

Detection of Nontuberculous Mycobacteria by Maldi-Tof Ms and Its Co-Infection with Mycobacterium Tuberculosis

Mahima Chouhan¹, Dr. Jayant Ramawat², Dr. Prabhu Prakash³

¹PhD Scholar, Department of Microbiology, Dr. S.N Medical College, Jodhpur.

²Senior Resident, Department of Microbiology, Dr. S.N Medical College, Jodhpur.

³Senior Professor & Head of Department, Department of Microbiology, Dr. S.N Medical College, Jodhpur.

Abstract

Introduction: Coinfection of MTB complex with NTM is increasingly recognised in TB endemic countries, which may remain undiagnosed or coexist with pulmonary TB, resulting in diagnostic and therapeutic challenges.

Methods: An observational study was conducted in a tertiary care centre, which included suspected NTM pulmonary disease patients' LJ culture isolates. Patients were of all age groups and both sexes. Detection of co-infections was via CBNAAT and/or LPA and MALDI-TOF Mycobacterial/Nocardia Kit.

Results: Among 55 patients with suspected NTM pulmonary disease, 10 NTM isolates (18.2%; 10/55) were identified by MALDI-TOF MS. The identified species were *Mycobacterium kansasii* (n=4; 40%), *Mycobacterium fortuitum* (n=4; 40%), and *Mycobacterium wolinskyi* (n=2; 20%). MTB–NTM coinfection was detected in 4/10 (40.0%) NTM-positive patients, corresponding to 4/55 (7.3%) of the total study population. Of these four coinfecting patients, two were MTB-positive by CBNAAT and rifampicin-sensitive, while the remaining two were positive by first-line LPA and were sensitive to both rifampicin and isoniazid. Among the coinfecting patients, *M. fortuitum* accounted for 2/4 (50%), while *M. kansasii* and *M. wolinskyi* accounted for 1/4 (25%) each.

Conclusion: NTM infection along with MTB–NTM coinfection was low among TB-suspected patients. Identification by the MALDI-TOF MS of suspected NTM pulmonary disease diagnosis enables rapid and accurate species identification and improves recognition of NTM infections in TB-endemic areas and potentially reduces morbidity.

Keywords: Tuberculosis, Nontuberculous mycobacteria, MTB–NTM coinfection, MALDI-TOF MS, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, *Mycobacterium wolinskyi*.

Introduction

Nontuberculous mycobacteria (NTM) are emerging opportunistic pathogens capable of causing a wide spectrum of infections, including surgical-site infections, post-injection abscesses, osteomyelitis, catheter-related bloodstream infections, and central nervous system infections. Pulmonary NTM disease occurs predominantly in individuals with underlying structural lung diseases such as chronic obstructive pulmonary disease (COPD), previous tuberculosis (TB), bronchiectasis, or in immunocompromised

patients, or receiving immunosuppressive therapy. (1). Coinfection of *Mycobacterium tuberculosis* complex (MTBC) and NTM has gained increasing attention in TB-endemic countries (1). The coexistence of both organisms in the same patient may complicate diagnosis and treatment, as routine molecular assays such as Cartridge-based Nucleic Acid Amplification Test (CBNAAT) are designed to detect MTBC but do not identify NTM species. Failure to recognise NTM may contribute to persistent symptoms, delayed clinical response, and inappropriate management (2,3).

Species-level identification of NTM has become increasingly important with the availability of rapid identification techniques. Matrix-Assisted Laser Desorption Ionisation–Time of Flight Mass Spectrometry (MALDI-TOF MS) has demonstrated high accuracy for identification of clinically significant mycobacterial species while substantially reducing turnaround time compared with conventional biochemical methods (4,5).

Materials and Methods

A prospective observational study was conducted in a tertiary care centre over a period of 6 months. The study included 55 randomised suspected NTM pulmonary disease patient's samples that were Lowenstein-Jensen (LJ) culture- and Mycobacteria Growth Indicator Tube (MGIT)- culture-positive and Mycobacterium Protein 64 (MPT64) test-negative. All the Pulmonary and Extrapulmonary samples of Suspected Tuberculosis patients are included in the study. Patients of all age groups and both sexes were included. Blood-stained samples will be excluded from the study. Potential confounders such as age, sex, smoking, diabetes, previous anti-TB treatment, antibiotic exposure, immunosuppression, underlying pulmonary disease, and urban/rural residence were considered during analysis. Analysis of recent TB infection records was also performed for these included patients to detect co-infections via either CBNAAT or Line Probe Assay (LPA). These 55 included samples were then processed via the MALDI-TOF Mycobacterial/Nocardia Kit. NTM detected in these MALDI-TOF results were included in further analysis of TB co-infections (Fig 1). Ethical approval was taken before starting the study; the IEC number is SNMC/IEC/2025/Plan/1042 dated 11.03.2025. For statistical analysis, SPSS software and for significance Fisher's exact and Mann-Whitney tests were used.

