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Review

Prevalence, Challenges in Diagnosis and Treatment of Non-Tuberculous Mycobacteria

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Abstract: Mycobacteria that cause tuberculosis and Non-Tuberculous Mycobacteria (NTM) are the two groups into which mycobacteria are divided. Almost all the NTM are ubiquitous in soil, water, air, etc. During recent years, studies have suggested that they are now considered globally evolved emerging pathogens. The NTM infection can cause self-limiting asymptomatic infections to life-threatening diseases, affecting multiple major organs. Several guidelines have been introduced for the treatment of pulmonary and extra-pulmonary NTM infections. Owing to its innate resistance, complexity, and resemblance of the organism to Mycobacterium Tuberculosis (MTB), the diagnosis and treatments have a high failure rate. Moreover, most healthcare workers lack knowledge of NTM infection, as the clinical symptoms and the microscopic morphology resemble MTB. This could lead to NTM becoming resistant to various antibiotics, and the treatment could become challenging in the future. This review article provides details on the prevalence of Tuberculosis (TB) and NTM in India and globally, information on the vulnerable population, complications caused, and means of diagnosis, treatment, and challenges associated with the diagnosis and treatment of NTM are discussed in detail. Additionally, information on various health complications associated with NTM and the challenges of NTM diagnosis is provided in detail. This article also throws light on co-infections associated with TB and NTM disease patterns that may lead to complications, along with treatment options for NTM are discussed in detail.

Keywords: co-infections, complications, *Mycobacterium tuberculosis*, Non-Tuberculous Mycobacteria (NTM), prevalence, treatment challenges

1. Introduction

1.1 *Non-tuberculous mycobacteria and its prevalence*

Although Non-Tuberculous Mycobacteria (NTM) are widely found in the environment, NTM infections are rare. Though Mycobacterium tuberculosis is thought to be more virulent than NTM, both immunocompetent and immunocompromised hosts can become ill from these organisms. Generally, NTM are free-living, aerobic, non-motile,

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ubiquitous organisms. There are more than 140 species of NTM identified to date.

Four groups of the *Mycobacterium* genus are recognized: (1) *Mycobacterium tuberculosis* complex, (2) *Mycobacterium leprae*, (3) slowly growing NTM (takes 7 days or more to grow), and (4) rapidly growing mycobacteria.

Historically, NTM have been classified based on their growth rate and pigmentation (Runyon 1959).

The slow-growing *mycobacteria* are further divided into the following categories according to the pigment they produce [1, 2].

Type 1- photochromogens (If the pigment is produced only upon exposure to light).

Type 2- scotochromogen (if the pigment is produced in the dark).

Type 3- nonchromogen (if the bacteria are not strongly pigmented).

Zhou et al. [3] carried out a systematic review and meta-analysis spanning 16 years (2006-2021), gathering global data from Medline, Embase, the Cochrane Library, and the Web of Science, and all the retrospective and prospective data without language restrictions were included. All the patients above the age of 18 who have been diagnosed with non-cystic fibrosis bronchiectasis were included in the study. The systematic review and meta-analysis contained information on 26,944 patients with non-Cystic Fibrosis (CF) bronchiectasis. Between 2006 and 2021, the prevalence of NTM with non-cystic fibrosis varied greatly but averaged about 10% worldwide. The prevalence of NTM infection varies from 1.0% to 25% in patients without cystic fibrosis, and it was reported that the prevalence in adults was 9.75% between 2006 and 2021. East Asia was found to have the highest pooled prevalence of NTM infection, at 7.50%. From 1990 to 2006, the prevalence of NTM was 5%. This demonstrates the rising trend in infections, with 90% of infections in bronchiectasis patients being caused by *Mycobacterium Avium* Complex (MAC), in line with previously published data [3].

A six-month study by Grigg et al., during 2019-2020 at four sites in the United States, reported that the annualized prevalence and incidence rates per 100,000 population were 7.5 and 4.8, respectively [4]. A study by Varghese et al. revealed that North America accounted for 33.4% of the cases of rare NTM infection between 1956 and 2018, with Europe and Asia accounting for 23.8% and 20.8% of the cases, respectively. The pulmonary sites accounted for 67% of all cases that were reported, with the remaining cases being extra-pulmonary or disseminated infections [5].

