Chapter 13

Overview of Non Tuberculosis Mycobacterial Lung Diseases

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Additional information is available at the end of the chapter

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Abstract

Nontuberculosis mycobacteria (NTM) are ubiquitous in nature and opportunistically infect different animals, including humans. Currently, NTM is emerging as an important cause of pulmonary infection among both immunocompromised and immunocompetent persons worldwide. The clinical relevance of pulmonary NTM varies among species while showing geographical heterogeneity in distribution as well as pathogenicity. The outcome of the respiratory NTM disease is a consequence of a complex interplay between microbial factors and host susceptibility. Furthermore, HIV infection, cystic fibrosis, cancer, underlying chronic lung disease and history of tuberculosis (TB) may be associated as risk factors for active nontuberculosis pulmonary diseases (NTMPD). The diagnosis of NTMPD requires the presence of symptoms, radiographic evidences, microscopic observations and definitive laboratory diagnostics. Lung infections resulted from a clinically significant NTM species should be treated with appropriate antimicrobial regimen.

Keywords: nontuberculosis mycobacteria, NTM, lung infections, NTM diagnosis, NTM infection

1. Introduction to genus *Mycobacterium*

The genus *Mycobacterium* was first proposed in 1896 by Lehmann and Neumann [1]. Currently, it contains about 160 species and it is likely that more will be discovered with recently developed more precise species identification techniques [2, 3]. Most species exist as free-living saprophytes and only minorities are successful as pathogens of higher vertebrates. The host-dependent mycobacteria are capable of reproducing *in vitro*. In contrast,

M. leprae and *M. lepraemurium* are uncultivable and require the intracellular milieu for survival and propagation [4].

The obligatory causative agents of the genus *Mycobacterium*, responsible for TB are classified into *Mycobacterium tuberculosis* complex (MTC). It comprises *M. tuberculosis*, *M.bovis*, *M. africanum, M. microti* [5], *M. canettii* [6], *M. caprae* [7], *M. pinnipedii* [8], *M. mungi* [9], *M. orygis* [10] and *M. suricattae* [11] species. *M. tuberculosis, M. africanum, M. canettii* and *M. orygis* cause TB primarily in human [4, 10], whereas *M. bovis* [12], *M. microti* [13], *M. caprae* [7] *M. pinnipedii* [8], *M. mungi* [9] and *M. suricattae* [11] infect cattle, domestic animals, goats, seals, mongooses and meerkats, respectively, and animal tuberculosis can also be zoonotic [14, 15]. However, geographical variation of the MTC species distribution has been identified. As an example, *M. africanum* is a common cause of human pulmonary TB (39%) as much as *M. tuberculosis* (55%) in West Africa [16]. In Ghana, 3% of pulmonary TB cases are represented by *M. bovis*, while 20% are *M. africanum* and 73% are *M. tuberculosis* [17].

Other medically important mycobacteria such as *M. avium, M. intracellulare* complex, *M. kansasii, M. marinum, M. fortuitum, M. chelonae* complex, *M. abscessus* and *M. scrofulaceum* are known as nontuberculosis mycobacteria (NTM) species or atypical mycobacteria or mycobacteria other than tuberculosis (MOTT). They are responsible for diseases including lymphadenitis in children, chronic pulmonary diseases, skin and soft-tissue diseases and infections of the skeletal system [18].

NTM are ubiquitous in nature and are widely distributed in water, soil and animals. Among prevailing NTM species, only a few species have a clinical impact on humans as opportunistic pathogens [19]. *M. avium* complex (MAC), *M. abscessus, M. kansasii, M. fortuitum, M. chelonae, M. szulgai, M. triviale* and *M. scrofulaceum* are common NTM species that cause pulmonary diseases in human [20]. Additionally, *M. riyadhense* was recently proposed as a causative agent of pulmonary NTM disease [21]. However, NTM are increasingly recognized as a significant cause of chronic human pulmonary infections in both immunocompromised and immunocompetent patients [3].

In contrast to TB, diseases caused by NTM have varied clinical manifestations, triggering a wide spectrum of infections with generally low virulence than TB [20]. Patients with underlying structural lung diseases such as chronic obstructive pulmonary diseases, cystic fibrosis, bronchiectasis, history of TB and chronic aspiration are more vulnerable to develop NTM lung disease [22]. Additionally, working in mining industry and advanced age are risk factors for NTM lung diseases. However, there is no evidence on animal-to-human (zoonosis) or human-to-human transmission of NTM, and human diseases are generally acquired from environmental exposure [22].

2. NTM species significant to lung diseases

NTM are emerging worldwide as significant causes of chronic pulmonary infection, while became a challenge for both clinicians and researchers in the past two to three decades [23]. However, isolation and discover of new NTM species from pulmonary clinical specimens have become frequent in the last years especially with the development of species identification techniques such as sequencing of 16S ribosomal DNA (rDNA) [24].

The pathogenicities of the different NTM species vary widely and show geographical heterogeneity. Commonly, most NTMPD infections are caused by the MAC, *M. abscessus*, *M. kansasii* [25, 26], *M. fortuitum, M. chelonae* [26], *M. szulgai, M. gordonae, M. vaccae and M. smegmatis* [20, 27].

MAC organisms are common in many environmental sites, including water and soil, and in animals as well as colonize in natural water sources, indoor water systems, pools and hot tubs [28]. Previously, MAC, a slow growing NTM species has been composed of *M. avium* and *M. intracellulare* but, with advance in genetic identification of species, MAC encompasses at least 10 species, i.e. *M. avium*, *M. intracellulare*, *M. arosiense*, *M. bouchedurhonense*, *M. chimaera*, *M. colombiense*, *M. marseillense*, *M. timonense*, *M. vulneris* and *M. yongonense*, as well as 4 subspecies, i.e. *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *hominissuis* and *M. avium* subsp. *paratuberculosis* [21, 25, 29]. MAC may cause progressive parenchymal lung disease and bronchiectasis in patients, particularly in middle-aged and elderly women without underlying lung diseases [30]. Fibrocavitary lung disease caused by MAC may associated with large cavities specially in late 1940s and early 1950s years, males who have a history of cigarette smoking and excessive alcohol use. Untreated form of this disease is generally progressive to extensive cavitary lung destruction and respiratory failure within 1–2 years. MAC lung disease also presents with nodular and interstitial nodular infiltrates frequently involving the right middle lobe or lingual, called as nodular bronchiectasis or nodular bronchiectatic disease [31]. Particular MAC species may have varying degrees of virulence and classifying MAC isolates into species level is important for identification of risk of clinical relapse/reinfection [32, 33].

