

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Table 2. Diagnostic performance of direct smear microscopy and MTB/RIF PCR compared with culture (2020--2023).

Test	n	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Smear microscopy	8,858	57	16	108	8,677	34.5 (27.3--42.3)	99.8 (99.7--99.9)	78.1 (66.9--86.9)	98.8 (98.5--99.0)
MTB/RIF PCR	4,096	107	61	10	3,918	91.5 (84.8--95.8)	98.5 (98.0--98.8)	63.7 (55.9--71.0)	99.7 (99.5--99.9)

TP: true positive; FP: false positive; FN: false negative; TN: true negative; PPV: positive predictive value; NPV: negative predictive value. Culture-confirmed *Mycobacterium tuberculosis* complex was used as the reference standard. Diagnostic performance estimates are presented as percentage with 95% confidence intervals calculated using the exact (Clopper–Pearson) method.

Table 3. Sensitivity and specificity of smear microscopy and MTB/RIF PCR according to specimen group.

Specimen group	Test	n	Sensitivity (%)	Specificity (%)
Pulmonary	Smear microscopy	4,430	50.5	99.9
	MTB/RIF PCR	2,591	97.6	98.4
Extrapulmonary	Smear microscopy	4,428	9.4	99.8
	MTB/RIF PCR	1,505	77.1	98.5

MTB/RIF PCR, Xpert MTB/RIF assay.

Table 4. Drug susceptibility patterns of *Mycobacterium tuberculosis* complex isolates (n = 160)

Test result	Antibiotics	n	%
Susceptible to four drugs	INH, RIF, EMB, SM	125	78.1
Single-drug resistance	INH	10	6.2
	RIF	1	0.6
	EMB	6	3.8
	SM	5	3.1
Resistance to two drugs	INH + RIF	1	0.6
	INH + SM	7	4.4
	INH + EMB	3	1.9
Resistance to three drugs	INH + RIF + SM	1	0.6
	INH + EMB + SM	1	0.6

INH: isoniazid; RIF: rifampicin; EMB: ethambutol; SM: streptomycin.

Table 5. Culture outcomes of TRACE-positive MTB/RIF PCR results among smear-negative patients.

Culture outcome	MTBC n (%)	NTM n (%)	No growth n (%)	Total n (%)
TRACE-positive cases	8 (27.6)	2 (6.9)	19 (65.5)	29 (100)

TRACE: very low-level positivity reported by the Xpert MTB/RIF assay; MTBC: *Mycobacterium tuberculosis* complex; NTM: non-tuberculous mycobacteria.

PCR were performed using two-proportion z-tests (equivalent to chi-square tests) on available tested subsets; p values <0.05 were considered statistically significant. Because not all patients underwent both tests simultaneously, comparisons were performed on the available subsets and did not represent fully paired analyses. Statistical analyses were performed using descriptive and inferential methods as appropriate.

RESULTS

Study population and culture results

Between January 2020 and December 2023, a total of 8,863 non-duplicate mycobacterial cultures were evaluated. Among these, 165 cultures (1.86%) were positive for *Mycobacterium tuberculosis* complex (MTBC). The annual MTBC positivity rate declined from 2.18% in 2020 to 1.31% in 2023 (Table 1).

In addition to MTBC, 21 non-tuberculous mycobacterial (NTM) isolates were identified during the study period. These isolates were excluded from subsequent analyses of diagnostic performance and drug resistance.

Diagnostic performance of direct smear microscopy and MTB/RIF PCR

Among patients who underwent both direct smear microscopy and culture (n = 8,858), smear microscopy demonstrated a sensitivity of 34.5% (95% CI: 27.3–42.3) and a specificity of 99.8% (95% CI: 99.7–99.9). The positive predictive value (PPV) was 78.1% (95% CI: 66.9–86.9), and the negative predictive value (NPV) was 98.8% (95% CI: 98.5–99.0).

Among patients with both MTB/RIF PCR and culture results available (n = 4,096), MTB/RIF PCR showed a substantially higher sensitivity of 91.5% (95% CI: 84.8–95.8) and a specificity of 98.5% (95% CI: 98.0–98.8), with a PPV of 63.7% (95% CI: 55.9–71.0) and an NPV of 99.7% (95% CI: 99.5–99.9) (Table 2). In subgroup-based comparisons,

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MTB/RIF PCR demonstrated significantly higher sensitivity than smear microscopy (p<0.001). Although the absolute difference in specificity was small, it also reached statistical significance (p<0.001).

When data were stratified by specimen type, smear microscopy exhibited markedly higher sensitivity for pulmonary samples (50.5%) than for extrapulmonary samples (9.4%). MTB/RIF PCR maintained high sensitivity in both specimen groups; however, diagnostic performance was higher in pulmonary specimens (97.6%) than in extrapulmonary specimens (77.1%) (Table 3).

Drug susceptibility patterns of MTBC isolates

Drug susceptibility testing was successfully performed for 160 of the 165 MTBC isolates. Resistance to at least one first-line antituberculosis drug was detected in 35 isolates (21.9%), whereas 125 isolates (78.1%) were fully susceptible to all tested agents.

The most frequently observed resistance was to isoniazid (14.4%), followed by resistance to streptomycin (8.8%), ethambutol (6.3%), and rifampicin (1.9%). Multidrug-resistant tuberculosis, defined as combined resistance to both isoniazid and rifampicin, was identified in two isolates (1.25%) (Table 4).

