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Detection of Mycobacterium tuberculosis and rifampicin resistance using the Xpert MTB/RIF assay among suspected tuberculosis patients at a tertiary-care hospital in Southern Punjab, Pakistan: a cross-sectional study

Taiba Ahmad
BUCH International Hospital
Raja Kamran Afzal
rajakamranafzal1@gmail.com

Combined Military Hospital (CMH)
Mehreen Aftab Khan
Multan Institute of Kidney Diseases (MIKD)
Saadia Latif
CMH Institute of Medical Sciences (CIMS)
Rashda Shabbir
CMH Institute of Medical Sciences (CIMS)
Waleed Nasir
One Brooklyn Health (OBH)

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Abstract

Background

Drug-resistant tuberculosis remains an important concern in high-burden settings, where delayed identification of resistance can complicate treatment and infection-control decisions. This study examined MTB detection by Xpert MTB/RIF and the frequency of molecularly detected rifampicin resistance among presumptive TB patients in a tertiary-care hospital in Southern Punjab, Pakistan.

Methods

A descriptive cross-sectional study was conducted in the Department of Microbiology, Combined Military Hospital, Multan, between 1 January and 31 December 2024. We included 170 adults aged 18–75 years with clinical or radiological findings suggestive of pulmonary or extrapulmonary TB. Clinical specimens were examined with the Xpert MTB/RIF assay. MTB detection and rifampicin resistance were summarized using frequencies, percentages and 95% confidence intervals.

Results

The 170 participants had a mean age of 45.8 ± 10.2 years; 95 (55.9%) were male and 75 (44.1%) were female. Pulmonary specimens accounted for 114 (67.1%) samples and extrapulmonary specimens for 56 (32.9%). MTB was detected in 136/170 participants (80.0%; 95% CI 73.2–85.8%). Detection was 96/114 (84.2%) in pulmonary specimens and 40/56 (71.4%) in extrapulmonary specimens. Among the 136 Xpert MTB-positive cases, rifampicin resistance was detected in 16 (11.76%; 95% CI 6.9–18.4%), while 120 (88.24%) had no molecular evidence of rifampicin resistance. The reported comparisons showed no statistically significant association between rifampicin resistance and age ($p = 0.42$) or sex ($p = 0.68$).

Conclusions

Xpert MTB/RIF identified MTB in a substantial proportion of presumptive TB patients and simultaneously identified rifampicin resistance in a subset of MTB-positive cases. The findings support rapid molecular testing in this clinical setting, but the single-centre design and absence of comprehensive drug-susceptibility testing restrict the extent to which the results can be generalized or used to characterize the wider resistance profile.

BACKGROUND

Tuberculosis (TB) continues to impose a substantial infectious-disease burden worldwide. The problem is compounded by drug-resistant disease, particularly in settings where rapid access to molecular

testing and comprehensive susceptibility testing is limited.

Pakistan carries a high burden of TB, and conventional diagnosis has historically relied heavily on sputum smear microscopy for acid-fast bacilli. Although microscopy remains useful because it is relatively inexpensive and widely available, it has limited sensitivity and cannot provide rapid information about drug resistance. Molecular assays can shorten the time between specimen collection and recognition of patients who require further evaluation for resistant TB.

Xpert MTB/RIF is a rapid nucleic-acid amplification assay capable of detecting M. tuberculosis complex DNA and molecular markers associated with rifampicin resistance. Its use has expanded the availability of rapid TB testing in many high-burden settings. Nevertheless, observed positivity and resistance proportions depend on the clinical population tested, specimen characteristics and local epidemiology.

Evidence from national and international studies provides broad estimates of TB detection and drug resistance, but data from individual tertiary-care centres remain useful for understanding local diagnostic patterns. Information from Southern Punjab is particularly relevant because referral hospitals may receive a heterogeneous population with both pulmonary and extrapulmonary disease.

Accordingly, this study was designed to quantify MTB detection among presumptive TB patients tested with Xpert MTB/RIF and to determine the proportion of Xpert MTB-positive cases in whom rifampicin resistance was detected at a tertiary-care hospital in Southern Punjab, Pakistan.

METHODS

Study design and setting

We conducted a descriptive cross-sectional study in the Department of Microbiology, Combined Military Hospital (CMH), Multan, from 1 January 2024 through 31 December 2024. The hospital is a tertiary-care facility serving patients from Multan and surrounding areas, including referred patients.

