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Original article

Impact of nontuberculous mycobacteria on the performance of Xpert MTB/RIF and Xpert MTB/RIF Ultra for the detection of tuberculosis and rifampin resistance: a diagnostic accuracy study

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Abstract

Background: The growing burden of nontuberculous mycobacteria (NTM) raises concerns regarding cross-reactivity in molecular tuberculosis (TB) diagnostics. In the current study, we evaluated whether high NTM loads affect *Mycobacterium tuberculosis* (MTB) detection or rifampin (RIF) resistance calls using Xpert MTB/RIF (Xpert) and Xpert MTB/RIF Ultra (Xpert Ultra).

Methods: In vitro spiking experiments were performed by mixing eight NTM species (1×10^6 colony-forming unit [CFU]/mL) with heat-inactivated MTB (RIF-susceptible H37Rv; RIF-resistant S450L) at 5×10^3 CFU/mL in pooled smear-negative sputum, before testing them in parallel using Xpert and Xpert Ultra. We also retrospectively analyzed 334 results from lower respiratory specimens, including 32 NTM-positive specimens tested using both assays, and assessed the presence of probe amplification in the NTM-confirmed specimens.

Results: In spiking experiments, both assays showed no NTM-related cross-reactivity: RIF-susceptible mixes were "RIF resistance not detected," S450L mixes were correctly resistant with preserved mutant melt peaks, and MTB-specific cycle threshold and melting peak temperature profiles were unchanged by NTM. Of the 334 clinical specimens, NTM was isolated from 32. Xpert classified all 32 as MTB-negative, and Xpert Ultra classified 31 of 32 as MTB-negative and one as "very low" positive in a patient with prior TB, consistent with residual nonviable DNA. In NTM-positive, Xpert/Xpert Ultra-negative specimens, neither assay showed probe amplification.

Conclusion: High-burden NTM did not compromise MTB detection or RIF-resistance determination using Xpert or Xpert Ultra. The assays demonstrated robust analytical specificity in mixed MTB–NTM contexts, supporting their use where NTM carriage is common.

Keywords: *Mycobacterium tuberculosis*, Nontuberculous mycobacteria, Rifampin, Xpert MTB/RIF, Xpert MTB/RIF Ultra

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Introduction

Background

The advent of the cartridge-based molecular assay Xpert MTB/RIF (Cepheid) and its upgraded version, Xpert MTB/RIF Ultra, has significantly advanced the diagnostic capability of *Mycobacterium tuberculosis* (MTB). Because the original Xpert showed limited sensitivity, especially in smear-negative or low-bacterial-load samples, the World Health Organization (WHO) recommended the use of the Xpert Ultra in 2017 [1]. Unlike the earlier version, which infers rifampin (RIF) resistance by comparing cycle threshold (Ct) delays among probes, Xpert Ultra utilizes melting temperature analysis with sloppy molecular beacon technology to detect mutations within the RIF resistance–determining region of the *rpoB* gene, enhancing diagnostic specificity and reducing false-positive resistance detection [2,3].

Previous studies have reported that Xpert can yield false-positive results for MTB detection due to its cross-reactivity with nontuberculous mycobacteria (NTM) [4,5], including erroneous RIF resistance reporting [4]. Similar evaluations have been conducted for Xpert Ultra, with several studies examining the cross-reactivity and probe amplification in the presence of various NTM species [6,7]. However, few studies have assessed the accuracy of RIF resistance calls or how high concentrations of NTM affect low-level MTB detection.

The global increase in NTM infections is also evident in South Korea [8]. At our institution, NTM comprise a substantial proportion of mycobacterial culture–positive specimens, underscoring the need to confirm that assays such as Xpert MTB/RIF and Xpert Ultra maintain both sensitivity for low-burden MTB and specificity for high NTM loads.

Objectives

In this study, we aimed to evaluate the performance of Xpert MTB/RIF and Xpert Ultra in detecting MTB in the presence of a high burden of common NTM species, and to determine whether the detection of RIF resistance is affected under these conditions. We also analyzed respiratory specimens that underwent parallel testing with both Xpert and Xpert Ultra and reviewed the probe reactions in the samples from which NTM was isolated.

