Original Article

# Nontuberculous mycobacteria: A report of eighteen cases from a tertiary care center in India

*Nitin Gupta[1], Ankit Mittal[1], Vettakkara Kandy Muhammed Niyas[1], Sayantan Banerjee[1], Yogiraj Ray[1], Parul Kodan[1], Sundeep Malla[2], Wasim Khot[1], Farhan Fazal[1], Binit Kumar Singh[1], Pankaj Jorwal[1], Neeraj Nischal[1], Manish Soneja[1], Naveet Wig[1]*

[1]*Department of Medicine, All India Institute of Medical Sciences, New Delhi, India,* [2]*Department of Radio-Diagnosis, All India Institute of Medical Sciences, New Delhi, India*

## ABSTRACT

**Context:** Nontuberculous mycobacteria (NTM) are ubiquitous mycobacteria present in environment and generally affect patients with either structural lung disease or immunosuppression and commonly involve lungs, lymph node, or skin. **Materials and Methods:** Between July 2016 and February 2019, 18 cases of NTM were diagnosed and their relevant clinical, diagnostic, and treatment details were recorded after taking informed consent. **Results:** We report 18 cases of NTM involving lungs (*n* = 11), skin and soft tissue (*n* = 3), joint (*n* = 2), genitourinary (*n* = 1), and central nervous system (*n* = 1). History of immunosuppression was present in two patients, whereas history of some form of intervention was seen in six patients. *Mycobacterium fortuitum* group (*n* = 5) was the most commonly isolated organism, followed by *Mycobacterium avium* complex (*n* = 4), *Mycobacterium abscessus* (*n* = 3), *Mycobacterium kansasii* (*n* = 2), and *Mycobacterium chelonae* (*n* = 1). In two patients, *M. chelonae* and *M. abscessus* were isolated in succession. Of these 18 patients, clinical response was present in 15 of the patients. Diagnosis and treatment of NTM in resource limited settings is extremely challenging. **Conclusion:** Most of the patients with NTM are misdiagnosed and are treated as tuberculosis in India, sometimes with a multidrug resistance regimen, which results in significant morbidity and mortality. We present these cases to shed some light on the epidemiology of NTM in this part of India.

**KEY WORDS:** *Mycobacterium abscessus, Mycobacterium avium complex, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium kansasii*

**Address for correspondence:** Dr. Manish Soneja, Department of Medicine, All India Institute of Medical Sciences, New Delhi, India.
E-mail: manishsoneja@gmail.com

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## INTRODUCTION

Nontuberculous mycobacteria (NTM) encompass all mycobacteria (more than 140 species), except the members of *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*.[1] NTM is ubiquitously present in the environment, most notably in water supplies. Their presence in tap water is attributed to their natural resistance to commonly used water disinfectants.[2] Because of this, routine exposure to NTM, most notably in the form of airborne particles, is extremely common. Considering the rarity of clinically significant NTM infection, it is safe to assume that certain risk factors are important for an individual to get an NTM infection. Two most important risk factors that are described in published literature are structural lung disease (cystic

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fibrosis, chronic obstructive pulmonary tuberculosis, past history of tuberculosis, etc.) and immunosuppression (HIV, transplantation, primary immunodeficiency, etc.).[3] Four common sites of NTM involvement are known: pulmonary, lymph node, skin, and bones/joints. Central nervous system including eye and ear involvement is considerably rare. The most common syndrome due to NTM is chronic pulmonary involvement followed by lymphadenitis. Cutaneous and bone NTM usually follow an invasive procedure and are a result of surgical site infection due to contaminated instruments. Disseminated NTM is usually described in immunosuppressed patients such as HIV/AIDS. NTM are classically classified based on the duration they take to grow on subculture on solid media into slow growers (>7 days) and rapid growers (<7 days).[1] The most important clinically relevant slow growers include *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, *Mycobacterium Marinum*, and *Mycobacterium Ulcerans*, whereas the most important clinically relevant rapid growers include *Mycobacterium abscessus* complex (MABC), *Mycobacterium fortuitum* complex, and *Mycobacterium chelonae*. There are very few reports of large series of clinical cases with follow-up from the Indian subcontinent. We report a series of eighteen cases of nontubercular mycobacteria to shed some light on the epidemiology of NTM infections in India.

