# THE DIVERSITY OF NON-TUBERCULOUS MYCOBACTERIA SPECIES IN OUR REGION.

**Ashish pal, Dr. Madhurendra Singh Rajput**

Research Scholar, Malwanchal University, Indore (M.P)

Associate Professor, Malwanchal University, Indore (M.P)

## ABSTRACT

Opportunistic infections caused by non-tuberculous mycobacteria have been seen in both immunocompromised and immunocompetent hosts in recent years. The purpose of this research is to learn how common NTM species are in TB patients (both pulmonary and extrapulmonary) in a tertiary care hospital in Pondicherry, to compare several phenotypic and genotypic approaches to NTM identification, and to find out what patterns their DSTs show.

**Key-words:** NTM, pulmonary, extrapulmonary, identification, patterns.

## INTRODUCTION

The role of non-tuberculous mycobacteria (NTM) as an opportunistic pathogen in pulmonary and extrapulmonary diseases has grown in recent years, despite its original classification as an environmental organism. Mycobacteria non-tuberculosis complex (NTM) includes organisms other than MTBC, lepromatosis, and leprae. Of the more than 170 NTM species known worldwide, around 25 are harmful, such as Mycobacterium avium complex (MAC), Mycobacterium gordonae, Mycobacterium xenopi, Mycobacterium fortuitum complex, Mycobacterium abscessus, and Mycobacterium kansasii.

Among the NTM species isolated from various nations throughout the globe, the most common ones were MAC, M. gordonae, and M. xenopi. But pulmonary disorders are more likely to show signs of MAC, M. kansasii, and M. abscessus. Nosocomial infections caused by non-human protozoan species, mainly Mycobacterium fortuitum and Mycobacterium chelonae, include peritonitis in peritoneal dialysis patients, abscesses at injection sites from contaminated syringes, infections of the mediastinum and pericardium following surgery, infections of the abdominal region following surgery, and infections of the sternal wounds following surgery. Clinical samples taken from patients in India, both in the lungs and elsewhere, have shown an incidence of NTM species ranging from 0.7% to 34%. The restricted examination of NTM species accessible in tertiary care facilities in south India is the reason for the low recorded occurrence.

Although MTBC and NTM species share certain clinical and phenotypic features, first-line antitubercular medication proved effective against NTM in some instances but had less of an impact on MTBC. Different protocols for NTM therapy were followed in accordance with the recommendations made by the American Thoracic Society (ATS) according to the species and kind of infecting bacteria.

All existing treatments for NTM are based on the use of a multidrug regimen including macrolides, which attach to the 50S ribosomal subunit and block the elongation process, therefore inhibiting protein synthesis. A three-drug macrolide-based regimen including rifampicin and ethambutol is suggested for the treatment of M. avium complex (MAC). Mycobacteria cell wall synthesis of arabinogalactan and lipoarabinomannan is impacted by ethambutol's inhibition of arabinosyl transferases and rifampicin's inhibition of DNA-dependent RNA polymerases. Particularly effective against MAC and rifamycin-resistant M. kansasii are fluoroquinolones, which are direct DNA synthesis inhibitors in bacteria. It is not advisable to use rifamycins to treat M. abscessus complex since this species is resistant to rifampicin, yet they are effective against MAC and M. kansasii.

## LITERATURE REVIEW

Singh (2016) The purpose of this article is to detail the authors' encounters with unusual mycobacterial infections in American patients who had cosmetic surgery and then returned home. From January 2010 through July 2015, we reviewed the medical records of patients who presented with infectious problems after cosmetic surgery. A comprehensive evaluation was conducted on patients who presented with mycobacterial infections after undergoing cosmetic surgery. Concerning fast- growing mycobacteria (RGM) associated with aesthetic operations, a comprehensive literature review was conducted. Three patients who had recently had cosmetic surgery came to our facility between 2010 and 2015 with Mycobacterium abscessus confirmed by culture. Everyone had surgery in a poor nation. The patients' ages ranged from 29 to 44 years old, with a mean of 36. It took up to sixteen weeks from the first sign to the final diagnosis. After undergoing surgical drainage and receiving combination antibiotics, every patient showed full recovery. We report a cohort of underdeveloped-world individuals who contracted mycobacteria after cosmetic surgery. This might be because of the endemic nature of the bacteria or because of insufficient sterile procedure or sterilisation.