In India, the reports of NTM infections are less due to a lack of awareness among the healthcare workers and limited diagnostic tools for the disease available in most healthcare centres. In 2001, a case of NTM pulmonary infection was reported for the first time. In 2013, a case of another pulmonary infection caused by NTM in a multidrug-resistant TB patient was reported. In 2014, a 2-year-old child with pulmonary complications was reported in Chennai. In 2017, a case of cervical lymphadenitis complication with NTM in a 15-year-old boy was reported in Punjab. In 2018, a case of a 9-year-old girl was reported from Mumbai, and in 2021, NTM complication in a patient with a history of TB treatment was reported from Chennai [5]. Based on research conducted by Ratnatunga et al., the isolation rates of NTM increased from 0.9% (2001-2010) to 1.6% (2011-2020), and it was reported that 1.1% of presumed tuberculosis patients in India had NTM infection [6]. Like other studies, in India too, most of the cases of NTM infection (76%) were isolated from pulmonary specimens and MAC was found to be the most isolated organism (19%), *M. chelonae* (10.3%), *M. fortuitm* (10%) and *M. abscessus* (15%) [6]. The prevalence of NTM rose from 19.6 cases per 100,000 people in 1994-1996 to 26.7 cases per 100,000 people in 2004-2006, according to a different study by Gopalaswamy et al. [7]. This indicates that there are many cases present in the Indian population, but the same could not be completely captured for various reasons, and this can be one of the major delays in achieving the target of eradicating TB [7].

2. Disease caused by NTM

The most common infections caused by NTM include the pulmonary and the extrapulmonary infections. Lymphadenitis has become one of the most common syndromes of chronic pulmonary infection. Invasive procedures often lead to cutaneous and bone NTM. Immunosuppressed patients usually are diagnosed with disseminated NTM [7]. The major organ infected by NTM is the lungs, causing pulmonary disease. In addition to this, it can cause extrapulmonary diseases, with lymph nodes, skin, or soft tissue being the main site of infection. The organism can also cause disseminated disease by traveling through the circulatory system [7].

2.1 *Pulmonary infection*

Pulmonary infection refers to the type of infection that involves the lungs and other parts of the respiratory system. NTMs are able to cause pulmonary infections, which mostly involve the lungs. The disease may mimic the clinical symptoms of tuberculosis. Most often, granulation is observed in the lobes of the lungs when observed by computed tomography [8].

In order to determine the prevalence of pulmonary NTM infection in the Gambia based on the national tuberculosis prevalence survey between December 2011 and January 2013, a cross-sectional study was carried out by Okoi [9]. The study is inclusive of 903 participants above the age of 15 with suspected NTM infection, who were negative for the *Mycobacterium Tuberculosis* Complex (MTBC) rapid identification assay. For the diagnosis of NTM infection, the American Thoracic Society/Infectious Disease Society of America guidelines were adhered to. The samples that did not show positivity even after 42 days of incubation were regarded as negative. Out of all the participants, the findings indicated that 229 had an NTM infection and 575 were confirmed positive for Acid-Fast Bacilli (AFB), with some isolates unable to be identified to the species level. Patients with NTM infection were mostly female, with a reported median age of 55. *M. fortuitum* (9.5%), *M. nonchromogenicum* (2.9%), and *M. avium* complex (71%) were the most frequently detected NTM isolates as a result obtained from phylogenetic analysis. It was also proved that patients who were older than 24 years of age were 4 times more susceptible to NTM infection. Also, patients who are above 65 years of age are also vulnerable to the disease, and the likelihood of infection was 1.5 times higher in females than in males [9].

2.2 *Extra-pulmonary infection*

Extra-pulmonary infections refer to an infection that involves any organ other than the lungs (pleura, lymph nodes, skin, bones, joints, gastrointestinal tract, meninges, etc). Skin and soft tissue infections, traumatic and surgical wound infections, or infections related to catheters and implants are examples of extra-pulmonary infections caused by NTM infections [10].

Case 1- a woman with type 2 diabetes mellitus, age 77. At the time of admission to the hospital, she had painful exudative and purulent ulceration of the left lower extremity. Routine laboratory work-up was performed, and the Magnetic Resonance Imaging (MRI) result reported an extended inflammation of the subcutaneous tissues. The debridement of the lesions submitted for microbiological analysis, failed to reveal the infectious agent on solid media and histological analysis but the blood culture bottle was positive after 2 days of incubation. Fine and beaded gram-positive branching bacilli and partially acid-fast Ziehl-Neelsen stain were observed under a microscope, which was identified to be *Nocardia brasiliensis* by Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) [10].

Case 2- a 63-year-old female who had ankylosing spondylitis and was under immunotherapy. The patient's right hand had a rapidly growing, painful mass, malodorous discharge, and surrounding edema for three months before admission. She was also subjected to routine laboratory work-up, and the MRI result revealed soft tissue inflammation on the hand's dorsal surface. Following microbiological and histological examination, the specimen was diagnosed; however, the results of the histological analysis did not identify the causative organism. On Lowenstein Jensen Media, growth was seen after 18 days of incubation. The microscopic analysis reported short, beaded gram-positive bacilli, and the Acid-Fast Bacilli (AFB) smear was positive by Ziehl-Neelsen staining. The infectious agent was identified to be *M. marinum* by MALDI-TOF mass spectrometry, and the result was confirmed using a commercially available Deoxyribonucleic Acid (DNA) strip assay [10].