## MATERIALS AND METHODS

Between July 2016 and February 2019, 18 cases of NTM were diagnosed based on available diagnostic criteria.[4] Clinical features, radiological features, method of microbiological diagnosis, treatment details, and outcome were recorded after taking informing consent. Microbiological diagnosis was established in our accredited laboratory in 15 of the patients. Ziehl–Neelsen (ZN) staining was done on the direct specimen followed by liquid culture on mycobacterium growth indicator tube (Becton Dickinson, Sparks, MD). This contains a liquid broth (modified Middlebrook 7H9) in conjunction with a fluorescence quenching-based oxygen sensor that detects mycobacterial growth. Line probe assay in the name of GenoType® mycobacterium common mycobacteria/additional species assay (Hain Lifescience, Nehren, Germany) was done on the positive cultures.[5] Diagnosis of NTM was established in an outside laboratory in three patients due to logistic reasons. Two patients were diagnosed by matrix-assisted laser desorption and ionization time of flight-mass spectrophotometry (MALDITOF-MS) and one patient was diagnosed by sequencing [Table 1].

## RESULTS

Of the 18 cases of NTM, 11 were male. The mean age was 41 ± 17 years. The state-wise distribution was as follows: Delhi ($n$ = 8), Bihar ($n$ = 2), Haryana ($n$ = 2), Uttar Pradesh ($n$ = 2), Madhya Pradesh ($n$ = 2), West Bengal ($n$ = 1), and Jammu and Kashmir ($n$ = 1). The localization of NTM was as follows: pulmonary ($n$ = 11), skin and soft tissue ($n$ = 3), joint ($n$ = 2), genitourinary ($n$ = 1), and central nervous system ($n$ = 1). The median duration of illness at presentation was 15 months (5.25–27 months). History of immunosuppression was present in two patients (HIV-1 and idiopathic CD4 lymphocytopenia-1). Six patients had a history of surgical intervention [Table 1]. All of these patients had rapidly growing mycobacteria as the causative agent. Constitutional symptoms such as fever, loss of appetite, and loss of weight were present in 13 patients [Table 1]. All except three patients had a history of receipt of conventional four-drug antitubercular therapy (ATT) for tuberculosis. The median duration of conventional ATT received before diagnosis was 6 months (5–30 months) [Table 1].

Of the 18 patients with culture positivity, culture isolates were speciated in 17 patients. Of the 17 patients, the same mycobacteria were cultured on multiple occasions in 15 patients. The organisms isolated on culture were *M. fortuitum* group ($n$ = 5), MAC ($n$ = 4), *M. abscessus* ($n$ = 3), *M. kansasii* ($n$ = 2), and *M. chelonae* ($n$ = 1). In two patients, two different species were detected at different times in the course of illness. Both the patients had *M. chelonae* and *M. abscessus* in succession [Table 1]. Most patients were on a total of four drugs at a time ($n$ = 9), followed by three drugs ($n$ = 7), five drugs ($n$ = 2), and six drugs ($n$ = 1). The mean number of drugs was 3.8 ± 0.9. Most commonly used drugs in the regimens included clarithromycin ($n$ = 11), amikacin ($n$ = 9), ethambutol ($n$ = 9), rifampicin ($n$ = 6), linezolid ($n$ = 5), levofloxacin ($n$ = 5), doxycycline ($n$ = 4), rifabutin ($n$ = 4), streptomycin ($n$ = 4), imipenem ($n$ = 3), azithromycin ($n$ = 3), isoniazid ($n$ = 2), moxifloxacin ($n$ = 1), and pyrazinamide ($n$ = 1). Of these 18 patients, two died and one was lost to follow-up. Clinical response was present in the rest of the patients [Table 1]. Those patients with clinical response were on specific therapy for an average duration of 13.7 ± 6.5 months.