Russell (2013) The significance of non-tuberculous mycobacteria (NTM), a class of adaptable opportunistic microorganisms, as a clinical threat is becoming more acknowledged. Using information from the Scottish Mycobacteria Reference Laboratory, we detail the features of NTM isolates across the country over a span of eleven years. The clinical relevance of isolates was assessed using microbiological criteria set by the American Thoracic Society. Time series analysis, correlations between species and body sites, variances in gender and age, regional variations, and a link between cystic fibrosis and Mycobacterium abscessus are all part of the data given. To guarantee the best possible monitoring of NTM illness, we stress the importance of uniform reporting requirements for NTM isolates.

According to Farnia (2018), bacteria may change their shape and display a wide variety of cellular structures. For proper bacterial activity, the shape of the cell is crucial. Bacteria may choose from a variety of morphologies that increase their food intake, motility, surface adhesion, chromosomal division symmetry, and secretion apparatus location. Certain components, including as peptidoglycan and proteins that resemble the cytoskeleton, have the ability to control and maintain the form of bacteria. When it comes to Mycobacterium tuberculosis, there are two main groups of reported morphological variations. One group includes rod-, V-, Y-, branched-, or bud-shaped variations; the other group includes round-, oval-, ultra-, spore-like, cell wall defiant, or L- shaped variations that can be observed occasionally under stress or environmental conditions. Pathogens may take several forms, from short rods to lengthy coccobacilli, depending on factors such as cell age and growth circumstances. In unfavourable environments, such as those with low oxygen levels or hunger, tubercle bacillus grew enlarged without producing vacuolar or globoid forms. Pathogens' transient lifestyles are controlled by environmental factors and dietary characteristics.

Velayati, (2014) Opportunistic infections known as nontuberculous mycobacteria (NTM) are ubiquitous in nature. Information on the distribution of these organisms in Iran is scarce. A survey of NTM publications published in Iran from 1992 to 2014 makes up this research. Twenty publications and fourteen case reports were found in this evaluation. Out of the 20 papers, 13 (or 65%) zeroed attention on NTM isolates found in clinical specimens, 6 (30%) on NTM isolates found in environmental samples, and 1 (or 5%) included both types of isolates. M. In both clinical (28%) and environmental (19%) isolated samples, fortuitum (229/997; 23%) was found to be the most common and rapidly growing mycobacteria (RGM) species ($P < 0.05$). In the group of mycobacteria that develop slowly, M. simiae (103/494; 21%) were more common in clinical samples compared to ambient samples, while M. yeast (44 out of 503; 9%). Fourteen of Iran's thirty-one provinces contributed to these statistics. The other seventeen provinces of Iran do not have any information on environmental or clinical NTM in the published data that is accessible at the moment. These findings highlight both the underestimating of NTM prevalence in Iran and the potential significance of NTM. There is a high rate of morbidity and death in Iran related to NTM, making it a serious clinical concern. Clinicians and researchers need to keep digging into environmental and clinical studies to fill gaps in our knowledge of NTM and how to treat and prevent it.

As pointed out by Loret (2019), non-tuberculous Mycobacterium species may be found in a wide variety of water settings. According to epidemiological research, the main sources of human contamination are natural or drinking waters. Therefore, it is critical to control non-tuberculous mycobacteria in water systems in order to avoid infection. This article provides a concise summary of the current literature on the causes of non-tuberculous mycobacteria in water systems (both natural and man-made), the efficacy of water treatment methods, and, from this, suggests strategies for reducing their prevalence in drinking water.

## RESEARCH METHODOLOGY

The Mahatma Gandhi Memorial Medical College ' institutional ethics committee in Indore has given its stamp of approval (No. IEC/C- P/48/2014).