Methods

Study design

We conducted an in vitro analytical interference study using NTM-spiked sputum, along with a retrospective, cross-sectional diagnostic accuracy study of clinical respiratory specimens. We compared Xpert MTB/RIF and Xpert MTB/RIF Ultra against culture-based identification as the reference standard. This study adheres to the Standards for Reporting Diagnostic Accuracy Studies guidelines (https://www.equator-network.org/reporting-guidelines/stard/).

Participants

This study was conducted at Samsung Medical Center, a tertiary-care hospital in Seoul, South Korea We assessed the possible cross-reactivity of RIF resistance detection in cases of coinfection with MTB and high bacterial loads of NTM species. Between August 2023 and May 2024, we tested 335 lower respiratory tract samples, comprising 273 sputum specimens, five endotracheal aspirates, 46 bronchoalveolar lavage fluids, and 11 bronchial washings, using both Xpert and Xpert Ultra in parallel. We then retrospectively analyzed specimens with available mycobacterial culture results in which NTM was isolated. Specimens from patients who had received anti-tuberculosis therapy within the preceding 6 months were excluded. In specimens that were culture-confirmed as NTM, we evaluated whether the presence of NTM affected MTB detection or the interpretation of RIF resistance.

Xpert and Xpert Ultra procedures

The Sample Reagent was added to the specimen at a 2:1 dilution, and 2 mL of the resulting mixture was added to each Xpert and Xpert Ultra cartridge. Both assays were performed in parallel according to the manufacturer's instructions [6,9]. When MTB was detected, the semiquantitative scale was assigned as very low, low, medium, or high. Xpert Ultra additionally provides a "trace" category, in which RIF resistance cannot be determined due to insufficient signal detection [10]. The results were analyzed using Xpert V6 and Xpert Ultra V4.

Mycobacterial stain and culture

Acid-fast bacilli (AFB) staining was performed using an auramine-rhodamine fluorescent stain, followed by confirmation with Ziehl-Neelsen staining. The staining results were graded according to the U.S. Centers for Disease Control and Prevention recommendations. Specimens were considered to be smear-positive if the samples had an AFB smear score of 1 or greater. Decontaminated samples were inoculated into mycobacterial growth indicator tubes (MGIT 960 system; Becton Dickinson) and 3% Ogawa agar (Shinyang) and cultured for 6 weeks. Cultures confirmed to be AFB-positive were further analyzed using the GREENCARE MTB/NTM detection kit (GC Medical Science), which is a real-time polymerase chain reaction (PCR) assay used to distinguish *M. tuberculosis* from NTM.

Cross-reactivity in RIF resistance detection with NTM species at high bacterial load

An AFB-negative sputum specimen pool was prepared for the spiking procedure and sputum specimens were confirmed to be negative by both smear and culture. Prior to spiking experiments, RIF-susceptible (H37Rv; ATCC 27294) and resistant (with S450L mutation) MTB strains were heat-inactivated at 80°C for 20 min [11,12]. As MTB grows in liquid culture as aggregates, we performed a thorough homogenization step to disperse the clumps and ensure a uniform suspension. Each isolate was pelleted and homogenized in 0.05% Tween 80 [13]. The suspension was transferred to screw-capped glass tubes containing 3-mm glass beads, vortexed vigorously for 3 min, and incubated for 30 min to obtain a supernatant free of visible clumps.

To assess the potential cross-reactivity in an environment with high NTM and low MTB concentrations, mock samples were prepared by spiking low levels of MTB and a high bacterial load of NTM species in AFB-negative sputum [14]. Specifically, eight NTM reference strains, including *M. avium, M. intracellulare*, *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense, M. chelonae, M. fortuitum*, *M. gordonae*, and *M. kansasii*, were used in addition to the MTB strains prepared by heat inactivation and homogenization.

The turbidity of MTB and NTM supernatants was adjusted to a 0.5 McFarland (approximately 1.0×10^7 colony-forming unit [CFU]/mL) [15,16]. Mock samples were prepared by mixing each NTM and MTB suspension in pooled AFB-negative sputum, yielding final concentrations of 1.0×10^6 CFU/mL (NTM) and 5.0×10^3 CFU/mL (MTB). Each spiked sample was tested in duplicate using Xpert and Xpert Ultra. The melting peak temperatures (*Tm*s) of the Xpert Ultra were recorded.

