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Research Article

The Incidence of Non-Tuberculous Mycobacteria (NTM) in TB Culture Samples in a South Indian Sub-Population: A Laboratory-Based Study

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Abstract: Non-Tuberculous Mycobacteria (NTM) are now considered globally evolved emerging pathogens, causing infections ranging from self-limiting asymptomatic to life-threatening infections affecting multiple major organs. Numerous directives have been implemented to treat NTM infections, but owing to their innate resistance, complexity, and resemblance to *Mycobacterium Tuberculosis* (MTB), the diagnosis and treatments have a high failure rate. The study was designed to estimate the incidence of MTB and NTM infection and identify the occurrence of NTM. In our study, a retrospective analysis of 6 years (January 2017 to December 2022) was done using laboratory data. All the samples received for Acid-Fast Bacilli (AFB) culture were included. Standard Auramine O and Ziehl Neelsen (ZN) staining, Lowenstein Jensen (LJ) medium, and an automated BacT Alert *Mycobacteria* Indicator Tube (MGIT) for inoculation. Differentiation between MTB and NTM was done using a TB antigen MPT64 rapid test kit. Anonymized data resulting from the generalization method was used for statistical analysis. Our study found that the incidence of NTM was 19.8% among the AFB-positive samples. The infection was found to be more common in males (60.6%) than in females (39.3%). The maximum number of samples received was sputum and BAL, indicating that the pulmonary infection was more than extra-pulmonary. The outcome is consistent with reports of pulmonary infections dominating worldwide. Our year-wise report shows that the cases of both MTB and NTM were found to be on the rising trend. Despite the low cases, it was noted that the number increased over time.

***Keywords*:** non-tuberculous mycobacteria (NTM), *Mycobacterium tuberculosis* (MTB), tuberculosis (TB)

1. Introduction

Mycobacteria are separated into two categories by Runyon and others: tuberculosis-causing *mycobacteria* and Non-Tuberculous Mycobacteria (NTM). Most of the species of NTM are rapid growers, while only some of them are slow growers. Almost all NTMs are environmental bacteria that are ubiquitously present in soil, water, air, etc. [1]. Although NTM infections lead to mortality and morbidity, they are opportunistic pathogens causing co-infections with other diseases [2]. However, certain studies have shown that these saprophytes have also started to affect immunocompetent

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children and adults [2]. The most common sites of infection include the pulmonary and extrapulmonary organs. Lymphadenitis has become one of the most common syndromes of chronic pulmonary infection. Invasive procedures often lead to cutaneous and bone NTM infections, and immunosuppressed patients usually are diagnosed with disseminated NTM infection [2].

The group of people who are prone to this infection includes patients who have lung diseases, either genetically or acquired (like Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD), previous pulmonary tuberculosis, non-CF bronchiectasis, and lung cancer) are more prone to this disease. The immunocompromised patients are at higher risk due to primary immune deficiency syndrome. Patients suffering from acquired immune deficiency syndrome, like AIDS, hematological malignancies, and hairy cell leukemia in particular, are also susceptible to NTM infection [2]. The most clinically important slow growers include *Mycobacterium Avium* Complex (MAC), *Mycobacterium marinum*, *Mycobacterium kansasii*, and *Mycobacterium ulcerans*, while the rapid growers include *Mycobacterium Abscessus* Complex (MABC), *Mycobacterium fortuitum* complex, and *Mycobacterium chelonae* [2]. The symptoms exhibited by NTM-infected patients are very similar to those of TB patients, and hence, NTM infections are mostly misdiagnosed as TB. Just like how the transmission of MTB is via aerosols of the organism, NTM is also transmitted in the same way [2].

A study by Zhou [3] on the global prevalence of NTM in adults with non-cystic fibrosis bronchiectasis, between 2006 to 2021, found that it was approximately 10% with great variations due to geographical locations. In patients with non-cystic fibrosis, the prevalence of NTM infection ranges from 1.0% to 25%, and between the year 2006 to 2021, the prevalence in adults was reported to be 9.75%. The pooled prevalence of NTM infection in East Asia was found to be the highest at 7.50%. This study also reported that the prevalence of NTM between 1990 to 2006 was 5%, showing an increasing trend of the infection. 90% of the infections in bronchiectasis patients were caused by MAC, which is like other data reported earlier.

