Non tuberculous mycobacteria: An emerging pathogen

Melreena Serra[1], Suchitra Shenoy[2], Vishak Acharya[3], Basavaprabhu A[4], Pooja Rao[5], Sruthi Vinayan[6]

[1]Ms Melreena Serra, Tutor, Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

[2]Dr Suchitra Shenoy M, Professor and HOD, Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

[3]Dr Vishak Acharya, Professor, Department of Pulmonary medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

[4]Dr Basavaprabhu A, Additional Professor, Department of Medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

[5]Dr Pooja Rao, Associate Professor, Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

[6]Dr Sruthi Vinayan, Senior Resident, Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

Corresponding author: Dr Pooja Rao, Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

Email id:pooja.keerthiraj@manipal.edu,

Ph: 7676523134

Abstract: Non-tuberculous mycobacteria (NTM) are saprophytes that are found in the environment comprising mycobacterial species other than the Mycobacterium tuberculosis complex (MTC). NTM can give rise to sporadic and epidemic forms of pulmonary and extrapulmonary infections of varying severity. Extrapulmonary disease involves lymphatic, skin/soft tissue infections, disseminated disease, and nosocomial origin post-surgery. These disease conditions mimic tuberculosis in all clinical parameters. Differentiating NTM and MTC using laboratory techniques is often challenging. More so, in isolating NTM the plausibility of it being a pathogen or a contaminant from the water source in the laboratory premises must be gauged. With the STOP TB campaign going strong it becomes imperative to distinguish NTM from MTC for proper treatment. In many instances where a suspected TB patient does not respond to treatment, it is often considered to be a drug-resistant strain, on the contrary NTM does not respond to

regular antitubercular drugs. Therefore, proper diagnosis and the appropriate treatment must be started at the earliest to reduce morbidity.

Keywords: Non tubercular mycobacteria, Drug-resistant TB, Extrapulmonary tuberculosis, MOTT

Background: NTM organisms are well documented to cause natural and nosocomial infections.[1,2] They cause diseases in various sites of the body and are on a rise globally due to the increasingly vulnerable patients, modern complex therapeutic procedures along with increasing diagnostic capabilities.[3,2,4] Lung disease due to NTM is the most common presentation in immunosuppressed individuals in comparison to extrapulmonary disease.[5,6] The risk of infection due to NTM increases with a decrease in cell-mediated immunity.[7] They are emerging infectious agents in India and the world over with pulmonary/extrapulmonary disease manifestations in both immunocompromised and immunocompetent patients.[8,9,10]

NTM was first described in the later part of the 19th century and recognized as a potential human pathogen in the 1930s. In 1950, around 2% of the patients initially diagnosed with tuberculosis failed to respond to routine treatments and were later diagnosed with the non-tuberculous disease by mycobacterial species.[6] They are found distributed worldwide and present in the environment in animals, vegetation, water, soil, and biofilms.[5] Pathogenic mycobacteria cause disease in two groups: tuberculosis due to the Mycobacterium tuberculosis complex and leprosy by *M. leprae*. Atypical or non-tuberculous mycobacteria (NTM), cause infections similar to that of M. tuberculosis but are generally opportunistic pathogens.[11]

A few NTM species are opportunistic pathogens in both humans and animals. They are species belonging to the Mycobacterium avium complex (MAC), comprising *M. avium, M. chimaera M. intracellulare,* the *M. chelonae–abscessus* complex (Mycobacterium *abscessus subsp. massiliense*., Mycobacterium *abscessus subsp. abscessus,* Mycobacterium *chelonae,* Mycobacterium *abscessus subsp. Bolletti*). Two strictly pathogenic NTM species are *M. ulcerans* and *M. marinum* causing infections of the skin and

mucous membrane. *M. smegmatis* and *M. gordonae*, are generally considered to be saprophytic and non-pathogenic.[11]

Epidemiology: A literature survey done by Rebecca et al concluded MAC to be predominant in East Asia and North America, while *M. xenopi, M. kansasii, and M. malmoense* are more common and increasing in prevalence in Europe since 2000.[12] A study on new species of genus Mycobacterium in Italy has stated that, in the coming years the species of the Mycobacterium genus despite being present in large numbers are destined to increase further.[3]

