https://doi.org/10.48047/AFJBS.6.15.2024.4013-4030

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

An overview of nontuberculous mycobacterial pulmonary disease

Nadeem Gul Dar[1], Dr. Geeta Gupta[2], Mudasir Khan[3], Dr. Nazia Khanum[4], Dr. Shagufta shahi[5], Dr. Zafar Nowshad Wani[6], Dr Rakesh Shukla[7], Dr. Shabir Ahmad Lone[8], Dr Sachin Kishore[9]

[1]PhD Scholar, Department of Microbiology, Santosh Medical College, Ghaziabad, Uttar Pradesh, India
[2]Professor, Department of Microbiology, Santosh Medical College, Ghaziabad, Uttar Pradesh, India
[3] PhD Scholar, Department of Microbiology, Santosh Medical College, Ghaziabad, Uttar Pradesh, India
[4] Microbiologist, Department of Microbiology, SVS Medical College Mahbubnagar, Telangana, India
[5] Director, State Tuberculosis Demonstration cum training center Srinagar, Jammu and Kashmir, India.
[6] Microbiologist, IRL Srinagar, Jammu and Kashmir, India
[7]Associate Professor, Department of Anatomy, Autonomous State Medical College Kaushambi, Uttar Pradesh, India
[8] Sher-i-Kashmir Institute of Medical Sciences, Srinagar Jammu and Kashmir, India.
[9]Associate Professor, Department of Microbiology, Autonomous State Medical College, Mirzapur, Uttar Pradesh, India

Address of Correspondence: Dr Sachin Kishore, Department of Microbiology, Autonomous State Medical College, Mirzapur, Uttar Pradesh, India
E-mail address- sachinkishore@ymail.com

Article History

Volume 6, Issue 15, 2024

Received : 03 May 2024

Accepted : 28 Aug 2024

Published: 07 Sep 2024

doi:10.48047/AFJBS.6.15.2024.4013-4030

Abstract:

Nontuberculous mycobacteria (NTM) are members of the Mycobacterium genus that are known for their acid-fast characteristics with thick lipid-rich cell walls. NTM cause infections in various sites, but pulmonary diseases are the most common manifestation in an immunocompetent individual. The prevalence of NTM-PD is not notifiable disease in many countries including Asia, Europe, and most of US. NTM is the most frequent cause of pulmonary disease in immunocompromised individuals. Infections caused by NTM have been classified into 4 clinical syndromes by IDSA namely pulmonary infections, lymphadenopathy, skin and soft tissue infections, and disseminated disease. Most clinical isolates are resistant to an extensive spectrum of antimycobacterial drugs in vitro, necessitating the use of many antibiotics in combination to treat these infections. We reviewed the literature on NTM -PD, including its microbiology, laboratory identification, environmental sources, transmission channels, risk factors, epidemiology, diagnosis, treatment options, and management challenges.

Introduction:

Mycobacteria are one of the oldest group of bacterial genera, they are aerobic and known for their acid-fast characteristics with thick lipid-rich cell walls (Cook GM et al.,2009) the most common species is Mycobacteria tuberculosis which is causative agent of Tuberculosis, which mainly causes pulmonary infection (Smith I, 2003) "Nontuberculous mycobacteria" (NTM) are members of the Mycobacterium genus that are not part of the M. TB complex, and typically exclude M. leprae. (American Thoracic Society guidelines, 1990) these are ubiquitous organism found worldwide in the environment, they are resistant to extremes of Ph, heat many antibiotics and disinfectants due to thick lipid-rich cell wall. (Falkinham J III. Ecology of nontuberculous mycobacteria 2013). NTM causes infections in various sites, but pulmonary diseases (NTM-PD) is the most common manifestation in an immunocompetent individual.

We reviewed the literature on NTM-PD, including its microbiology, laboratory identification, environmental sources, transmission channels, risk factors, epidemiology, diagnosis, treatment options, and management challenges.

Non tuberculous Mycobacteria species(NTM):

With 142 species of mycobacteria in the environment, it is critical to divide them into groups for accurate and prompt diagnosis and treatment. The Mycobacterium tuberculosis complex is the most well-known group. This category causes tuberculosis in humans and animals. It comprises M. tuberculosis, M. bovis, M. africanum, M. microti, and M. canelti (Perez-Martinez I et al.,2008). The second group comprises just Mycobacterium leprae, the bacterium that causes leprosy in humans.

All other mycobacterial species are classed as NTMs. This category comprises around 130 species. This group is also known as atypical mycobacteria, environmental mycobacteria, and mycobacteria other than tuberculosis (MOTT) (Le Dantec C et al.,2002 and Bland CS et al.,2005).

Because this category contains so many species, it may be further divided into two subcategories: rapidly growing mycobacteria (ROM) and slow growing mycobacteria (SGM). NTM are categorized into two groups: saprophytes (generally non-pathogenic) and opportunistic pathogens that infect the host (Wagner, D. and Young, L.S 2004).