In a 6-month study by Grigg [4] during 2019-2020 at four sites in the US, the annualized prevalence and incidence rates per 100,000 population were 7.5 and 4.8, respectively. Another study has reported that the prevalence of NTM increased from 19.6 cases per 100,000 people during the year 1994-1996 to 26.7 cases per 100,000 people during the year 2004-2006 [5]. A study by Varghese [6] reported that most of the cases of rare NTM infection during the period of 1956-2018 were reported from North America (33.4%), followed by Europe (23.8%) and Asia (20.8%). 67% of the total cases reported were observed at pulmonary sites, while the remaining were extrapulmonary or disseminated infections.

In India, the reports of NTM infections are less due to a lack of awareness among healthcare workers and limited diagnostic tools for the disease. In 2001, a case of NTM pulmonary infection was reported. In 2013, a case of another pulmonary infection caused by NTM in a multidrug-resistant TB patient was reported. In 2014, a 2-year-old child with pulmonary complications was reported in Chennai. In 2017, a case of cervical lymphadenitis complication with NTM in a 15-year-old boy was reported in Punjab. In 2018, a case of a 9-year-old girl was reported from Mumbai, and in 2021, NTM complication in a patient with a history of TB treatment was reported from Chennai [7]. According to a study by Ratnatunga [8], the isolation rates of NTM increased from 0.9% (2001-2010) to 1.6% (2011-2020), and the prevalence of NTM infection with presumptive TB patients in India was reported to be 1.1%. Like other studies, in India too, most of the cases of NTM infection (76%) were isolated from pulmonary specimens and MAC was found to be the most isolated organism (19%) followed by *M. chelonae* (10.3%), *M. fortuitm* (10%) and *M. abscessus* (15%) [9]. The research gaps revolve around the limited commercial identification tools, the Lack of data regarding drug sensitivity testing to predict treatment outcomes, and the Development of non-culture-based rapid diagnostic tests for early detection. The classical method for the identification of NTM has a longer and expensive turnaround time. Moreover, not all laboratories are equipped with expensive instruments like MALDI-TOF and molecular diagnostic tools.

2. Methods

Anonymized data resulting from generalized methods were requested from the Neuberg Anand reference laboratory, Bangalore, collected during the period between January 2017 to December 2022. The data included results of smear microscopy analysis, AFB culture results, and SD Bioline TB Ag rapid test results for differentiating *M. tuberculosis* complex and non-tuberculous mycobacteria. The data also included demographic information like age and sex. All samples that were received during the study period for AFB culture were included in the study.

The methodology used during the processing of samples for AFB cultures was standard Auramine O and Ziehl Neelsen (ZN) staining for Acid-fast bacilli microscopy (Figures 1 & 2). For culturing, non-sterile samples were processed by the N-Acetyl L-Cysteine (NALC) decontamination process. The decontaminated sediments were inoculated on a slant of Lowenstein-Jensen (LJ) Medium and an automated BacT Alert *mycobacteria* indicator tube (MGIT). Sterile samples were processed without decontamination.