A study by Sundeep et al between 2016 to 2019 on 18 cases of NTM involving lungs, joints, skin and soft tissue, genitourinary, central nervous system isolated *Mycobacterium avium complex,* Mycobacterium fortuitum group, *Mycobacterium kansasii, Mycobacterium chelonae, Mycobacterium abscessus,* while *M. abscessus*, and *M. chelonae* was isolated from 2 patients. History of immunosuppression was known in two patients and surgical intervention in six other patients.[13] Numo et al in their study concluded that 71% of NTM-associated infections presented with pulmonary manifestations while 29% presented with disseminated infection.[6]

Taxonomy: Mycobacteria grow as branched filaments belonging to the phylum Actinobacteria, family Mycobacteriaceae, the order Actinomycetales, and a unique genus, called Mycobacterium. NTM are microaerophilic or aerobic, with optimal growth rates at temperatures 25 to 50°C, depending on the species.[5,11] Advances in molecular techniques have led to the identification of approximately 180 NTM species to date of which only about 60 species are pathogenic.[5,9,14]

Transmission and Pathogenesis:

Human-to-Human transmission has been documented recently in the cases of *Mycobacterium abscessus* developing in subjects with cystic fibrosis. Outside of CF, human-to-human transmission is rarely reported.[15,16] Pulmonary infections by NTM are presumed to be contracted by aerosol inhalation from domestic/institutional hot water systems, natural surface water, sprinklers, showers, sauna (hot tub lung)

& soil.[17] Implantation infection- Skin & soft tissue infections (SSTI) as with mycobacterium abscess occur by inoculation.[18]

Risk factors

Fleshner et al observed that across multiple populations with an elevated rate of mortality, PNTM remains a serious public health concern.[19] A specific morphotype of individuals has more predisposition to develop NTM infections. This has been referred to as lady Windermere syndrome named after a character in Oscar Wilde's play. The protagonist is a tall thin sophisticated Caucasian lady, who tends to suppress a cough as a part of social etiquette and this predisposes her to chronic microaspiration into the middle lobes pre-disposing to NTM infections.[20] Significant risk factors for mortality include fibro cavitary, thoracic skeletal abnormalities, pulmonary hypertension, rheumatoid arthritis, and the use of immunomodulatory drugs.[12] The conditions that predispose to NTM include chronic lung diseases, such as cystic fibrosis, COPD, and alpha-1 antitrypsin deficiency; however, association with bronchiectasis continues to remain enigmatic.[10]

Clinical Presentations

Patients with NTM infection can have varied clinical symptoms but chronic cough with purulent sputum and hemoptysis is a common presentation. With advancing disease, the patient may have systemic symptoms like weight loss, malaise, and fatigue.[17] It is important to suspect NTM infection as it is often complicated by symptoms of coexisting or chronic lung diseases such as bronchiectasis, COPD, cystic fibrosis, and pneumoconiosis. There should be a high index of suspicion in suspected tuberculosis patients as NTM isolation rates are as high as 8% in them. The symptoms of NTM pulmonary disease may be nonspecific and variable. In those subsets of patients with disproportionate, unexplained symptoms or frequent exacerbations of COPD, bronchiectasis, or post-TB fibrosis NTM must be suspected. [17,16]

Skin or soft-tissue infection is the commonest manifestation of extrapulmonary NTM disease, which develops usually after trauma, surgery, or cosmetic procedures, which may expose the skin with less

integrity to saprophytic mycobacteria in the water, soil, or contaminated medical devices. [Fig. 1].[21]Clinical presentations of NTM diseases involving the musculoskeletal system is rare and vary depending on the immune status of the patient. Vertebral involvement is seen among immunocompromised patients with disseminated disease.[21] In non-HIV infected adults *M. tuberculosis* is responsible for >90% of mycobacterial lymphadenitis, whereas in children MAC is responsible for nearly 80% of culture-proven lymphadenitis.[22]