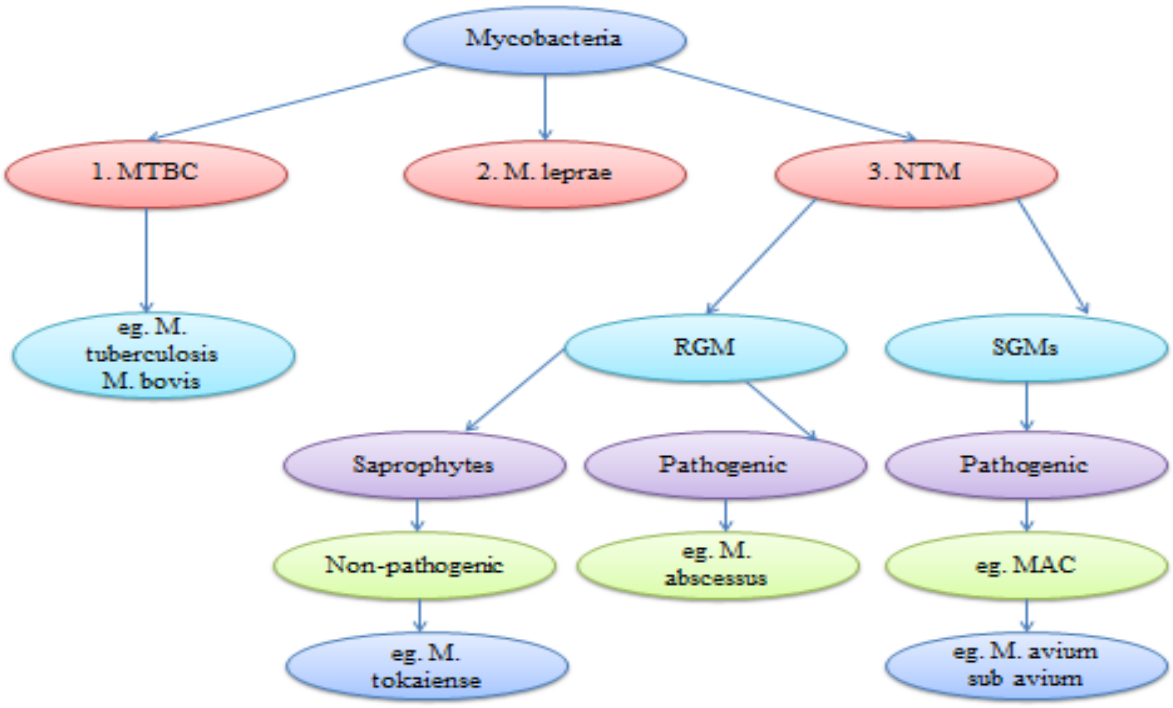

Figure 1.- Categorization of Mycobacteria

Over 130 species and subspecies of NTM have been identified which although closely related have distinct epidemiologic features. NTM were classified in mid 1950s in 4 groups based on growth rates and pigment output known as Runyon's categorization (Table 1).

Table 1: Runyon's Classification of Non-tuberculous Mycobacteria

Runyon's Class	**Description**	**Growth**	**Pigment Production**	**Species examples**
I	Photochromogens	Slow growing	Yellow-orange pigment production when exposed to light	M. kansasii
II	Scotochromogens	Slow growing	Yellow-orange pigment production with or without light	M. gordonae

III	Nonchromogens	Slow growing	None	M. xenopi
IV	Rapid Growers (form colonies in under 7 days)	Rapid growing	None	M. chelonae

The M. avium complex (MAC) and M. abscessus (MAB) complex are the leading causes of NTM-PD globally (Prevots DR and Marras TK 2015). MAB organisms are rapidly growing mycobacteria with 3 subspecies such as M abscessus subspecies abcessus, MAB organisms are rapidly growing mycobacteria. MAC is a slow-growing group of mycobacteria with 12 species (M. avium, Mycobacterium intracellulare, Mycobacterium chimaera, Mycobacterium colombiense, Mycobacterium arosiense, Mycobacterium vulneris, Mycobacterium bouchedurhonense, Mycobacterium timonense, Mycobacterium marseillense, Mycobacterium yongonense, Mycobacterium paraintracellulare, and Mycobacterium lepraemurium).. Mycobacterium xenopi, Mycobacterium fortuitum, and Mycobacterium kansasii are the next most prevalent causes of NTM-PD, after MAC and MAB [9]. Other significant species responsible for NTM-PD are Mycobacterium malmoense, Mycobacterium simiae, and Mycobacterium szulgai (Yan M et al.,2023).

Epidemiology:

As NTM -PD is not notifiable disease in many countries including asia, Europe and most of US (Schildkraut JA et al.,2020 and Van Ingen J et al.,2021) estimating the prevalence has major challenges. The true burden of the disease cannot be captured due to absence of this systemic surveillance, and as the diagnosis is criteria based, number of reports of pathogen isolation does not represent total prevalence of clinical disease. Thus the prevalence varies widely across the world ranging from 23-37 cases/100000 in USA to 33-65 cases/100000 persons in japan. (Adjemian J et al.,2012, Prevots DR et al., 2010 and Morimoto Ket al.,2014).

According to one systemic review, MAC species account for 34% to 61% of all isolates worldwide, with the largest numbers seen in North America and Oceania (Zweijpfenning SMH et al.,2018).

In another systemic review of 47 studies involving 285,681 NTM isolates from 18 countries, 82% observed an increase in infection and 68% reported an increase in illness. The yearly

rate of change for NTM infection and illness per 100,000 populations was 4.0% and 4.1%, respectively (Dahl VN et al.,2022).

The observed increase in NTM infection and disease could be attributed to increased physician awareness, improved detection methods, increased exposure, increased host susceptibility, and complex medical and surgical procedures (Haworth CS et al.,2017 and Ahmed I et al.,2020).