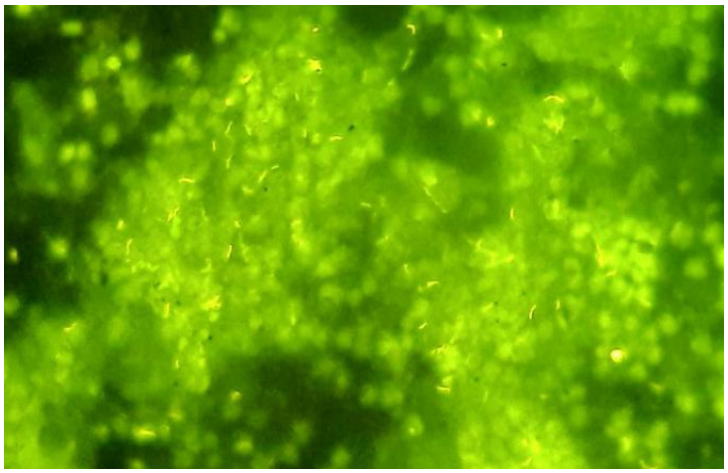

Figure 1. Microscopic representation of Acid-fast bacilli under Auramine O stain

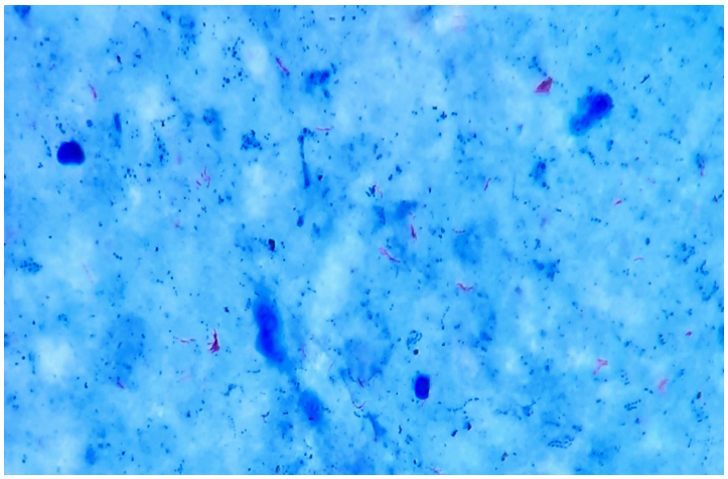

Figure 2. Microscopic representation of Acid-fast bacilli under Zheil-Neelsen staining

Method of data anonymization: The Department of Data Science uses a generalization method for data anonymization. The generalization method involves the exclusion of some parts of the data purposely to make it less/identifiable. When the data is retrieved from the database, sensitive patients are excluded, and a new pseudo-identification number is given to each patient after shuffling the data multiple times. This robust process ensures the privacy and security of the sensitive information of the patient, and the records cannot be traced back to the database.

2.1 *Ethical clearance reference number*

NAALM/EC/5.2/05-2023, valid for 2 years. The ethical clearance committee experts have recommended providing

consent documents to the study participants, and the ethics committee has the right to monitor the study with prior intimation.

2.2 *Case definition*

A sample was considered positive, for MTB if the AFB microscopy, culture, and MPT64 rapid test kit were positive whereas a sample was regarded as MOTT (*Mycobacteria* other than MTB) if the smear microscopy and culture were positive but MPT64 rapid test was negative (Figure 3a & 3b).

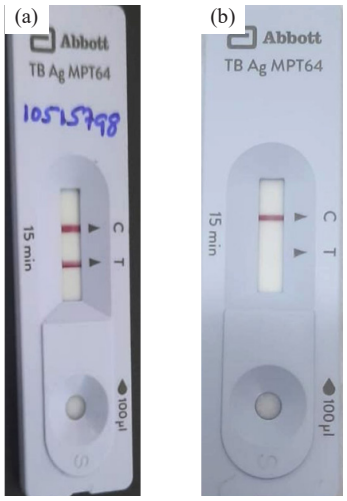

Figure 3. The results of the MPT64 rapid Ag kit. (a) Positive MPT64 test suggesting the presence of *M. tuberculosis* (b) Negative MPT64 test suggesting MOTT

2.3 *Coverage population*

The samples of the patients received in the laboratory for culture only are included in the study. It was not possible to collect all the data from the entire state. Therefore, this study is based on a single laboratory dataset where most of the samples were received from Bangalore.

2.4 *Data analysis*

Values are expressed in numbers and percentages. Year-wise distribution of both NTM and MTB was analyzed. Age and gender distribution of both infections were also analyzed. In the case of NTM infections, the major site of infection was also analyzed based on the samples positive for the infection.

3. Results

A total of 3,375 samples were received for AFB culture, out of which 86.7% were negative, whereas 13.2% were positive beeped TB cultures. Of the total positive samples, 80.0% were found to be MTB, and 19.8% were found to be NTM (Figure 4). Out of the total MTB-positive cases, 58.38% were males while 41.62% were females, indicating that tuberculosis is more prevalent in males as compared to females (Figure 5). From the data analyzed for the year-wise distribution of MTB and NTM, it was found that the number of cases each year increased with time (The graphical representation of Figure 6 and Figure 7 shows the number of year-wise cases of both MTB and NTM). In the case of MTB, it was observed that the infection was more prevalent in the age group between 20 to 30 indicating

that the younger generations are at more risk followed by people in the age group from 30 to 60 years of age (Figure 8 represents the graphical representation of the age-wise distribution of MTB), and in case of NTM infections, it was observed that the age group between 60 to 70 had the greatest number of cases indicating that the elderly people were at more risk, unlike the cases of MTB (Figure 9 represents the graphical representation of the age-wise distribution of NTM). The gender-wise analysis of NTM data also revealed that the infections caused by the organism are dominantly more prevalent in males (60.6%) than in females (39.3%) (Figure 10). Also, among the samples of NTM, pulmonary samples were more common than extra-pulmonary samples (Figure 11). Figures 12 and 13 represent how both MTB and NTM resemble each other in the culture media and the microscopy, but the rapid Ag tests differentiate between the two.

