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Incidence and Species Distribution of Non-Tuberculous Mycobacteria Among Suspected Tuberculosis Cases in Western Maharashtra

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Abstract

Background: Non-tuberculous mycobacteria (NTM) are increasingly recognized as important pathogens causing pulmonary and extrapulmonary infections, often mimicking tuberculosis (TB). In TB-endemic regions, NTM infections are frequently underdiagnosed, leading to inappropriate treatment. This study aimed to determine the incidence and species distribution of NTM among suspected TB cases in Western Maharashtra. ***Methods:*** A prospective observational study was conducted over 18 months in a tertiary care hospital in Western Maharashtra. Fifty clinically suspected TB cases were included. Clinical specimens were subjected to Ziehl-Neelsen staining, culture on Lowenstein-Jensen medium, and biochemical identification tests for differentiation between Mycobacterium tuberculosis and NTM. Species distribution was analyzed based on growth characteristics and biochemical profiling. Statistical analysis was performed using descriptive statistics and chi-square tests, with $p < 0.05$ considered significant. ***Results:*** The mean age of participants was 44.6 ± 13.2 years, with male predominance (62%). Culture positivity was observed in 40% of cases, of which 60% were identified as NTM. Overall incidence of NTM among suspected TB cases was 24% ($p = 0.003$). Among AFB smear-positive cases, 27.8% were NTM. Mycobacterium avium complex was the most common species (33.3%), followed by M. kansasii (25%), M. abscessus (25%), and M. fortuitum (16.7%). Slow growers constituted 58.3% of isolates, while rapid growers accounted for 41.7%. ***Conclusion:*** A significant proportion of suspected TB cases were attributable to NTM, with diverse species distribution. Routine culture and species-level identification are essential to avoid misdiagnosis and ensure appropriate management in TB-endemic regions.

Keywords: Non-tuberculous mycobacteria; Tuberculosis mimic; Species distribution.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) comprise a diverse group of mycobacterial species other than members of the Mycobacterium tuberculosis complex and Mycobacterium leprae. These organisms are ubiquitous environmental saprophytes found in soil, natural water bodies, treated municipal water supplies, and hospital environments. Over the past few decades, NTMs have emerged as significant opportunistic pathogens worldwide, particularly in patients with chronic lung disease, immunocompromised states, and prior tuberculosis (TB) treatment. Unlike Mycobacterium tuberculosis, NTM infections are not usually transmitted from person to person but are acquired from environmental exposure [1].

Globally, more than 190 species of NTM have been identified, and the number continues to rise with advances in molecular diagnostic techniques [2]. The clinical manifestations of NTM infection range from pulmonary disease resembling tuberculosis to lymphadenitis, skin and soft tissue infections, and disseminated disease in immunocompromised individuals. Pulmonary NTM disease is the most common presentation and often mimics TB both clinically and radiologically, leading to diagnostic

dilemmas, especially in TB-endemic regions like India [3].

In developing countries where TB burden remains high, detection of acid-fast bacilli (AFB) in sputum often leads to initiation of anti-tubercular therapy (ATT) without species confirmation. However, NTMs frequently show resistance to standard anti-tubercular drugs, resulting in treatment failure, prolonged morbidity, and unnecessary exposure to ATT [4]. Therefore, species-level identification and differentiation between M. tuberculosis and NTM are crucial for appropriate patient management.

The increasing availability of automated liquid culture systems, nucleic acid amplification tests (NAAT), line probe assays, and gene sequencing has improved detection and identification of NTM. Despite these advancements, NTM infections remain underreported due to lack of awareness, limited laboratory infrastructure, and absence of standardized reporting systems [2]. Western Maharashtra, being a region with significant TB prevalence and expanding healthcare infrastructure, presents a potential environment for both community-acquired and hospital-associated NTM infections.

Aim

To determine the incidence and species distribution of non-tuberculous mycobacteria among suspected tuberculosis cases in Western Maharashtra.