3. NTM co-infection and complications

Co-infections may be defined as the simultaneous infection of a host by multiple numbers of pathogens. This may include bacteremia, fungemia, sepsis, septic shock, etc. Patients who have underlying diseases like lung disorder, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), organ transplant, and immunocompromised patients are at high risk of being infected by multiple organisms at the same time [11].

A retrospective analysis for the characteristics of NTM-infected patients with HIV/AIDS in South Korea by

Lee [11], from January 2000 to March 2021, comprises 34 NTM cases from the data analyzed from 5 hospitals. This study delivers an updated understanding of the epidemiology, clinical characteristics, microbiological features, and antibiotic sensitivity of NTM disease in HIV/AIDS patients. All the samples, including lymph node biopsy, sputum, Bronchoalveolar Lavage (BAL), pleural fluids, epidural abscess, intestinal lymph nodes, and sigmoid biopsy, were analyzed, and the NTM-positive samples were included in the study. Pulmonary, extra-pulmonary, and disseminated infections were involved in this study. Using the Infectious Diseases Society of America (ISDA)/American Thoracic Society (ATS) guideline [12], the specimens were processed and stained. The identification of NTM isolates was done by Polymerase Chain Reaction (PCR), and a restriction fragment length polymorphism method based on the *rpo*B gene was used. The broth microdilution method was used to determine the antibiotic susceptibility, and Minimal Inhibitory Concentrations (MICs) for both oral and parenteral antibiotics were also analyzed. By reviewing electronic medical records, various information on age, sex, initial $CD4^{+}$T cell counts, viral load, past and current Combination Antiretroviral Therapy (cART) regimens, isolated NTM species, antimicrobial susceptibility test results, NTM treatment regimens, and outcomes were collected [11].

The result obtained reported that 51 cases of HIV co-infection with NTM were reported, out of which 17 patients were not included in the study, because they did not meet the American Thoracic Society (ATS) criteria [12]. Therefore, only 34 patients participated in this study. 20 cases were diagnosed to be pulmonary infections, while 14 were extra-pulmonary infections. The average age of extra-pulmonary NTM-infected patients was 35, whereas 45 in the case of pulmonary infection, and the most infected area was found to be the lymph node. The $CD4^{+}$T cell count was found to be lower in the case of extra-pulmonary NTM infection than in the pulmonary NTM-infection. It was also observed that the pulmonary NTM infected patients had a lower body mass index than the extra-pulmonary infection. Most of the patients in this study were under cART at the time of NTM diagnosis. MAC was the most commonly isolated pathogen, followed by *Mycobacterium intracellulare* (6) and *Mycobacterium kansasii* (3). The results of the testing of only 14 samples for antibiotic susceptibility showed that MAC was mostly resistant to moxifloxacin, linezolid, ethambutol, and rifampin, but susceptible to clarithromycin. *M. intracellulare* was resistant to linezolid and moxifloxacin, but susceptible to clarithromycin. Isoniazid and p-amino salicylate resistance was demonstrated by *M. kansasii*. For the average duration of treatment, the pulmonary disease groups were treated for 16 months, and the extra-pulmonary disease group was treated for 19 months; a total of 41.2% of the patients were treated completely. However, in the case of pulmonary NTM infection, treatment failure was found to be 20% [11].

4. Who are at risk for NTM infection

The NTMs are opportunistic pathogens; most people have been exposed to this organism in day-to-day life, but they affect only certain groups of people. The groups of people who are more prone to this infection are the patients who have lung diseases either genetically or acquired, like cystic fibrosis, Chronic Obstructive Pulmonary Disease (COPD), non-CF bronchiectasis, alpha-1 antitrypsin deficiency, previous pulmonary tuberculosis, and lung cancer. The immunosuppressed patients, due to primary immune deficiency syndrome, are at high risk. Individuals with hematological malignancies, hairy cell leukemia, and acquired immune deficiency syndrome, such as AIDS, are also more vulnerable to NTM infection [6]. Additionally, it was discovered that Body Mass Index (BMI), living situation, and educational attainment were separate risk factors for the infection [13].

5. NTM infection: Symptoms and disease pattern

The signs and symptoms of the pulmonary NTM infection mimic the clinical symptoms of TB, including fever, productive cough, and weight loss. In certain cases, hemoptysis is also seen, and a few of the patients required long-term oxygen therapy. In radiological findings, patients show extensive fibro-cavitary disease [14].