Analysis

Descriptive statistics were applied to all assays.

Results

Cross-reactivity in RIF resistance detection with NTM species at high bacterial load

To explore the possibility of cross-reactivity, mock sputum samples were prepared by spiking eight NTM reference strains (*M. avium*, *M. intracellulare*, *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, and *M. kansasii*) at 1×10 CFU/mL together with heat-inactivated MTB at 5×10 CFU/mL in duplicate. Both the Xpert and Xpert Ultra assays showed no cross-reactivity in the detection of RIF resistance (Table 1). All samples spiked with RIF-susceptible MTB strains yielded RIF resistance results of "Not detected" in both assays. All MTB strains with the S450L mutation correctly reported resistance, with no alteration in mutant *Tm* shifts.

We analyzed individual probe Ct values and *Tm* (Table 2), demonstrating that even at NTM loads of 1.0×10^6 CFU/mL, MTB-specific probe amplification and *Tm* profiles remained indistinguishable from those of MTB-only controls. In both RIF-susceptible and RIF-resistant samples, the presence of any of the eight NTM species did not produce shifts in the Ct values or *Tm* patterns.

Comparison of the Xpert and Xpert Ultra for the analysis of NTM-positive clinical specimens

Among the 335 respiratory specimens, one was excluded due to an invalid culture result, leaving 334 samples for further evaluation. NTM was isolated from 32 specimens, comprising 28 sputum, three bronchoalveolar lavage, and one bronchial washing fluid.

Xpert correctly classified all 32 NTM-positive specimens as MTB-negative (specificity 100%, 95% confidence interval [CI]: 89.1%–100%), and Xpert Ultra classified 31 of 32 as MTB-negative (specificity

96.9%, 95% CI: 83.8%–99.9%). One sample was called "very low" positive by Xpert Ultra; this specimen originated from a patient with prior pulmonary TB treatment whose MTB PCR was positive 2 months earlier, despite negative cultures. This isolated discordance likely represents the detection of residual, non-viable MTB DNA rather than a false-positive result due to NTM. Overall, both assays demonstrated excellent specificity in the presence of NTM. When we further analyzed individual probe Ct values for "MTB not detected" results, neither Xpert nor Xpert Ultra showed amplification in any probe.

Table 1. Assessment of cross-reactivity in RIF resistance detection by Xpert and Xpert Ultra with NTM species at high bacterial load

NTM strain (1.0×10^6 CFU/mL equivalent)	MTB (5.0×10^3 CFU/mL equivalent)	Xpert result				Xpert Ultra result			
		1st		2nd		1st		2nd	
		MTB	RIF resistance	MTB	RIF resistance	MTB	RIF resistance (Mutant *Tm*, rpoB3/rpoB4-A)	MTB	RIF resistance (Mutant *Tm*, rpoB3/rpoB4-A)
M. avium (KMRC 0136-41011)	RS	D, Low	ND	D, Low	ND	D, Very low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.7/73.8)	D, Trace	Indeterminate
M. intracellulare (ATCC 13950)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.7/73.8)	D, Low	D (73.7/73.8)
M. kansasii (ATCC 12478)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.7/73.7)	D, Low	D (73.4/73.8)
M. abscessus subsp. *abscessus* (ATCC 19977)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.4/73.7)	D, Low	D (73.2/73.6)
M. abscessus subsp. *massiliense* (CCUG 48898)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.5/73.8)	D, Low	D (72.8/73.3)
M. gordonae (ATCC 14470)	RS	D, Low	ND	D, Medium	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.6/73.7)	D, Low	D (73.5/73.8)
M. chelonae (ATCC 35752)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (73.8/73.8)	D, Low	D (73.5/73.9)
M. fortuitum (KMRC 0136-60004)	RS	D, Low	ND	D, Low	ND	D, Low	ND	D, Very low	ND
	RR	D, Low	D	D, Low	D	D, Low	D (72.7/73.3)	D, Low	D (73.4/73.7)

Abbreviations: RIF, rifampin; NTM, nontuberculous mycobacteria; CFU, colony-forming unit; MTB, *Mycobacterium tuberculosis*; KMRC, Korea Mycobacterium Resource Center; ATCC, American Type Culture Collection; CCUG, Culture Collection University of Göteborg; D, detected; ND, not detected; RS, rifampin-susceptible reference strain (H37Rv, ATCC 27294); RR, rifampin-resistant clinical isolate with S450L mutation.