MICROBIAL CHARACTERISTICS AND PHYSICAL PROPERTIES OF MYCOBACTERIA

The genus Mycobacterium is classified under the family Mycobacteriaceae of order Actinomycete. They have distinct cell wall, acid fast and high genmic C+G content (55-70%) (Howard ST et al.,2000 and Turenne CY et al.,2007). Mycobacteria are gram positive bacilli with cell wall thicker than other bacteria due to high lipid content accounting around 40% of total dry weight (Ovi MO, 2008). The cell wall has 2 layers held together by linking polysaccharide arabinogalactan. The outer layer is impermeable made up of mycolic acid while inner layer is made up of peptidoglycan (McMurray DN, 4th edition, 1996). [figure 2]

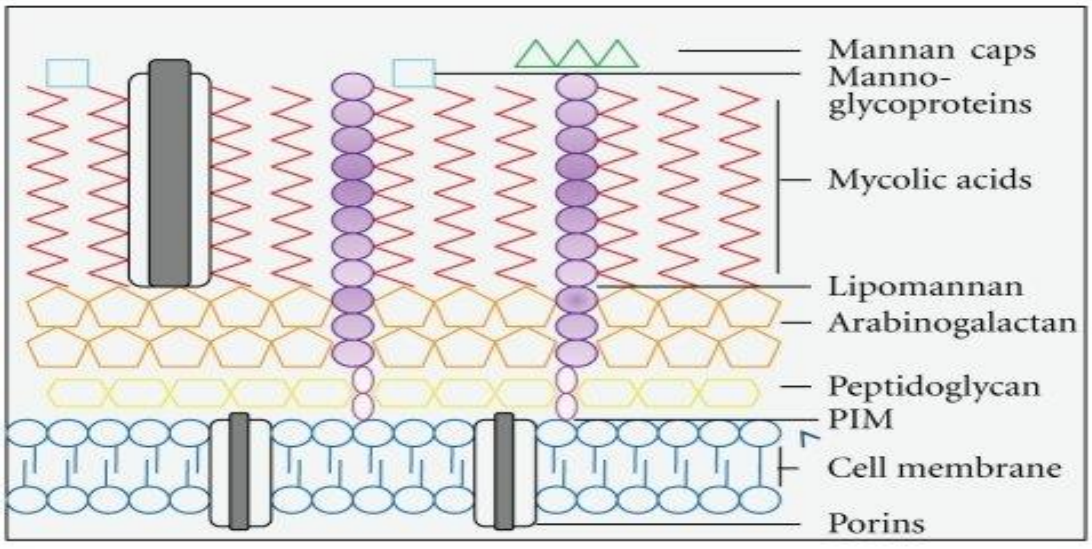

Figure 2- Complex cell wall structures of mycobacteria

The presence of mycolic acid or mycolates makes the cell acid fast and give appearance of waxy slender curve rods.

IDENTIFICATION OF MYCOBACTERIA

Mycobacteria identification is a laborious, expensive, and time-consuming process. First, clinical samples are analysed microscopically with the Ziehl-Neelsen (ZN) staining method.

The ZN stain's primary benefit is its ability to immediately screen materials for the presence of mycobacteria without the necessity for culture. It is one of the initial steps in the diagnostic procedure. Once a sample is deemed ZN positive the next process is to decontaminate and culture the specimen.

Mycobacteria are difficult to grow taking upto months to grow (Nieminen T et al.,2006). Isolation from clinical samples requires selective media to grow such as Lowenstein-Jensen (egg-based) or conventional lab media such as Middlebrook 7H10 and 7H11 (Lee ES et al.,2008 and Turenne CY et al.,2001). The incubation time varies depending on type of species but ideally for unknown samples its 28°C - 37°C (Tortoli E et al.,2003). All non-sterile samples should be decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NaCl-NaOH) in combination with 5% oxalic acid, or 0.001% Cetylpyridinium chloride monohydrate (CPC) to eliminate fast growing bacteria from overshadowing the growth of mycobacteria (Lee ES et al.,2008).

Many laboratories use automated in vitro diagnostic methods such as BACTEC™ or MGIT 960™ optimized for the rapid identification of mycobacteria from clinical specimens (Somoskovi A et al.,2000 and Rodrigues C et al.,2009). Drawback of these are that they are costly thus may not be available in smaller laboratories with limited economical resources (Martin A et al.,2009).

There are currently multiple commercial systems that employ a variety of methods to identify mycobacteria species in clinical laboratories. Such are the INNOLiPA mycobacteria test (Innogenetics), the Cobas Amplicor PCR system (Roche), the HAINTM DNA probe (Hain Lifesciences), and the BD ProbTec strand displacement amplification (Becton Dickinson) (Williams'J K et al.,2007).

Subsequent analysis requires identification of a definite species using a range of phenotypic and genotypic tests.