Patient selection: This research included patients whose diagnosis of NTM illness was validated by the American Thoracic Society. The patient's full demographic information, as well as their clinical and radiological records, were gathered throughout the enrollment process.

We followed the RNTCP protocol when we gathered the samples. For three days in a row, the microbiology lab received sputum, bronchial washings, body fluids, aspirates (including pleural tap, ascitic tap, gastric aspirates, synovial fluid, pus, discharge, drains, and cerebrospinal fluid, or CSF), and an early morning whole urine sample. The containers were sterile and screw- capped.

Presumptive identification of NTM:

The following criteria must be met in order to demonstrate that the strains of NTM isolated and identified from clinical specimens are etiological agents of clinical importance; this is because NTM species are present everywhere in the environment and might potentially contaminate laboratory results.

a). Isolating the same patient isolate many times.

b) Confirmatory favourable results from imaging and clinical examinations; and c). Appropriate histological status.

In contrast to Mycobacterium tuberculosis, NTM found in lung samples need special testing to rule out the possibility that they are a colonizer or contaminant rather than the actual pathogen responsible for lung illness. Criteria for diagnosing NTM lung disease isolates have been published by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA).

## RESULTS

Among the NTM patients surveyed, 224 had a smoking history (or 64.49%), 69 had quit smoking within the last three months, and 57 had quit within the last six months; nevertheless, 86 were chronic smokers (or 24.14 percent) who were still smoking when they contracted the illness. Even among those who never smoked, 118 out of 342 participants (or 34.50%) had an NTM infection, whereas 12 out of 342 participants (or 3.50%) had a history of occasional smoking.

**Table: 1. History of risk factor, smoking:**

| Smoking duration | No. of NTM isolated Pulmonary | Percentage | No. of NTM isolatedetrapulmonary | Percentage |
|---|---|---|---|---|
| Non smoker | 118 | 34.50% | 77 | 71.29% |
| Occasional smoker | 12 | 3.50% | 3 | 2.77% |
| Smoking discontinued for a period of 3 months | 69 | 20.17% | 18 | 16.66% |

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|---|---|---|---|---|
| Smoking discontinued for a period of 6 months | 57 | 16.66% | 22 | 20.37% |
| Chronic Smoker | 86 | 24.14% | 28 | 24.92% |
| Total | 342 | | 108 | |

Pulmonary symptoms accounted for the majority of NTM infection cases (76%), however a small percentage (16.6%) of patients had a combination of respiratory and systemic symptoms, such as coughing up blood, hemoptysis, thoracic discomfort, fever, decreased appetite, and night sweats. Prior tuberculosis therapy was documented in 103/450 (22.88%) individuals.

The ATS has determined that 450 people have NTM disease. In cases of lung infection with a duration of more than one to two months, the most prevalent symptom was cough, which was reported by 324 out of 342 patients (94.73%).

In cases of pulmonary infection, this is followed by a fever of 342/342 (100%) and in cases of extrapulmonary infection, 87/108 (80.55%).

Chest pain (249/342) in the lungs and 14/108 in the rest of the body, shortness of breath (198/342) in the lungs and hemoptysis (8/108 in the rest of the body) were all seen.

Weight loss (312/342), lack of appetite (237/342), and thoracic discomfort (146/342) are symptoms of a pulmonary infection, but with an extrapulmonary illness, these symptoms are more common. Some studies have shown that thoracic discomfort affects 16% of patients, while a lack of appetite affects 62% and weight loss affects 91%.