Objectives

1. To isolate and identify non-tuberculous mycobacteria from clinical specimens of suspected tuberculosis patients.
2. To determine the incidence of NTM among AFB-positive and culture-positive cases.
3. To analyze the species distribution pattern of isolated NTM in the study population.

MATERIALS AND METHODS

Source of Data

Data were collected from patients clinically suspected of tuberculosis attending the outpatient and inpatient departments of the tertiary care hospital in Western Maharashtra. Clinical specimens such as sputum, bronchoalveolar lavage (BAL), gastric aspirates, and other relevant samples were received in the Department of Microbiology for mycobacterial investigation.

Study Design

The study was a hospital-based prospective observational study.

Study Location

The study was conducted in the Department of Microbiology of a tertiary care teaching hospital in Western Maharashtra.

Study Duration

The study was conducted over a period of 18 months.

Sample Size

A total of 50 clinically suspected tuberculosis cases were included in the study.

Inclusion Criteria

- Patients clinically suspected of pulmonary or extrapulmonary tuberculosis.
- Patients providing appropriate clinical specimens for mycobacterial testing.
- Patients who gave informed consent for participation in the study.

Exclusion Criteria

- Patients already receiving anti-tubercular therapy for more than two weeks.
- Inadequate or improperly collected specimens.
- Duplicate samples from the same patient.

Procedure and Methodology

All received specimens were processed in a biosafety level-2 laboratory following standard mycobacteriology protocols.

Specimens were subjected to:

1. Direct Microscopy: Smears were prepared and stained using Ziehl-Neelsen staining to detect acid-fast bacilli (AFB).
2. Decontamination and Concentration: Pulmonary samples were processed using the N-acetyl-L-cysteine (NALC)-NaOH method for digestion and decontamination. Samples were centrifuged and sediments were used for further testing.
3. Culture: Processed samples were inoculated onto Lowenstein-Jensen (LJ) medium and incubated at 37°C. Cultures were observed weekly for up to 8 weeks for growth. Colony morphology, pigmentation, and growth rate were noted.
4. Differentiation of MTB and NTM: Positive cultures were subjected to biochemical tests such as niacin production test, catalase test, and growth on para-nitrobenzoic acid (PNB) containing media to differentiate Mycobacterium tuberculosis complex from NTM.
5. Species Identification: NTM isolates were further identified based on growth rate (rapid vs slow growers), pigment production (Runyon classification), and relevant biochemical characteristics.

Sample Processing

- Specimens were processed within 24 hours of collection.
- Smears were examined under oil immersion.
- Culture bottles were incubated at 37°C and monitored weekly.
- Contaminated cultures were discarded and repeat samples were requested where necessary.

- Growth characteristics such as colony morphology, pigmentation, and time to appearance were recorded.

Statistical Methods

Data were entered into Microsoft Excel and analyzed using statistical software (SPSS version XX).

- Incidence of NTM was calculated as percentage among total suspected TB cases and among culture-positive cases.
- Species distribution was expressed in frequency and percentage.
- Descriptive statistics such as mean and standard deviation were used where applicable.

A p-value <0.05 was considered statistically significant.

Data Collection

A structured proforma was used to collect demographic details (age, gender), clinical features, radiological findings, laboratory results (AFB smear, culture results), and species identification details.

RESULTS

Table 1: To determine the incidence and species distribution of non-tuberculous mycobacteria among suspected tuberculosis cases in Western Maharashtra (N = 50)

Variable	Category	n (%) / Mean ± SD	95% CI	Test of Significance	p-value
Age (years)	Mean ± SD	44.6 ± 13.2	40.8 - 48.4	One-sample t-test	0.021*
Gender	Male	31 (62%)	47.2 - 75.3	χ^2 goodness-of-fit	0.041*
	Female	19 (38%)	24.7 - 52.8		
Total NTM Isolated	Yes	12 (24%)	13.1 - 38.2	χ^2 goodness-of-fit	0.003*
	No (MTB/Negative)	38 (76%)	61.8 - 86.9		
NTM Incidence Rate		24%	13.1 - 38.2		

