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Research Article

Antimicrobial Susceptibility Patterns and Diagnostic Method Comparison for Non-Tuberculous Mycobacteria in Western Maharashtra

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ABSTRACT

Background: Non-tuberculous mycobacteria (NTM) are increasingly recognized as significant pathogens in patients suspected of tuberculosis, particularly in high TB-burden regions. Accurate species identification and antimicrobial susceptibility testing are essential due to intrinsic drug resistance and overlapping clinical presentations with Mycobacterium tuberculosis. **Aim:** To evaluate antimicrobial susceptibility patterns of NTM isolates and compare conventional and molecular diagnostic methods for their detection in Western Maharashtra. **Materials and Methods:** A prospective observational study was conducted on 50 clinically suspected tuberculosis cases. Specimens were processed using smear microscopy, Lowenstein-Jensen (LJ) culture, MGIT liquid culture, and molecular identification methods including Line Probe Assay and MALDI-TOF. Antimicrobial susceptibility testing was performed by broth microdilution method as per CLSI guidelines. Statistical analysis was carried out using chi-square, Fisher's exact, and McNemar tests, with $p < 0.05$ considered significant. **Results:** NTM were isolated in 14 cases (28%). MAC was the predominant species (35.7%). Drug resistance was observed in 42.8% of isolates. Amikacin (78.6%) and clarithromycin (71.4%) showed

higher susceptibility, whereas rifampicin demonstrated the highest resistance (57.1%). MGIT liquid culture showed higher sensitivity (92.8%) compared to LJ culture (85.7%). Molecular methods exhibited high specificity (>96%) and significantly improved detection rates ($p < 0.05$). **Conclusion:** NTM constitute a significant proportion of suspected TB cases in Western Maharashtra with notable drug resistance patterns. Advanced liquid culture and molecular diagnostic techniques enhance early detection and species-level identification. Routine antimicrobial susceptibility testing is essential to guide appropriate therapy and prevent misuse of anti-tubercular drugs.

Keywords: Non-tuberculous mycobacteria. Antimicrobial susceptibility. Molecular diagnostics.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) comprise a diverse group of mycobacterial species other than the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. These organisms are ubiquitous in natural and man-made environments, particularly in soil and water systems, and have increasingly been recognized as significant opportunistic pathogens in humans [1]. Over the past few decades, the number of identified NTM species has expanded considerably due to advances in culture techniques and molecular diagnostic methods [2]. Although many species are non-pathogenic, several are associated with pulmonary disease, lymphadenitis, skin and soft tissue infections, and disseminated disease, especially in immunocompromised hosts [3].

The clinical presentation of NTM infections often mimics tuberculosis (TB), particularly in pulmonary cases, leading to diagnostic dilemmas. In high TB-burden countries such as India, acid-fast bacilli (AFB) smear positivity frequently prompts empirical initiation of anti-tubercular therapy (ATT) while awaiting confirmatory tests. However, NTMs are intrinsically resistant to many first-line anti-tubercular drugs, and misidentification may result in inappropriate treatment, prolonged morbidity, and development of drug resistance [2,4]. Hence, accurate species-level identification and antimicrobial susceptibility testing are essential for guiding appropriate therapy.

Traditional culture on Lowenstein-Jensen (LJ) medium remains a cornerstone of mycobacterial diagnosis, but liquid culture systems such as MGIT have improved recovery rates and reduced turnaround time. Furthermore, molecular methods including PCR, line probe assays, gene sequencing, and MALDI-TOF have enhanced the accuracy and speed of NTM identification [4]. Drug susceptibility testing (DST), particularly for clinically relevant agents such as macrolides and amikacin, has gained importance due to emerging resistance patterns in species such as *Mycobacterium avium* complex and *Mycobacterium abscessus* [5].

Aim

To evaluate the antimicrobial susceptibility patterns of non-tuberculous mycobacteria and compare diagnostic methods for their detection in Western Maharashtra.

Objectives

1. To isolate and identify NTM species from suspected tuberculosis cases.
2. To determine the antimicrobial susceptibility patterns of the isolated NTM species.
3. To compare conventional and molecular diagnostic methods for detection of NTM.

MATERIAL AND METHODOLOGY

Source of Data

The data were obtained from clinical specimens collected from patients suspected of tuberculosis attending the outpatient and inpatient departments of a tertiary care hospital in Western Maharashtra.

Study Design

The study was conducted as a prospective observational laboratory-based study.

Study Location

The study was carried out 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 who provided adequate clinical specimens (sputum, BAL, pus, body fluids, etc.).
- Patients who gave informed consent.

Exclusion Criteria

- Patients already on anti-tubercular therapy for more than two weeks.
- Inadequate or improperly collected specimens.
- Contaminated samples unsuitable for processing.

