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PHENOTYPIC IDENTIFICATION AND DRUG RESISTANCE PROFILE OF MYCOBACTERIA IN A TERTIARY CARE HOSPITAL IN WESTERN RAJASTHAN

Microbiology

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ABSTRACT

Tuberculosis (TB) remains a significant public health burden in India. The emergence of drug-resistant TB and non-tuberculous mycobacteria (NTM) complicates diagnosis and treatment. This study aimed to phenotypically identify Mycobacterium species in patients suspected of having tuberculosis in a tertiary care hospital setting. Out of 150 clinically suspected pulmonary and extrapulmonary samples, 64 were MGIT positive. Among these, MPT64 antigen testing and ZN staining confirmed 42 MTB-positive cases, while the remaining were screened for NTM using MPCR-ULFA. Early identification of MTB and NTM is crucial for effective TB control and management.

KEYWORDS

Mycobacterium tuberculosis, Non-tuberculous mycobacteria (NTM), MGIT, MPT64, MPCR-ULFA

INTRODUCTION

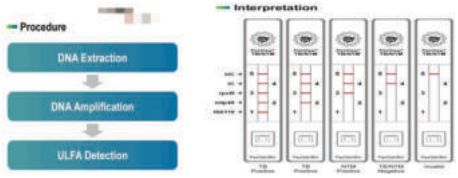
Tuberculosis (TB), caused by the Mycobacterium tuberculosis complex (MTBC), continues to be one of the leading causes of infectious mortality worldwide, especially in high-burden countries like India [1]. While pulmonary TB predominates, extra-pulmonary TB is also clinically significant [2]. Additionally, the rise in non-tuberculous mycobacteria (NTM) has added complexity to TB diagnosis and management. In recent years, non-tuberculous mycobacteria (NTM) have emerged as important opportunistic pathogens. These environmental mycobacteria are often misdiagnosed as M. tuberculosis due to similar clinical features [3]. NTM infections are increasingly reported among immunocompromised individuals and those with chronic lung disease [7]. Without accurate identification, NTMs can lead to misdiagnosis and inappropriate treatment.

MATERIALS AND METHODS

This hospital-based prospective study was carried out between April and June 2025 at the TB C&DST Laboratory, Department of Microbiology, IDI Block, Kamla Nehru Chest Hospital, Dr. S.N. Medical College, Jodhpur, Rajasthan. A total of 150 clinical specimens were collected from patients with a presumptive diagnosis of tuberculosis. These included both pulmonary and extrapulmonary samples such as sputum, bronchial alveolar lavage (BAL), pleural fluid, pus, and cerebrospinal fluid (CSF). Samples like urine, stool, foul smell, or fungal contamination were excluded.

All specimens were processed in a Biosafety Level 3 (BSL-3) laboratory following strict NTEP (National Tuberculosis Elimination Programme) protocols. Specimens were decontaminated using the standard N-acetyl-L-cysteine–sodium hydroxide (NALC–NaOH) method. After decontamination, sedimented material was inoculated into the BACTEC MGIT 960 liquid culture system and Lowenstein–Jensen (LJ) medium for growth and isolation of mycobacteria. ZN staining was performed for the detection of acid-fast bacilli, and positive cultures were further tested using MPT64 antigen detection to confirm the presence of the Mycobacterium tuberculosis complex.

Drug susceptibility testing (DST) was performed on MPT64-positive samples using the Line Probe Assay (LPA), which included DNA extraction, PCR amplification, and hybridization steps. For samples that were ZN-positive, MGIT-positive, and MPT64-negative, Multiplex PCR–Universal Lateral Flow Assay (MPCR-ULFA) was employed to identify non-tuberculous mycobacteria (NTM). Solid culture using Lowenstein-Jensen (LJ) medium was also performed for NTM identification. Strict quality control measures and decontamination procedures were adhered to throughout the study.



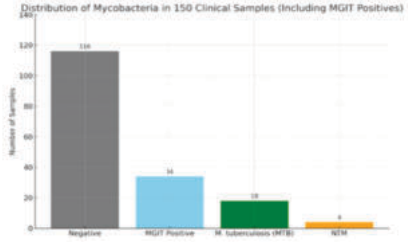
RESULTS

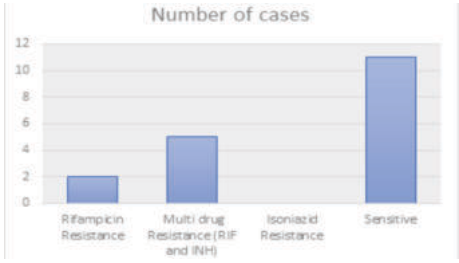
Out of the 150 clinical samples processed during the study period, 34 samples (22.66%) were found positive by MGIT culture. Among these, 18 isolates were identified as Mycobacterium tuberculosis (MTB), while 4 isolates (2.66%) were confirmed as non-tuberculous mycobacteria (NTM). Age-wise distribution revealed that the highest prevalence of mycobacterial infection (45.3%) occurred in the 21–40 years age group, indicating that young adults were the most affected demographic. Interestingly, a greater number of samples were obtained from female patients (72%) compared to males (28%).