Tap water is likely the major reservoir for *M. kansasii* causing human pulmonary disease [34]. Genotypic studies in Netherland and France have shown that isolates recovered from the patients have similar genotype to isolates from drinking water source and the environment [35, 36]. DNA sequencing of *M. kansasii* has confirmed the presence of seven subspecies, which are related to human infections [37], while subtype 1 is the predominant in human lung infections [37–39]. Clinical symptoms of *M. kansasii* lung disease are generally identical to those associated with pulmonary TB. Chest radiographic abnormalities are also very similar to reactivation of pulmonary TB, including cavitary infiltrates with an upper lobe predilection. Also, *M. kansasii* may show noncavitary or nodular/bronchiectatic lung disease [40], which is similar to clinical presentation of MAC.

Some studies have been strengthened that drinking water may be the source of infection of *M. abscessus* lung diseases [41]. The common clinical symptoms of *M. abscessus* and *M. fortuitum* infection are similar to other NTM respiratory pathogens, especially MAC, including cough and easy fatigability. Disease caused by *M. genavense* commonly has been recognized in acquired immunodeficiency syndrome (AIDS) patients, while observed also in HIV-negative patient with pulmonary nodules [42].

Although most NTM lung infections are caused by common organisms, other NTM species such as *M. flavescens, M. mucogenicum* [26, 43], *M. colombiense, M. genavense, M. holsaticum, M. kumamotonense, M. lentiflavum, M. mantenii, M. marseillense, M. monacense, M. neoaurum, M. parascrofulaceum, M. phocaicum, M. saskatchewanense, M. seoulense, M. septicum, M. setense, M. shimoidei, M. stomatepiae, M. szulgai, M. triplex* [44], *M. xenopi, M. malmoense, M. immunogenum* [45], *M. scrofulaceum, M. terrae complex, M. engbaeki, M. shimoidei, M. gilvum, M marinum, M. interjectum subspecies, M. heckeshornense, M. branderi* and *M. chromogen* [24] may cause pulmonary disease in both immunocompetent and immunocompromised patients.

Isolation of multiple NTM species from respiratory specimens has also been recorded. In Taiwan, two patients of 298, one had five isolates of MAC and one isolate of *M. fortuitum*, while another patient had 11 isolates of MAC and one isolate of *M. gordonae* [27]. Thus, the pathogenic significance of a NTM specimen must be determined in the context of a patient's clinical presentation.

3. Prevalence and current epidemiology of pulmonary NTM disease

In Western societies, most laboratories report a dramatically greater prevalence of NTM than TB [46]. However, the prevalence of NTM pulmonary infections, which based on laboratory records, should be coupled with clinical characteristics [47] as only approximately half of people with positive NTM cultures fulfilled clinical criteria for active infection [48]. Studies form North America, Australia, South Korea, Japan and Taiwan have shown the continued increase in NTM prevalence since 2000. The annual prevalence in North America and Australia ranges from 3.2 to 9.8 per 100,000 and is generally higher than in Europe. In Queensland, Australia, cases of pulmonary disease rose from 2.2 to 3.2 per 100,000 population [49] *during 1999–2005.* Furthermore, in Australia, the annual percent of NTM isolation has increased steadily every year, and the incidence rate of patients with NTM lung disease was 1.82 per 100,000 in 2006 and increased to 4.38 per 100,000 in 2010 [50], while the same changed from 9.4 per 100,000 in 2009 to 36.1 per 100,000 in 2016 [51]. In Africa and the Middle East, prevalence of NTM ranges from 4 to 15% among suspected TB cases and 18% to 20% among suspected multidrug-resistant TB (MDR-TB) cases [52]. The prevalence rate of NTMPD in Germany was increased from 2.3 to 3.3 cases per 100,000 population from 2009 to 2014, and this was strongly association with advanced age and chronic obstructive pulmonary disease [53]. The prevalence of NTM isolation approximately was doubled from 2005 (6%) to 2013 (11%) in Hawaii, USA [26], while in Oregon, USA, the estimated prevalence of NTMPD was 8.6 per 100,000 [48]. By 2014, in Japan, the incidence rate of NTMPD was 14.7 cases per 100,000 person, which was ≈2.6 times higher than the same reported in 2007 and current incidence rate of NTMPD may exceed that of TB in Japan [48]. The general prevalence of NTM was 477 per 100,000 in Zambia with the regional variation of rate of prevalence within the country [54]. In Korea, the rates of recovery of NTM from clinical specimens and the number of patients with NTM lung infections increased significantly between 2009 and 2015 [55].

While some species such as MAC and *M. abscessus* are commonly implicated worldwide, others (e.g., *M. malmoense, M. xenopi*) are regionally important [23]. Generally, MAC is predominant in North America and East Asia, whereas *M. kansasii, M. xenopi* and *M. malmoense* are more common in Europe [52]. In Hawaii, USA, the most prevalent species was MAC, *M. fortuitum* group and *M. abscessus* [26]. Even though isolation of slowly growing mycobacteria (SGM) is frequent in most of the European and Western countries, rapidly growing mycobacteria (RGM) species such as *M. fortuitum* and *M. abscessus* are more prevalent in Gulf Cooperation Council (GCC) except in few countries [56]. As examples, *M. fortuitum* was the predominant course of NTM lung disease in Middle East during 1984–2014 [57]. Furthermore, *M. fortuitum* and *M. abscessus* are predominant in Saudi Arabia, while MAC is the most common species in Oman [56]. However, it has been observed in Saudi Arabia that rare species are going to be prominent, alarming diversity of clinically relevant NTM's causing pulmonary infections [58].

Country (no. of infections tested)	NTM species (%)								
	M. abscessus	*M. avium*	*M. chelonae*	*M. fortuitum*	*M. gordonae*	*M. kansasii*	*M. triviale*	*M. scrofulaceum*	*M. szulgai*
India (15)	–	–	–	40	–	33	20	–	7
Hong Kong (28)	–	54	14	–	4	4	–	–	4
South Korea (131)	39	50	2	3	–	4	–	–	0
Japan (1064)	–	81	0.6	2	–	14	–	–	0.5
Thailand (132)	–	43	5	5	–	17	–	8	–
Singapore (15)	–	60	7	–	–	27	–	7	–
Taiwan (302)	19	43	10	10	–	9	–		–

Table 1. NTM species causing pulmonary infections in Asian region during 1971–2007 [20].

The most frequent NTM species were *M. intracellulare* followed by *M. avium* subspecies in South Africa by 2010 [59], while *M. kansasii* is the more frequently associated among definite or probable active TB patients. Also, *M. avium-intracellulare* complex was the prominent course of NTM lung infections in Greece during 2007–2013 [60].

Furthermore, clinical relevance of pulmonary NTM species shows not only the geographically heterogeneous but also the time-to-time variations. As examples, MAC was the main cause of pulmonary diseases in India during the period of 1971–2007 [20]. But, according to the recent publications, *M. abscessus* was the predominate species followed by *M. intracellulare* [61, 62] in India, even both of the species were not recorded till 2007 [20] (**Table 1**). *M. intracellulare* followed by *M. kansasii* were most common NTM species related to the NTMPD in China from 2004 to 2009 [63] and it changed in 2010–2015 period, as *M. kansasii* was replaced by *M. abscessus* [64, 65]. Similar observation had been in Japan where, dramatic increases of pulmonary *M. abscessus* incidence had been occurred [48] comparative to period of 2001–2007.