6. Detection/laboratory diagnostic methods for NTM infection

To effectively treat NTM infection, the American Thoracic Society [12] has stated that the best way for the treatment of NTM infection is to identify them at species level, because every species has different susceptibility and resistance to a particular antibiotic so that multi drug resistant by the organism can be prevented as this would lead to the treatment becoming challenging. The 2020 Infectious Disease Society of America (IDSA)/American Thoracic Society (ATS) guidelines state that to diagnose pulmonary NTM infection, a patient must have respiratory symptoms, chest radiography findings (CT scan), and the isolation of NTM from BAL or two different sputum samples. Moreover, the clinical and the microbiological criteria as stated by the ATS/ISDA guidelines should be met [14]. Since non-tuberculous mycobacteria belong to the genus *Mycobacteria*, the organism exhibits certain characteristics that mimic the characteristics of *Mycobacterium tuberculosis*. Both show the property of acid fastness, the disease caused by them shows similar clinical symptoms, and also the radiological findings are quite similar in both cases [15]. So, differentiating the clinical manifestation of these two groups is often challenging. Therefore, to reduce the misdiagnosis and the rate of mortality by this organism, the species should be correctly identified so that appropriate and effective treatment can be provided [15]. The list of different methods/tests that are currently available for diagnosis of TB and NTM is listed in Table 1, and recent approaches used with precision diagnosis, along with their limitations, are mentioned in Table 2.

Table 1. Comparison of different methods available for diagnosis of NTM and TB samples

Details	MTB	NTM	Reference
Basic conventional microbiological tests	Staining-Zheil Neelsen staining Culture-solid and liquid culture, Mycobacteria Growth Indicator Tube (MGIT) system	Staining-Zheil Neelsen staining Culture- solid and liquid culture, MGIT system with PNB	[1, 4, 6, 15, 16]
Rapid diagnostic tests	TB Ag MPT64 rapid card test	TB Ag MPT64 rapid card test, High Performance Liquid Chromatography (HPLC)	
Nucleic acid amplification tests	Amplified Mycobacterium Tuberculosis Direct Test (MTD), Xpert MTB-Rif system, loop -mediated isothermal amplification	PCR sequencing, (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) PCR-RFLP), Accuprobe analysis, Anyplex MTB/NTM detection	
Line probe assays	Inno-LiPA Mycobacteria assay, Genotype Mycobacterium common Mycobacteria (CM) and Additional Species (AS) assays	Inno-LiPA Mycobacteria assay, Genotype Mycobacterium CM and AS assays	
Other methods	MALDI-TOF Next generation sequencing	MALDI-TOF Next generation sequencing	

Even if culturing remains the gold standard for the isolation of mycobacteria, it is a laborious process with increased turnaround time. Therefore, the more rapid, highly sensitive, and effective method for the diagnosis of lung disease turns out to be Computed Tomography (CT scan). This method of diagnosis provides a swift assessment of different parts of the lungs- the lobes, trachea, and bronchi. The imaging attributes of NTM consist of exudation, cavities, and nodules, whereas cavities, lung consolidation, patchy lung nodules, mediastinal lymphadenopathy, lymph node calcification, and pleural effusion are seen in the case of TB. Hence, similar imaging features of both infections result in debate of the accuracy about the diagnostic method [16, 17].

Studies have reported that MALDI-TOF can be one of the reliable methods that can be used to identify NTM at the species level [16, 18]. In certain laboratories, conventional phenotypic tests like colony morphology, growth rate, and biochemical tests are also employed. In addition to this, chromatography techniques like HPLC, Gas-Liquid chromatography, and thin-Layer Chromatography (TLC) are also used [18]. The rapid methods for the identification of NTM species rely on molecular-based methods. The Food and Drug Administration (FDA) has approved the use of a

genetic acridinium-ester-labeled non-radioactive DNA probe based on rRNA detection that is commercially available (AccuProbe; GeneProbe Inc., San Diego, CA). The line probe-based assay, the INNO genetics Line Probe Assay (INNO-LiPA) test, is also commercially available. Polymerase restriction endonuclease analysis uses the PCR-based 65 kDa heat shock protein (hsp 65) [16], rpo b-gene, the 16S rRNA gene, the 16S-23S rDNA space region, and the DNA-J gene [16].

A study by Wang [19], evaluated the effectiveness of metagenomic next-generation sequencing for identification of pulmonary NTM isolates as the currently available culture-based method is time consuming and has a high possibility for misdiagnosis. The retrospective observational study was done at the Second Clinical Hospital of Chongqing Medical University, China. Routine samples were collected and examined by conventional microbiological tests as well as by metagenomic Next Generation Sequencing (mNGS) and radiological presentations. The mNGS results were validated using fluorescence quantitative Polymerase Chain Reaction (qPCR). The result obtained reported that a total of 1,362 patients' samples were analyzed using mNGS, and 12 patients were diagnosed with pulmonary NTM infection. Of the 12 patients, 8 have at least one underlying illness. The radiological findings also reported multiple nodules, bronchiectasis, and cavitary opacities. The NTM isolates were identified to be *M. kansasii* (4), *M. abscessus* (3), *M. intracellulare* (2), *M. fortuitum* (2), and *M. avium* (1). The patients were under a general NTM treatment regimen, which was adjusted according to the result of mNGS later on. After proper treatment, depending on the species isolated as per mNGS, the symptoms disappeared. The mNGS turnaround time was found to be 1 to 4 days, which is much shorter than the culturing process. This could lead to earlier diagnosis and improved prognoses of patients with NTM infection [19].