Participants and sampling

Eligible participants were adults aged 18–75 years who had clinical symptoms and/or radiological findings considered suggestive of pulmonary or extrapulmonary TB. Consecutive non-probability sampling was used. Patients who had received antituberculosis treatment for more than two weeks, those from whom a suitable specimen was unavailable or contaminated, and those who declined participation were excluded.

The study enrolled 170 participants. The source manuscript reports that the sample size was calculated using Daniel's formula for a single population proportion, with an anticipated Xpert detection proportion of 85%, a 95% confidence level (Z = 1.96), and an absolute precision of 5.4%. The displayed calculation

was not present in the source document and should be inserted from the original protocol before submission.

Specimen collection and Xpert testing

The study included respiratory specimens (sputum and bronchoalveolar lavage) and extrapulmonary specimens, including pleural fluid and cerebrospinal fluid. Specimens were handled according to the laboratory's routine procedures and tested using the automated Xpert MTB/RIF assay. The assay was used to identify M. tuberculosis complex DNA and molecular evidence of rifampicin resistance.

The source manuscript does not state the exact Xpert instrument, assay/cartridge version, manufacturer, specimen-processing volumes, or detailed internal quality-control procedure. These technical details should be added from the laboratory records before submission. The final manuscript should also report the number of invalid, error and no-result assays and explain how such results were handled.

Outcome measures

Outcome measures
The principal outcome was the proportion of tested presumptive TB patients in whom MTB was detected by Xpert MTB/RIF. The secondary outcome was the proportion of Xpert MTB-positive cases with molecular evidence of rifampicin resistance. Because an independent reference standard was not described, these proportions are presented as MTB detection and rifampicin-resistance detection rates rather than measures of diagnostic sensitivity, specificity or accuracy.

Rifampicin resistance was interpreted on the basis of the molecular result generated by the Xpert assay. Since the study did not assess isoniazid resistance or perform comprehensive phenotypic drug-susceptibility testing, detection of rifampicin resistance alone was not used to classify individual participants as having multidrug-resistant TB.

Statistical analysis

Data were analysed using SPSS version 25.0. Continuous variables were summarized using means and standard deviations, and categorical variables using frequencies and percentages. The principal proportions were accompanied by 95% confidence intervals. Associations between rifampicin resistance and demographic variables were assessed using the tests reported in the study dataset. Because only 16 rifampicin-resistant cases were identified, subgroup analyses had limited statistical power. A two-sided p value below 0.05 was considered statistically significant if this threshold was prespecified in the study protocol.

Ethical considerations

Ethical approval

was obtained from the Institutional Ethical Review Board (IERB), Combined Military Hospital (CMH), Multan (reference number CMH Multan: File No.13/Trg/2021, dated 15-09-2021). Informed consent to participate was obtained from all participants before enrolment, and patients who declined to participate were excluded. All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki.

RESULTS

Participant characteristics

All 170 eligible participants were included in the reported analysis. Mean age was 45.8 ± 10.2 years. Males comprised 95 (55.9%) participants and females 75 (44.1%). Of the specimens tested, 114 (67.1%) were pulmonary and 56 (32.9%) were extrapulmonary (Table 1).

Table 1
Baseline demographic and clinical characteristics of participants

Variable	Category	Frequency (n)	Percentage (%)
Age (years)	18–45	81	47.6%
	46–75	89	52.4%
Mean age (± SD)	Entire cohort	45.8 ± 10.2	—
Sex	Male	95	55.9%
	Female	75	44.1%
Specimen type	Pulmonary (sputum/BAL)	114	67.1%
	Extrapulmonary (fluids)	56	32.9%

MTB detection

MTB was detected by Xpert MTB/RIF in 136 of 170 participants, corresponding to 80.0% (95% CI 73.2–85.8%). The detection proportion was 84.2% (96/114) for pulmonary specimens and 71.4% (40/56) for extrapulmonary specimens (Table 2). These values describe the proportion of specimens in this selected clinical cohort that yielded an MTB-positive Xpert result and should not be interpreted as assay sensitivity or community prevalence.

Table 2
MTB detection by Xpert MTB/RIF according to specimen category

Specimen type	Total evaluated (N)	MTB detected (n)	MTB detection rate (%)
Pulmonary	114	96	84.2%
Extrapulmonary	56	40	71.4%
Total	170	136	80.0%

Rifampicin resistance

Among the 136 cases with MTB detected by Xpert, 16 (11.76%; 95% CI 6.9–18.4%) showed molecular evidence of rifampicin resistance, whereas 120 (88.24%) did not (Table 3). The latter category represents absence of Xpert-detected rifampicin resistance and should not be taken as equivalent to a complete phenotypic susceptibility profile.