Results

Among the 55 MGIT culture-positive and MPT64 antigen-negative specimens subjected to MALDI-TOF MS, 10 (18.2%; 95% CI: 9.1–30.9%) were identified as NTM. The mean age of NTM-positive patients was 40.0 ± 13.7 years, with a median age of 41.5 years (IQR: 28.25–46.75 years). The age distribution did not significantly deviate from normality (Shapiro–Wilk $W=0.964$, $p=0.827$). Eight (80.0%, 8/10) patients were male, and two (20.0%, 2/10) were female. *Mycobacterium kansasii* and *Mycobacterium fortuitum* were each identified in four (40.0%, 4/10) isolates, while *Mycobacterium wolinskyi* was identified in two (20.0%, 2/10) isolates. Microbiological evidence of MTB–NTM coinfection was detected in 4/10 (40.0%; 95% CI: 12.2–73.8%) NTM-positive patients, corresponding to 4/55 (7.3%; 95% CI: 2.0–17.6%) of the total study population. Two of the four coinfecting patients were identified by CBNAAT and two by first-line LPA. All CBNAAT-positive patients were rifampicin-sensitive, while both patients tested by first-line LPA were sensitive to rifampicin and isoniazid. No significant association was observed between sex and MTB–NTM coinfection (Fisher's exact test, $p=1.000$), and age did not differ significantly between coinfecting and non-coinfecting patients (Mann–Whitney $U=15.0$, $p=0.593$). (Table 1).

Discussion

In the present study, 10 NTM isolates (18.2%) were identified among 55 suspected NTM pulmonary disease patients' LJ culture isolates, which were subjected to the MALDI-TOF MS Mycobacterial/Nocardia Kit. Among these, four patients (40%) fulfilled the diagnostic criteria for both pulmonary tuberculosis and NTM pulmonary disease, demonstrating microbiologically confirmed MTB–NTM coinfection. These findings highlight the importance of considering NTM in patients undergoing evaluation for pulmonary tuberculosis, particularly when culture characteristics & therapeutic prognosis are inconsistent with *Mycobacterium tuberculosis* complex (MTBC).

Our findings are consistent with those reported by Sharma et al., who demonstrated the presence of clinically significant NTM among patients suspected of tuberculosis and drug-resistant tuberculosis and emphasised that MIGIT culture-positive, MPT64-negative isolates require further species identification to avoid misdiagnosis and inappropriate anti-tubercular therapy [1]. Similarly, Bui et al. described a patient with pulmonary tuberculosis and *Mycobacterium avium* complex infection and highlighted the importance of differentiating true NTM disease from colonisation using clinical, radiological, and microbiological criteria [2,3-5].

In the present study, all NTM cases fulfilled the diagnostic criteria recommended by the American Thoracic Society / European Respiratory Society / European Society of Clinical Microbiology and Infectious Diseases / Infectious Diseases Society of America (ATS/ERS/ESCMID/IDSA) Clinical Practice Guideline for NTM pulmonary disease, while pulmonary tuberculosis was confirmed according to the National Tuberculosis Elimination Programme (NTEP) diagnostic algorithm using CBNAAT and/or first-line line probe assay, supporting the diagnosis of true MTB–NTM coinfection rather than simple coexistence of organisms [2,6-8].

Among the ten NTM isolates, four (40%, 4/10) were associated with MTB coinfection. Although this proportion appears higher than that reported by Lin et al. (19.3% n=85/450, among patients with NTM disease) and Bonnet et al. (3.1%, 4/124 among NTM patients), direct comparison should be interpreted cautiously because of differences in study design, study populations, diagnostic algorithms, and the denominator used for calculating coinfection rates [9,10]. The relatively higher proportion observed in the present study may be explained by the selective inclusion of MPT64-negative culture isolates, species identification being performed only on the MALDI-TOF-tested subset, the small sample size, and the study being conducted at a tertiary referral tuberculosis centre, where diagnostically challenging cases are more frequently encountered.[11-13]

The predominant NTM species identified were *Mycobacterium kansasii* and *Mycobacterium fortuitum*, followed by *Mycobacterium wolinskyi*. *M. kansasii* is one of the most important slowly growing NTM causing pulmonary disease and frequently mimics pulmonary tuberculosis both clinically and radiologically [7]. *M. fortuitum* is a rapidly growing mycobacterium commonly associated with skin and soft-tissue infections but is increasingly recognized as a cause of pulmonary disease, particularly in patients with underlying structural lung disease or previous tuberculosis [14]. Although *M. wolinskyi* is rarely isolated from respiratory specimens, its identification in the present study demonstrates the diversity of clinically relevant NTM species and highlights the importance of accurate species-level identification, as antimicrobial susceptibility patterns and treatment regimens vary considerably among NTM species [2,14].