Furthermore, in Korea, *M. intracellulare* followed by *M. avium* was predominate species during 2009–2015, while it was *M. avium* followed by *M. abscessus* in earlier (**Table 1**) [55].

4. Host-pathogen interactions

Unlike TB, the mode of transmission of NTM to humans has not been defined. Bathroom showers have been implicated as a primary source of exposure to aerosolized NTM. Even though animals are potential reservoir for NTM infections, zoonosis is not properly evident yet. However, drinking untreated water and living in close contact with cattle or other domestic animals may lead of infection in human [66]. In USA, NTM diseases are more

associated with densely populated areas, suggesting the infective source as urban municipal water supply [67], while Japan suggesting the soil as the source for more patients who were farmers and gardeners [68]. Furthermore, characteristic gradient clustering of the ratios of *M. avium* and *M. intracellulare* has been observed in Japan, suggesting that environmental factors strongly affect the epidemiology of NTMPD [69].

The outcome of the respiratory NTM disease is a result of a complex interplay between microbial factors like particle size, number of organisms and duration of contact and host susceptibility factors such as immunity, genetic background, lung damages and chronic lung disease. The clinical presentation of NTM lung infections may be varied, including hypersensitivity pneumonitis (HP)-like granulomatous lung disease, cavitary (TB-like) disease and nodular bronchiectasis. A hypersensitivity pneumonitis (HP)-like granulomatous lung disease, with nontuberculous mycobacteria can be triggered by inhalation of NTM with hot water aerosols (hot-tub lung) from sources such as hot tubs/spas, showers and indoor swimming pools. This may have been the primary source of MAC infections in middle-aged women with subacute presentation of respiratory complaints and HIV patients in the United States [70]. While MAC is the most common NTM causing "hot tub lung," *M. fortuitum* has also been rarely implicated [71]. Physicians need to be alerted to the possibility of hot tub lung being caused by various NTM species other than MAC. Furthermore, a case study has been confirmed *M. gordonae* as a potential pathogen in humidifier lungs [72].

Rarely, with underlying lung disease or smoking or prior TB, cavitary disease could be caused by multiple NTM species especially by MAC. This condition is different from the typical presentation of MAC pulmonary infections as they may have upper lobe cavity, as well as TB-like symptoms [73, 74]. Nodular bronchiectasis, which is often present with older nonsmoking female, is associated mostly with MAC. Sometimes, mixed infections of MAC and *M. abscessus* may lead to nodular bronchiectasis [75, 76]. In really, solitary pulmonary nodules (SPN) due to MAC infection also have been identified in some studies [74, 77, 78]. However, clinical outcome of the NTM diseases basically depends on the interactions between NTM and the host (**Figure 1**).

4.1. Host factors

Immunosuppressed hosts who may be associated with immunosuppressive HIV infection, hematological and lymphoproliferative malignancy, stem cell and solid organ transplant and inflammatory disorders treated with biologicals are highly vulnerable for pulmonary infections caused by *M. avium* and other nontuberculous species. Defense against *Mycobacterium* species

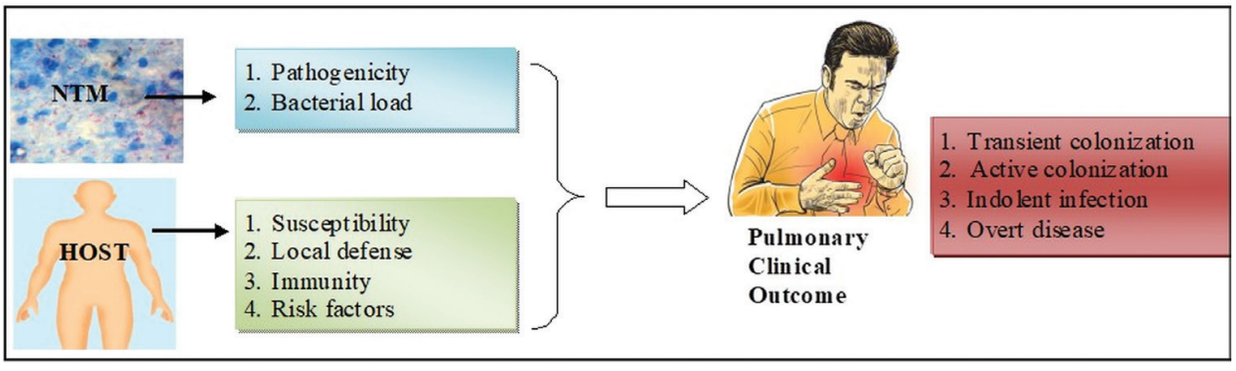

Figure 1. Interactions between NTM and the host that determine the clinical outcome.

is mediated by mononuclear phagocytes' ability to kill mycobacteria and secrete interleukin-12 (IL-12), augmented by interferon-gamma (IFNγ) secreting lymphocytes such as $CD4^{+}$T cells. Human natural killer cells (NK) are important in host defense against *Mycobacterium* as it secretes cytokines that induce macrophages to inhibit the growth of bacteria within macrophages [79, 80]. Cytokines that induce IL-32 (newly described pro-inflammatory cytokine), such as interferon-gamma, IL-18, IL-12, granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha, have considerable importance in mycobacterial immunity [81]. The alliance formed between IL-12 and IFN-gamma is essential for protective immunity against mycobacteria in human [82]. Therefore, genetic deficiencies in immunity mediated by IL-12 or IFN-gamma are highly susceptible to mycobacteria NTM infections in both individuals and familial clusterings of disease [79, 83].

IL-32 is expressed in multiple cell types in the lungs but particularly in the airway epithelial cells of patients with MAC pulmonary disease. Human airway epithelial cells (BEAS-2B) infected with *M. avium* produce IL-32 by a nuclear factor-kappa B-dependent mechanism. In both BEAS-2B cells and human monocyte-derived macrophages, exogenous IL-32 significantly reduced the growth of intracellular *M. avium* by increased apoptosis of infected cells. Thus, IL-32 not only facilitates host defense against MAC organisms but may also contribute to the airway inflammation associated with MAC pulmonary disease [81].

In immune evasion mechanism of *M. avium* subsp. paratuberculosis (MAP), bacteria are survived in macrophages by activation of mitogen-activated protein kinase (MAPK) pathway that leads to inhibition of antimicrobicidal activity of macrophages and over expression of IL-10. High levels of IL-10 in paratuberculosis promote the survival of MAP by reducing bactericidal activity of defense cells. Therefore, the pathways involved in the upregulation of IL-10 such as MAPK can be vital for developing a therapeutic strategy for the control of paratuberculosis [84]. A monogenic disorders conferring susceptibility to NTM infection are called as Mendelian Susceptibility to Mycobacterial Disease (MSMD) conditions, which are extremely rare and predominantly affecting children. Genetic disorders, which affect the immune response to mycobacterial infection, are known to result from disorders in genes of ISG15, IL-12B, IL12RB1, IFNGR1, IFNGR2, STAT1, IRF8, ISG-15, GATA2, NADPH and oxidase complex subunit genes such as CYBB [85].