Table 2. Different methods available for diagnosis of NTM infection and identification of the species with details

NTM	Principle	limitations	References
Decontamination method	N-Acetyl-L-Cysteine (NALC)- decontamination process	negatively influence recovery and viability bacteria in the samples	[14]
HPLC	Based on the No. of Carbon atom on the mycolic acid cell wall		[16]
PCR-RFLP	*hsp*65 specific for NTM		
Nucleic acid probes	16s RNA gene based	NAATS- Sensitivity 81%	[15, 19, 20]
Line probe assay	Reverse hybridization		
Gene sequencing	Single and multiple conserve sequencing and whole genome sequencing		
MALDI-TOF	Based on the conserved protein sequences		[18]
Culture	AFB culture on Lowenstein Jensen medium or middlebroke broth MGIT	LJ media- sensitivity- 80-85% False positive- 2% to 4 % MGIT- sensitivity- 90% Specificity- 100%	[1, 4, 20]
Staining	Auramine O, Zheil neelsen staining	ZN staining sensitivity- 60-70% Auramine O-around 80%	
MPT64	Negative	Specificity- 100% Sensitivity- 98.6 %	[21]
Conventional confirmatory test	MPT64 ag negative, AFB smear and culture positive with phenotypic or genotypic methods		

7. Misdiagnosis of NTM infection

When the diagnosis is made using clinical symptoms, sputum smear microscopy, and rarely, radiological findings, NTM is frequently misdiagnosed as MTB. Moreover, due to poor laboratory infrastructures, the criteria laid down by

the American Thoracic Society/Infectious Disease Society of America to diagnose pulmonary NTM disease are often neglected and hence misdiagnosed as TB, and anti-TB drugs have been administered based on the identification of Acid-Fast Bacilli (AFB). This failure in treatment leads to the second-line drug treatment of TB. Simultaneously, the NTM disease proceeds as they have an innate resistance towards conventional anti-TB drugs. This results in mortality and morbidity, and also the rising cases of NTM infection. Moreover, certain non-mycobacteria like *Nocardia* have also been isolated from smear and culture techniques of acid-fast bacilli [22, 23].

A case study of 18 NTM cases in a tertiary care center by Gupta [24], between July 2016 to February 2019 at the Department of Medicine, All India Institute of Medical Sciences, New Delhi, India reported that, after taking the consent of the patients, the clinical and radiological features, microbiological methods of diagnosis and treatment regime and outcome of the patients were recorded. Of the 18 cases, 11 were males, and the age range was from 24-58, and the sites of infection were 11 pulmonary, 3 skin and soft tissue, 2 joints, 1 genitourinary, and 1 central nervous system [24].

Most of the symptoms shown by the patients were fever, weight loss, and loss of appetite, and the mean duration of illness was 15 months. The result reported that of the 18 positive specimens, 17 were speciated. Different NTM strains, including *M. fortuitum* group, MAC, *M. abscessus*, *M. kansasii*, and *M. chelonae*, were isolated. Of the 11 pulmonary cases, 8 had cavitary disease and 3 had a nodular pattern. It was noted that microbiological diagnosis is required for the diagnosis of NTM, as clinical and radiological findings are nonspecific. It was discovered that in HIV patients, MAC was the most frequent organism causing lung illness, lymphadenitis, and disseminated infection. In this study, only 2 patients were immunosuppressed, and most of the patients were under anti-TB therapy. The outcome of the treatment is affected by the initiation of the treatment and the choice of drugs [20]. Therefore, in India, being a TB endemic country, the diagnosis of NTM is frequently misdiagnosed as pulmonary TB and treated with anti-TB drugs, which results in increased morbidity [24].

8. Treatment and antibiotic susceptibility of NTM infection

In cases of pulmonary NTM, the treatment of MAC recommended includes a 3-drug macrolide-based regimen to achieve a result of 12 weeks of culture negativity. Infection caused by *M. kansasii* is treated using combined therapy of rifampicin, isoniazid, and ethambutol. Initially, intravenous treatment needs to be done, followed by oral therapy when there is clinical improvement [25]. So far, in India, there is not a single drug approved by the FDA for the treatment of NTM. Moreover, speciation is not readily available, and the antibiotic susceptibility of NTM is not known, which makes it difficult the treat and predict its response [1]. Also, the effectiveness of the treatment given doesn't solely depend on the drug but also on the host immune response, the organs infected, or the severity of the disease. Another factor that plays a role during the treatment of NTM is the time of initiation and the choice of treatment given. Misdiagnosis of NTM with MTB, with the treatment of multi-drug-resistant therapy, has increased the risk of mortality in patients having the disease, and also the organisms tend to develop multi-drug resistance, which makes the treatment challenging [26]. Various treatment (antibiotic) options and approaches are listed in Table 3.