Drug resistance profiling using Line Probe Assay (LPA) among the MTB-positive samples showed that 2 cases (11.11%) were resistant to rifampicin, while no resistance to isoniazid was detected. However, 5 samples (27.77%) exhibited multidrug resistance (MDR), indicating resistance to both rifampicin and isoniazid. Furthermore, all four NTM isolates showed ZN positivity and MGIT culture positivity but tested negative for the MPT64 antigen, thereby ruling out M. tuberculosis. These were subsequently confirmed as NTM using the MPCR-ULFA method, underscoring the importance of molecular assays in accurate species differentiation and appropriate clinical management.

This demographic trend is consistent with the high exposure risk and active lifestyle of individuals in this age group, contributing to increased susceptibility and disease burden. NTM infections were more frequently observed in older patients, particularly above 50 years, suggesting an age-related predisposition, possibly linked to chronic lung conditions or immunocompromised states.

The use of advanced molecular diagnostics allowed for rapid detection of both MTB and NTM, as well as timely identification of strains, underscoring the importance of such methods in clinical decision-making.





Here is a bar graph representing the key results from the study, including the total number of samples, MGIT culture positives, confirmed M. tuberculosis (MPT64-positive) cases, non-tuberculous mycobacteria (MPCR-ULFA-positive) cases and the Drug resistance profile of MTB cases.

DISCUSSION

The present study highlights the significance of phenotypic identification and drug resistance profiling of Mycobacterium species in a tertiary care setting in Western Rajasthan. Among the 150 clinically suspected TB cases, 22.6% were culture-positive by MGIT 960, with 18 cases confirmed as Mycobacterium tuberculosis and 4 as non-tuberculous mycobacteria (NTM). The presence of NTM (2.66%) underlines the need for species-level identification before initiating anti-TB therapy, as misdiagnosis can lead to inappropriate treatment [6].

These findings reflect the growing clinical relevance of NTM, which are often misdiagnosed as tuberculosis due to overlapping clinical presentations. This misdiagnosis can lead to inappropriate treatment, prolonged morbidity, and increased healthcare costs.

This study confirms the ongoing burden of Mycobacterium tuberculosis infection among young adults, with the highest prevalence in the 21–40 years age group, consistent with previous findings by Hatolkar et al. [5]. The emergence of non-tuberculous mycobacteria (NTM) in TB-suspected cases, though less frequent, is clinically significant. In our study, 2.66% of cases were identified as NTM, comparable to prior studies reporting rates ranging from 0.3% to 1.04% in India [5,8]. These findings emphasize the need for molecular differentiation, as NTMs require different treatment protocols and are often resistant to standard anti-TB drugs [6].

Rapid detection of drug resistance using Line Probe Assay (LPA) allowed timely identification of rifampicin resistance and multidrug-resistant TB (MDR-TB). This aligns with the results of Sharma et al., who also reported the effectiveness of LPA in the early diagnosis of resistance [8]. The role of MPCR-ULFA in confirming NTM highlights its utility as a diagnostic tool when conventional tests yield inconclusive results [5,6].

Given the challenges of differentiating MTB from NTM through traditional methods such as smear

CONCLUSION

This study emphasizes the critical importance of early phenotypic and molecular identification of mycobacterial infections and their drug resistance profiles. Early and accurate identification of Mycobacterium species and their resistance profiles is crucial in effective TB management. The detection of NTM in 4% of culture-positive cases reinforces the need for improved diagnostic differentiation between MTB and NTM. Multidrug resistance in nearly one-fifth of MTB isolates calls for vigilant drug susceptibility testing before initiating therapy. Incorporating rapid diagnostic tools such as MPCR-ULFA into routine TB diagnostics can significantly improve treatment outcomes, especially in non-responders to standard therapy. Differentiating NTM from TB is crucial due to differences in clinical management and antimicrobial therapy. Regular surveillance, strengthened laboratory infrastructure, and clinician awareness are crucial for effectively managing and controlling both TB and NTM infections.

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