Diseases and therapies that reduce cell-mediated immunity increase the risk of NTM disease. Acquired immunodeficiency virus (AIDS), cancer and organ transplants have been associated with NTM disease. The use of immunosuppressive drugs, including anti-TNF biologics, is also a risk factor for NTMPD [86]. NTM are often found in sputum cultures of patients with cystic fibrosis as they undergo lung transplantation followed by immunosuppressive medications. Therefore, effective medical treatment may need to control NTM after lung transplantation. The post-transplant infections can be associated with *M. abscessus*, which not affect for the survival of the patient in pre-transplantation stage. Therefore, sputum culture positivity for NTM before lung transplantation should not preclude transplantation, but should be treated in order to minimize the risk for recurrence after transplantation [87]. There is a possibility of co-existing pulmonary NTM infection in patients with lung cancer and disseminated NTM infection in patients with hematologic cancer [88, 89]. A study has suggested that anti-NTM therapy should be introduced only with worsening of symptoms under careful consideration as anti-NTM treatment is long and anti-mycobacterial drugs have extensive effects on anti-cancer drugs [90].

Also female sex, age, post-menopausal waning of endogenous estrogen levels, coeliac disease and exposure to use of dietary phytoestrogens can be risk factors for NTM lung diseases [91] while oral corticosteroids treatment in rheumatoid arthritis patients is also a comorbidity of NTM disease [92]. However, another study has showed that bronchiectasis and NTM lung disease are risk factors for breast cancer in women, and this phenomenon will open a new pathway for investigation of common pathophysiologic links of NTMPD [93].

4.2. Microbial factors

Aside from host factors, microbial factors such as virulence and microbial dose of exposed would be considerable factors for progression of NTM lung diseases. The critical exposure dose and relationship between quantitative mycobacterial exposure and disease are yet to be known. However, it may vary with the host susceptibility. Although exposure is common, disease is unusual, as most of NTM species are nonpathogenic and pathogenicities are varied according to the NTM species. Only few are highly pathogenic in human in descending order of pathogenicity, *M. malmoense*, *M. szulgai*, *M. kansasii*, *M. abscessus*, *M. Xenopi*, *M. avium* and *M. simiae*/*M. chelonae* and *M. intracellulare*. Even though MAC account for the plurality of pulmonary isolates as well as disease worldwide, the clinical relevance of NTM isolation from respiratory specimens appears to vary by geographic region, presumably due to variability in both environmental microbial distribution and the prevalence of host risk factors [23].

5. Diagnosis of NTM lung infections

Unlike TB, the isolation of NTM in pulmonary specimens does not equate with disease. The guidelines published in 2007 by American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) (ATS/IDSA) have specified that both clinical and microbiologic criteria must be met for the confirmation of diagnosis of pulmonary NTM disease [22]. Also, correct species identification is vital as NTM species differ in their clinical relevance. Correct diagnosis and choice of treatment regimen are needed as to prevent misdiagnosis, which direct chronic disease, antimicrobial resistance and death [94]. However, identification of all clinically obtained NTM isolates, especially from sputum, may not be needed. For instance, the exact identification of a pigmented rapidly growing mycobacteria isolated in low numbers from only one of multiple sputum specimens collected from patient undergoing therapy for MAC lung disease may not be necessary as it would not likely be clinically significant [22]. The diagnosis requires the presence of symptoms, radiographic abnormalities or chest high resolution computed tomography (HRCT) scan in the absence of cavitations, three or more sputum specimens for acid fast bacilli (AFB) analysis and exclusion of other disorders such as TB and lung malignancy. According to the ATS/IDSA guidelines, the criteria apply for definitive diagnosing of nontuberculous mycobacterial lung disease is following [22].

Clinical (both required)

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph or an (HRCT) scan that shows multifocal bronchiectasis with multiple small nodules.
2. Appropriate exclusion of other diagnoses.

Microbiologic.

1. Positive culture results from at least two separate expectorated sputum samples. (If the results from the initial sputum samples are nondiagnostic, consider repeat sputum AFB smears and cultures.) (or)
2. Positive culture results from at least one bronchial wash or lavage (or)
3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM.
4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination.
5. Patients who are suspected of having NTM lung disease but who do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded.
6. Making the diagnosis of NTM lung disease does not, *perse*, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients.

5.1. Clinical and radiographic based diagnosis

Delaying of diagnosis of NTM lung diseases is frequent due to the slow growing rate, misdiagnosed as TB or other AFB-positive bacilli and low index of clinical suspicion. The clinical symptoms, such as chronic cough, increased sputum production, dyspnea, low-grade fever, malaise and weight loss, are often nonspecific and overlapping clinical characteristics with pulmonary TB [95].

Radiological imaging and observing of radiological patterns, including miliary pulmonary pattern, nodular lesions, cavitary lesions, pleural effusion, abdominal adenopathy and splenic hypoechoic, is important when NTM lung disease is suspected in AIDS patients [96]. HRCT scanning allows early detection and better differentiation between colonization and invasive infection that are not visible on the chest X-ray [97]. In CT features; pleural effusion and nodules are significantly more common in patients with pulmonary TB (PTB) while bronchiectasis combined with cystic changes are significantly more common in patients with NTM lung infections [98]. Bronchiectasis in the right middle lobe or left lingual segment and thin-walled cavity with a diameter of more than 3 cm are the frequent chest CT features in patients with NTM-LD [99]. Furthermore, cavities associated with adjacent pleural thickening, ill-defined satellite tree-in-bud nodules or fewer noncavitary nodules in CT findings are highly suggestive of NTM disease rather than TB [58, 100].

Also, NTM lung infection can present itself with different radiological patterns, while two main patterns, fibrocavitary form and nodular bronchiectatic form, have been observed frequently [100]. The fibrocavitary form is usually characterized by upper lobe cavities with areas of increased opacity and with or without calcification (**Figure 2**) [98, 101].