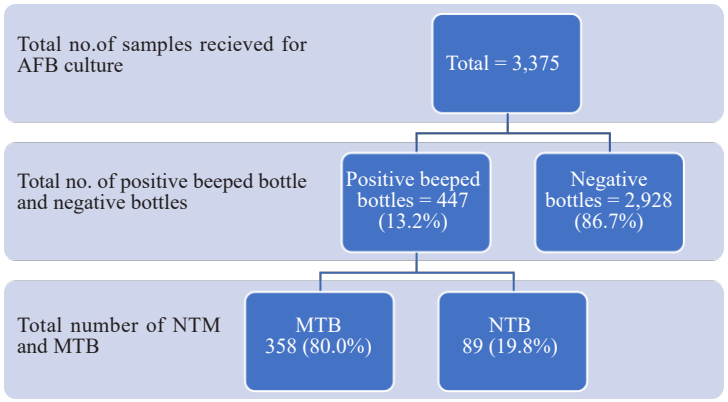

Figure 4. Mycobacterial analysis resulted from the data analysis

Figure 5. Distribution data of MTB in different gender

Figure 6. Graphical representation of year-wise distribution data of MTB

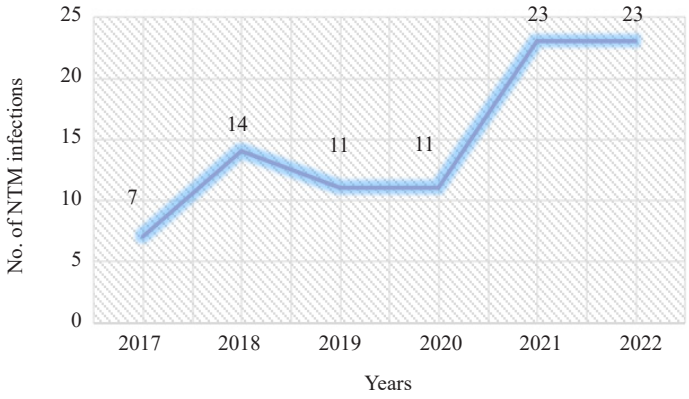

Figure 7. Graphical representation of year-wise distribution data of NTM infection

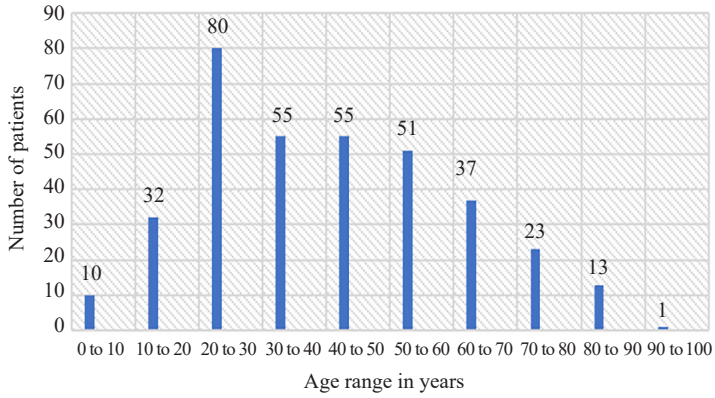

Figure 8. Graphical representation of age-wise distribution data of MTB

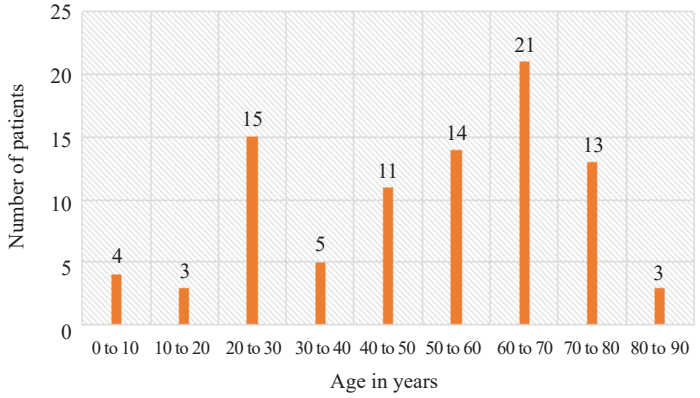

Figure 9. Graphical representation of age range distribution data of NTM infection