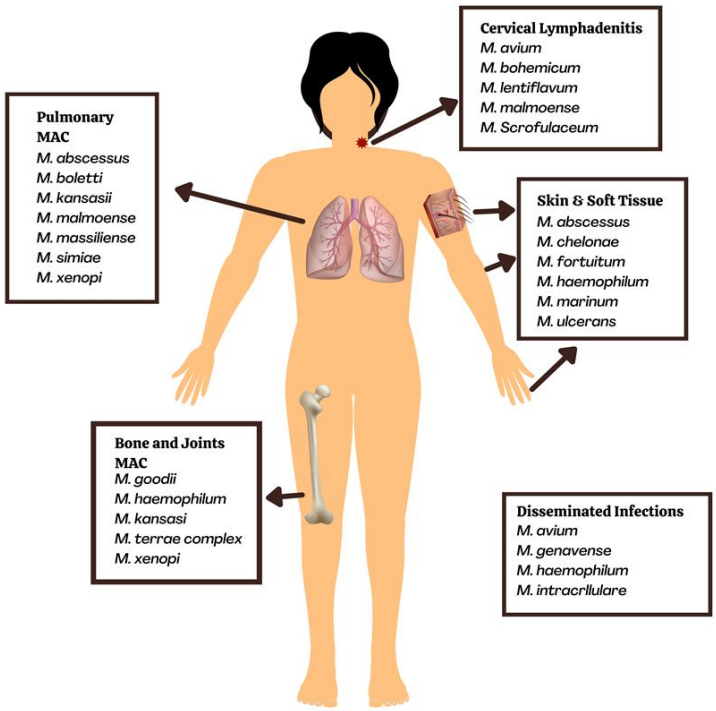

Fig-1. Body sites affected by NTM species[23]

Diagnosis: (Table 1)

Diagnosis of NTM infections requires correlation of the clinical manifestations, radiological findings and microbiological evidence. Identification of NTM species is another vital component for the diagnosis as it

helps in the assessment of clinical significance of the isolate, prognosis, and expected antibiotic susceptibility pattern.[24] Acid fast bacterial (AFB) staining helps in the identification of mycobacteria. It is rapid, simple, and inexpensive but lacks sensitivity and has poor specificity, especially in areas where NTM and MTC coexist. Therefore, culturing of clinical specimens on a solid and/or liquid media helps to distinguish between NTM and MTC. Culturing of mycobacteria is considered a "gold standard" diagnostic method and it is preferred for identifying the growth rate of different species. Culturing on solid media helps to distinguish between slowly growing mycobacteria (SGM) and rapidly growing mycobacteria (RGM). Most pathogenic mycobacteria belong to SGM. The Mycobacterium terrae complex belongs to SGM whereas the members of the M. chelonae–abscessus complex belong to RGM. However, AFB culture will not differentiate between tuberculous and nontuberculous mycobacteria.[25,26,27]

In the case of cavitary lung disease in areas where MTC infection is endemic, it is important to rule out NTM by sputum culture if the smear is positive. [28] As NTM can be environmental contaminants in sputum samples, more than one positive sputum culture is recommended for diagnosis, and the same NTM species (or subspecies in the case of M. abscessus) should be isolated from two or more sputum culture (collected one week apart) in order to meet the disease criteria.[29]

Source of culture in cases of extrapulmonary tuberculosis (EPTB) is determined by site of involvement, such as biopsy in skin infections, blood cultures in dessiminated infections, bone marrow biopsy in cases of cytopenias.[29] It is difficult to diagnose EPTB based on smear positivity or culture due to low bacillary load. For the same reason of low bacillary load, the diagnosis of EPTB or HIV-TB co-infection or smear-negative PTB is a great challenge to all the clinicians and laboratory scientists the world over.[25]

Conventional biochemical tests like niacin accumulation test, catalase estimation, nitrate reduction, arylsulfatase test, and growth in MacConkey agar media, are commonly used to identify mycobacterial species, but they are tedious and time-consuming.[9] Due to the different treatment regimens for different NTM strains, identification of specific NTM species is crucial. From a literature survey done by Radha et

al, it is found that the p-nitro benzoic acid test (PNB) which discriminates MTC from NTM is an important biochemical test used routinely in clinical laboratories.[9,27]

Identifying the causative agent is imperative in the diagnosis and management of any infection or detection of an outbreak. NTM identification was based on cultural traits and standard biochemical test results for many years. At present they are obsolete in most places due to the time consumed and the slow growth of the organisms.[3] As the Mycobacteria are slow growers, there is a delay in the conventional species-specific identification and proper patient management. Though most Mycobacterial infections are caused by MTC strains, NTM infections are increasing mainly among immunocompromised individuals.[30] In both MTB and NTM the characteristic histological finding is necrotizing granulomatous lesion along with chronic inflammation around the bronchus, cavitation, or bronchiectasis.[25]

In a study done by Reena et. al., the identification of NTM from positive growth on the media was done using GenoType Mycobacteria CM. The assay allows the simultaneous molecular genetic identification of 14 of the most common NTM species and the MTC from cultivated samples.[22] Gurupreet et al opined that phenotypic methods are not a replacement for molecular methods and are time-consuming and molecular methods should be used to confirm the diagnosis of atypical strains in case of emergency.[1]