A study carried out by Muñoz-Muñoz [20] to find out the in vitro activity of several beta-lactam antibiotics against the non-tuberculous mycobacteria. All the β-lactam chemical classes are included, and 32 agents, including β-lactamase inhibitors, were selected for this study. Using the MIC assay, the sensitivity of these antibiotics was checked against 22 *M. kansasii* strains. The ATCC 12478 reference strain of Mycobacterium was utilized in the Middlebrook 7H9 and Cation-adjusted Mueller-Hinton Broth (CAMHB) base media. The MIC determination was done using a broth micro-dilution assay in a 96-well plate. According to the results, the first and third generation cephalosporins, along with penicillin and clavulanate, proved to be the most effective against the NTM. The only antibiotic that was found to be inactive was penicillin; higher values were found for second and fourth-generation cephalosporins. Additionally, it was reported that the most effective beta-lactams against a panel of clinical strains were cefadroxil and amoxicillin with clavulanate. When clavulanate was added, penicillins such as amoxicillin had up to four times the activity against the majority of strains [20]. Time-kill assays were used to confirm the β-lactam antibiotics' antimicrobial activities against *M. kansasii*, and the time-kill kinetics and MIC results agreed. The MIC values determined in this study were based on the growth inhibition with a growth limit of detection of 107 cells/mL after 6 days of incubation. The evaluation to integrate b-lactams into standard treatment showed that it was discovered that the quadruple combination of amoxicillin-

clavulanate or cefadroxil was superior to the conventional ones. In the case of an isoniazid-containing regimen and a clarithromycin-containing regimen, the amoxicillin-clavulanate contribution to the bactericidal activity of the currently available therapies was better at 1X and 2X concentrations, respectively. Therefore, the oral medications that are easily available, amoxicillin-clavulanate and cefadroxil, could be a promising alternative for the treatment of *M. kansasii* [20].

Table 3. Available antibiotics options for treatment of MTB and NTM infections of various types

Treatment option	TB	Reference
Drug sensitive (1st line)	Isoniazid, rifampicin, pyrazinamide, ethambutol	
2nd line	Cycloserine, Ethionamide, Streptomycin, Amikacin/kanamycin, Capreomycin, Levofloxacin, Moxifloxacin	
Multi and extremely drug resistant	In addition, bedaquiline, delamanid	
	NTM	
ATS/ IDSA guidelines		[6, 20, 25, 27, 28]
M. abscessus	Macrolide, Intravenous amikacin; streptomycin or cefotaxime	
M. avium complex	Clarithromycin or Azithromycin, Ethambutol, Rifampin	
M. kansasii	Rifampicin sensitive- Rifampin, Ethambutol, Isoniazid, Pyridoxine Rifampicin resistant- three drug regimen resulted from drug sensitivity testing	
M. malmoense	Isoniazid, Ethambutol, Rifampicin with quinolones or macrolides	
M. xenopi	Isoniazid, Rifabutin or Rifampin, Ethambutol, and Clarithromycin with moxifloxacin	

To assess the in vitro efficacy of rifamycin derivatives against clinical NTM isolates, Kim [25] conducted another study. Rifampin, rifapentine, rifaximin, and rifabutin are examples of rifamycin derivatives. 311 clinical isolates of the five major pathogenic NTM are evaluated. 311 non-duplicated patients receiving treatment for pulmonary NTM infection provided the isolates. Strains 57 and 44 are resistant to aminoglycosides and macrolides, respectively, were also confirmed by mutation analysis and included in the study. By using the broth microdilution method, the in vitro drug susceptibility testing was done by measuring the MIC [25]. Rifabutin was found to have the lowest Minimum Inhibitory Concentration (MIC) against all non-target Mold species, including *M. avium* complex, *M. abscessus*, and *M. kansasii*. Rifabutin's in vitro activity also demonstrated sensitivity to NTM strains resistant to aminoglycosides and macrolides. Therefore, rifabutin can be one of the promising antibiotics for the treatment regimen of NTM diseases, especially in the case of infection caused by the drug-resistant strains. Additionally, the results of the study indicated that rifamycin antibiotics differ in their in vitro activities for each NTM species and that rifapentine or rifaximin may be used as a treatment for NTM infections in the lungs that are brought on by MAC [25].