## DISCUSSION

Most reports from India are laboratory data of culture isolates [Table 2].[6-16] Because NTM are commonly known to colonize or contaminate nonsterile specimens, mere isolation does not establish disease and these reports although informative may not be the true representation of the epidemiology of NTM in India. Diagnosis of NTM infection, therefore, requires relevant clinical background. On the other hand, because the clinical features and radiological findings are often so nonspecific, microbiological diagnosis becomes mandatory to make a diagnosis of NTM.

Pulmonary involvement is the most common NTM syndrome known worldwide. There are two major forms of pulmonary NTM: fibrocavitary and nodular bronchiectasis.[17] Of the 11 patients with pulmonary

**Table 1: Demography, clinical features, diagnosis, treatment, and response of patients with nontuberculous mycobacteria**

| Age/sex/residence | DOI | Site of involvement | IDD/procedure | F/A/W | cATT | Radiology | Culture | Treatment, duration and response |
|---|---|---|---|---|---|---|---|---|
| 65/male/Delhi | 24 | Pulmonary | | Yes | Yes (9) | Unilateral, cavitary, mediastinal LNs | *M. chelonae* (LPA) | LeACS for 1 year, clinical response present but no culture conversion |
| 47/female/Bihar | 60 | Pulmonary | | Yes | Yes (30) | Bilateral cavitary | *M. fortuitum* group (LPA) | RbELiCA for 1.5 years, clinical response and culture conversion present |
| 33/male/Haryana | 68 | Pulmonary | | No | Yes (48) | Bilateral nodular/ bronchiectasis | MAC (*M. intracellulare*) (LPA) | RiEC for 2 years, clinical response and culture conversion (history of relapse) |
| 45/female/Delhi | 6 | Pulmonary | | Yes | Yes (6) | Bilateral cavitary | *M. kansasii* (LPA) | RiEC for 1.5 years, clinical response and culture conversion |
| 30/female/Uttar Pradesh | 60 | Pulmonary | | Yes | Yes (36) | Bilateral nodular | *M. chelonae* (first) and *M. abscessus* (s) (LPA) | ACLiED for 4 years, died |
| 53/female/Haryana | 36 | Pulmonary | | Yes | Yes (30) | Bilateral cavitary/ collapse/consolidation/ bronchiectasis | MAC (*M. intracellulare*) (LPA) | ARiCE for 3 months, died |
| 56/male/Delhi | 8 | Pulmonary | | Yes | Yes (3) | Unilateral cavitary | *M. kansasii* (LPA) | RiHE for 10 months, clinical response and culture conversion |
| 52/male/Delhi | 12 | Pulmonary | | Yes | Yes (6) | Unilateral cavitary | MAC (*M. intracellulare*) (LPA) | RiAzE for 9 months, clinical response and culture conversion |
| 45/male/Delhi | 3 | Pulmonary | | Yes | Yes (6) | Unilateral cavitary | *M. abscessus* (LPA) | CRbMA for 1 year, clinical response and culture conversion |
| 48/female/Delhi | 24 | Pulmonary | | Yes | Yes (6) | Bilateral cavitary | *M. abscessus* (first) and *M. chelonae* (second) (LPA) | ACLiLe - same regimens used for both the organisms for 12 months, clinical response and culture conversion |
| 35/male/Delhi | 3 | Pulmonary | HIV (baseline CD4-5/mcl) | Yes | No | Bilateral nodules, ground glass opacities | MAC (*M. chimaera*) (MALDI-TOF) | HRiZESAz for 6 months, clinical response and culture conversion |
| 78/male/Jammu and Kashmir | 18 | Joint | Bilateral TKR | No | Yes (7) | Bilateral, FDG avid uptake in knee joint | *M. abscessus* (LPA) | RbCAI for 8 months, response present |
| 25/male/Delhi | 18 | Joint | Idiopathic CD4 lymphocytopenia arthroscopy | Yes | Yes (9) | Chronic osteomyelitis of femur involving the hip joint and extending up to knee joint | *M. abscessus* (LPA) | AzRbEAS for 2.5 years, response present |
| 13/female/West Bengal | 1 | CNS | VP shunt placement and appendectomy | Yes | Yes (1) | Communicating hydrocephalus wit peri-ventricular ooze Large uniloculated cyst in abdomen enclosing the shunt | *M. fortuitum* group (LPA) | ILiALe for 1 year 4 months, response present |
| 22/male/Uttar Pradesh | 1 | SSTI | RTA, intramuscular NSAID abuse | No | No | Large gluteal abscess | *M. fortuitum* group (LPA) | IliD, initial response present but lost to follow up |
| 26/female/Delhi | 10 | SSTI | Laparoscopic cholecystectomy | No | No | Large hypoechoic lesion in the anterior abdominal wall | *M. fortuitum* group - *M. senegalense* (MALDI-TOF) | LeDCS for 7 months, response present |
| 24/male/MP | 6 | SSTI | | Yes | Yes (2) | Large multi-septate collection involving left chest wall | NTM (not speciated) (target gene sequencing) | CRiE for 1 year, response present |