The mycolic acids found in the cell wall of mycobacteria may be investigated for identification purposes by chromatographic methods. The High-performance liquid chromatography (HPLC) method where mycolic acids are analyzed, is highly species-specific and has shown to differentiate 95.6% of NTM species. Both gas-liquid chromatography (GLC) and thin-layer chromatography have very limited value as diagnostic tests but are widely used in research laboratories.[3,20]

Molecular methods like polymerase chain reaction (PCR) can play an important role in accurate and rapid diagnosis of extrapulmonary tuberculosis.[17]A multiplex PCR developed in three different studies, Duplex PCR assay by Kim et al have highlighted the importance of rapid detection of Mycobacteria from primary cultures and also the differentiation of MTC and NTM. Rapid identification and differentiation of species contribute significantly to early treatment.[2,26,27,28] PCR is precise and do not need BioSafety Level 3

(BSL-3) laboratory therefore it has become a common detection method in EPTB diagnosis. Real-time PCR (qPCR) which has high sensitivity, reproducibility, accuracy, less false positives due to reduced contamination is widely used in laboratory diagnoses of infectious diseases.[18]

From a literature survey done by Huang et al, it was found that NTM species differentiation can be done by PCR restriction analysis (PRA) using specific restriction enzymes MspI, HaeIII, and BstEII.[30] The ambiguous patterns of PRA and the increasing shared patterns between different species of NTM may result in misidentification as stated by Enrico Tortoli. [3]

From different studies, it was found that sequencing provides the minute details for gene sequence analysis. This is currently considered the gold standard for identifying Nontubercular mycobacterial species. But a qualified database is essential for alignment and comparison. The disadvantage of single gene sequencing is we may not be able to distinguish between closely related NTM species.[27,31,32]

MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time Of Flight) is a protein extraction protocol using pulses of laser light to vaporize the matrix proteins from which specific mass-to-charge ratios are determined are specific for NTM species.[33,34] Eventhough it is cheaper and simpler than commercial nucleic acid amplification assays or whole genome sequencing, it requires pure isolates and is marred by the limited spectra of library in the instrument.[24]

Imaging techniques

The first imaging tool used for evaluating pulmonary lesions such as nodules and opacities due to cavities is Chest radiography. Along with the clinical suspicion, the associated radiographic changes are important criteria to diagnose NTM-PD. Nodular bronchiectatic and fibrocavitary patterns are the two major types of radiological manifestations. Other radiological patterns, such as consolidation, mass-like lesions, and diffuse infiltration, are less common.[35]

In the case of pulmonary infections, the radiographic pattern cannot differentiate the fibrocavitary lesions caused by MTC from those caused by NTM. Most patients with NTM disease, therefore, end up receiving

therapy for TB or other common infections for months to years. The culture-proven cases of NTM are also ignored as possible contaminants grown in the culture. Due to this most patients who are infected with NTM may receive anti-tubercular therapy or other antibiotics for months. NTM in conjunction with nodular bronchiectasis is best appreciated on high-resolution chest computed tomography (HRCT).[8,17]

A study by Marc et al stated that the chest X-ray is often normal or nonspecific, therefore it is ideal to have computerized tomography (CT) imaging of the thorax of all patients with suspected NTM-PD. A study published in 2019 stated that the CT examination can be done on high clinical suspicion and non-confirming chest radiography.[8]

Diagnosis of NTM-PD is a challenge because of the nonspecific clinical signs and symptoms, large variation in the radiological findings, and most important difficulty in isolating the organisms. Adding to the dubiety, NTM can be found in the lungs without causing any tissue damage.[8]

Table 1: Diagnostic criteria for NTM-PD[29]

Clinical & radiological criterion	• Pulmonary symptoms • Radiologic cavitary opacities or Nodular lesions on chest radiograph, or a high-resolution computed tomography scan that shows multiple small nodules of bronchiectasis • And Appropriate exclusion of other diagnoses.
Microbiological criterion	Culture positive from at least two separate expectorated sputum samples Or At least one bronchial wash or lavage culture positive result

	Or Culture positive for NTM by sputum or BAL and mycobacterial histologic features in the Transbronchial or other lung biopsy specimen

Table 2: The decision to initiate therapy for NTM is decided on basis of these factors[38]
▶ Patient's age, general condition, co-morbidities, and symptoms.
▶ Willingness of patient & discussion of risk/ benefits / outcomes.
▶ Nature of the disease radiologically
▶ Severity or extent of lung damage
▶ Microbiological load
▶ The species of NTM isolated *M.gordonae*- Rarely pathogenic *M. kansasii*- Always pathogenic *M. xenopi*- highly pathogenic *M. abscessus*- Mostly pathogenic