Table 2. Cycle threshold values and melt peak temperatures of Xpert MTB/RIF and Xpert MTB/RIF Ultra assays

Concentration (CFU/mL equivalent)		Xpert MTB/RIF						Xpert MTB/RIF Ultra									
		Ct value (1st/2nd)						Ct value (1st/2nd)						*Tm* (1st/2nd)			
NTM strain (1.0×10^6)	MTB (5.0×10^3)	SPC	Probe A	Probe B	Probe C	Probe D	Probe E	SPC	IS1081/6110	rpoB1	rpoB2	rpoB3	rpoB4	rpoB1	rpoB2	rpoB3	rpoB4
-	RS	22.8	24.2	24.9	24.5	24.8	25.7	25.6	18.0	25.5	24.7	26.8	28.9	69.4	73.1	75.9	67.3
	RR	22.7	22.1	23.5	22.9	23.1	0.0	27.0	19.0	23.8	24.2	27.1	23.1	69.4	74.3	73.4	73.8
M. avium	RS	21.6/25.3	24.7/26.9	25.0/27.9	24.6/27.2	25.0/27.6	26.4/28.3	26.0/26.0	18.2/18.3	25.2/25.6	24.6/24.9	26.5/27.0	28.3/28.7	69.5/69.5	73.3/73.4	76.0/76.1	67.5/67.5
(KMRC 0136-41011)	RR	22.2/24.7	24.1/25.3	24.7/26.6	24.3/25.8	24.4/26.1	0.0/0.0	25.0/0	20.6/23.5	25.4/0.0	24.9/0.0	28.1/0.0	24.7/0.0	69.3/-	74.4/-	**73.7/-**	**73.8/-**
M. intracellulare	RS	23.8/22.2	25.7/23.6	26.5/24.3	25.7/24.1	26.4/24.6	27.3/25.7	26.0/25.2	22.3/19.5	29.4/26.7	29.0/26.0	30.5/28.0	31.6/30.0	69.4/69.4	73.2/73.2	75.9/75.9	67.2/67.4
(ATCC 13950)	RR	23.2/23.8	24.7/25.4	25.7/26.3	25.1/26.1	25.4/26.3	0.0/0.0	25.7/25.5	20.8/19.4	25.7/23.4	25.4/23.6	28.7/26.6	24.9/22.4	69.4/69.5	74.3/74.4	**73.7/73.7**	**73.8/73.8**
M. kansasii	RS	23.5/23.4	25.0/24.5	26.0/25.4	25.2/24.6	25.6/25.2	26.8/26.4	25.1/25.8	17.5/18.5	24.7/26.0	23.5/25.0	26.0/27.5	27.8/29.3	69.3/69.3	73.2/73.1	75.9/75.8	67.4/67.3
(ATCC 12478)	RR	23.5/24.0	26.4/23.4	27.0/24.5	26.5/23.7	26.7/24.2	0.0/0.0	25.6/26.0	21.2/20.3	26.3/25.7	25.7/25.0	29.2/28.1	25.4/24.1	69.2/69.4	74.2/74.2	**73.7/73.4**	**73.7/73.8**
M. abscessus	RS	22.0/23.7	24.0/24.9	24.8/25.8	24.2/25.3	24.5/25.6	25.8/26.6	24.8/25.2	18.3/18.1	23.8/25.7	23.6/25.3	26.0/27.4	30.5/31.1	69.6/69.4	73.4/73.2	76.1/75.9	67.6/67.4