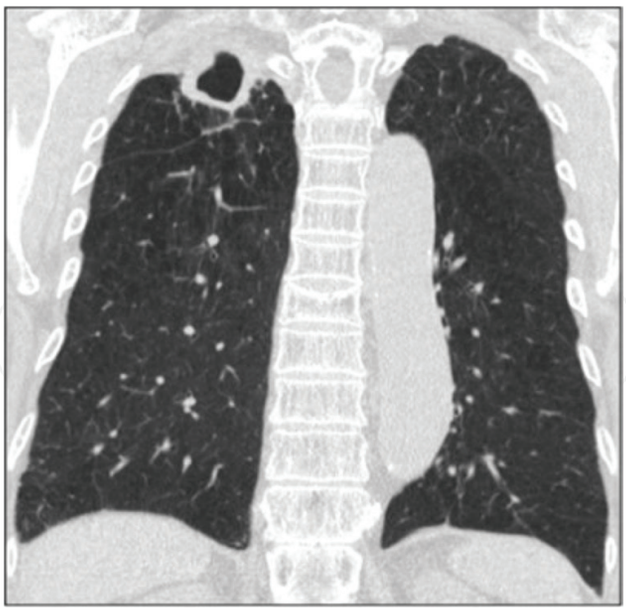

Figure 2. CT of fibrocavitary form of *M. intracellulare* pulmonary disease with a large cavity in the right upper lobe [101].

In the nodular bronchiectatic form: bilateral, multilobar bronchiectasis, especially in the middle and lower lung fields, with small nodules are the frequently observed chest CT features (**Figure 3**) [99, 101].

Even though there are no characteristic radiographic patterns for individual NTM species, centrilobular, peribronchovascular nodules, bronchiectasis, consolidation, tree-in-bud, pleural thickening and pleural adhesion are commonly observed CT findings in patients with MAC infection [102]. A recent study has shown that cavities are more common in patients with *M. malmoense*, while consolidations are mostly found among patients with an MAC and nodules are frequent in *M. kansasii* patients [103]. However, due to the presence of considerable overlap of the clinical symptoms and radiographic appearances of PTB and NTM lung diseases, the isolation and identification of causative organisms are mandatory for correct diagnosis of patients with AFB-positive sputum specimens [76].

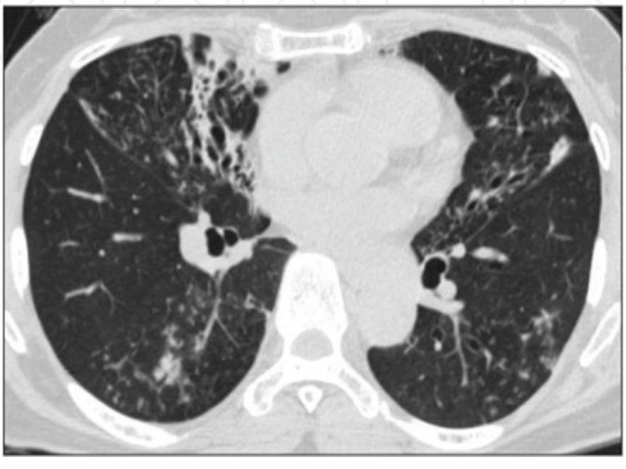

Figure 3. CT of nodular bronchiectatic form of *M. intracellulare* pulmonary disease with severe bronchiectasis in the right middle lobe and the lingular segment of the left upper lobe [101].

5.2. Laboratory diagnosis

The initial laboratory identification of the genus *Mycobacterium* can be made by microscopic observation for the presence of AFB. The definitive diagnosis demands the recovery of *Mycobacterium* species on a culture medium, followed by species identification tests. Although numerous novel, rapid and direct molecular methods have been developed, culture remains the gold standard for identification of *Mycobacterium* species from clinical specimens [104].

5.2.1. AFB smear microscopy

AFB staining, such as fluorochrome technique, Ziehl-Neelsen method or Kinyoun stain, which initially adopted for identification of *Mycobacterium tuberculosis* complex (MTBC), is satisfactory for NTM also. However, Smear microscopy cannot use for differentiation of MTBC form NTM, hence the presence of AFB can lead to a false-positive diagnosis of TB. The burden of organisms in clinical material is usually reflected by the number of organisms seen on stained smears. Since NTM are present in the environment, especially in water sources, the careful collection of high-quality respiratory specimens is necessary to avoid contamination. However, environmental contamination, which usually involves small numbers of organisms, rarely results in a positive smear examination. Semi-quantitative analysis of smears can be useful for diagnostic purposes and fluorochrome smears are graded from 1 (1–9 organisms per 10 high-power fields) to 4 (90 organisms per high-power field) [101, 105, 106].

5.2.2. Mycobacterium culture and species identification by conventional methods

Isolation of *Mycobacterium* by culturing is a primary requirement in conventional species identification and indirect drug susceptibility testing of NTM. The general microbiological measures of growing clinical material on a selective or differential culture media and subculturing to obtain pure cultures cannot be applied to *Mycobacterium*. Genus *Mycobacterium* will not grow on simple, chemically defined media and it requires special, enriched, selective media. Also, slow replication rate is a characteristic feature in culturing of *Mycobacterium, hence* culturing is time-consuming [107]. Generally, an AFB-positive sputum will require 3 weeks for producing visible colonies of *Mycobacterium* on solid medium [4]. However, NTM species, such as *M. fortuitum, M abscessus* and *M. chelonae,* are considered as rapid growers as they grow into visible colonies within 3–5 days of incubation [19, 22].

As per ATS/IDSA guidelines, both solid and liquid cultures are required for NTM species identification. Even though mycobacteria produce more rapid cultures with high yield in broth media than those on solid media, solid cultures need to proceed simultaneously as they allow observing of colony morphology, growth rates and mixed infections (more than one mycobacterial species), which are important factors in identification of the NTM species. Also, broth media cultures alone may not be sufficient for better diagnosis of NTM species due to the bacterial overgrowth and high chance for the contaminations from other bacteria and fungus [22].

In conventional culture techniques, Lowenstein-Jensen (LJ) media and agar-based Middlebrook media (7H10 and 7H11) are used as the common solid media, while 7H9 medium used as the liquid/broth media. BACTEC MGIT 960 system is a fully automated, nonradiometric system that is suitable for the detection of growth of TB and other mycobacteria with the shorter

detection time ~2 weeks [108]. The recently introduced, microchannel electrical impedance spectroscopy (m-EIS) has ability to detect *M. smegmatis* with initial loads of 1000 CFU/ml within 20 h, while commercial BACTEC MGIT 960 system need 41.7 h for the same [109].

Species, such as *M. haemophilum*, *M. genavense*, *M. avium* subsp. paratuberculosis (formerly *M. paratuberculosis*) and *M. ulcerans*, are required special supplementation for recovery on culture media. *M. haemophilum* grows only on media supplemented with iron-containing compounds such as ferric ammonium citrate, hemin or hemoglobin [110]. *M. genavense* and *M. avium* subsp. paratuberculosis require mycobactin J, and *M. ulcerans* may be optimally recovered with egg yolk supplementation [22].