Regarding the extent of resistance, 22 isolates (13.8%) exhibited resistance to a single drug, 11 isolates (6.9%) to two drugs, and 2 isolates (1.25%) to three drugs. No isolate demonstrated resistance to all four first-line antituberculosis agents.

TRACE-positive MTB/RIF PCR results

In the cohort of 8,863 patients, TRACE-level MTB/RIF PCR positivity was observed in 29 cases. Culture confirmed MTBC in 8 of these TRACE-positive cases (27.6%), while non-tuberculous mycobacteria were identified in 2 cases (6.9%). No mycobacterial growth was detected in the remaining 19 cases (65.5%) (Table 5).

DISCUSSION

In this four-year retrospective study, we evaluated the diagnostic performance of direct smear microscopy and MTB/RIF PCR, compared with culture-confirmed *Mycobacterium tuberculosis* complex (MTBC), and analyzed first-line drug resistance patterns in a tertiary care setting. Our findings demonstrate a declining trend in culture-confirmed tuberculosis over the study period, together with the markedly superior sensitivity of MTB/RIF PCR compared with smear microscopy, consistent with previous reports [1,4].

The overall sensitivity of direct smear microscopy in our cohort was 34.5%, in line with earlier studies reporting substantial variability depending on specimen quality, bacillary burden, and laboratory expertise [6,9,14]. Despite its limited sensitivity, smear microscopy maintained excellent specificity (99.8%), supporting its continued role as a rapid and inexpensive method for identifying highly infectious cases. However, the low sensitivity underscores the considerable risk of missed diagnoses when smear microscopy is used as a standalone diagnostic tool.

In contrast, MTB/RIF PCR demonstrated high sensitivity (91.5%), high specificity (98.5%), and a very high negative predictive value (99.7%). These findings highlight the clinical value of molecular testing as a reliable rule-out method, particularly in patients with smear-negative tuberculosis [15]. The relatively lower positive predictive value of MTB/RIF PCR likely reflects the low prevalence of culture-confirmed tuberculosis in our study population, as well as the detection of non-viable bacilli or residual mycobacterial DNA in previously treated patients [16].

In addition to MTBC isolates, a subset of culture-positive isolates were identified as non-tuberculous mycobacteria (NTM). Although these isolates were excluded from diagnostic performance and resistance analyses, their identification remains clinically relevant. NTM are increasingly recognized as opportunistic pathogens, particularly in individuals with chronic lung disease, immunosuppression, or structural airway abnormalities [17,18]. Importantly, NTM infections may yield smear-positive but MTB/RIF PCR–negative results, which can be misinterpreted as false-negative molecular testing [15,19,20]. These findings emphasize the continued importance of culture-based methods to accurately differentiate MTBC from NTM in routine clinical practice.

The low rate of culture confirmation among TRACE-positive, smear-negative cases (27.6%) further supports the need for cautious interpretation of TRACE results. Such findings may represent residual DNA from non-viable organisms, early infection, or cross-reactivity with NTM rather than active tuberculosis, particularly in low-prevalence settings [15,17–20].

Analysis of drug susceptibility patterns revealed that 21.9% of MTBC isolates were resistant to at least one first-line antituberculosis drug. Isoniazid resistance was the most frequently observed resistance pattern (14.4%), whereas rifampicin resistance was relatively low (1.9%). The multidrug-resistant tuberculosis (MDR-TB) rate of 1.25% observed in our cohort is lower than that reported in many national and international studies, suggesting that MDR-TB is currently uncommon in our region; nevertheless, continued surveillance remains essential [11,12].

The observed decline in MTBC positivity between 2020 and 2023 may reflect improvements in public health interventions, enhanced case detection strategies, or changes in healthcare utilization during the COVID-19 pandemic. However, the persistence of isoniazid resistance highlights the ongoing need for routine drug susceptibility testing, even in settings with declining tuberculosis incidence.

Limitations

This study has several limitations. First, the retrospective design limited access to complete clinical data and precluded re-

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analysis of archived specimens. Second, drug susceptibility testing could not be performed for all culture-positive isolates because of technical constraints. Finally, the single-center design may limit the generalizability of the findings.

Although overall drug resistance rates were relatively low, the persistence of isoniazid resistance underscores the importance of ongoing resistance monitoring. The integration of rapid molecular diagnostics with conventional methods remains essential for effective tuberculosis management.

CONCLUSION

In this four-year retrospective analysis, MTB/RIF PCR demonstrated substantially higher sensitivity and an excellent negative predictive value compared with direct smear microscopy for the diagnosis of culture-confirmed tuberculosis. Although smear microscopy retains high specificity, its limited sensitivity underscores the risk of missed diagnoses when used as a standalone test. Despite relatively low overall drug resistance rates, the persistence of isoniazid resistance highlights the importance of routine drug susceptibility testing. These findings support the integration of rapid molecular diagnostics with conventional culture-based methods to improve the accuracy and timeliness of tuberculosis diagnosis and management.

Ethics Committee Approval: Ethical approval was obtained from the Marmara University Faculty of Medicine Ethics Committee (Protocol No: 09.2025.25-0982, Date: 10.12.2025).

Informed Consent: Due to the retrospective nature of the study, informed consent was waived.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that they have no conflict of interest.

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