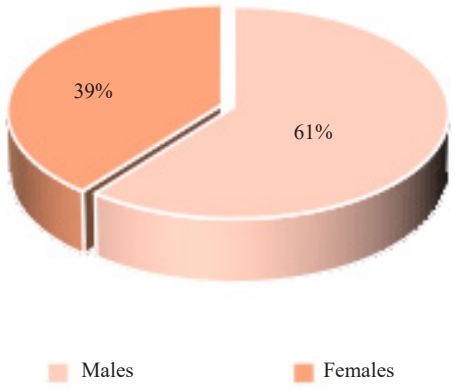

Figure 10. Distribution data of NTM in different gender

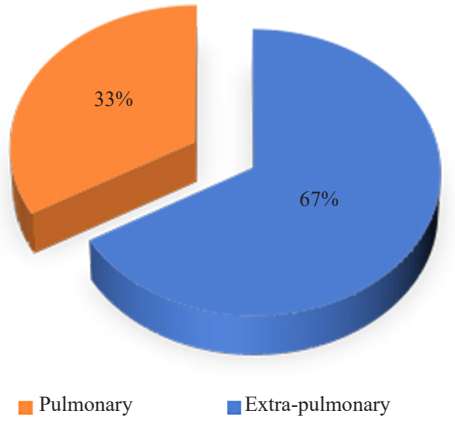

Figure 11. Specimen distribution data of NTM positive samples

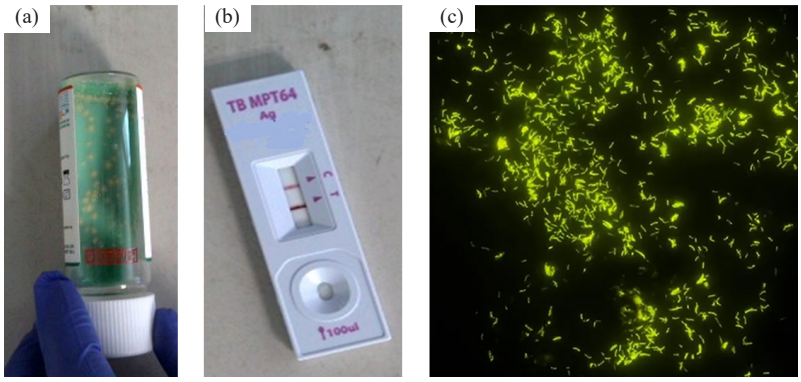

Figure 12. Images of organism growth, MPT-64 card-based positive result, and staining of an organism with auramine. (a) Growth on L J media; (b) MPT-64 antigen card positive and (c) organisms with Auramine stain

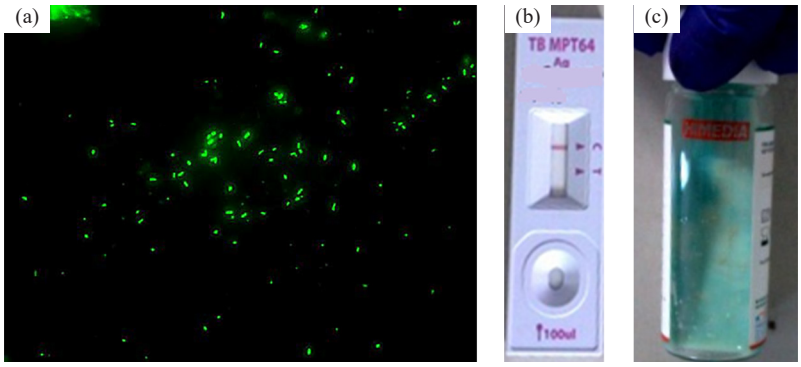

Figure 13. (a) Images of auramine O stain of NTM, (b) MPT-64 antigen card negative, and (c) Growth on L J media

4. Discussion

The emergence of non-*tuberculous mycobacterial* infection is rapidly rising in the global scenario [1]. With a highly diverse species spectrum and pathogenic potentials, they can cause pulmonary and extra-pulmonary diseases in both immunocompetent and immunocompromised patients [10]. This rapidly rising case may be due to frequent host-pathogen interactions. Johansen [1] has suggested that the most dominant place of inhabitation by NTM is the water supply, although they are present ubiquitously in the environment. So, their inhabitation in the tap water has exposed them to different kinds of water disinfectants and hence made them resistant [2].

Recent studies have also mentioned that the mode of entry for NTMs from environmental sources was through ingestion and inhalation of contaminated food or aerosols, injured skin, etc. [2]. Another study has reported that the pathogenic NTMs, such as *M. avium, M. kansasii, M. lentifavum,* and *M. abscessus,* were isolated from household water and aerosols [11]. A study was conducted in Northern Tanzania, with 120 healthcare providers, by asking a set of questionnaires regarding the prevalence of NTM. 79 participants were not aware of the existence of NTM infection, and 77 believed that NTM pulmonary infection is clinically similar to TB. And only 19 participants in the study could correctly define NTM. This shows that the existence of such infection is not aware to most of the healthcare providers [10].