Treatment (Table 2)

TB patients with NTM co-infections may be treated incorrectly, due to inaccurate sputum smear microscopy and Xpert™ M. tuberculosis/rifampin results.[26] A challenging aspect of NTM pulmonary disease is, that just based on clinical and laboratory diagnostic criteria treatment need not be initiated as the following are to be considered: anti-mycobacterial treatment is prolonged, difficult to tolerate, and long-term response rates are variable and uncertain. In some cases, "watchful waiting" may be the best course of action. However, NTM infection being indolent, most progress over time, and most of them eventually require treatment.[34] Infectious Diseases Society of America (IDSA) treatment guidelines for nontuberculous mycobacterial pulmonary disease in 2020 have suggested initiation of treatment in patients who meet the diagnostic criteria of NTM pulmonary disease especially in sputum positive and/or cavitary lung disease.[29]

As the organism is slow growing we may have to start empiric treatment and then change over to specific treatment based on the invitro drug susceptibility testing. Pulmonary disease due to MAC is treated with macrolides (preferably azithromycin-based) and amikacin after susceptibility testing. Empiric treatment is not advocated. In macrolide-susceptible MAC pulmonary disease, a 3 drug regimen (including a macrolide and ethambutol) is adviced. The frequency being 3 times per week macrolide-based regimen in noncavitary nodular/bronchiectatic patients and in cavitary or severe/advanced nondular bronchiectatic cases daily regimen. The duration of treatment should continue for at least 12 months after culture conversion. Addition of macrolide in the treatment regimen is associated with increase in rate of sputum conversion and reduction in mortality. In macrolide-resistant MAC pulmonary disease or in patients with cavitary or advanced/severe bronchiectatic, parenteral amikacin or streptomycin can be included in the initial treatment regimen.[29]

M. kansasii pulmonary disease is treated with rifampicin after the DST results. A regimen of 3 oral drugs, including rifampicin, ethambutol, and either isoniazid or a macrolide, is suggested by the IDSA guidelines for treatment success. The drugs may be given daily or three times a week depending on the

combination.[29] *M.xenopi* treatment is based on the clinical presentation as there is no single drug recommended for treatment.[15] As high mortality is associated with M. xenopi causative disease, the patients should be treated aggressively, preferably with 3 drugs: rifampicin, ethambutol, and either a macrolide and/or a fluoroquinolone (moxifloxacin).[29]

The drug susceptibility testing (DST) for NTM diseases is difficult and controversial when compared to TB because the results observed in in vitro susceptibility testing and the in vivo outcomes are comparable. But DST is important to devise a suitable treatment strategy. Molecular tests are used to detect any mutation in the genes responsible for the susceptibility to anti-TB drugs. Though these methods are specific and rapid it must be noted that genotypic resistance may not always express phenotypic resistance. Therefore, it is necessary to conduct phenotypic assays.[27]

Delays in starting treatment lead to poor clinical outcomes.[27] In patients who have localized involvement of the lung (cavitary disease, severe bronchiectasis) and can tolerate surgery, resection of the diseased lung is advocated to reduce the burden of organisms.It can also be opted in cases of failed medical management and in the presence of drug resistant isolates after weighing risks against benefits.[29]

Treatment of disseminated NTM disease usually requires 6 to 12 months after immune restoration. In case of macrolide-sensitive *M. abscessus* and *M.massiliense* along with macrolides (clarithromycin), amikacin, cefoxitin/imipenem, moxifloxacin, clofazamine, minocycline, and cotrimoxazole may be given. In all other species, macrolide is the mainstay of the treatment along with quinolone and minocycline.[28]

Monitoring of patients under observation

No set guidelines to determine the optimal frequency of monitoring for disease progression are determined. Appropriate frequency depends on the extent of the disease. Sputum cultures should be obtained every 2–3 months and repeat imaging every 6 months. Development of new radiographical features such as cavitation or worsening nodularity would be a sign of progressive disease with increasing bacterial load & smear conversion to positivity.[17]

Conclusion: Misdiagnosis of most of the NTM cases results in them being treated as tuberculosis or as drug-resistant tuberculosis in India. It increases morbidity and mortality significantly. This significantly contributes to the development of drug resistance and perpetuates the eradication of the disease leading to greater public health issues. Tools that can differentiate TB and NTM infections accurately and simultaneously are the need of the hour specifically in resource-limited settings where it is extremely challenging.

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