**Table:2.** Clinical manifestations of NTM disease:

| S. No | Clinical Signs & Symptoms | Pulmonary (n=342) | | Extrapulmonary (n=108) | |
|---|---|---|---|---|---|
| | | Number | Percentage | Number | Percentage |
| 1 | Cough | 324 | 94.73% | 18 | 16.66% |
| 2 | Sputum Production | 198 | 57.89% | 6 | 4.55% |
| 3 | Shortness of breath | 249 | 72.80% | 14 | 12.96% |
| 4 | Haemoptysis | 41 | 11.98% | 8 | 7.40% |
| 5 | Fever | 342 | 100% | 87 | 80.55% |

| | | | | | |
|---|---|---|---|---|---|
| 6 | Night sweats | 178 | 52.04% | 28 | 24.9% |
| 7 | Weight loss | 312 | 91.22% | 91 | 84.25% |
| 8 | Loss of appetite | 237 | 69.29% | 62 | 57.40% |
| 9 | Thoracic pain | 146 | 42.69% | 16 | 14.81% |
| 10 | Past history of ATT | 84 | 24.56% | 19 | 17.59% |

**Radiological manifestations of NTM:** Fibrosis, nodularity, and bronchiectatis are the most common clinical manifestations, while unilateral lung involvement is the most common. 14.35% pleural effusion and 11.11% cervical lymphadenopathy.

**Table:3.** X-ray findings of lung in NTM infections:

| **Type of lesion** | **Lung involvement** | **Total number** | **Percentage** |
|---|---|---|---|
| Lung involved | Unilateral | 209 | 61.11% |
| | Bilateral | 133 | 38.88% |
| Cavitation | Present | 194 | 56.72% |
| | Absent | 148 | 43.27% |
| Fibrosis | Present | 235 | 68.71% |
| | Absent | 107 | 31.28% |
| Nodularity | Present | 210 | 61.40% |
| | Absent | 132 | 38.89% |
| Bronchiectitis | Present | 203 | 59.35% |
| | Absent | 139 | 40.64% |
| Pleural effusion | Present | 46 | 13.45% |
| | Absent | 296 | 86.54% |
| Cervical lymphadenopathy | Present | 38 | 11.11% |
| | Absent | 304 | 88.88% |

Unilateral lung involvement was more common on chest X-rays of NTM infected patients compared to bilateral. Although cervical lymphadenopathy was seen in 38 out of 342 individuals (11.11%), fibrosis was the most prevalent manifestation (235/342, or 68.71%). Pleural effusion was detected in 46 out of 342 patients, or 13.45% of the total.

**Fig.1: Chest X-Ray** (1) **Fig:2: Chest-Ray**(2)

**Fig.1: Chest X-Ray** (1) **Fig:2: ChestX-Ray** (2)

**Fig: 2:** Chest X-ray showing left lower lobe broncheactasis.

**Fig: 3:** Chest X-ray showing right lower lobe dense homogenous opacity with air brobchogram-Pneumonia.

**Fig.3: Chest X-Ray** (3) **Fig.4: Chest X-Ray (4)**

**Fig: 3**: Left upper lobe fibrotic strands with cystic lesions suggestive of left upper lobe traction bronchiectasis.

**Fig:4:** left upper lobe dense homogenous opacities with mass effect.

**Fig.5: Chest X-Ray (5)** **Fig.6: Chest X-Ray (6)**

**Fig:5: Chest X-ray showing homogenous opacity with air bronchogram in right upper lobe suggestive of right upper lobe pneumonia.**

**Fig 6: Chest X-ray showing bilateral central brochiectasis.**

**Fig.7: Chest X-Ray (7)** **Fig.8: ChestX-Ray (8)**

**Fig 7:** Chest X-ray showing left upper lobe fibrosis.

**Fig:8:** Chest X-ray showing cystic changes in the left lingual – broncheactasis.

Of the 450 patients studied, 203 (or 59.35%) had bronchiectasis, 176 (51.46%) had chronic obstructive pulmonary disease (COPD), and 37 (10.81%) had post-TB sequelae. Of the 424 patients surveyed, 94.22% had concomitant conditions; 26 individuals, or 4.77 percent, did not.

The significance of NTM in antimicrobial treatment and the need to identify them at the species level in patients with pulmonary and systemic disorders are both brought to light by these results.