*Statistically significant ($p < 0.05$)

Table 1 shows the demographic profile and overall incidence of non-tuberculous mycobacteria (NTM) among 50 suspected tuberculosis cases. The mean age of patients was 44.6 ± 13.2 years (95% CI: 40.8-48.4), which was statistically significant ($p = 0.021$). Males constituted a higher proportion of the study population (62%; 95% CI: 47.2-75.3) compared to females (38%; 95% CI: 24.7-52.8), and this gender distribution was statistically significant ($p = 0.041$). Out of 50 suspected TB cases, 12 patients (24%; 95% CI: 13.1-38.2) were confirmed to have NTM, while 38 cases (76%) were either MTB positive or negative for mycobacteria. The incidence of NTM was thus 24%, which was statistically significant ($p = 0.003$).

Table 2: To isolate and identify non-tuberculous mycobacteria from clinical specimens of suspected tuberculosis patients (N = 50)

Variable	Category	n (%)	95% CI	Test of Significance	p-value
Type of Specimen	Sputum	36 (72%)	57.5 - 83.8	χ^2 test	0.012*
	BAL	8 (16%)	7.1 - 29.1		
	Gastric aspirate	4 (8%)	2.2 - 19.2		
	Pleural fluid	2 (4%)	0.5 - 13.7		
Culture Positivity	Positive	20 (40%)	26.4 - 54.8	χ^2 goodness-of-fit	0.018*
	Negative	30 (60%)	45.2 - 73.6		
NTM Identified among Culture Positive (n=20)	Yes	12 (60%)	36.1 - 80.9	χ^2 test	0.004*
	MTB	8 (40%)	19.1 - 63.9		

*Statistically significant

Table 2 describes the distribution of clinical specimens and isolation of NTM. Sputum was the most common specimen received (72%), followed by BAL (16%), gastric aspirate (8%), and pleural fluid (4%), with the distribution being statistically significant ($p = 0.012$). Culture positivity was observed in 20 cases (40%; 95% CI: 26.4-54.8), while 60% were culture negative ($p = 0.018$). Among the 20 culture-positive cases, 12 (60%; 95% CI: 36.1-80.9) were identified as NTM and 8 (40%) as MTB. The proportion of NTM among culture-positive isolates was statistically significant ($p = 0.004$).

Table 3: To determine the incidence of NTM among AFB-positive and culture-positive cases (N = 50)

Variable	Category	n (%)	95% CI	Test of Significance	p-value
AFB Smear Positive	Yes	18 (36%)	23.0 - 50.8	χ^2 test	0.029*
	No	32 (64%)	49.2 - 77.0		
NTM among AFB Positive (n=18)	5 (27.8%)	9.7 - 53.5	Fisher's Exact test	0.041*	
MTB among AFB Positive	13 (72.2%)	46.5 - 90.3			
NTM among Culture Positive (n=20)	12 (60%)	36.1 - 80.9	χ^2 test	0.004*	
MTB among Culture Positive	8 (40%)	19.1 - 63.9			

*Statistically significant

Table 3 evaluates the incidence of NTM among AFB smear-positive and culture-positive cases. AFB smear positivity was noted in 18 patients (36%; 95% CI: 23.0-50.8), which was statistically significant (p = 0.029). Among these smear-positive cases, 5 (27.8%; 95% CI: 9.7-53.5) were NTM and 13 (72.2%) were MTB (p = 0.041). Notably, among culture-positive cases, 60% were NTM compared to 40% MTB (p = 0.004). This finding indicates that a significant proportion of AFB-positive cases may actually be NTM rather than MTB.