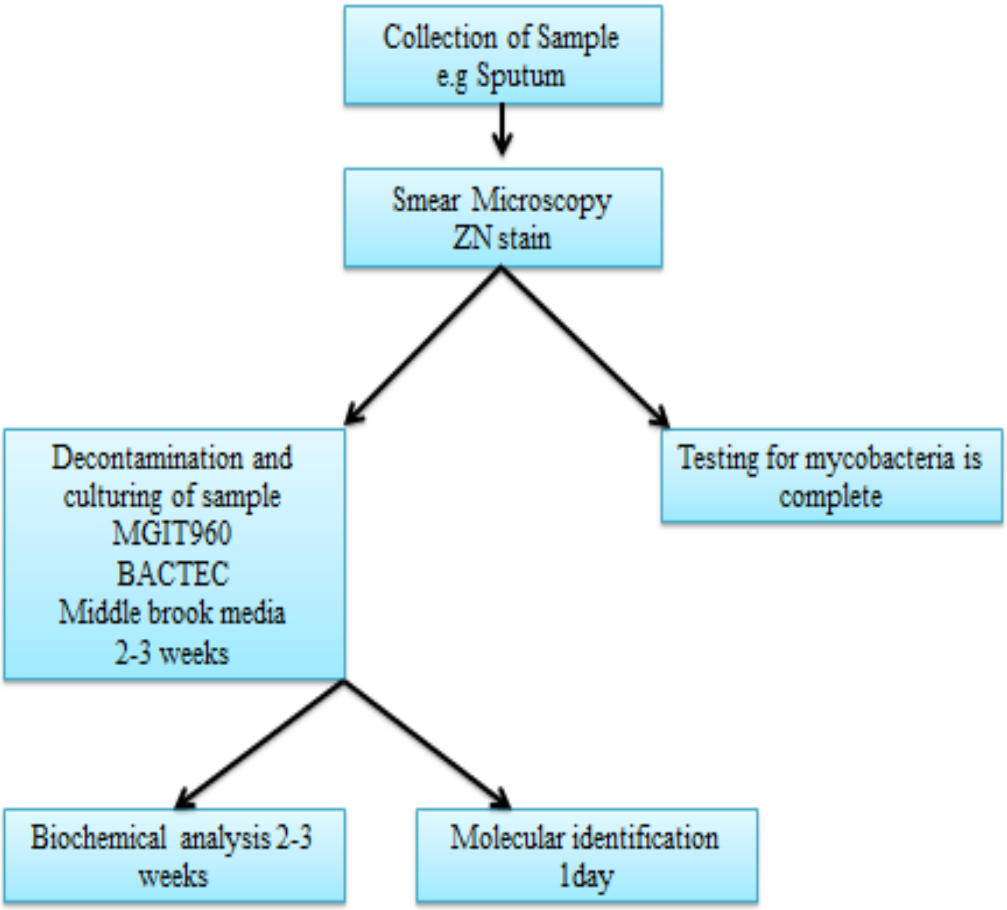

Figure 3- Brief outline of Clinical Analysis of Mycobacteria

Phenotypic identification of NTM

Phenotypic identification includes several methods such as phenotypic studies, biochemical tests and high-performance liquid chromatography (HPLC). Traditional phenotypic studies built on Runyons classification, include growth rate, colonial morphology, pigmentation, biochemical profiles, and gas-liquid chromatography of short chain fatty acids (Tateishi Y et al.,2009). The drawback of this method is its inability to distinguish between closely related species of mycobacteria such as M. abscessus and M. chelonae, which may lead to prescribing incorrect antimicrobial treatment in patients.

Biochemical tests used for identification of mycobacterial species include nitrate reduction, catalase production, catalase inactivation at 68^0C, hydrolysis of Tween, reduction of potassium tellurite, tolerance of 5% NaCl, iron uptake and urease production (Springer B et al.,1996 and Hall, Leslie et al.,2006). Drawback of these tests are these are cumbersome,

laborious and time-consuming, with many tests taking 2-6 weeks for completion (Therese KL et al.,2009).

High-performance liquid chromatography (HPLC) was proposed in 1985, with the principle based on the analysis of mycolic acids present in the cell wall. The method reduced the time from several weeks to days, and became the standard for the Centre for Disease Control (CDC) in 1990 (Butler WR et al.,2001).

Molecular identification of NTM

Recently many molecular methods have been developed which has drastically decreased the identification time from weeks to hours and identifies up to species level. This often requires either a manual PCR-based approach, such as 16S rRNA gene analysis, or an automated probe-based assay, such as the HAINTM test. This technique is especially suitable for large-scale high-throughput screening in a clinical laboratory.

A DNA STRIP® is coated with highly specific probes which are complementary to selective species specific nucleic acid sequences. There are various test kits available for identifying mycobacteria species, including Genotype Mycobacteria CM/AS and Genotype® Mycobacteria MTBDRs / MTBDR Plus. This method can detect 44 species of mycobacteria, including all clinically significant species, as well as discriminate between species pairs with identical 16S rRNA sequences (Daley P et al.,2008).

Recently, PCR has become the main diagnostic tool in many clinical and research laboratories, due to its ability to produce rapid and reliable results.

16S rRNA gene analysis to a species level for mycobacteria

In the 1980s, a new method of recognizing microorganisms emerged. It was thought that phylogenetic connections might be inferred by comparing conserved portions of the genetic code (Winthrop KL, 2010). The 16S rRNA gene is the most extensively utilized DNA segment for taxonomic purposes today. Sequencing of the 16s rRNA gene can be performed using specific primers such as 285F and 264R (Mirsaeidi M et al.,2014) or commercial kits like MicroSeq 500 16S rDNA protocol kit (Richter E et al.,2006 and Therese KL et al.,2009). The most valuable nucleotide sequences in the mycobacterial 16S rRNA gene include those shared by all members of the genus Mycobacterium (Boddinghaus, B et al.,1990) as well as

highly variable areas with species-specific variability. The 16S rRNA-based genetic study of mycobacterial taxonomy and phylogeny focuses on two highly variable sequences known as region A and B. Slow growers have one copy of the 16S rRNA gene, whereas quick growers have two, with the exception of M abscessus and M. chelonae (Tortoli E, 2003).

