Section: Microbiology

Original Research Article

DETECTION OF NON-TUBERCULOUS MYCOBACTERIA IN AFB SMEAR-POSITIVE BUT MTBC MOLECULAR-NEGATIVE CLINICAL SPECIMENS: A RETROSPECTIVE OBSERVATIONAL STUDY FROM CENTRAL INDIA

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Received *: 17/03/2026*
Received in revised form *: 11/05/2026*
Accepted *: 29/05/2026*

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DOI: 10.70034/ijmedph.2026.2.752

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (2); 4605-4608

ABSTRACT

Background: In high tuberculosis-burden settings such as India, acid-fast bacilli (AFB) positivity in clinical specimens is frequently presumed to indicate infection with Mycobacterium tuberculosis complex (MTBC). However, non-tuberculous mycobacteria (NTM) also exhibit acid-fastness and can cause both pulmonary and extrapulmonary disease, posing a diagnostic challenge when rapid molecular assays for MTBC yield negative results. This study evaluated the occurrence of NTM among AFB smear-positive clinical specimens that tested negative for MTBC by rapid molecular assays at a tertiary care center in Central India.

Materials and Methods: This retrospective observational study was conducted in the Department of Microbiology at a tertiary care teaching hospital in Central India from May 2024 to May 2026. A total of 12,732 clinical specimens were screened for AFB positivity during the study period (6,644 in 2024–25 and 6,088 in 2025–26). Rapid molecular assays (GeneXpert/CBNAAT and/or Truenat) identified MTBC in 489 specimens. Specimens that were AFB-positive by fluorescent microscopy and/or Ziehl–Neelsen staining but negative for MTBC by these assays were further tested using the Bio-Speedy MTB/NTM qPCR Kit for the detection of NTM and Mycobacterium avium complex (MAC). Lowenstein–Jensen culture was performed on all such specimens. Laboratory and clinical data were retrieved retrospectively from institutional records.

Results: Sixteen AFB smear-positive but MTBC molecular-negative specimens met the inclusion criteria. These comprised both pulmonary and extrapulmonary samples, including sputum, pleural fluid, cerebrospinal fluid, ascitic fluid, pus, aspirates, and endometrial tissue. Multiplex real-time PCR detected NTM in all 16 specimens: MAC in 8 cases, non-MAC NTM in 4 cases, and Mycobacterium species without definitive speciation in 4 cases. Lowenstein–Jensen culture yielded growth in only two specimens; both culture-positive isolates were identified as MAC and correlated with the multiplex PCR results. Six patients had already been started on anti-tubercular therapy prior to NTM detection.

Conclusion: NTM should be considered in AFB smear-positive specimens that test negative for MTBC by rapid molecular assays such as GeneXpert and Truenat. Routine incorporation of NTM-targeted molecular assays in such cases can improve diagnostic accuracy and help avoid inappropriate anti-tubercular therapy in high TB-burden settings.

Keywords: Non-tuberculous mycobacteria; NTM; Mycobacterium avium complex; MAC; GeneXpert; Truenat; acid-fast bacilli; multiplex real-time PCR; tuberculosis.

INTRODUCTION

Tuberculosis (TB) continues to impose a substantial public health burden in India despite notable improvements in diagnostic capabilities. The widespread adoption of rapid molecular assays such as GeneXpert MTB/RIF and Truenat has significantly enhanced the early detection of Mycobacterium tuberculosis complex (MTBC). Nevertheless, acid-fast bacilli (AFB) positivity on smear microscopy is not specific to MTBC, as non-tuberculous mycobacteria (NTM) also stain acid-fast and are capable of causing both pulmonary and extrapulmonary infections.[1]

NTM represent a diverse group of environmental mycobacteria that are increasingly recognized as opportunistic pathogens. Pulmonary disease is the most frequent manifestation, although extrapulmonary involvement of lymph nodes, pleura, central nervous system, soft tissues, and genitourinary sites is being reported with growing frequency. In high-TB-burden countries, smear-positive cases are often empirically treated as TB without further speciation, leading to potential misdiagnosis and unnecessary exposure to anti-tubercular drugs.[2]

The increasing reliance on MTBC-specific molecular tests has highlighted a subset of patients who remain AFB smear-positive yet test negative for MTBC. These cases raise the possibility of NTM infection and underscore the need for targeted NTM diagnostics. Data on the burden of NTM in India, particularly from routine laboratory settings in Central India, remain limited.[3]

The present study was undertaken to determine the occurrence of NTM among AFB smear-positive but MTBC molecular-negative clinical specimens received at a tertiary care center in Central India.

MATERIALS AND METHODS

Study Design and Setting: This retrospective observational study was performed in the Department of Microbiology at a tertiary care teaching hospital in Central India between May 2024 and May 2026.

Sample Screening and Inclusion Criteria During the study period, 12,732 clinical specimens were examined for AFB positivity. Rapid molecular testing identified MTBC in 489 specimens. Specimens were included if they fulfilled all of the following criteria: (1) AFB positivity by fluorescent microscopy and/or Ziehl–Neelsen staining; (2) negative for MTBC by GeneXpert/CBNAAT and/or Truenat assay; and (3) further evaluated for NTM using multiplex real-time PCR. Exclusion criteria included inadequate specimen volume, incomplete laboratory records, and repeat samples from the same patient. The summary of sample screening is depicted in [Table 1].

Laboratory Processing Specimens were processed according to standard microbiological protocols. Smear microscopy was performed using fluorescent and/or Ziehl–Neelsen staining. MTBC-specific rapid molecular testing was conducted with GeneXpert or Truenat platforms. AFB-positive, MTBC-negative specimens were subsequently tested with the Bio-Speedy MTB/NTM qPCR Kit for detection of NTM and MAC. Lowenstein–Jensen (LJ) culture was performed for all included specimens, with biochemical characterization attempted for any isolates that grew.