In most of the literature for NTM infection, the disease being misdiagnosed as *Mycobacterium tuberculosis* complex is quite commonly portrayed. The misdiagnosis of NTM as MTB occurs often when the diagnosis is based on clinical symptoms, sputum smear microscopy [12], and rarely with radiological findings. Moreover, due to poor laboratory infrastructures, the criteria laid down by the American Thoracic Society/Infectious Disease Society of America [13] to diagnose pulmonary NTM disease are often neglected. Since the disease is misdiagnosed, first-line anti-TB drugs have been administered based on the identification of Acid-Fast Bacilli (AFB) [12, 14]. Often, the failure of

the first-line drug treatment and second-line drug treatments of TB are usually done leading to the pathogens becoming resistant to the drugs. Simultaneously, the NTM disease proceeds as they have innate resistance towards conventional anti-TB drugs. This results in mortality and morbidity, and the rising cases of NTM infection, while the treatment becomes challenging [14]. Therefore, to reduce the risk of misdiagnosis and missed diagnosis of NTM infection, providing awareness among healthcare providers is crucial [10].

In our study, a retrospective analysis was carried out to estimate the number of NTMs from the TB culture samples received during the study period. The period of retrospective analysis is between January 2017 to December 2022. All the samples that were requested for AFB culture are included in the study. Our study has shown that out of 3,375 specimens received for AFB culture, 358 were found to be MTB, and 89 were found to be NTM. Out of the total MTB-positive samples, 209 were male and 149 were female patients, indicating that TB is predominant in male patients in our retrospective study. In the case of NTM infection, 54 patients were male, whereas 35 were female. Our study has also come across that the most common positive samples received for culturing were sputum and BAL fluid in both MTB and NTM cases, indicating that pulmonary infections by both the organisms were more prevalent in the study population.

A study by Varghese [6], who reported the global update on rare NTM infection in humans, also reported that the majority of the NTM cases (67%) were seen in pulmonary sites, which were observed in our study. According to our study, the age range for maximum NTM infection was found to be 60 to 70 years of age. Reports from recent studies show that the patients who are at risk of NTM infection include patients whose lungs are already affected by certain other diseases, immune-suppressed patients, etc. [2]. Therefore, with aging, a person's immune system also weakens, and hence elderly patients are at more risk, whether it be an opportunistic infection or a pathogenic infection.

In another study conducted with 18 NTM cases, male patients were dominant with the infection, and the patients belonged to the age group above 41. The study conducted was to check the prevalence of NTM in India. Delhi had the greatest number of cases. It has been reported that NTM infections are mostly pulmonary infections worldwide. This study also proved to be the same, as most cases have pulmonary involvement. All the patients involved in this study have shown to have some underlying infections, and hence NTM causes the complication as an opportunistic pathogen [2].

Our study showed that male patients were more dominant than female patients. A study conducted in China reported that, in Europe, the incidence of NTM is higher in men as compared to women, but in North America, women had a higher risk of the infection. Post-menopausal women, especially those with low BMI and a tall and thin body shape, were reported to have a higher risk of the infection [3]. A study also showed that MTB co-infection with NTM, as well as co-infection by two different NTM species, is also possible. As TB is a disease, the factors affecting it are poor socio-economic conditions, malnutrition, and a weak immune system. This is one of the major factors that leads to the co-infection of NTM with MTB, which can be misdiagnosed as multidrug-resistant TB. Therefore, environmental, socio-economic status, and geographical locations also play an important role in the prevalence of the disease [15].

To effectively treat NTM infection, the American Thoracic Society has stated that the best way is to identify them at the species level because every species has different susceptibility and resistance to a particular antibiotic [14]. However, in our study, the speciation is not done due to a lack of advanced molecular diagnostic equipment, but the differentiation between NTM and MTB is done so that the treatment regimen can be effective. In our study, the MTB is confirmed by using the MPT64 Ag rapid test kit. The negative MPT64 Ag rapid test kit samples, which are acid-fast smear positive, and culture positive are considered NTM or MOTT so that misdiagnosis is avoided. Also, the effectiveness of the treatment given doesn't solely depend on the drug but also the host's immune response, on the organs infected, or the severity of the disease. Other factors that play a role during the treatment of NTM are the time of initiation and the choice of treatment given [2].