Microscopic observation of ZN-stained smear prepared from culture will provide evidence only for the presence of mycobacteria, purity of the culture and cord formation. These basic characters are not sufficient for definitive species level identification. The conventional taxonomic differentiation of the genus *Mycobacterium* is based on phenotypic characters of the cultures and biochemical properties of bacteria. The characters of rapid growth, pigmentation (scotochromogens, photochromogens or nonchromogens), ability to grow in PNB incorporated media and creamy like watery colonies indicate the presence of NTM [107]. Several biochemical tests based on the properties of the genus *Mycobacterium*, including nitrate reductase, niacin production, catalase activity, production of arylsulfatase and urease, tween 80 hydrolysis, growth in the presence of 5% NaCl and MacConkey agar without crystal violet and the use of mannitol, inositol and sorbitol, may adequate to identify majority of clinically relevant mycobacterial species [107].

5.2.3. Molecular-based identification methods

Biochemical analysis and phenotypic characters may occasionally fail to arrive at a definitive identification. Because of differences in antimicrobial susceptibility at species level that determine treatment options, precise species identification of the NTM is required and only determination of merely as groups, such as *M. chelonae* (or/and *M. abscessus*) group, is not recommended [22]. To fulfill this requirement, rapid accurate and cost-effective molecular-based techniques, both *in-house* and commercial kits, with satisfied sensitivity and specificity were developed during last years. Currently, molecular methods especially assays based on the principle of nucleic acid amplification which allows a speedy and precise identification of the *Mycobacterium* species in <24 h have been developed.

Real-time PCR, DNA sequencing, probe hybridization, multiplex PCR and polymorphism analysis of restriction fragments (PCR-RFLP) are commonly used for differentiation of NTM species related to lung infections [111, 112]. The real-time PCR assays are advantageous because of its rapidity and high sensitivity. Furthermore, the specificity of the real-time PCR can be enhanced by combination with HPLC, which is a useful tool to discriminate NTM at the species level, although it requires specific equipment and technical expertise [113]. Furthermore, multiplex real-time PCR assay combined with melting curve analysis is also an accurate, rapid and effective tool for the mycobacterial identification from cultures [114]. The commercial form of real-time PCR Light cycler® *Mycobacterium* detection assay, which based on the 16S ribosomal RNA (rRNA), has shown 100% sensitivity and 99% specificity for differentiation of MTBC and *M. avium* from sputum samples [115, 116].

Several commercial kits, which are based on PCR amplification of selected fragment of 16S or 23S rRNA gene or 16S–23S rRNA spacer region, followed by reverse hybridization on nitrocellulose membrane strips such as GenoType *Mycobacterium* CM/AS (Hain Lifescience, Nehren, Germany) [117–120] and INNO-LiPA Mycobacteria (LiPA; Innogenetics, Zwijnaarde, Belgium) [121, 122] are available for identification of common pathogenic NTM species with high sensitivity and specificity. Genus *Mycobacterium,* MTBC and 16 NTM species are identified by INNO-LiPA mycobacteria assay, and it is based on the nucleotide variations in the 16S–23S rRNA spacer region (**Figure 4**).

Mixed populations easily identified with this assay and fully automated processing of the strips is possible using TENDIGO™ and Auto-LiPA 48. GenoType *Mycobacterium* CM kit identifies

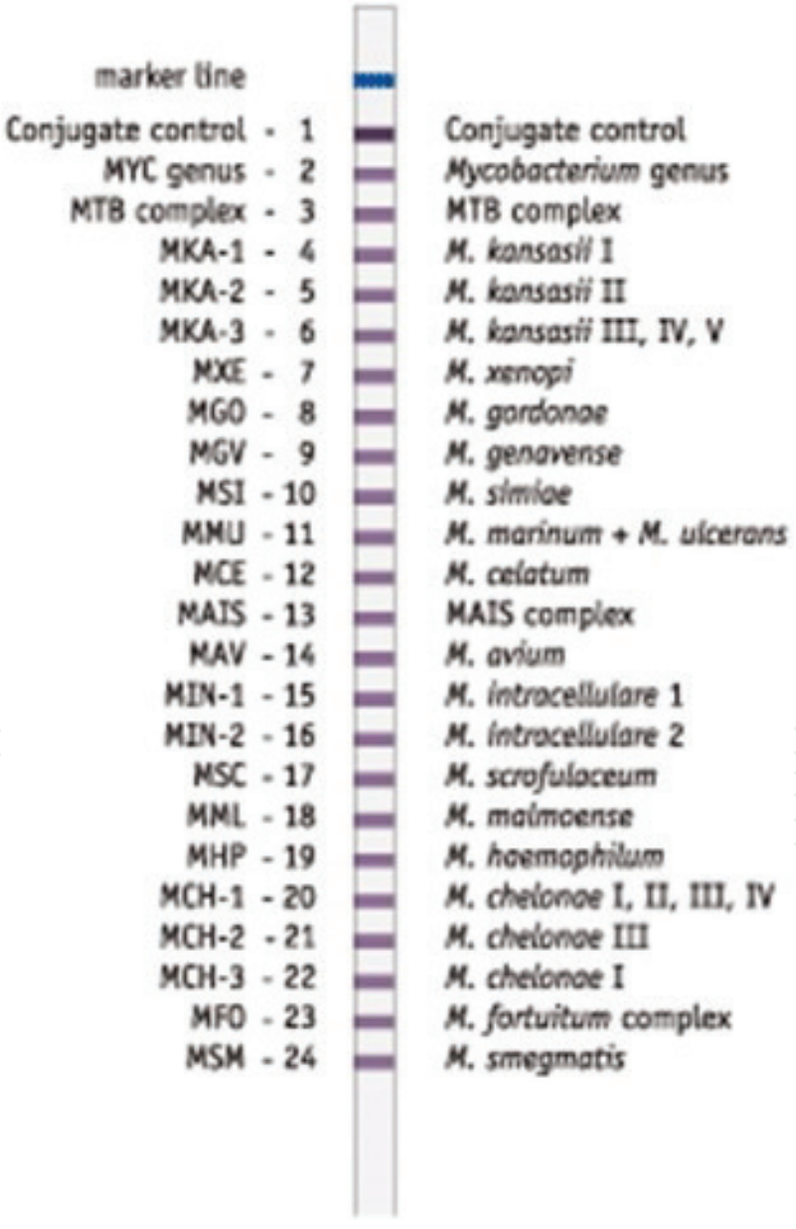

Figure 4. Location of the different probes on the INNO-LiPA Mycobacteria v2 strip.

the MTBC and differentiates of 27 clinically relevant NTM, while GenoType *Mycobacterium* AS kit enables the differentiation of 19 additional NTM species (**Figures 5** and **6**).