Table 3
Rifampicin-resistance status among Xpert MTB-positive cases

Xpert MTB/RIF result	Frequency (n)	Percentage (%)
Rifampicin resistance detected	16	11.76%
Rifampicin resistance not detected	120	88.24%
Total MTB-positive cases	136	100.0%

Demographic comparisons

The mean age was 44.2 ± 9.1 years among rifampicin-resistant cases and 46.1 ± 10.4 years among those without detected rifampicin resistance; the reported comparison was not statistically significant ($p = 0.42$). Within the 136 MTB-positive participants, rifampicin resistance was identified in 10/76 (13.2%) males and 6/60 (10.0%) females ($p = 0.68$) (Table 4). The small number of resistant cases limits the strength of these subgroup comparisons.

Table 4
Rifampicin resistance according to demographic characteristics among Xpert MTB-positive cases (n = 136)

Demographic factor	Rifampicin resistant (n = 16)	Rifampicin not detected (n = 120)	χ^2 / t-test value	p-value
Age (mean ± SD)	44.2 ± 9.1	46.1 ± 10.4	t = 0.809	0.42
Male	10 (13.2%)	66 (86.8%)	$\chi^2 = 0.170$	0.68
Female	6 (10.0%)	54 (90.0%)	—	—
Note: Table 4 includes only MTB-positive participants (76 males and 60 females).				

Tables

Figure 1. MTB detection rate according to specimen category. showing 84.2% detection for pulmonary specimens and 71.4% for extrapulmonary specimens.

DISCUSSION

This study found that MTB was detected by Xpert MTB/RIF in 80.0% of presumptive TB patients tested at a tertiary-care hospital in Multan. Rifampicin resistance was identified in 16 of the 136 MTB-positive cases, giving a molecular resistance proportion of 11.76%. MTB detection was more frequent in pulmonary than extrapulmonary specimens, while the available demographic comparisons did not demonstrate statistically significant differences in rifampicin resistance by age or sex.

The 80.0% MTB detection proportion should be interpreted in relation to the study population rather than as a measure of the intrinsic diagnostic performance of Xpert MTB/RIF. Participants were selected because they had clinical or radiological features suggestive of TB and were evaluated in a tertiary-care hospital. Consequently, the result reflects the yield of MTB detection in this selected group and cannot be extrapolated directly to the general population. Similar studies have demonstrated the value of rapid molecular assays for detecting MTB and rifampicin resistance in presumptive TB populations (14, 15).

The detection of rifampicin resistance in 11.76% of MTB-positive cases is clinically relevant because identification of resistance at the time of initial molecular testing can prompt timely evaluation and management for drug-resistant TB. However, the finding should not be interpreted as a regional prevalence estimate. The study was performed at one tertiary-care centre, and the participants may differ from patients in other healthcare settings. Moreover, Xpert-based detection of rifampicin resistance does not establish the complete drug-susceptibility pattern of an isolate (16).

The higher MTB detection proportion in pulmonary specimens (84.2%) than in extrapulmonary specimens (71.4%) may partly reflect differences in specimen quality and bacillary burden. Respiratory specimens are generally more amenable to molecular detection when organisms are present at higher concentrations, whereas extrapulmonary disease can be paucibacillary. The present study did not directly quantify bacillary load, so this explanation remains a plausible interpretation rather than a finding demonstrated by the dataset.

No significant association was observed between rifampicin resistance and either age or sex. The result should be interpreted cautiously because only 16 participants were classified as rifampicin resistant. With this small resistant subgroup, the study may not have had sufficient statistical power to identify modest demographic associations. Factors such as previous TB treatment, treatment adherence, healthcare exposure and household transmission may influence resistance patterns, but these variables were not collected in the present study and therefore cannot be evaluated from these data (19).