subsp. *abscessus* (ATCC 19977)	RR	23.2/22.7	23.9/24.4	25.0/25.3	24.3/24.8	24.5/25.2	0.0/0.0	25.4/25.3	21.1/20.0	26.1/25.3	25.7/25.0	29.1/27.6	25.0/27.3	69.3/69.2	74.2/74.1	**73.4/73.2**	**73.7/73.6**
M. abscessus	RS	22.2/23.5	26.4/25.5	25.1/26.2	24.6/26.0	25.0/26.0	26.3/27.3	26.3/25.9	17.8/19.4	24.0/28.5	24.2/27.7	25.6/30.0	27.7/31.6	69.3/69.4	73.1/73.1	75.8/75.9	67.4/67.3
subsp. *massiliense* (CCUG 48898)	RR	23.0/21.7	25.8/23.1	26.5/24.0	26.1/23.5	26.3/23.8	0.0/0.0	25.1/28.8	20.0/19.9	23.8/28.9	24.2/28.2	27.3/31.6	22.8/26.0	69.3/69.1	74.2/73.4	**73.5/72.8**	**73.8/73.3**
M. gordonae	RS	21.7/21.7	22.5/21.6	23.6/22.7	22.7/21.8	23.3/22.4	24.2/23.3	26.1/25.0	18.4/18.5	25.5/24.8	24.8/24.3	26.9/26.0	29.0/28.0	69.3/69.3	73.1/73.2	75.8/75.9	67.4/67.4
(ATCC 14470)	RR	23.8/24.0	24.7/26.4	25.7/27.1	25.0/26.8	25.3/27.0	0.0/0.0	25.2/27.6	22.2/20.3	27.2/25.5	26.8/25.2	30.1/28.8	26.1/25.5	69.3/69.4	74.4/74.3	**73.6/73.5**	**73.7/73.8**
M. chelonae	RS	22.6/22.7	24.4/23.5	24.9/24.5	24.5/23.6	25.1/24.2	26.1/25.2	24.7/25.4	17.7/19.0	25.0/25.9	24.1/25.0	26.1/27.0	28.5/28.7	69.4/69.2	73.2/73.0	75.9/75.7	67.4/67.3
(ATCC 35752)	RR	23.3/23.8	24.3/25.8	25.1/26.5	24.6/26.3	24.9/26.4	0.0/0.0	24.4/25.4	21.5/19.9	25.6/24.8	25.2/24.1	28.3/28.0	24.5/23.6	69.4/69.4	74.5/74.3	**73.8/73.5**	**73.8/73.9**
M. fortuitum	RS	23.2/24.8	25.4/25.7	26.1/26.7	25.4/26.1	26.0/26.4	27.2/27.5	25.7/27.1	17.7/21.0	24.2/29.3	23.8/28.5	26.0/30.6	28.6/31.0	69.2/69.3	73.0/73.1	75.7/75.8	67.3/67.2
(KMRC 0136-60004)	RR	21.7/22.5	23.6/23.7	24.4/24.6	23.7/24.4	24.3/24.5	0.0/0.0	28.3/26.2	19.5/20.6	27.0/26.2	26.9/25.8	31.1/29.3	24.9/25.3	69.0/69.3	73.3/74.2	**72.7/73.4**	**73.7/73.7**