Data Collection and Ethical Considerations Demographic, clinical, and laboratory data including specimen type, smear results, molecular assay findings, culture results, and anti-tubercular therapy status were extracted retrospectively from laboratory registers and hospital records. The study protocol was approved by the Institutional Ethics Committee. As it was retrospective and utilized anonymized data, the requirement for informed consent was waived.

RESULTS

Sixteen AFB smear-positive but MTBC molecular-negative specimens fulfilled the inclusion criteria. These specimens included both pulmonary and extrapulmonary samples such as sputum, pleural fluid, cerebrospinal fluid, ascitic fluid, pus, aspirates, and endometrial tissue.

Multiplex real-time PCR demonstrated NTM positivity in all 16 specimens. MAC was detected in 8 cases, non-MAC NTM in 4 cases, and Mycobacterium species without definitive speciation in the remaining 4 cases. LJ culture was performed on all specimens, but growth was obtained in only two; both culture-positive isolates were identified as MAC and were concordant with the multiplex PCR results. Six patients had already been initiated on anti-tubercular therapy before NTM was detected [Figure 1]. The summary of the results is depicted in [Table 2].

Table 1: Overview of AFB screening and study inclusion (May 2024 – May 2026)

Parameter	Value
Total clinical specimens screened for acid-fast bacilli	12,732
Specimens screened in 2024–25	6,644
Specimens screened in 2025–26	6,088
MTBC positive by GeneXpert/CBNAAT or Truenat	489
AFB smear-positive but MTBC molecular-negative specimens included and tested for NTM	16

Table 2: Molecular detection and culture results of NTM among AFB smear-positive, MTBC molecular-negative specimens (N = 16)

Result	Number (%)
Mycobacterium avium complex (MAC) by multiplex qPCR	8 (50.0)
Non-MAC non-tuberculous mycobacteria	4 (25.0)
Mycobacterium species (without definitive speciation)	4 (25.0)
Positive growth on Lowenstein–Jensen culture	2 (12.5)
Culture-positive isolates identified as MAC	2 (100.0)
Patients already initiated on anti-tubercular therapy prior to NTM detection	6 (37.5)

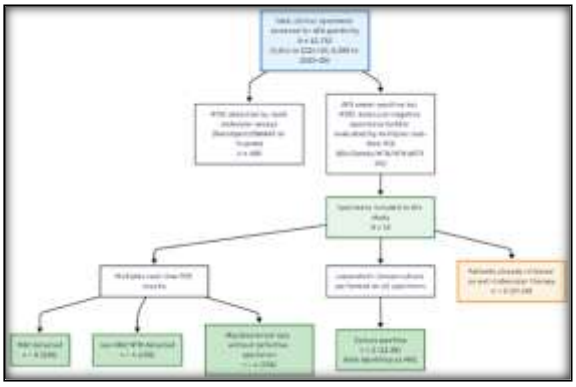

Figure 1: Flow diagram of specimen selection and laboratory findings for the detection of non-tuberculous mycobacteria (NTM) in AFB smear-positive but MTBC molecular-negative clinical specimens (May 2024 – May 2026).

DISCUSSION

The present study documents the presence of NTM in AFB smear-positive clinical specimens that tested negative for MTBC by rapid molecular assays in a high-TB-burden tertiary care setting in Central India. In resource-limited, high-prevalence regions, smear positivity is routinely interpreted as presumptive TB, often prompting empirical anti-tubercular therapy. However, NTM can produce identical microscopic and clinical features, leading to diagnostic confusion and inappropriate treatment.[2,4]

All included specimens in this study were AFB-positive yet negative by MTBC-specific assays (GeneXpert and Truenat). Subsequent multiplex real-time PCR confirmed NTM in every case, highlighting the diagnostic gap that exists when only MTBC-targeted tests are employed. Both pulmonary and extrapulmonary specimens were represented, underscoring the broad clinical spectrum of NTM infections, which are frequently under-recognized in extrapulmonary sites.[5]

MAC accounted for half of the detections, consistent with its prominence in global and Indian literature on clinically significant NTM. Culture positivity was low (only 2/16 specimens), likely due to low bacillary load, prior antimicrobial exposure, or the fastidious nature of certain NTM species. Importantly, the two culture-positive isolates aligned with MAC detection by PCR, supporting the reliability of the molecular findings.[6]

A clinically relevant finding was that six patients had already commenced anti-tubercular therapy prior to NTM confirmation. This observation illustrates the risk of misdiagnosis and unnecessary drug exposure, which may delay appropriate NTM-specific management.[1]

Limitations of the study include its retrospective design, lack of detailed clinical and radiological correlation, absence of species-level identification for all isolates, and non-utilization of liquid culture systems (e.g., MGIT) or sequencing. Despite these constraints, the study provides real-world laboratory evidence of NTM in smear-positive, MTBC-negative specimens from Central India and aligns with findings from similar regional studies.

CONCLUSION

Non-tuberculous mycobacteria should be actively considered in AFB smear-positive specimens that test negative for MTBC by rapid molecular assays. Integration of NTM-specific molecular testing in this diagnostic niche can enhance accuracy, facilitate timely appropriate therapy, and reduce inappropriate exposure to anti-tubercular drugs in high TB-burden settings. Prospective studies with comprehensive species identification, liquid culture, and detailed clinical correlation are warranted to further elucidate the epidemiology and clinical impact of NTM infections in India.

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