An important strength is the inclusion of both pulmonary and extrapulmonary clinical specimens during a defined one-year study period. The study also reflects routine molecular testing in a tertiary-care microbiology service, providing information on the population encountered in that setting. Nevertheless,

several limitations affect interpretation. First, the single-centre cross-sectional design prevents assessment of treatment outcomes or the temporal origin of resistance. Second, consecutive non-probability sampling limits representativeness and may have resulted in a clinically enriched cohort. Third, an independent reference standard such as culture was not described, preventing assessment of Xpert sensitivity and specificity. Fourth, the study did not include comprehensive drug-susceptibility testing or isoniazid resistance assessment, so the 16 rifampicin-resistant cases cannot be assumed to represent MDR-TB. Finally, the small number of resistant cases restricts the statistical power of demographic comparisons.

Future multicentre studies should combine rapid molecular testing with culture and comprehensive drug-susceptibility testing, while also collecting information on previous TB treatment, adherence and relevant exposure history. Longitudinal surveillance could then determine how resistance patterns change over time and whether molecularly detected resistance is associated with clinical outcomes. Where appropriate and ethically feasible, genomic methods could further characterize transmission and resistance mechanisms (23).

CONCLUSION

Xpert MTB/RIF detected M. tuberculosis in 80.0% of presumptive TB patients evaluated at this tertiary-care hospital, with detection proportions of 84.2% in pulmonary and 71.4% in extrapulmonary specimens. Rifampicin resistance was detected in 11.76% of MTB-positive cases. These findings illustrate the value of rapid molecular testing for identifying MTB and rifampicin resistance in a tertiary-care setting. Because the study was single-centre and did not include an independent reference standard or comprehensive drug-susceptibility testing, the results should not be interpreted as estimates of regional TB prevalence, assay accuracy or the overall burden of MDR-TB.

Abbreviations

BAL: Bronchoalveolar lavage; MTB: Mycobacterium tuberculosis; RR-TB: rifampicin-resistant tuberculosis; TB: tuberculosis; Xpert MTB/RIF: Xpert MTB/RIF assay.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethical Review Board (IERB), Combined Military Hospital (CMH), Multan (reference number CMH Multan: File No.13/Trg/2021, dated 15-09-2021). Informed consent to participate was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Clinical Trial Number

Clinical trial number not applicable.

Funding

No funding source was availed.

Authors' contributions

TA and RKA conceived and designed the study. TA and RKA developed the methodology and supervised the overall research process. TA, RKA, MAK, SL, RS and WN contributed to investigation and data collection. TA and MAK contributed to data curation and formal analysis. TA and RKA drafted the original manuscript. All authors contributed to critical review and revision of the manuscript and approved the final version.

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Not applicable.

Data Availability

Yes. This study used and generated research data from 170 adult patients with presumptive tuberculosis who were evaluated using the Xpert MTB/RIF assay. The dataset included demographic characteristics, specimen type, "Mycobacterium tuberculosis" detection results, and rifampicin-resistance results. The data were analyzed to determine MTB detection rates and the frequency of rifampicin resistance among MTB-positive specimens.