*Contd...*

**Table 1: Contd...**

| Age/sex/residence | DOI | Site of involvement | IDD/procedure | F/A/W | cATT | Radiology | Culture | Treatment, duration and response |
|---|---|---|---|---|---|---|---|---|
| 38/male/Bihar | 24 | Genitourinary | PCNL | No | Yes (5) | Unilateral diffuse urothelial scarring LNs (renal/para-aortic) | *M. fortuitum* group (LPA) | CDLe for 1 year, response present |

DOI: Duration of illness at presentation, IDD: Immunodeficiency disorders, F/A/W: Fever/loss of appetite/significant weight loss, CNS: Central nervous system, SSTI: Skin and soft tissue infection, TKR: Total knee replacement, PCNL: Percutaneous nephrolithotomy, VP: Ventriculoperitoneal, RTA: Road traffic accident, MAC: *Mycobacterium avium* complex, LPA: Line probe assay, LNs: Lymph nodes, NTM: Nontuberculous mycobacteria, Drugs - A: Amikacin, Az: Azithromycin, C: Clarithromycin, D: Doxycycline, E: Ethambutol, H: Isoniazid, I: Imipenem, Le: Levofloxacin, Li: Linezolid, M: Moxifloxacin, Ri: Rifampicin, Rb: Rifabutin, S: Septran, Z: Pyrazinamide, Procedure: History of invasive procedure preceding the onset of disease, cATT: History of receipt of antitubercular drugs (and duration) for suspected *Mycobacterium tuberculosis* before the diagnosis of NTM, MALDI-TOF: MS matrix-assisted laser desorption ionization mass spectrophotometry - time of flight,. *M. abscessus*: *Mycobacterium abscessus, M. fortuitum*: *Mycobacterium fortuitum, M. intracellulare*: *Mycobacterium intracellulare, M. kansasii*: *Mycobacterium kansasii, M. chelonae*: *Mycobacterium chelonae, M. chimaera*: *Mycobacterium chimaera, M. senegalense*: *Mycobacterium senegalense, M. avium*: *Mycobacterium avium,* FDG: Fluorodeoxy glucose

NTM in our series, eight patients had cavitary disease (bilateral – 3 and unilateral – 5), whereas the rest three had nodular/bronchiectatic pattern. All but one patient with pulmonary NTM in our series had a prior history of ATT intake due to presumed pulmonary tuberculosis. MAC is the most common etiology of pulmonary NTM and is also responsible for lymphadenitis and disseminated infection in patients with HIV. *Mycobacterium avium* complex includes *M. avium*, *Mycobacterium intracellulare* and several other species closely related to *M. intracellulare* like *Mycobacterium chimaera*, *Mycobacterium indicus pranii*, *M. Marseillense*, etc.[18] It is interesting to note that *M. chimaera* is reported to be less virulent than *M. avium* and *M. intracellulare. M. kansasii* is the second most common cause of lung disease worldwide and is usually believed to be a true pathogen in most cases.[19]

Rapid growers such as MABC (*M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *Bolletii, and M. abscessus* subsp. *massiliense*), *M. fortuitum* group (including *Mycobacterium senegalense*, *Mycobacterium septicum*, and *Mycobacterium brisbanense*), and *M. chelonae* are important causes of pulmonary infection and surgical site infection.[20] Rapid growers are commonly health care associated (prosthetic devices, postinjection abscesses, cosmetic, and laparoscopic procedures) because of their ubiquitous presence in water and ability to form biofilms.[11,21,22] History of procedure, prosthetic device, intramuscular injection, or road traffic accident was present in six of our patients.