However, other subgroups of mycobacteria have evolved in recent years, such as MAC, which the 16S gene cannot detect beyond the species level. As a result, new molecular approaches with increased discriminating strength have emerged. These include insertion sequence (IS) profiling and Sequevar analysis using the hsp65 gene.

Insertion Sequence (IS) profiling

IS are mobile DNA elements that may cause a variety of genomic rearrangements, including deletions, inversions, duplications, and replicon fusions, by transposition within the genome. Most IS are 800-2,500bp long, have one or more open frames, and encode a transposon that facilitates element mobility (Richardson ET et al.2009). This is valuable for tracking lineages within a species, such as MAC, and it has also been used to measure virulence within isolate populations. For example, IS 1245 is thought to be a virulence factor in M. avium, subsp. Hominissuis (Alcaide F et al.2010).

Several species-specific IS have been utilized to type distinct mycobacteria strains, including IS901 (Guerrero ET et al.1995), IS1245 (Alcaide F et al.2010), IS900 (Alcaide F et al.2010), and IS 1311 (Alcaide F et al.2010). In addition to 16S rRNA gene sequencing for detecting NTM, IS or internal transcribed spacer sequences were deemed to be useful for identification and profiling.

hsp65 gene and Sequevar analysis to a sub-species level

hsp65 gene profiling has proven to be effective for molecular typing of mycobacterial isolates, particularly MAC. It was first considered since the hsp65 gene is found in all mycobacteria. However, hsp65 gene profiling is thought to be more variable than 16S rRNA gene sequences, making it potentially useful for identifying genetically related species (Plikaytis BB et al.1998).

TRANSMISSION OF NON TUBERCULOUS MYCOBACTERIA(NTM):

Unlike mycobacteria tuberculosis, NTM is not transmitted person to person (Spagnolo P et al.2008), it is acquired through environmental contamination. Soil, plants, air and dust are possible sources of NTM transmission. Transmission of NTM pathogen to human occurs through inhalation of aerosolized NTM contaminated water droplets and soil (Kamal A et al.2023).

On inhalation the mycobacteria infection is caused, whether they lodge in the nose or go via the trachea and bronchi to the alveoli. Clumps of two or three bacilli can penetrate the alveolar airways and be taken up by macrophages, triggering pathogenesis. Mycobacteria are thought to be incapable of penetrating undamaged skin, although they may infiltrate through microscopic abrasions and are swiftly phagocytosed by polymorphs and macrophages. M. chelonae and M. ulcerans infections can start in such areas (Uslan DZ et al.2006).

Risk factors:

Presence of certain risk factors increases the likelihood of development of NTM-PD. Underlying structural airway diseases such as Bronchiectasis, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pneumoconiosis, prior TB, pulmonary alveolar proteinosis, α-1-antitrypsin anomalies, and oesophagal motility disorders provide conditions which are favourable to colonisation by NTM (Chan ED and Iseman MD et al.2013). Among other risk factors are old age, female gender, immune system deficiencies and immunosuppressive medications. Role of genetics remain unclear, presence of NTM-PD has been reported among families indicating there may be genetic contribution to it (Colombo RE et al.,2010 and Kobashi Y et al.,2006).

INFECTIONS WITH NON TUBERCULOUS MYCOBACTERIA[NTM]

Infections caused by NTM has been classified into 4 clinical syndromes by IDSA namely pulmonary infections, lymphadenopathy, skin and soft tissue infections and disseminated disease. The most common among these are the pulmonary infections.

IDSA guidelines for NTM isolation from lung specimen include:

1. Symptoms of Lung disease or abnormalities observed radiologically, such as nodular or cavitary opacities on chest radiograph or multifocal, nodular bronchiectasis on high-resolution computed tomography (CT) scan;

2. Appropriate exclusion of other diagnoses; and

3. NTM-positive cultures from at least two separately expectorated sputum samples or from at least one bronchial wash (McCarthy KD et al.2012).

NTM-PD is the most frequent NTM related diseases (Cooksey RC et al.,2008 and Johnson MM et al.,2014). NTM has been frequently isolated from patients with cystic fibrosis indicating that cystic fibrosis is one of the major risk factors for NTM lung infections. Highest morbidity and mortality is reported in CF lung disease (Hill UG et al.2012). Other lung infections include bronchiectasis (Amalakuhan B et al.2014), hypersensitivity pneumonitis (Spagnolo P et al.2008) and Chronic Obstructive Pulmonary Disease (Sethi S 2010).

TREATMENT:

Treatment regimens for NTM-caused infections remain mostly unknown, and the outcomes have been disappointing, despite breakthroughs in laboratory testing and the availability of new antimicrobials. Long-term regimens, side effects, and pharmaceutical interactions all lead to patient noncompliance and suboptimal therapy, which reduce therapeutic efficacy (Griffith DE et al.2007).

Treatment is based on the site of infection and the subspecies identified. Most clinical isolates are resistant to an extensive spectrum of antibiotics in vitro, including antimycobacterial drugs, necessitating the use of many antibiotics in combination to treat these infections. Clarithromycin, azithromycin, ethambutol, and rifampicin are among the drugs used to treat MAC infections.