The promising molecular diagnostic tools for the differentiation of NTM and MTB identification at the species level include 16S rRNA, rpoB, hsp65, and ITS. The commercially available kits can only identify 20 species of NTM. Also, a positive result does not always mean that the patient has been exposed to the pathogen, as it can be environmental exposure or contamination during sample collection and processing, as NTM are ubiquitously present in the environment. So, correlation with the patient's clinical diagnosis plays a major role as a deciding factor [16].

4.1 *Limitations*

The sample included in this study is from a single diagnostic center and, hence, a limited number of patients. Since we are not speciating about the non-*tuberculous mycobacteria*, we do not know the number of pathogenic and non-pathogenic organisms from the number of NTM indicated in the study. In the study, MPT64-positive strains are considered as MTB complex. The sensitivity of MPT64 in detecting MTB complex is not 100%. According to the kit used, the specificity and sensitivity of the rapid antigen kit are 100% and 98.6% respectively [17] A study by Sutantangjai [18] suggested that the immuno-chromatographic card sensitivity and specificity are 100% when solid and liquid cultures were used however, the sensitivity of the card is not applicable in case of detection of MTB in sputum samples treated with sputum solvent. Another study by N'Dira Sanoussi [19] reported that for the detection of L5 MTB lineage, the sensitivity of the immune-chromatographic card is low. Therefore, this rapid testing kit can be considered as a first screening test rather than confirmation, and the confirmation should be done by another reliable identification method.

4.2 *Relevance for clinical practice*

This study provides information regarding the incidence of NTM in Bangalore so that misdiagnosis of NTM infection as TB can be avoided, and further identification and speciation of the NTM will help in proper diagnosis and an appropriate treatment regimen so that morbidity and mortality can be avoided. The treatment regimen for NTM infections is different from that of MTB, and the organism has an inherent anti-TB drug resistance property. So, to get a proper and effective treatment outcome, differentiation of the two organisms is crucial. In developing countries like India, also a TB-burden country, the diagnosis of MTB itself is challenging. Therefore, for the appropriate treatment of NTM infection, special attention and training are required because the two cases require different treatment regimens. So, it is important to create awareness and provide information regarding the prevalence of such diseases to avoid unnecessary or inappropriate treatment, which could become a burden later on. Therefore, this study is done to document the NTM reports in Bangalore to elucidate the importance of the infection and to establish the need for research in future studies.

5. Conclusion

In our study, the total number of samples received for acid-fast bacilli culture was 3,375. Out of these total samples, 86.7% were found to be negative and 13.2% were found to be positive. Of the positive cases, 80.0% were found to be *Mycobacterium tuberculosis* complex, and 19.8% were non-*tuberculous mycobacteria*. Therefore, it can be considered that if the differentiation of MTB and NTM is not done, then 19.8% of NTM infections could have been misdiagnosed as MTB, which could cause morbidity and mortality. The misdiagnosis would lead to inappropriate treatment, and the patient may be exposed to different types of anti-TB drugs, which could make the pathogens resistant to antibiotics while the disease progresses, leading to mortality. The NTMs are reported to be resistant to the first line of anti-TB drugs because of their innate property. Moreover, most healthcare workers lack the idea of NTM infection as the clinical symptoms and the microscopic morphology resemble MTB. This could lead to patients being exposed to a second line of anti-TB drugs or being regarded as multidrug or extremely drug-resistant. Since the response of the NTM to the anti-TB drugs is not quite effective, to treat the infection for a proper clinical outcome, the patients are exposed to many different kinds of drugs. This could lead NTM to become resistant to various available antibiotics, and the treatment could become challenging. This may lead to the infection becoming a burden like that of tuberculosis, especially in developing countries where the diagnosis of TB itself is still challenging. This may lead to the infection becoming a burden like that of tuberculosis, especially in developing countries where the diagnosis of TB itself is still challenging. The results of our study can be used to distinguish TB from NTM, which will help to provide the right treatment for each person infected with TB.

Acknowledgments

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Conflicts of interest

The authors declare no competing financial interest.