The first step in the microbiological diagnosis of NTM is visualization of acid-fast bacilli in ZN stain. Subtle differences have been described to differentiate between *M. tuberculosis* and NTM on ZN stain, but conclusive diagnosis of NTM cannot be established on microscopy alone. Classically, the standard of diagnosis for NTM has been culture followed by biochemical identification, but due to the cumbersome nature of tests, most laboratories have moved on to molecular identification following culture. The most common molecular methods for speciation of NTM are 16SrRNA sequencing, line probe assay, and MALDITOF-MS.[5,23]

Recommended treatment for MAC is a three-drug macrolide-based regimen with an aim to achieve at least 12 months of negative cultures in cases of pulmonary NTM. *M. kansasii* is usually responsive to treatment and requires a combination of rifampicin, isoniazid, and ethambutol. For rapid growers, combination therapy is recommended for a total of at least 4–6 months. Intravenous drugs should be a part of the initial combination until there is clinical improvement after which oral combination therapy can be used. The treatment of NTM is usually guided by the ATS/IDSA guidelines and the British thoracic guidelines, but the adherence to these guidelines is often difficult.[4,24] Except for liposomal inhaled amikacin, which is not available in India, there are no other Food and Drug Administration-approved drugs for NTM.[25] Treatment of NTM is largely based on small observational studies and expert opinion. Furthermore, the speciation of NTM is often not available at the first go. More often than not, the diagnosis of NTM is usually based on the negativity of immunochromatography test that detects antigens specific for *M. tuberculosis* complex. The speciation if available is often delayed that results in administration of empiric treatment regimens. Furthermore, drug susceptibility of NTM is not available in most centers and it is difficult to predict response without susceptibility. This results in use of more drugs than recommended in absence of early response because of fear of nonsusceptibility. For this reason, most of our patients were on three to four drugs at a time. Microbiological cure is often very difficult to achieve, and therefore, duration of treatment in most cases has to be decided on a case-to-case basis. The average duration in our series was more than a year. The treatment outcome in NTM depends on the underlying host factors, organ involved, and the disease severity. In our series, history of immunosuppression was present in only two patients. The time of initiation and choice of treatment regimens also have considerable bearing on the outcomes. Favorable outcome of NTM infections varies with the definitions of cure used in different studies. In a systematic review, proportion of sputum culture conversion was around 61% for MAC (on triple