Resistance in NTM arises due to mainly five mechanisms - Decreased uptake or impermeability, decreased uptake or impermeability, Enzymatic inactivation, Modification of the target site and reduced pro-drug activating enzyme activity (Zhang Y et al., 2004).

M. kansasii is resistant to several antibiotics, however its sensitivity to antimycobacterial drugs varies in vitro. The current treatment for pulmonary infections consists of ethambutol,

rifampicin, and isoniazid. Treatment is often recommended to last 18 months, with the patient monitored for an additional 12 months to ensure that no relapse occurs (Wong KS et al.2008). M. abscessus is resistant to several antibiotics, both in vivo and in vitro. Consequently, clinically important isolates must be tested. They are frequently susceptible to amikacin, cefoxitin (pulmonary infections), clarithromycin, and azithromycin (skin and soft tissue infections). (De Groote MA et al., 1763)

Conclusion

Nontuberculous mycobacteria (NTM) represent a significant and growing challenge in the field of infectious diseases. This review has highlighted the complex nature of NTM infections, particularly pulmonary disease, which remains the most common manifestation in immunocompetent individuals. The increasing prevalence of NTM-PD, coupled with the lack of mandatory reporting in many countries, underscores the need for improved surveillance and epidemiological data collection.

The classification of NTM infections into four clinical syndromes by IDSA provides a useful framework for understanding the diverse presentations of these infections. However, the extensive drug resistance exhibited by many NTM isolates poses a significant challenge in treatment, often necessitating complex, multi-drug regimens.

This review has identified several key areas requiring further attention. First, there is a need for standardized diagnostic criteria and treatment protocols across different regions to ensure consistent patient care. Second, the environmental sources and transmission channels of NTM require more in-depth investigation to develop effective prevention strategies. Third, the increasing incidence of NTM infections in both immunocompromised and immunocompetent individuals calls for a better understanding of risk factors and host-pathogen interactions.

Future research should focus on developing more effective and less toxic treatment regimens, improving diagnostic techniques, and exploring potential vaccine candidates. Additionally, the impact of climate change and human activities on NTM ecology and transmission should be investigated.

In conclusion, NTM infections, particularly NTM-PD, represent an emerging public health concern that demands increased attention from clinicians, researchers, and policymakers.

Addressing the challenges in diagnosis, treatment, and management of NTM infections will require a coordinated, multidisciplinary approach to improve patient outcomes and limit the spread of these increasingly prevalent pathogens.

References

1. Adjemian J, Olivier KN, Seitz AE, et al. Prevalence of nontuberculous mycobacterial lung disease in U.S.Medicare beneficiaries. Am J Respir Crit Care Med 2012; 185: 881–886.
2. Ahmed I, Tiberi S, Farooqi J, et al. Non-tuberculous mycobacterial infections-A neglected and emerging problem. Int J Infect Dis. 2020 Mar;92S:S46–S50
3. Alcaide F, Esteban J. Cutaneous and soft skin infections due to non-tuberculous mycobacteria. Enfermedades infecciosas y microbiología clínica. 2010 Jan 1;28:46-50.
4. Amalakuhan B, Maselli DJ, Martinez-Garcia MA. Update in bronchiectasis 2014. American Journal of Respiratory and Critical Care Medicine. 2015 Nov 15;192(10):1155-61.
5. American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am Rev Respir Dis 1990; 142: 940–953.)
6. Bland CS, Ireland JM, Lozano E, Alvarez ME, Primm TP. Mycobacterial ecology of the Rio Grande. Applied and Environmental Microbiology. 2005 Oct;71(10):5719-27.
7. Boddinghaus, B. Rogall, T. Flohr, T. Blocker, H. and Bottger E.C. 1990. Detection and identification ofmycobacteria by amplification ofrRNA. J Clin Microbiol. 28(8); 1751-1759
8. Butler WR, Guthertz LS. 2001 Mycolic acid analysis by high-performance liquid chromatography for identification of Mycobacterium species Clin Microbiol Rev.(4);704-26
9. Chan ED, Iseman MD. Underlying host risk factors for nontuberculous mycobacterial lung disease. Semin Respir Crit Care Med. 2013 Feb;34(1):110–123. doi: 10.1055/s-0033-1333573.
10. Colombo RE, Hill SC, Claypool RJ, et al. Familial clustering of pulmonary nontuberculous mycobacterial disease. Chest. 2010 Mar;137(3):629–634. doi: 10.1378/chest.09-117382.