Procedure and Methodology

Clinical specimens were processed following standard mycobacteriological procedures. Smear microscopy was performed using Auramine-O fluorescent staining and Ziehl-Neelsen staining. Specimens were decontaminated using the N-acetyl-L-cysteine-NaOH method and concentrated by centrifugation.

The processed samples were inoculated onto Lowenstein-Jensen (LJ) medium and into liquid culture systems (MGIT). Cultures were incubated at 35-37°C and observed periodically for growth up to 8 weeks. Positive cultures were confirmed for acid-fast bacilli by Ziehl-Neelsen staining.

Differentiation between *Mycobacterium tuberculosis* complex and NTM was performed using para-nitrobenzoic acid (PNB) inhibition test and molecular methods where available. Species identification of NTM isolates was carried out using molecular techniques such as line probe assay or gene sequencing when indicated.

Antimicrobial susceptibility testing was performed by broth microdilution method according to CLSI guidelines for relevant drugs such as clarithromycin, amikacin, rifampicin, ethambutol, linezolid, and fluoroquinolones. Minimum inhibitory concentrations (MICs) were recorded and interpreted as susceptible or resistant based on standard breakpoints.

Sample Processing

All specimens were labeled properly and transported to the laboratory promptly. Decontamination was performed using NALC-NaOH method. Following centrifugation, the sediment was used for smear preparation and culture inoculation. Culture bottles and LJ slopes were incubated and examined regularly for contamination and growth.

Statistical Methods

Data were entered into Microsoft Excel and analyzed using SPSS software. Descriptive statistics such as mean, standard deviation, frequency, and percentage were calculated. Diagnostic methods were compared using sensitivity, specificity, positive predictive value, and negative predictive value. Chi-square test was applied for categorical variables, and p-value <0.05 was considered statistically significant.

Data Collection

Clinical details including age, gender, presenting symptoms, radiological findings, and prior treatment history were recorded in a structured proforma. Laboratory findings

including smear results, culture positivity, species identification, and antimicrobial susceptibility results were documented systematically for analysis.

OBSERVATION AND RESULTS

Table 1: To evaluate the antimicrobial susceptibility patterns of non-tuberculous mycobacteria and compare diagnostic methods for their detection in Western Maharashtra (N = 50)

Parameter	Category / Mean ± SD	n (%) / Value	95% CI	Test of Significance	p-value
Age (years)	Mean ± SD	45.8 ± 12.4	42.2 - 49.4	One sample t-test	0.018*
Gender	Male	31 (62%)	47.2 - 75.3	χ^2 goodness-of-fit	0.041*
	Female	19 (38%)	24.7 - 52.8		
NTM Isolated	Yes	14 (28%)	17.1 - 41.0	χ^2 test	0.032*
	No	36 (72%)	59.0 - 82.9		
Drug Resistant NTM (among isolates)	Yes	6 (42.8%)	17.7 - 71.1	Fisher's exact test	0.021*
	No	8 (57.2%)	28.9 - 82.3		

Table 1 presents the demographic characteristics, NTM isolation rate, and drug resistance profile among 50 suspected tuberculosis cases. The mean age of patients was 45.8 ± 12.4 years (95% CI: 42.2-49.4), which was statistically significant (p = 0.018), indicating that middle-aged adults constituted the major affected group. Males predominated with 31 cases (62%) compared to females 19 (38%), and this gender distribution was statistically significant (p = 0.041). Non-tuberculous mycobacteria were isolated in 14 cases (28%; 95% CI: 17.1-41.0), which was significant (p = 0.032), indicating a notable burden of NTM among suspected TB patients. Among the isolated NTM strains, drug resistance was observed in 6 cases (42.8%; 95% CI: 17.7-71.1), which was statistically significant (p = 0.021).

Table 2: To isolate and identify NTM species from suspected tuberculosis cases (N = 50)

Parameter	Category	n (%)	95% CI	Test of Significance	p-value
Culture Positivity	Positive	18 (36%)	23.4 - 50.9	χ^2 goodness-of-fit	0.014*
	Negative	32 (64%)	49.1 - 76.6		
NTM Species Distribution (n=14)	MAC	5 (35.7%)	12.8 - 64.9	χ^2 test	0.027*
	M. kansasii	3 (21.4%)	4.7 - 50.8		

	M. abscessus	4 (28.6%)	8.4 - 58.1		
	M. fortuitum	2 (14.3%)	1.8 - 42.8		
Smear Positivity	AFB Positive	20 (40%)	26.4 - 54.8	χ^2 test	0.022*
	AFB Negative	30 (60%)	45.2 - 73.6		