**Table 2: Nontuberculous mycobateria studies from India**

| Study name | Type of study, place and year of publication | Clinical details | Treatment details | Test for confirmation | Most commonly isolated NTM species | Remarks |
|---|---|---|---|---|---|---|
| Umrao *et al*. | Prospective (laboratory based), Lucknow, 2016 (*n*=263) | No | No | LPA | *M. abscessus* (31.3%) followed by *M. fortuitum* (22%), *M. intracellulare* (13.6%), *M. chelonae* (9.1%) | Risk factor identified: Male sex and age >55 years<br>Pulmonary four times more common than extra pulmonary |
| Desikan *et al*. | Retrospective (laboratory based), Bhopal, 2016 (*n*=38) | No | No | LPA | 53.8% were *M. abscessus*; 38.4% *M. intracellulare*, 3.8% *M. kansasii* and one 3.8% *M. fortuitum* | Male >female<br>15.8% mortality |
| Jesudason *et al*. | Retrospective (laboratory based), Vellore, 2005 (*n*=173) | No | No | Biochemical tests | *M. chelonae* (46%) and *M. fortuitum* (41%) | Male >female<br>Yield: Tissue >pus >sputum |
| Jain *et al*. | Retrospective (laboratory based), Delhi, 2014 (*n*=13) | Yes | No | Polymerase chain reaction assay and biochemical tests | *M. kansasii* (4), *M. chelonae* (3), *M. avium* (1) and *M. fortuitum* (1) | Pulmonary >extrapulmonary<br>Risk factors identified: Preexisting lung disease, COPD, past history of TB, chronic smoking, diabetes mellitus, steroids and malignancy |
| Sairam B *et al*. | Retrospective (patient based), Delhi, 2018 (*n*=34) | Yes | Yes | Not clear | *M. abscessus* (5), *M. intracellulare* (8), *M. kansasii* (7), *M. fortuitum* (1), M. chelone (1) | Pulmonary >extrapulmonary<br>Risk factors identified: Past history of TB, immunocompromised state<br>No macrolide resistance detected |
| Krishnappa *et al*. | Retrospective (patient based), 2017, Vellore (*n*=24) | Yes | Yes | Not clear | Only 9 were culture positive - *M. fortuitum* (4) and *M. chelonae* (3) and others (2) | Chronic nonhealing surgical scar with discharge (most common with laparoscopic cholecystectomy) |
| Maurya *et al*. | Prospective (laboratory based), 2015, Lucknow (*n*=62) | No | No | Biochemicals and LPA | *M. fortuitum* (27.5%) and *M. intracellulare* (20.9%), *M. abscessus* (14.6%), *M. chelonae* (12.9%), *M. avium* complex (8.1%), *M. kansasii* (4.8%) | Only extrapulmonary samples |
| Shah *et al*. | Retrospective (patient based), 2010, Pune (*n*=7) | Yes | Yes | Biochemicals | *M. fortuitum* (4) and *M. chelonae* (3) | |
| Sharma *et al*. | Prospective study (laboratory based), 2018 (*n*=26) | Yes | No | Sequencing | Pulmonary: *M. intracellulare* 62.5%, Extrapulmonary: *M. intracellulare* 6.5%, *M. abscessus* 2.6%, *M. avium* 1.3% | History of tuberculosis was present in 31% and radiological features of upper lung lobe involvement in 60% |
| Garima *et al*. | Retrospective (laboratory based), 2012, Delhi (*n*=44) | No | No | Sequencing | *M. kansasii* group (11), *M. fortuitum* (10), *M. avium* (7), *M. intracellulare* (9), *M. abscessus* (1) | Pulmonary >extrapulmonary |
| Gundavda *et al*. | Retrospective (patient based), 2017, Mumbai (*n*=4) | Yes | Yes | Not mentioned | *M. fortuitum* (1) *M. chelonae* (1) | All were musculoskeletal NTM cases |
| Gupta *et al*. (present study) | Patient based study, 2019, Delhi (*n*=18) | Yes | Yes | LPA | *M. fortuitum* group (*n*=5), MAC (*n*=4), *M. abscessus* (*n*=3), *M. kansasii* (*n*=2), *M. chelonae* (*n*=1). In two patients, *M. chelonae* and *M. abscessus* were isolated in succession | Lungs (*n*=11), skin and soft tissue (*n*=3), joint (*n*=2), genitourinary (*n*=1) and CNS (*n*=1). History of immunosuppression was present in two patients while history of some form of intervention was seen in six patients |

MAC: *Mycobacterium avium* complex, CNS: Central nervous system, LPA: Line probe assay, COPD: Chronic obstructive pulmonary disease, NTM: Nontuberculous mycobacteria, *M. avium*: *Mycobacterium avium*, *M. abscessus*: *Mycobacterium abscessus*, *M. chelonae*: *Mycobacterium chelonae*, *M. fortuitum*: *Mycobacterium fortuitum*, *M. intracellulare*: *Mycobacterium intracellulare*, *M. kansasii*: *Mycobacterium kansasii*

drug regimen), 41% for *M. abscessus*, and 80% for *M. kansasii*.[26,27] A favorable clinical outcome in terms of resolution of clinical symptoms was reported in 15 of 18 patients. In patients with pulmonary NTM, eight out of eleven patients showed culture conversion.

## CONCLUSION

India is highly endemic for tuberculosis, but the epidemiology of NTM in India is not well defined. Most of the patients with NTM are misdiagnosed and are

treated as tuberculosis, sometimes with a multidrug resistance regimen, which results in significant morbidity and mortality. Diagnosis and treatment of NTM in resource-limited settings is extremely challenging. There is an urgent need for increased suspicion, better diagnostic facilities that provide drug-susceptibility testing, and newer evidence-informed treatment guidelines.

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