11. Cook GM, Berney M, Gebhard S, et al. Physiology of mycobacteria. Adv Microb Physiol. 2009;55:81–182, 318–9. doi: 10.1016/S0065- 2911(09)05502-7.
12. Cooksey RC, Jhung MA, Yakrus MA, Butler WR, Adékambi T, Morlock GP, Williams M, Shams AM, Jensen BJ, Morey RE, Charles N. Multiphasic approach reveals genetic diversity of environmental and patient isolates of Mycobacterium mucogenicum and Mycobacterium phocaicum associated with an outbreak of bacteremias at a Texas hospital. Applied and environmental microbiology. 2008 Apr 15;74(8):2480-7
13. Dahl VN, Molhave M, Floe A, et al. Global trends of pulmonary infections with nontuberculous mycobacteria: a systematic review. Int J Infect Dis. 2022 Dec;125:120–131.
14. Daley P, Petrich A, May K, Luinstra K, Rutherford C, Chedore P, Jamieson F, Smieja M. 2008. Comparison of in-house and commercial 16S rRNA sequencing with high-performance liquid chromatography and genotype AS and CM for identification of nontuberculous mycobacteria. Diagn Microbiol Infect Dis. 2008 Jul;61(3):284-93. Epub 2008 May
15. De Groote MA, Huitt G. Infections due to rapidly growing mycobacteria. Clin Infect Dis42: 1756–1763.
16. Falkinham J III. Ecology of nontuberculous mycobacteria—where do human infections come from? Semin Respir Crit Care Med 2013; 34: 095–102
17. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. American journal of respiratory and critical care medicine. 2007 Feb 15;175(4):367-416.
18. Guerrero. C, Bernasconi, C. Burki, D. Bodmer, T. and Telenti, A. 1995. A novel insertion element from Mycobacterium avium, IS 1245, is a specific target for analysis ofstrain relatedness
19. Hall, Leslie and. Roberts. Glenn D 2006. Non-molecular identification of nontuberculous mycobacteria in the clinical microbiology laboratory; What's the real deal? Clinical Microbiology Newsletter. Vol 28, Issue 10, Pg 73-80.
20. Haworth CS, Banks J, Capstick T, et al. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax. 2017 Nov;72(Suppl 2):ii1–ii64. doi: 10.1136/thoraxjnl-2017-21092792.

21. Hill UG, Floto RA, Haworth CS. Non-tuberculous mycobacteria in cystic fibrosis. Journal of the Royal Society of Medicine. 2012 Jun;105(2_suppl):14-8.
22. Howard ST, Byrd TF. The rapidly growing mycobacteria: saprophytes and parasites. Microbes and infection. 2000 Dec 1;2(15):1845-53.
23. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. Journal of thoracic disease. 2014 Mar;6(3):210.
24. Kamal A. Hamed a and Glenn Tillotsonb. A narrative review of nontuberculous mycobacterial pulmonary disease: microbiology, epidemiology, diagnosis, and management challenges. EXPERT REVIEW OF RESPIRATORY MEDICINE 2023, VOL. 17, NO. 11, 973–988.
25. Kobashi Y, Yoshida K, Niki Y, et al. Sibling cases of Mycobacterium avium complex disease associated with hematological disease. J Infect Chemother. 2006 Oct;12(5):331–334. doi: 10.1007/s10156- 006-0461-Z
26. Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V. Occurrence of mycobacteria in water treatment lines and in water distribution systems. Applied and Environmental Microbiology. 2002 Nov;68(11):5318-25.
27. Lee ES, Lee MY, Han SH, Ka JO. Occurrence and molecular differentiation of environmental mycobacteria in surface waters. Journal of microbiology and biotechnology. 2008;18(7):1207-15.
28. Martin A, Fissette K, Varaine F, Portaels F, Palomino JC. 2009. Thin layer agar compared to BACTEC MGIT 960 for early detection of Mycobacterium tuberculosis. J Microbiol Methods. (1): 107-8
29. McCarthy KD, Cain KP, Winthrop KL, Udomsantisuk N, Lan NT, Sar B, Kimerling ME, Kanara N, Lynen L, Monkongdee P, Tasaneeyapan T. Nontuberculous mycobacterial disease in patients with HIV in Southeast Asia. American journal of respiratory and critical care medicine. 2012 May 1;185(9):981-8.
30. McMurray DN. Mycobacteria and nocardia. Medical Microbiology. 4th edition. 1996.
31. Mirsaeidi M, Farshidpour M, Ebrahimi G, Aliberti S, Falkinham JO. Management of nontuberculous mycobacterial infection in the elderly. European Journal of Internal Medicine [Internet]. 2014 Apr [cited 2019 Nov 16];25(4):356–63. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4067452/

32. Morimoto K, Iwai K, Uchimura K, et al. A steady increase in nontuberculous mycobacteriosis mortality and estimated prevalence in Japan. Ann Am Thorac Soc 2014; 11: 1–8.
33. Nieminen T, Pakarinen J, Tsitko I, Salkinoja-Salonen M, Breitenstein A, Ali-Vehmas T, Neubauer P. 16S rRNA targeted sandwich hybridization method for direct quantification of mycobacteria in soils. Journal of microbiological methods. 2006 Oct 1;67(1):44-55.
34. Ovi MO. Mycobacterial diseases. Infectious Diseases of Wild Mammals. 2008 Feb 28;340.
35. Perez-Martinez I, Ponce-De-Leon A, Bobadilla M, Villegas-Sepulveda N, Perez-Garcia M, Sifuentes-Osornio J, González-y-Merchand JA, Estrada-García T. A novel identification scheme for genus Mycobacterium, M. tuberculosis complex, and seven mycobacteria species of human clinical impact. European Journal of Clinical Microbiology & Infectious Diseases. 2008 Jun;27:451-9.
36. Plikaytis BB, Crawford JT, Shinnick TM. 1998. IS 1549 from Mycobacterium smegmatis forms long direct repeats upon insertion. J Bacteriol. 180(5): 1037-43
37. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2015 Mar;36(1):13–34
38. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med 2010; 182: 970–976.
39. Richardson ET, Samson D, Banaei N. 2009. Rapid Identification of Mycobacterium tuberculosis and Nontuberculosis Mycobacteria by Multiplex, Real-Time PCR. J Clin Microbiol. May; 47(5); 1497-502.
40. Richter E, Rusch-Gerdes S, Hillemann D. 2006 Evaluation ofthe GenoType Mycobacterium Assay for identification ofmycobacterial species from cultures. J Clin Microbiol. May;44(5); 1769-75
41. Rodrigues C, Shenai S, Sadani M, Sukhadia N, Jani M, Ajbani K, Sodha A, Mehta A. 2009. Evaluation ofthe bactec MGIT 960 TB system for recovery and identification of Mycobacterium tuberculosis complex in a high through put tertiary care centre. Indian J Med Microbiol.(3):217-21
42. Schildkraut JA, Gallagher J, Morimoto K, et al. Epidemiology of nontuberculous mycobacterial pulmonary disease in Europe and Japan by Delphi estimation. Respir med. 2020 Nov;173:106164