Table 2 demonstrates the culture positivity and species distribution of NTM among the 50 suspected TB cases. Culture positivity was observed in 18 cases (36%; 95% CI: 23.4-50.9), which was statistically significant (p = 0.014), emphasizing the importance of culture in diagnosis. Among the 14 confirmed NTM isolates, the most common species identified was Mycobacterium avium complex (MAC) in 5 cases (35.7%), followed by M. abscessus in 4 cases (28.6%), M. kansasii in 3 cases (21.4%), and M. fortuitum in 2 cases (14.3%). The species distribution was statistically significant (p = 0.027), suggesting heterogeneous NTM prevalence. Smear positivity for AFB was observed in 20 cases (40%; 95% CI: 26.4-54.8), which was also significant (p = 0.022)

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Table 3: To determine the antimicrobial susceptibility patterns of the isolated NTM species (n = 14 isolates)

Drug	Susceptible n (%)	Resistant n (%)	95% CI (Resistance)	Test of Significance	p-value
Clarithromycin	10 (71.4%)	4 (28.6%)	8.4 - 58.1	Fisher's exact test	0.031*
Amikacin	11 (78.6%)	3 (21.4%)	4.7 - 50.8	Fisher's exact test	0.042*
Linezolid	9 (64.3%)	5 (35.7%)	12.8 - 64.9	χ^2 test	0.048*
Moxifloxacin	8 (57.1%)	6 (42.9%)	17.7 - 71.1	χ^2 test	0.021*
Rifampicin	6 (42.9%)	8 (57.1%)	28.9 - 82.3	χ^2 test	0.018*

Table 3 outlines the antimicrobial susceptibility patterns among the 14 isolated NTM strains. Clarithromycin showed susceptibility in 71.4% of isolates, with resistance noted in 28.6% (p = 0.031). Amikacin demonstrated the highest susceptibility (78.6%), with resistance in 21.4% (p = 0.042), suggesting it remains an effective agent. Linezolid showed 35.7% resistance (p = 0.048), while moxifloxacin demonstrated 42.9% resistance (p = 0.021). Rifampicin exhibited the highest resistance rate at 57.1% (p = 0.018), indicating poor efficacy against NTM isolates

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Table 4: To compare conventional and molecular diagnostic methods for detection of NTM (N = 50)

Diagnostic Method	Positive n (%)	95% CI	Sensitivity (%)	Specificity (%)	Test of Significance	p-value

LJ Culture	14 (28%)	17.1 - 41.0	85.7%	94.4%	McNemar χ^2	0.036*
MGIT Liquid Culture	16 (32%)	20.0 - 46.3	92.8%	91.6%	McNemar χ^2	0.018*
Line Probe Assay	15 (30%)	18.7 - 44.6	89.2%	96.0%	McNemar χ^2	0.022*
MALDI-TOF	14 (28%)	17.1 - 41.0	90.0%	97.2%	McNemar χ^2	0.027*

Table 4 compares conventional and molecular diagnostic methods for NTM detection among 50 cases. LJ culture detected 14 cases (28%; sensitivity 85.7%, specificity 94.4%; p = 0.036). MGIT liquid culture showed slightly higher positivity at 16 cases (32%) with superior sensitivity (92.8%) but slightly lower specificity (91.6%), and this difference was statistically significant (p = 0.018). Line Probe Assay detected 15 cases (30%) with high specificity (96.0%) and sensitivity of 89.2% (p = 0.022). MALDI-TOF demonstrated comparable detection (28%) with high specificity (97.2%) and sensitivity (90.0%) (p = 0.027). The statistically significant McNemar χ^2 values indicate meaningful differences among diagnostic modalities, with MGIT and molecular techniques showing improved detection rates compared to conventional solid culture methods.

DISCUSSION

Demographic Profile and Incidence of NTM (Table 1): In the present study, the mean age of patients was 45.8 ± 12.4 years, with a statistically significant predominance of middle-aged adults (p = 0.018). Similar findings were reported by Gopalaswamy R et al. (2020)[1], who observed that the majority of NTM pulmonary infections occurred in individuals between 40-60 years of age. Likewise, Nejad SS et al. (2025)[2] noted increasing NTM prevalence among middle-aged and elderly populations, attributing it to structural lung disease and environmental exposure.

Male predominance (62%) in our study was statistically significant (p = 0.041). Comparable male preponderance has been reported in Indian studies by Maya TG et al. (2022)[3], who found 65% of NTM cases in males, possibly due to higher occupational exposure and smoking habits. However, some Western studies such as le Roux AJ et al. (2024)[4] reported female predominance in nodular bronchiectatic NTM disease, suggesting regional variation.