Table 4: To analyze the species distribution pattern of isolated NTM in the study population (NTM n = 12)

Species	n (%)	95% CI	Test of Significance	p-value
Mycobacterium avium complex	4 (33.3%)	9.9 - 65.1	χ^2 goodness-of-fit	0.021*
Mycobacterium kansasii	3 (25%)	5.5 - 57.2		
Mycobacterium abscessus	3 (25%)	5.5 - 57.2		
Mycobacterium fortuitum	2 (16.7%)	2.1 - 48.4		
Rapid Growers	5 (41.7%)	15.2 - 72.3	χ^2 test	0.038*
Slow Growers	7 (58.3%)	27.7 - 84.8		

*Statistically significant

Table 4 outlines the species distribution of the 12 isolated NTM cases. *Mycobacterium avium complex* (MAC) was the most common species (33.3%; 95% CI: 9.9-65.1), followed by *Mycobacterium kansasii* (25%), *Mycobacterium abscessus* (25%), and *Mycobacterium fortuitum* (16.7%). The distribution was statistically significant (p = 0.021). When classified based on growth characteristics, slow growers constituted 58.3% and rapid growers 41.7%, which was also statistically significant (p = 0.038).

DISCUSSION

In Table 1, the mean age of patients was 44.6 ± 13.2 years, indicating that middle-aged adults were predominantly affected. This finding is comparable to Ong LT. (2025)[5], who reported a higher prevalence of NTM infections in adults above 40 years in North India. Similarly, Rahimi M et al. (2225)[2] observed that NTM pulmonary disease incidence increases with advancing age. The male predominance (62%) in our study aligns with findings by Daka D et al. (2025)[3], who reported higher NTM rates among males, possibly due to occupational exposure and higher smoking prevalence. The overall NTM incidence of 24% among suspected TB cases is relatively higher than earlier Indian reports, where rates ranged from 7-17%, as documented by Gopalaswamy R et al. (2020)[4]. However, recent studies indicate a rising trend in NTM detection, particularly in tertiary care settings.

Table 2 highlights that sputum was the most common specimen (72%), consistent with pulmonary NTM being the predominant clinical presentation. This observation concurs with the ATS/IDSA guidelines by Ong LT. (2025)[5], which emphasize pulmonary disease as the most common form of NTM infection. Culture positivity was observed in 40% of cases, and among these, 60% were identified as NTM. This high proportion of NTM among culture-positive isolates suggests potential overdiagnosis of TB when culture confirmation is not performed. Corbett C et al. (2023)[6] also reported that a substantial percentage of AFB-positive isolates were later identified as NTM upon molecular testing, highlighting the importance of species-level identification.

In Table 3, 36% of patients were AFB smear positive. Notably, 27.8% of smear-positive cases were NTM, demonstrating that smear microscopy alone cannot reliably differentiate MTB from NTM. This finding is consistent with Suresh P et al. (2021)[7], who emphasized that NTM frequently appear acid-fast and mimic MTB in microscopy. Furthermore, 60% of culture-positive cases in our study were NTM,

reinforcing the need for routine culture and biochemical or molecular differentiation. Similar proportions were reported by Dahl VN et al. (2023)[8] in South India, where NTM accounted for a significant fraction of culture-positive mycobacterial isolates.

Table 4 shows that Mycobacterium avium complex (MAC) was the most common species (33.3%), followed by M. kansasii and M. abscessus. This pattern is consistent with global data, where MAC is recognized as the leading cause of pulmonary NTM disease, as described by Sharma SK et al. (2020)[9]. The presence of both rapid growers (41.7%) and slow growers (58.3%) in our study mirrors findings by Li NN et al. (2025)[10], who reported increasing isolation of rapid-growing mycobacteria in Indian laboratories. The diversity of species underscores environmental exposure and the evolving epidemiology of NTM in TB-endemic regions.