**Table:4.** Past history and associated medical condition in NTM infections.

| **Predisposing factor** | **Number** | **Percentage** |
|---|---|---|
| Post TB history | 37 | 10.81% |
| COPD | 176/342 | 51.46% |
| Broncheatitis | 203/342 | 59.35% |
| Asthma | 48/342 | 14.03% |
| Diabetes | 14/450 | 4.09% |
| History of surgery | 9/450 | 2.63% |
| HIV | 2 | 0.58% |
| Under corticosteroids | 2 | 0.58% |

**Table: 5.** Culture identification of Mycobacterial species in BacT/ALERT Vials.

| Cultures | | Number | Percentage |
|---|---|---|---|
| Culture in BacT/ALERT MP | | 19,436 | |
| Total number of Samples | Pulmonary | 16190 | 83.29% |
| | Extrapulmonary | 3246 | 16.70% |
| Positive cultures | Pulmonary | 5964/16190 | **36.83%** |
| | Extrapulmonary | 397/3246 | **12.23%** |
| Culture identification of Pulmonary specimens | MTB | 5622/5864 | **94.87%** |
| | NTM | 342/5864 | **4.83%** |
| Culture identification of | MTB | 289/397 | **72.97%** |

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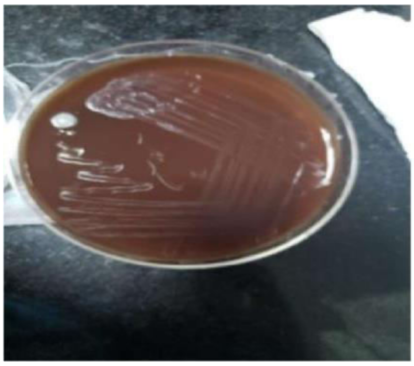
|---|---|---|---|
| extrapulmonary specimens | NTM | 108/397 | **27.20%** |

**Table:6.** Repeated isolation of NTM isolates from culture positive cases.

| Specimens | Culture positive in Bac T/Alert | Culture positive in LJ medium with PNBA | SD Bioline MPT 64 Rapid test |
|---|---|---|---|
| Pulmonary Specimens | 5964 | 342/5964(4.73%) | 5620/5964(94.23%) |
| Extrapulmonary Specimens | 397 | 107/397(26.95%) | 289/397(72.79%) |
| **Total** | **6361** | **449** | **5909** |

Out of the 5964 cultures that tested positive for AFB in the lung specimens, 344 were found to be negative by the fast test, whereas 5620 were shown to be MTBC by the SD bioline immunochromatography test. Hsp65 RFLP, 16S– 23S rRNA ITS RFLP, and hsp65 gene sequencing were all used to validate each of the 344 isolates. Three hundred forty-two lung isolates were found to be nontuberculous mycobacteria, according to all molecular techniques.



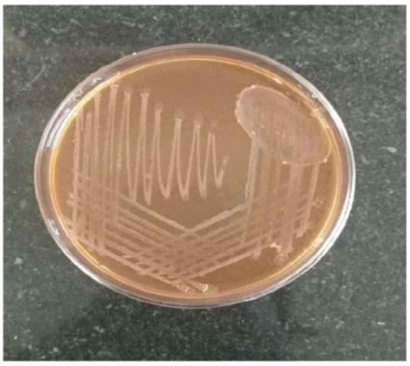


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**Fig: 9 Fig: 10**

**Fig.10: NTM growth on Blood agar. Fig.11: NTM growth on MacConkey agar.**

Mycobacteriosis is becoming more common, even in those with healthy immune systems, according to recent studies. we want to find out how often NTM species are in TB cases (both pulmonary and extrapulmonary), where those species are most commonly found, how well various phenotypic and genotypic methods work for NTM identification, and lastly, what patterns of antibiotic susceptibility can be uncovered.



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## CONCLUSION

Although TB cases are on the decline worldwide, there has been an uptick in the proportion of infections caused by non-tuberculous mycobacteria in both immunocompromised and immunocompetent humans in recent years. An rise in immunocompromised states and the development of more precise diagnostic tools may be contributing factors to the rising incidence of NTM infections. Lung, lymph gland, bone, skin, and wound infections are among the many human illnesses caused by NTM.

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