Another retrospective study on the Antimicrobial Susceptibility Test (AST) by Kim [27] reported that while *M. avium* was resistant to clarithromycin, *M. intracellulare* was susceptible to it. Amikacin susceptibility was discovered for *M. intercellulare* and *M. avium*. The isolates of *M. kansasii* were sensitive to clarithromycin and rifampin [27].

8.1 *Challenges in treatment*

While the available 3-drug regimen of NTM infection- ethambutol and rifampicin, along with a macrolide (either clarithromycin or azithromycin), for 12 months after negative sputum culture, there are reports of relapse in patients within 6 to 12 months after the completion of the drug course. This is due to the lack of a standardized treatment plan because of the genetic variability of NTM species [7]. Another reason for ineffective treatment, as the study suggests, is the pharmacokinetic interactions of the drugs involved. Rifampicin significantly reduces serum concentrations of

macrolides. When used at the same time, clarithromycin and azithromycin concentrations were also decreased by up to 68% and 23%, respectively, when used with rifampicin [28]. While the already available drug treatment options posed ineffective, the development of new drugs are also hindered due to the following reason-antibiotic resistance (acquired or intrinsic, ability to form biofilm, colony morphology of the organism (e.g. *M. abscessus* successful treatment depends on its colony morphology), unusual cell wall structure (provides intrinsic and extrinsic resistance as well as viability and survival advantage during pathogenesis), L-forms (ability to switch from cell walled NTM into cell wall deficient forms) [28].

9. Recent developments in NTM management and diagnosis

In recent literature studies, the whole genome sequencing technique with higher discriminatory power would be beneficial for differentiating between MAC species and fully identifying NTM [26]. The promising molecular diagnostic tools for the differentiation of NTM and MTB identification at the species level include 16S rRNA, rpoB, hsp65, and Internal Transcribed Spacer (ITS) [16]. The commercially available kits can only identify 20 species of NTM. Also, a positive result does not always mean that the patient has been exposed to the pathogen, as it can be environmental exposure or contamination during sample collection and processing, as NTM are ubiquitously present in the environment [29]. So, correlation with the patient's clinical diagnosis plays a major role in deciding a factor [30].

A study by Oliva [18] shows that MALDI-TOF can be one of the reliable methods that can be used to identify NTM at the species level. This advantage can be useful in implementing this technology for routine identification of NTM in many laboratories for rapid diagnosis, so that treatment according to the antibiotic susceptibility of the species can be started at the earliest, and misdiagnosis and missed diagnosis can be avoided [18]. So, the identification of the species of NTM that is causing the disease proved to be crucial. One of the promising molecular diagnostic tools for early and accurate detection for PTB and Non-Tuberculous Mycobacterial Pulmonary Disease (NTM-PD) is the metagenomic next generation sequencing as it identifies the organism to species level and also helps to differentiate the two and it is also one of the quickest detection methods which can change the delay and misdiagnosis of the patients [19].

A study on the antibiotic susceptibility testing reported that the combination of first and third-generation cephalosporins and penicillin plus clavulanate proved to be the most effective against the NTM. Additionally, it was reported that the most effective beta-lactams against a panel of clinical strains were cefadroxil and amoxicillin with clavulanate. When clavulanate was added, penicillin, such as amoxicillin, had up to four times the activity against the majority of strains. The oral medications cefadroxil and amoxicillin-clavulanate are readily available, well-tolerated by most people, and have very few adverse effects. Therefore, these drugs can be considered as substitutes and are a promising alternative for the treatment of infections caused by *M. kansasii* [20, 25, 31].

The very first therapy that was introduced to treat refractory pulmonary infection by MAC is the Amikacin Liposome Inhalation Suspension (ALIS). This therapy was approved by the US, EU, and Japan and has been reported to have high efficacy as it provides target delivery to the lungs and penetrates the macrophages and biofilm [20, 25, 31]. Last but not least, the culture-based diagnosis of the disease requires longer turnaround time and laborious work; a non-culture-based rapid diagnostic method should be developed for early detection, and less toxic and more efficient drugs should be discovered so that side effects of the treatment are avoided [14]. According to a study by Conyers et al., bedaquiline, linezolid, and phage therapy are the new available drug options for the treatment of NTM infections. Moreover, resistance to bacteriophages has not been reported, and it is well tolerated by patients [28].