Values in bold denote mutant *Tm*.

Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; CFU, colony-forming unit; Ct, cycle threshold; *Tm*, melting peak temperature; NTM, nontuberculous mycobacteria; SPC, specimen processing control; KMRC, Korea Mycobacterium Resource Center; ATCC, American Type Culture Collection; CCUG, Culture Collection University of Göteborg; RS, rifampin-susceptible reference strain (H37Rv, ATCC 27294); RR, rifampin-resistant clinical isolate with S450L mutation.

Discussion

Key results

In spiking experiments, high-load NTM did not cause false-positive results for MTB nor did they affect rifampin-resistance determinations in either Xpert assay. The expected mutant melting peaks associated with the S450L mutationwere preserved. Among 335 respiratory specimens, one was excluded, and 32 were culture-confirmed as NTM. Both Xpert and Xpert Ultra assays correctly classified all NTM-positive specimens as MTB negative, with no probe amplification detected in any NTM-positive, MTB-negative sample. No analytical interference was observed, and clinical specificity reached 100% in these specimens.

Interpretation/comparison with previous studies

Previous studies have reported NTM-induced cross-reactivity, false RIF resistance calls, and amplification of specific probes for the Xpert assay. Pang et al. [4] suggested that high concentrations of NTM species could induce false-positive MTB results in Xpert. Notably, *M. abscessus* and *M. smegmatis* also produced false-resistant results for RIF, although our previous study failed to reproduce these findings [15]. Previous studies have assessed the analytical specificity of Xpert Ultra using high-concentration NTM isolates and reported no cross-reactivity with MTB [6,7]. However, studies evaluating whether high NTM concentrations interfere with the detection of low-level MTB or the accuracy of RIF resistance calls remain scarce [6]. In our study, Xpert Ultra reliably detected MTB in the presence of a high NTM burden. Furthermore, when assessing RIF resistance using the S450L mutation as a representative of RIF-resistance-conferring *rpoB* mutations, we found no alteration in *Tm*s due to co-existing NTM. The use of mock samples in our study reinforced the robustness of Xpert Ultra for the determination of RIF resistance in mixed MTB-NTM environments.

Previous studies have reported that probe amplification is often observed in the Xpert Ultra and Xpert MTB/RIF assays for NTM species. Tang et al. reported that Xpert probe A was amplified in the presence of *M. intracellulare* [17]. Similarly, a weak rpoB2 probe signal was observed for most NTM species in a validation study of the Xpert Ultra assay [6] and in a study conducted by Opperman et al. [7] in South Africa. Alemu et al. [18] also demonstrated that weak probe amplification, mainly for rpoB2 and Probe C in the Xpert Ultra and Xpert assays, was common for NTM species. In their cohort of 990 patients with diabetes mellitus and chronic kidney disease, all 87 clinical specimens from which NTM was isolated in the culture yielded negative Xpert Ultra results; however, probe amplification was observed in 12.6% of these cases. In contrast, none of the 32 culture-positive NTM specimens in our study showed probe amplification attributable to NTM in either assay. Moreover, Alemu et al. [18] reported that among the 990 specimens, 97 (9.8%) were Xpert Ultra–negative, yet exhibited probe amplification, and 13.4% of these yielded NTM in culture. In our study, only one of the 334 specimens (0.3%) showed weak rpoB2 amplification (Ct, 35.9; data not shown), and this specimen was culture-negative. Variability in population characteristics and other laboratory factors, including assay versions and NTM strains used, are likely to influence how often weak probe signals are detected, which may explain the interstudy differences. We used the latest versions of the assays, Xpert

version 6 and Xpert Ultra version 4, whereas the versions used in the previous studies were not identical. Moreover, because only a limited number of strains per species were used, we cannot exclude the possibility of probe amplification attributable to NTM. Nevertheless, our results suggest that while weak probe signals can occur in Xpert Ultra-negative specimens, their utility as an indicator of NTM may vary across settings, and further assessment in larger studies would be informative.

Limitations

One limitation of our study was the relatively small number of NTM-positive clinical specimens (n = 32), reflecting the inherent constraint of evaluating non-selective clinical specimens. Additional limitations of our study include the in vitro nature of mock samples and the focus on a single RIF-resistance mutation; moreover, the use of heat-inactivated MTB strains in spiking experiments may not fully replicate clinical conditions where viable MTB coexist with NTM in mixed infections. Future studies should incorporate clinical specimens with live MTB, a broader array of RIF-resistance-conferring *rpoB* mutations, and variable NTM species. Nevertheless, our findings support the use of Xpert Ultra as a reliable tool for the simultaneous detection of MTB and RIF resistance, even in regions where NTM carriage is common.

Conclusion

Even under substantial NTM loads, both Xpert and Xpert Ultra maintained accurate detection of MTB and RIF resistance. These results indicate strong analytical specificity for mixed MTB–NTM samples, reinforcing the suitability of these assays in settings with frequent NTM isolation.

Ethics statement

This study was approved by the Institutional Review Board of Samsung Medical Center (approval number: 2024-09-068). The requirement for informed consent was waived due to the use of anonymized data.

Conflicts of interest

Hee Jae Huh has been an editorial board member of the *Annals of Clinical Microbiology* since September 2024. However, she was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

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Data availability

The datasets generated during the current study are available from the corresponding author upon request.

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