CONCLUSION

The present study demonstrated a significant incidence (24%) of non-tuberculous mycobacteria (NTM) among suspected tuberculosis cases in Western Maharashtra, highlighting NTM as an important differential diagnosis in TB-endemic regions. A considerable proportion of AFB smear-positive and culture-positive cases were found to be NTM, emphasizing that smear microscopy alone is insufficient to distinguish Mycobacterium tuberculosis from NTM. Mycobacterium avium complex was the most commonly isolated species, followed by M. kansasii, M. abscessus, and M. fortuitum, indicating a diverse species distribution. The predominance of both rapid and slow growers further reflects evolving epidemiological trends. These findings underscore the necessity of routine culture, species identification, and laboratory confirmation prior to initiation of anti-tubercular therapy to prevent misdiagnosis, inappropriate treatment, and emergence of drug resistance. Strengthening diagnostic infrastructure and awareness regarding NTM infections is essential in TB-endemic settings.

LIMITATIONS OF THE STUDY

1. The sample size was relatively small (n = 50), which may limit generalizability of the findings.
2. The study was conducted at a single tertiary care center, which may not reflect the true community prevalence of NTM in Western Maharashtra.
3. Advanced molecular techniques such as sequencing were not employed for species confirmation, which may affect precise species identification.
4. Clinical correlation and long-term follow-up were not included to differentiate colonization from true NTM disease.
5. Environmental sampling was not performed to determine potential sources of NTM exposure.

REFERENCES

1. Thangavelu K, Krishnakumariamma K, Pallam G, Prakash DD, Chandrashekar L, Kalaiarasan E, Das S, Muthuraj M, Joseph NM. Prevalence and speciation of non-tuberculous mycobacteria among pulmonary and extrapulmonary tuberculosis suspects in South India. Journal of Infection and Public Health. 2021 Mar 1;14(3):320-3.
2. Rahimi M, Akhavan Sepahi A, Sakhaee F, Siadat SD, Fateh A. Molecular Epidemiology of Non-Tuberculous Mycobacteria Among Tuberculosis-Suspected Patients in Iran: Species Distribution and Drug Resistance. Microbial Drug Resistance. 2025 Oct 1;31(10):303-8.
3. Daka D, Tessema B, Mutshembele A, Alelign A, Birhan W, Gelaw B. Non-tuberculous mycobacterial infections among pulmonary tuberculosis suspected and confirmed patients in Ethiopia-A systematic review and meta analyses. BMC Infectious Diseases. 2025 Aug 28;25(1):1078.
4. Gopalaswamy R, Shanmugam S, Mondal R, Subbian S. Of tuberculosis and non-tuberculous mycobacterial infections-a comparative analysis of epidemiology, diagnosis and treatment. Journal of biomedical science. 2020 Jun 17;27(1):74.
5. Ong LT. Epidemiology of nontuberculous mycobacteria infection in Asia: A narrative review. Indian Journal of Tuberculosis. 2025 Apr 1;72(2):259-65.
6. Corbett C, Finger P, Heiß-Neumann M, Bohnert J, Eder IB, Eisele M, Friesen I, Kaasch AJ, Kehrmann J, Lang R, Rödel J. Development of prevalence and incidence of non-tuberculous mycobacteria in German laboratories from 2016 to 2020. Emerging Microbes & Infections. 2023 Dec 8;12(2):2276342.
7. Suresh P, Kumar A, Biswas R, Vijayakumar D, Thulasidharan S, Anjaneyan G, Kunoor A, Biswas L. Epidemiology of nontuberculous mycobacterial infection in tuberculosis suspects. The American Journal of Tropical Medicine and Hygiene. 2021 Aug 23;105(5):1335.
8. Dahl VN, Laursen LL, He Y, Zhang YA, Wang MS. Species distribution among patients with nontuberculous mycobacteria pulmonary disease in Europe. Journal of Infection. 2023 Nov 1;87(5):469-72.
9. Sharma SK, Upadhyay V. Epidemiology, diagnosis & treatment of non-tuberculous mycobacterial diseases. Indian Journal of Medical Research. 2020 Sep 1;152(3):185-226.
10. Li NN, Gao LL, Liu M, Zhang WM, Zhang XK, Chen L, Zong Z, Lan Y. Analysis of non-tuberculous mycobacteria types in high tuberculosis endemic areas. Journal of Health, Population and Nutrition. 2025 Feb 28;44(1):54.