Direct sequence analysis of amplified 16S rRNA gene is a promising rapid and accurate method for species determination of nontuberculous mycobacteria [123], and in last decades, novel NTM species related to pulmonary infections were identified by this technique. In addition to that, several gene targets, including *rpoB* gene [124–126], *secA1* gene [127] and *hsp65*

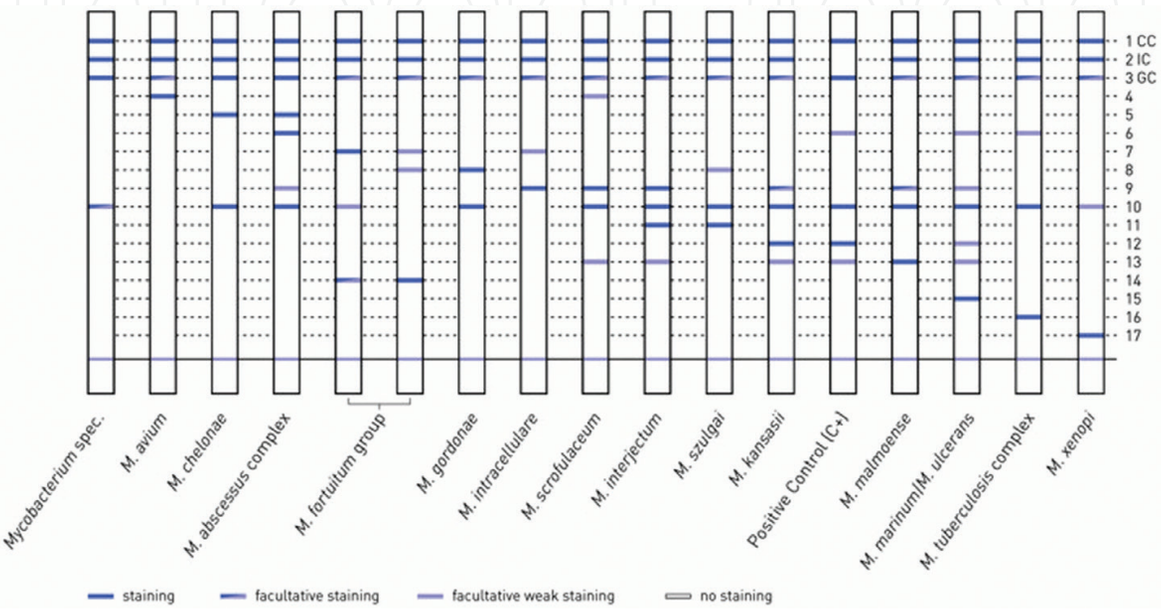

Figure 5. Location of the different probes on the GenoType *Mycobacterium* AS strip.

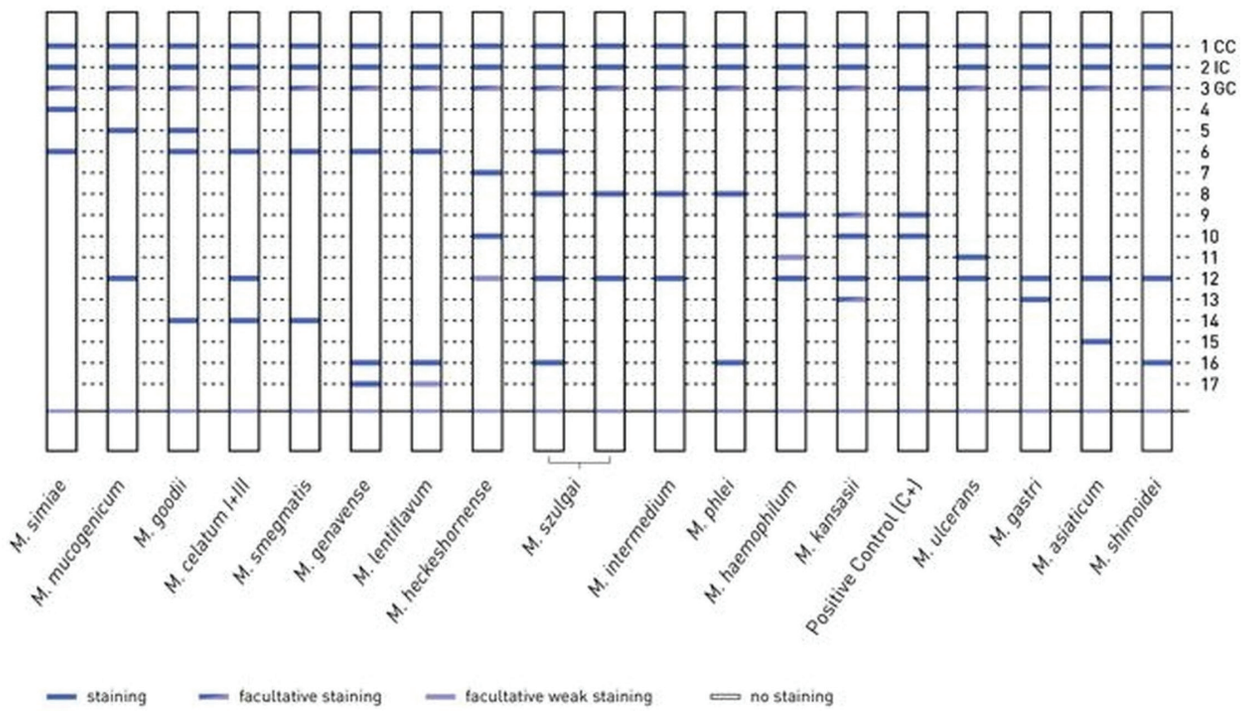

Figure 6. Location of the different probes on the GenoType *Mycobacterium* CM strip.

gene [128], have used for NTM species identification by DNA sequencing. Also, *gyrB*-based microarray [129], mycobacteria mobility shift assay (MMSA) [130], biochip assay system [131] and multiplex SNaPshot assay [132] have been proven as rapid detection methods to identify closely related mycobacterial species with satisfied level of sensitivity and specificity, which may be useful in the diagnosis and effective management of NTM lung disease [129–132].

6. Antimicrobial susceptibility testing for NTM

The laboratory susceptibility testing of pulmonary infective NTM species are based on the ATS/IDSA and Clinical and Laboratory Standards Institute (CLSI) guidelines. CLSI has recommended broth microdilution method as the gold standard for laboratories where antimicrobial susceptibility testing of NTM is performed [133]. There are no current recommendations for a specific method of *in vitro* susceptibility testing for fastidious NTM species and some less commonly isolated NTM species. Validation and quality control should be in place for susceptibility testing of antimicrobial agents with all species of NTM. According to the diagnostic guidelines for nontuberculous mycobacteria which are recommended by the ATS [22], only the Clarithromycin should be tested for susceptibility for new, previously untreated MAC isolates and susceptibility testes for other drugs are not recommended. Also, MAC isolates from patients who fail macrolide treatment or prophylaxis regimens should be tested to clarithromycin susceptibility. Isolates of *M. kansasii* that show susceptibility to rifampin will also be susceptible to rifabutin. Therefore, previously untreated *M. kansasii* strains should be tested *in vitro* only to rifampin. The rifampin resistant of *M. kansasii* isolates should be tested against a panel of secondary agents, including rifabutin, ethambutol, isoniazid, clarithromycin, fluoroquinolones, amikacin and sulfonamides. Unless the patient fails treatment after several months, *M. marinum* isolates do not require susceptibility testing.