10. Conclusion

TB, one of the nation's burdens, has been reported by studies that a prevalence of NTM infections that is even higher than the cases of TB in certain countries and regions. This rapidly rising case may be due to frequent host-pathogen interaction, as many studies have suggested that the most dominant place of inhabitation by NTM is the water supply, although they are present ubiquitously in the environment [29]. So, their inhabitation in the tap water has exposed them to different kinds of water disinfectants and hence made them resistant [29]. Recent studies have also mentioned that the mode of entry for NTM infections was through ingestion and respiration of contaminated food or

aerosols, injured skin, etc., from environmental sources. In most of the literature for NTM infection, the disease being misdiagnosed as *Mycobacterium tuberculosis* complex is quite commonly portrayed. When the diagnosis is made using clinical symptoms, sputum smear microscopy, and rarely, radiological findings, NTM is frequently misdiagnosed as MTB. Furthermore, because of inadequate laboratory facilities, the criteria established by the American Thoracic Society and the Infectious Disease Society of America for the diagnosis of pulmonary Non-Tuberculosis Mycobacteria (NTM) are frequently disregarded, leading to incorrect diagnoses of TB and the administration of anti-TB medications based on the detection of Acid-Fast Bacilli (AFB). This failure in diagnosis and treatment has often led to the second-line drug treatment of TB. Simultaneously, the NTM disease proceeds as they have an innate resistance to conventional anti-TB drugs. This results in mortality and morbidity, and also the rising cases of NTM infection. This has led to challenging treatments in the case of the rising NTM infection scenario. Therefore, to lower the danger of misdiagnosis and missed diagnosis of NTM infection, providing awareness among healthcare providers is crucial. Moreover, the rise in the cases of NTM infection may be due to the cessation of the TB vaccination programs in developed countries. As discussed before, the commercially available TB vaccine, Bacille Calmette-Guérin (BCG) vaccine, has been shown to provide partial protection against some of the NTM infections [32, 33]. Recombinant vaccines and vaccines specific for the pathogens are still under study. One of the promising vaccines for *M. ulcerans* using software is currently under study. As mentioned by Zimmermann et al. [33], it has been suggested that glutathione possesses antibiotic qualities by boosting the ability of immune cells to fight and survive illness. This can be considered when designing a vaccine. Moreover, most of the infections caused by NTM have a rather long duration of therapy with multiple drug regimens. Therefore, preventive measures like vaccines can be used to prevent the disease while discovering the cure in the long run. Certain pulmonary infections caused by fungi, namely bronchomycosis, aspergillosis, etc., may also cause co-infections with pulmonary NTM. In addition, patients who were exposed to the recent wave of COVID-19 have a high chance of developing opportunistic infections as the main organ for both the infections are lungs [34]. The summary of factors associated with the outcome of both TB and NTM is mentioned in Table 4.

Table 4. Comparison of different factors associated with MTB and NTM infections infection to outcome

Details	TB	NTM	References
Species distribution	Limited strains are reported at different places with geographical variation	Specie distribution depends on the regional variation, with climatic conditions as one of the factors	[20]
Growth rate	Slow growers	Rapid and slow growers	[25]
Mode of transmission in humans	Via aerosols from the infected person in the form of droplet nuclei with the bacilli	Only in the case of cystic fibrosis, patients infected with *M. abscesses*	[20]
Virulence	Highly virulent	Low virulent	[25]
Pathogenicity	Strictly pathogenic	Opportunistic pathogens	[15]
Interaction with the host	Obligate	Opportunistic	[25]
Latent infection	Not reported	Latent TB may lead to other active infections	
Risk factor	Underlying lung disease, cystic fibrosis, COPD	Both healthy and infected lungs, malnutrition, smoking, cancer etc	
Morphology under the microscope	Absence of serpentine cords	Presence of serpentine cords	[20]
Identification	Depends on the culturalresult, as smear mimics MTB	Depends on both the smear and the culture result	
Treatment guidelines	ATS/European Respiratory Society (ERS)/European Society of Clinical Microbiology and Infectious Diseases (ESCMID)/IDSA guidelines must be followed	National guidelines must be followed	

Table 4. (cont.)

Details	TB	NTM	References
Epidemiology	Ubiquitous to the environment	Not commonly found in the environment	[15]
Availability of vaccine	BCG	None	[20, 32]
Causative organism	*Mycobacterium tuberculosis* complex	*Mycobacterium abscesses* complex (most common)	[15]
Disease caused	Pulmonary, extra-pulmonary and disseminated	Pulmonary, extra-pulmonary and disseminated	
Antibiotic resistance	MDR strains and XDR strains are resistant to specific antibiotics	Have innate properties of resistance against first line of anti-TB drugs	[28]
Interaction with host defense system	Phagocytosed by alveolar macrophages and formation of granuloma	Phagocytosed by alveolar macrophages and formation of granuloma	[29]
Resistance property	Adaptive resistance due to exposure of different antibiotics	Innate resistance against anti-TB drugs	
habitat	Symptomatic infected person	Environment- soil, air, predominant in water	[6]
Gender predominance	Male	Male or female	
Radiological diagnosis	X-ray	X-ray; Positron Emission Tomography (PET)/CT or High-Resolution Computed Tomography (HRCT)	
Sample for diagnosis	Sputum, BAL, aspirates	Sputum, BAL, CSF, tissues, fluids	

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Conflicts of interest

The authors declare no competing financial interest.

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