References

1. CRyPTIC Consortium. A data compendium associating the genomes of 12,289 Mycobacterium tuberculosis isolates with quantitative resistance phenotypes to 13 antibiotics. PLoS Biol. 2022;20(8):e3001721.
2. Vīksna A, Sadovska D, Berge I, Bogdanova I, Vaivode A, Freimane L, et al. Genotypic and phenotypic comparison of drug resistance profiles of clinical multidrug-resistant Mycobacterium tuberculosis isolates using whole genome sequencing in Latvia. BMC Infect Dis. 2023;23(1):638.
3. Köser CU, Georghiou SB, Schön T, Salfinger M. On the consequences of poorly defined breakpoints for rifampin susceptibility testing of Mycobacterium tuberculosis complex. J Clin Microbiol. 2021;59(4):e02328–20.
4. Xiao Y-X, Liu K-H, Lin W-H, Chan T-H, Jou R. Whole-genome sequencing-based analyses of drug-resistant Mycobacterium tuberculosis from Taiwan. Sci Rep. 2023;13(1):2540.
5. Yusoof KA, Garcia JI, Schami A, Garcia-Vilanova A, Kelley HV, Wang S-H, et al. Tuberculosis phenotypic and genotypic drug susceptibility testing and immunodiagnostics: a review. Front Immunol. 2022;13:870768.
6. Vasiliauskaitė L, Bakuła Z, Vasiliauskienė E, Bakonytė D, Decewicz P, Dziurzyński M, et al. Detection of multidrug-resistance in Mycobacterium tuberculosis by phenotype- and molecular-based assays. Ann Clin Microbiol Antimicrob. 2024;23(1):81.
7. Liu D, Huang F, Zhang G, He W, Ou X, He P et al. Whole-genome sequencing for surveillance of tuberculosis drug resistance and determination of resistance level in China. Clin Microbiol Infect. 2022;28(5):731.e9-731.e15.
8. Zhao B, Liu C, Fan J, Ma A, He W, Hu Y, et al. Transmission and drug resistance genotype of multidrug-resistant or rifampicin-resistant Mycobacterium tuberculosis in Chongqing, China. Microbiol Spectr. 2022;10(5):e02405–21.
9. Tong E, Zhou Y, Liu Z, Zhu Y, Zhang M, Wu K, et al. Bedaquiline resistance and molecular characterization of rifampicin-resistant Mycobacterium tuberculosis isolates in Zhejiang, China. Infect Drug Resist. 2023;16:6951–63.
10. Sadovska D, Nodieva A, Pole I, Vīksna A, Ķimsis J, Ozere I, et al. Discordance between phenotypic and WGS-based drug susceptibility testing results for some anti-tuberculosis drugs: A snapshot study of paired Mycobacterium tuberculosis isolates with small genetic distance. Infect Drug Resist. 2024;17:3289–307.
11. He W, Tan Y, Liu C, Wang Y, He P, Song Z, et al. Drug-resistant characteristics, genetic diversity, and transmission dynamics of rifampicin-resistant Mycobacterium tuberculosis in Hunan, China, revealed by whole-genome sequencing. Microbiol Spectr. 2022;10(1):e01543–21.
12. Qadir M, Faryal R, Khan MT, Khan SA, Zhang S, Li W, et al. Phenotype versus genotype discordant rifampicin susceptibility testing in tuberculosis: implications for a diagnostic accuracy. Microbiol Spectr. 2024;12(1):e01631–23.
13. Reta MA, Tamene BA, Abate BB, Mensah E, Maningi NE, Fourie PB, et al. Mycobacterium tuberculosis drug resistance in Ethiopia: an updated systematic review and meta-analysis. Trop Med

Infect Dis. 2022;7(10):300.
14. Selfegna S, Alelign A. Detection of Mycobacterium tuberculosis and rifampicin resistance using GeneXpert MTB/RIF assay at enat hospital, Central Ethiopia. Tuberculosis Res Treat. 2022;2022(1):1250404.
15. da Silva MP, Cassim N, Ndlovu S, Marokane PS, Radebe M, Shapiro A, et al. More than a decade of GeneXpert® Mycobacterium tuberculosis/rifampicin (Ultra) testing in South Africa: Laboratory insights from twenty-three million tests. Diagnostics (Basel). 2023;13(20):3253.
16. William A, Raı Y, Kaur R. Evaluation of Rifampicin-resistant Tuberculosis in Pediatric Patients by GeneXpert MTB/RIF. J Microbiol Infect Dis. 2021;11(02):81–7.
17. Ramachandra V, Mani R, Muthaiah M, Soundappan G, Frederick A. Assess the Diagnostic Accuracy of GeneXpert to Detect Mycobacterium tuberculosis and Rifampicin-Resistant Tuberculosis among Presumptive Tuberculosis and Presumptive Drug Resistant Tuberculosis Patients. Microbiol Res. 2024;15:91–108.
18. Wobudeya E, Bonnet M, Walters EG, Nabeta P, Song R, Murithi W, et al. Diagnostic advances in childhood tuberculosis—improving specimen collection and yield of microbiological diagnosis for intrathoracic tuberculosis. Pathogens. 2022;11(4):389.
19. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P, et al. A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. Eur Respir J. 2021;58(5):2100526.
20. Walker TM, et al. Web Annex D. Review of the diagnostic accuracy of targeted next-generation sequencing technologies for detection of drug resistance among people diagnosed with TB. WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection. 3rd ed. Geneva: World Health Organization; 2024. p. 508.
21. Adepoyibi T. Communicable and non-communicable disease prevention: three public health challenges. University of New South Wales (Australia); 2022.
22. Soni A, Mishra R, Nath A, Kapoor P. Evaluation of Xpert MTB/RIF and GenoType MTBDRplus for the Detection of Rifampicin-Resistant Tuberculosis: A Cross-Sectional Diagnostic Accuracy Study at a Tertiary Care Center in India. Cureus. 2025;17(9):e91825.
23. McDowell A, Anjorin O, van der Westhuizen H-M, Engel N, Subramanian A, Delrooz N et al. WHO standard: universal access to rapid tuberculosis diagnostics: web annex B: stakeholder perspectives on barriers and enablers to the implementation of molecular WHO-recommended rapid diagnostic tests for tuberculosis. 2023.