The isolation rate of NTM was 28%, which is comparable to findings of Wang H et al. (2025)[5], who reported NTM prevalence ranging from 20-32% among suspected TB cases in tertiary care centers. The significant proportion of drug-resistant NTM (42.8%) in our study aligns with observations by Zhou L et al. (2020)[6], who emphasized the rising antimicrobial resistance trends among NTM species globally.

Culture Positivity and Species Distribution (Table 2): Culture positivity was observed in 36% of suspected TB cases (p = 0.014). This finding is consistent with Bhalla GS et al. (2021)[7], who reported culture positivity rates between 30-40% in suspected TB cohorts in India.

Among the 14 NTM isolates, Mycobacterium avium complex (MAC) was the most common species (35.7%), followed by M. abscessus (28.6%), M. kansasii (21.4%), and M. fortuitum (14.3%). Similar species distribution has been documented by Sharma SK et al. (2020)[8], where MAC was identified as the most frequent pathogen worldwide. Indian data by Kim KJ et al.

(2022)[9] also demonstrated MAC and M. abscessus as predominant isolates.

Smear positivity (40%) was higher than confirmed NTM isolation, highlighting the inability of smear microscopy to differentiate NTM from MTBC. This observation supports findings by Calado Nogueira de Moura V et al. (2023)[10], who stressed that smear-positive cases may often represent NTM, leading to misdiagnosis as drug-resistant TB.

Antimicrobial Susceptibility Patterns (Table 3): Clarithromycin susceptibility was 71.4%, with 28.6% resistance ($p = 0.031$). This is comparable to global macrolide resistance rates of 20-30% reported by Zhou L et al. (2020)[6]. Amikacin showed the highest susceptibility (78.6%), similar to findings by Tan J et al. (2024)[11], who noted that amikacin remains one of the most effective agents against MAC and rapid growers.

Rifampicin resistance was highest (57.1%), reinforcing that traditional anti-tubercular drugs have limited role in NTM therapy. This is consistent with Bhalla GS et al. (2021)[7], who emphasized intrinsic resistance of many NTM species to rifampicin. High resistance to fluoroquinolones and linezolid observed in our study parallels patterns described by Gopalaswamy R et al. (2020)[1], indicating emerging multidrug resistance among NTM.

Comparison of Diagnostic Methods (Table 4): MGIT liquid culture demonstrated higher sensitivity (92.8%) compared to LJ culture (85.7%), with statistically significant differences ($p = 0.018$). Similar superiority of liquid culture systems was reported by Wang H et al. (2025)[5], who showed improved recovery rates with MGIT compared to solid media.

Line Probe Assay and MALDI-TOF showed high specificity (>96%), consistent with findings by Mazzarelli A et al. (2024)[12], who recommended molecular methods for rapid species identification. Rahimi M et al. (2025)[13] also emphasized the importance of molecular diagnostics in differentiating NTM from MTBC, especially in high TB burden settings.

CONCLUSION

The present study highlights a significant burden of non-tuberculous mycobacteria (NTM) among suspected tuberculosis cases in Western Maharashtra, with an isolation rate of 28%. Middle-aged males constituted the majority of affected individuals. Mycobacterium avium complex (MAC) emerged as the most common species, followed by M. abscessus, M. kansasii, and M. fortuitum, indicating heterogeneous species distribution in the region.

Antimicrobial susceptibility testing revealed variable resistance patterns among isolates. Amikacin and clarithromycin demonstrated relatively higher susceptibility rates, whereas rifampicin exhibited the highest resistance, reaffirming the limited role of conventional anti-tubercular drugs in NTM management. The presence of drug-resistant strains in nearly 43% of isolates underscores the necessity of performing routine drug susceptibility testing before initiating therapy.

In diagnostic comparison, MGIT liquid culture showed superior sensitivity compared to conventional Lowenstein-Jensen culture. Molecular methods such as Line Probe Assay and MALDI-TOF demonstrated high specificity and rapid identification capability, supporting their role in early and accurate species-level detection. The study emphasizes the importance of incorporating advanced diagnostic techniques and antimicrobial susceptibility testing in routine laboratory practice to prevent misdiagnosis, inappropriate treatment, and emerging resistance in high TB-burden settings.

LIMITATIONS OF THE STUDY

1. The sample size (N = 50) was relatively small, which may limit generalizability of findings to the broader population.
2. The study was conducted in a single tertiary care center, and regional environmental variations were not evaluated.
3. Long-term clinical follow-up and treatment outcomes of NTM-infected patients were not assessed.
4. Molecular characterization was limited to species identification and did not include detailed gene mutation analysis for resistance mechanisms.
5. Environmental sampling was not performed to identify possible sources of NTM transmission.

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