43. Sethi S. Infection as a comorbidity of COPD. European Respiratory Journal. 2010 Jun 1;35(6):1209-15.
44. Smith I. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Rev. 2003 Jul;16 (3):463–496.
45. Somoskovi A, Kodmon C, Lanstos A, Bartfai Z, Tamasi L, Fiizy J,.2000. Comparison ofrecoveries of Mycobacterium tuberculosis using the automated BACTEC MGIT 960 System, BACTEC 460 TB System and LowensteinJensen Medium. J Clin Microbiol 38:2395-7
46. Spagnolo P, Richeldi L, du Bois RM. Environmental triggers and susceptibility factors in idiopathic granulomatous diseases. InSeminars in respiratory and critical care medicine 2008 Dec (Vol. 29, No. 06, pp. 610-619). © Thieme Medical Publishers.
47. Springer B, Stockman L, Teschner K, Roberts GD, Bottger. 1996 TwoLaboratory Collaborative Study on Identification of Mycobacteria: Molecular versus Phenotyic Methods. Journal ofClinical Microbiology. Feb; 34(2): 296- 303.
48. Tateishi Y, Hirayama Y, Ozeki Y, Nishiuchi Y, Yoshimura M, Kang J, Shibata A, Hirata K, Kitada S, Maekura R, Ogura H, Kobayashi K, Matsumoto S. 2009. Virulence of Mycobacterium avium complex Strains Isolated from Immunocompentent Patients. Microbial pathogenesis. Jan;46(l);6-12
49. Therese KL, Bartell J, Deepa P, Mangaiyarkarasi S, Ward D, Dajcs J, Madhavan HN, Stroman D. 2009 DNA sequencing by Microseq kit targeting 16S rRNA gene for species level identification ofmycobacteria Indian J Med Res. 2009 Feb; 129(2): 176-81
50. Therese KL, Bartell J, Deepa P, Mangaiyarkarasi S, Ward D, Dajcs J, Madhavan HN, Stroman D. 2009 DNA sequencing by Microseq kit targeting 16S rRNA gene for species level identification ofmycobacteria Indian J Med Res. 2009 Feb; 129(2): 176-81
51. Tortoli E. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clinical microbiology reviews. 2003 Apr;16(2):319-54.
52. Turenne CY, Tschetter L, Wolfe J, Kabani A. Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous Mycobacterium species. Journal of Clinical Microbiology. 2001 Oct 1;39(10):3637-48.
53. Turenne CY, Wallace Jr R, Behr MA. Mycobacterium avium in the postgenomic era. Clinical microbiology reviews. 2007 Apr;20(2):205-29.

54. Uslan DZ, Kowalski TJ, Wengenack NL, Virk A, Wilson JW. Skin and soft tissue infections due to rapidly growing mycobacteria: comparison of clinical features, treatment, and susceptibility. Archives of dermatology. 2006 Oct 1;142(10):1287-92.
55. Van Ingen J, Obradovic M, Hassan M, et al. Nontuberculous mycobacterial lung disease caused by Mycobacterium avium complex - disease burden, unmet needs, and advances in treatment developments. Expert Rev Respir Med. 2021 Nov;15 (11):1387–1401.
56. Wagner, D. Young, L.S 2004 Nontuberculosis Mycobacterial Infections: A Clinical Review. Infection vol 32, number 5.
57. Williams'J K, C. L. Ling, C. Jenkins, S. H. Gillespie^ and T. D. McHugh 2007 A paradigm for the molecular identification ofMycobacterium species in a routine diagnostic laboratory J Med Microbiol 56 (2007), 598-602; DOI; 10.1099/jmm.0.46855-0
58. Winthrop KL. Pulmonary Disease Due to Nontuberculous mycobacteria: an Epidemiologist's View. Future Microbiology. 2010 Mar 8;5(3):343–5.
59. Wong KS, Lin TY, Huang YC, Chiu CY. Nontuberculous mycobacterial pulmonary disease in an immunocompetent adolescent. Respiratory care. 2008 Jul 1;53(7):908-11.
60. Yan M, Brode SK, Marras TK. The other nontuberculous mycobacteria: clinical aspects of lung disease caused by less common slowly growing nontuberculous mycobacteria species. Chest. 2023 Feb;163(2):281–291.
61. Zhang Y, Vilchèze C, Jacobs JR WR. Mechanisms of drug resistance in Mycobacterium tuberculosis. Tuberculosis and the tubercle bacillus. 2004 Dec 13:115-40.
62. Zweijpfenning SMH, Ingen JV, Hoefsloot W. Geographic distribution of nontuberculous mycobacteria isolated from clinical specimens: a systematic review. Semin Respir Crit Care Med 2018; 39: 336–342.