Figures

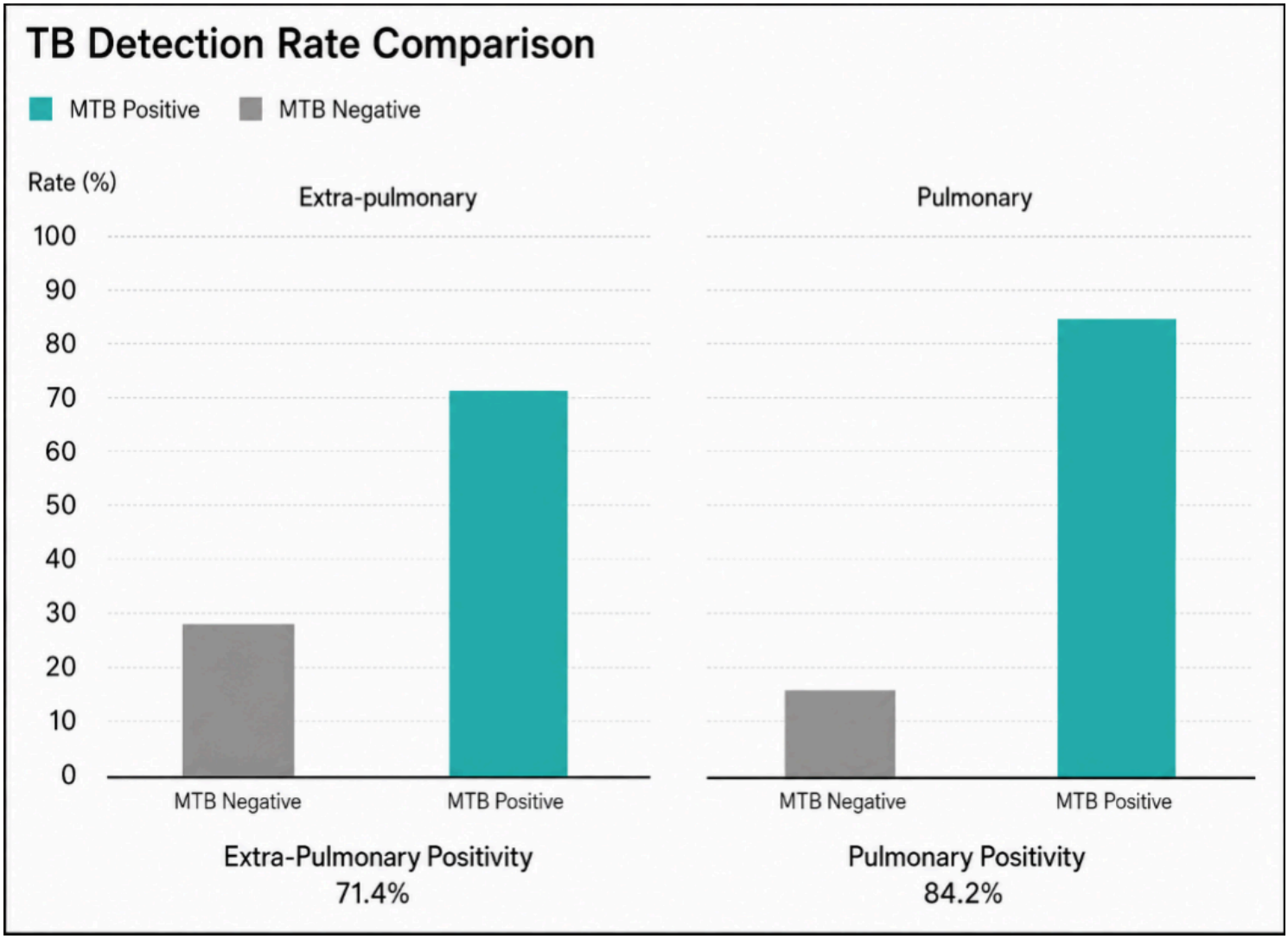


Figure 1

MTB detection rate according to specimen category. showing 84.2% detection for pulmonary specimens and 71.4% for extrapulmonary specimens.