Species identification by MALDI-TOF MS enabled rapid and reliable identification of all NTM isolates recovered in the study. Conventional smear microscopy cannot differentiate MTBC from NTM, while

molecular assays such as CBNAAT and line probe assay are designed primarily for the detection of MTBC and drug resistance [14]. Therefore, culture-based species identification remains essential for recognising clinically significant NTM infections. Previous studies have demonstrated that MALDI-TOF MS provides accurate species-level identification of mycobacteria with shorter turnaround times than conventional phenotypic methods, making it a valuable adjunct in routine mycobacteriology laboratories [4,5].

Overall, the present study demonstrates that MTB–NTM coinfection can occur among patients with suspected pulmonary tuberculosis in Western Rajasthan. Although only a small number of coinfecting patients were identified, this study provides valuable baseline data from a region where published evidence remains limited and emphasises the importance of incorporating species-level identification into routine diagnostic workflows to facilitate accurate diagnosis and appropriate patient management.[13]

References

1. Sharma M, Malhotra B, Tiwari J, Bhargava S. Profile of Nontuberculous Mycobacteria in Patients Suspected of Tuberculosis and Drug-Resistant Tuberculosis. *J Lab Physicians*. 2020;12(3):177-181.
2. Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ Jr, Andrejak C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: An official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Clin Infect Dis*. 2020;71(4):e1-e36.
3. Bui TI, Rowe J, Eberly AR. Colonization or coinfection? A case report of *Mycobacterium avium* complex detected in a patient with pulmonary tuberculosis. **ASM Case Reports**. 2025.
4. Buckwalter SP, Olson SL, Connelly BJ, et al. Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of *Mycobacterium* species and *Nocardia* species. *J Clin Microbiol*. 2016;54:376-384.
5. Body BA, Beard MA, Slechta ES, et al. Evaluation of the VITEK MS v3.0 system for identification of mycobacteria and *Nocardia* species. *J Clin Microbiol*. 2018;56:e00237-18.
6. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175(4):367-416.
7. World Health Organization. WHO Operational Handbook on Tuberculosis. Module 3: Diagnosis. Geneva: WHO; 2021.
8. Lin CK, Yang YH, Lu ML, et al. Incidence of nontuberculous mycobacterial disease and coinfection with tuberculosis in a tuberculosis-endemic region: a population-based retrospective cohort study. *Medicine*. 2020;99(52):e23775. doi:10.1097/MD.00000000000023775
9. Bonnet M, San KC, Pho Y, et al. Nontuberculous mycobacteria infections at a provincial reference hospital. *Cambodia Emerg Infect Dis*. 2017;23(7):1139–1147. doi:10.3201/eid2307.170060
10. Yilmaz N, Ucar EY, Saglam L. *Mycobacterium tuberculosis* and nontuberculous mycobacteria coinfection of the lungs. *Turk Thorac J*. 2017;18(1):23–26. doi:10.5152/TurkThoracJ.2017.16034
11. Izadi N, Derakhshan M, Samiei A, Ghazvini K. Co-infection of long-standing extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) and non-tuberculosis mycobacteria: a case report. *Respir Med Case Rep*. 2015;15:12–13. doi:10.1016/j.rmcr.2014.11.006
12. Kelgaonkar A, Nayak S, Routray N, Behera HS. **Combined PCR Positivity for *Mycobacterium tuberculosis* Complex and Nontuberculous *Mycobacterium* Species in Patients Treated for Tubercular Panuveitis.** *Ocul Immunol Inflamm*. 2022;30(7-8):1837-1842. doi:10.1080/09273948.2021.1914472.

13. Central TB Division. National Tuberculosis Elimination Programme (NTEP): Technical and Operational Guidelines. Ministry of Health and Family Welfare, Government of India.
14. Brown-Elliott BA, Wallace RJ Jr. Clinical and Taxonomic Status of Pathogenic Rapidly Growing Mycobacteria. Clin Microbiol Rev. 2002;15:716–746.