References

[1] Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of *Mycobacterium abscessus*. *Nature Reviews Microbiology*. 2020; 18(7): 392-407.
[2] Soneja M, Gupta N, Mittal A, Muhammed Niyas V, Banerjee S, Ray Y, et al. Nontuberculous mycobacteria: A report of eighteen cases from a tertiary care center in India. *Lung India*. 2020; 37(6): 495-500.
[3] Zhou Y, Mu W, Zhang J, Wen SW, Pakhale S. Global prevalence of non-tuberculous mycobacteria in adults with non-cystic fibrosis bronchiectasis 2006-2021: A systematic review and meta-analysis. *BMJ Open.* 2022; 12(8): e055672.
[4] Grigg C, Jackson KA, Barter D, Czaja CA, Johnston H, Lynfield R, et al. Epidemiology of pulmonary and extrapulmonary nontuberculous mycobacteria infections at 4 US emerging infections program sites: A 6-month pilot. *Clinical Infectious Diseases*. 2023; 77(4): 629-637.
[5] 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; 27(1): 74.
[6] Varghese B, Al-Hajoj S. A global update on rare non-tuberculous mycobacteria in humans: Epidemiology and emergence. *International Journal of Tuberculosis and Lung Disease*. 2020; 24(2): 214-223.
[7] Rajendran P, Padmapriyadarsini C, Mondal R. Nontuberculous mycobacterium: An emerging pathogen: Indian perspective. *International Journal of Mycobacteriology*. 2021; 10(3): 217-227.
[8] Ratnatunga CN, Lutzky VP, Kupz A, Doolan DL, Reid DW, Field M, et al. The rise of non-tuberculosis mycobacterial lung disease. *Frontiers in Immunology*. 2020; 11: 303.
[9] Sharma S, Upadhyay V. Epidemiology, diagnosis & treatment of non-tuberculous mycobacterial diseases. *Indian Journal of Medical Research.* 2020; 152(3): 185-226.
[10] Maya TG, Komba E, Mensah GI, Mnyambwa NP, Doulla B, Mfinanga S, et al. Non-tuberculous mycobacterial pulmonary disease: Awareness survey of front-desk healthcare workers in Northern Tanzania. *PLOS Global Public Health*. 2023; 3(1): e0000741.
[11] Choi JY, Sim BR, Park Y, Yong SH, Shin SJ, Kang YA. Identification of nontuberculous mycobacteria isolated from household showerheads of patients with nontuberculous mycobacteria. *Scientific Reports*. 2022; 12(1): 8648.
[12] Karamat A, Ambreen A, Ishtiaq A, Tahseen S, Rahman MA, Mustafa T. Isolation of non-tuberculous mycobacteria among tuberculosis patients, a study from a tertiary care hospital in Lahore, Pakistan. *BMC Infectious Diseases.* 2021; 21(1): 381.
[13] Infectious Diseases Society of America. *Nontuberculous Mycobacterial (NTM) Diseases Practice Guidelines*. Available from: https://www.idsociety.org/practice-guideline/nontuberculous-mycobacterial-ntm-diseases/ [Accessed 22 March 2025].
[14] Thumamo BD, Yeboah-Manu D, Ofori S, Guemdjom PW, Teyim PM, Lawson L, et al. Prevalence of non-tuberculous mycobacteria among previously treated TB patients in the Gulf of Guinea, Africa. *IJID Regions*. 2022; 3: 287-292.
[15] Wang J, Xu H, Wang X, Lan J. Rapid diagnosis of non-tuberculous mycobacterial pulmonary diseases by metagenomic next-generation sequencing in non-referral hospitals. *Frontiers in Cellular and Infection Microbiology*. 2023; 12: 1083497.
[16] Wang J, Chen Z, Xu Y, Qiu W, Chen S, Pei H, et al. Screening and drug resistance analysis of non-tuberculous mycobacteria in patients with suspected pulmonary tuberculosis on the Hainan island, China. *Infection and Drug*

Resistance. 2023; 16: 463-476.
[17] Abbott Global Point of Care. *Bioline TB Ag MPT64 Rapid*. Available from: https://www.globalpointofcare.abbott/ww/en/product-details/bioline-tb-ag-mpt64-rapid.html [Accessed 22 March 2025].
[18] Sutantangjai M, Faksri K, Chaicumpar K, Chaimanee P, Lulitanond, Namwat W. Evaluation of an immunochromatographic test kit for detecting Mycobacterium tuberculosis complex in sputum samples and on solid and in liquid cultures. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 2014; 45(2): 357-364.
[19] Sanoussi CN, Jong M, Odoun M, Arekpa K, Ligali MA, Bodi O, et al. Low sensitivity of the MPT64 identification test to detect lineage 5 of the *Mycobacterium tuberculosis* complex. *Journal of Medical Microbiology*. 2018; 67(12): 1718-1727.