The *in vitro* susceptibility patterns of some NTM such as *M. kansasii, M. marinum* and *M. fortuitum* are closely parallel to the clinical response to therapeutic agents. But, MAC, *M. abscessus* and *M. simiae* have limited evidences for the correlation between *in vitro* susceptibility results and clinical response in the treatment of pulmonary disease caused by these agents [134]. Furthermore, antimicrobial susceptibility patterns of rapidly growing mycobacteria (RGM) including isolates of the *M. fortuitum* group, *M. chelonae* and *M. abscessus* provide taxonomical value also in addition to the evidence of drug resistance [135].

According to the recent publications, the microplate Alamar Blue assay [136] and tetrazolium Microplate Assay [137] have also shown reliable results to the recommended microdilution method. However, molecular assays have not yet been able to replace time-consuming culture-based susceptibility methods in the mycobacteriology laboratory.

7. Treatment of NTM lung infections

After determination of the clinical significance of a NTM species, patient should be treated with appropriated antimicrobial regimen. The duration of treatment for most pulmonary NTM pathogens is based on treatment recommendations. Frequently encountered species

such as MAC and *M. kansasii* are treated 12 months of negative sputum cultures while on therapy. For disseminated disease, treatment duration for most NTM pathogens is the same as for disseminated MAC infection.

Treatment recommendations for infrequently encountered NTM are made on the basis of only a few reported cases. As recommendations for routine *in vitro* susceptibility testing of NTM isolates are limited, the clinician should use in vitro susceptibility data with an appreciation for its limitations. Empiric therapy for suspected NTM lung disease is not recommended. Furthermore, there are no widely accepted criteria for choosing patients with NTM lung disease for resectional surgery. In generally, surgery could be considered based on risk/benefit perspective in case of NTM infections that are more difficult to treat medically [22].

7.1. *M. avium* complex (MAC)

Drug therapy for MAC lung disease should be a combination of several antibiotics (**Table 2**), and the optimal therapeutic regimen has yet to be established [22].

Special recommendations of drug regimens are needed for patients with intolerance to first-line agents, a macrolide-resistant MAC cases or failed prior drug therapy. The macrolides should never be used as monotherapy for treatment of MAC lung disease. The duration of the treatment is 12 months of negative sputum cultures while on therapy; hence continuous observation of AFB in sputum of the patient is required throughout the treatment [22, 138]. The addition of intramuscular streptomycin to standard regimen for the first 3 months of treatment for MAC pulmonary disease improves the rate of culture conversion, even though clinical response and radiological outcome are not significantly improved. An intermittent (3× per week) oral antibiotic regimen should not be used in individuals with severe MAC pulmonary disease or in individuals with a history of treatment failure [138].

Major risk factors for macrolide-resistant MAC disease are inappropriate prescription patterns and deviations from the standard treatment due to adverse drug reactions [139]. More

Drug	Initial therapy for nodular/bronchiectatic disease	Initial therapy for cavitary disease	Advance or previously treated disease
Macrolide	Clarithromycin 1000 mg TIW or azithromycin 500–600 mg TIW	Clarithromycin 500*–1000 mg/d or azithromycin 250–300 mg/d	Clarithromycin 500*–1000 mg/d or azithromycin
Ethambutol	25 mg/kg TIW	15 mg/kg/d	15 mg/kg/d
Rifamycin	Rifampin 600 mg TIW	Rifampin 450*–600 mg/d	Rifabutin 150*–300 mg/d or rifampin 450*–600 mg/d
IV aminoglycoside	None	Streptomycin or amikacin or none	Streptomycin or amikacin

Notes: IV = intravenous; TIW = three times weekly.*Lower dose for weight 50 kg.

Table 2. Recommended antimicrobial combination [138].

effective therapy is essential to treat and prevent macrolide-resistant with MAC lung disease [140]. Antibiotic treatment associated with rifampicin, ethambutol and isoniazid or a quinolone with streptomycin or amikacin and surgical resection of disease can be used in macrolide-resistant MAC diseases [31, 101, 138, 140]. Furthermore, the addition of moxifloxacin can improve the outcomes of patients with macrolide-resistant [141]. However, recent study has shown that continuation of macrolides or the addition of a new quinolone or injectable aminoglycoside to therapy with rifampicin and ethambutol would not improve clinical outcome

Species	Drug regimen	Duration
M. kansasii – Rifampicin-sensitive	Rifampicin 600 mg daily + Ethambutol 15 mg/kg daily + Isoniazid 300 mg (with pyridoxine 10 mg) daily or Azithromycin 250 mg daily or Clarithromycin 500 mg twice daily	12 months after culture conversion.
M. malmoense	*Non-severe disease:*	
	Rifampicin 600 mg daily + Ethambutol 15 mg/kg daily + Azithromycin 250 mg daily or Clarithromycin 500 mg twice daily	Minimum of 12 months after culture conversion
	Severe M. malmoense-pulmonary disease:	
	Rifampicin 600 mg daily +Ethambutol 15 mg/kg daily + Azithromycin 250 mg daily or Clarithromycin 500 mg twice daily + consider intravenous amikacin for up to 3 months or nebulized amikacin	Minimum of 12 months after culture conversion
M. xenopi	*Non-severe*	
	Rifampicin 600 mg daily + Ethambutol 15 mg/kg daily + Azithromycin 250 mg daily or Clarithromycin 500 mg twice daily + Moxifloxacin 400 mg daily or Isoniazid 300 mg (+ pyridoxine 10 mg) daily	Minimum of 12 months after culture conversion
	Severe	
	Rifampicin 600 mg daily + Ethambutol 15 mg/kg daily + Azithromycin 250 mg daily or Clarithromycin 500 mg twice daily + Moxifloxacin 400 mg daily or Isoniazid 300 mg (+ pyridoxine 10 mg) daily + consider intravenous amikacin for up to 3 months or nebulized amikacin	Minimum of 12 months after culture conversion
M. abscessus Clarithromycin sensitive isolates or inducible macrolide-resistant cases	*Initial phase*:	
	≥1 month iv amikacin 15 mg/kg daily or 3× per week + iv tigecycline 50 mg twice daily and where tolerated iv imipenem 1 g twice daily and where tolerated oral clarithromycin 500 mg twice daily or oral azithromycin 250–500 mg daily	
	Continuation phase:	
	Nebulized amikacin + oral clarithromycin 500 mg twice daily or azithromycin 250–500 mg daily +1–3 of the following antibiotics guided by drug susceptibility results + patient tolerance: oral clofazimine 50–100 mg daily, oral linezolid 600 mg daily or twice daily (with pyridoxine 50 mg daily), oral minocycline 100 mg twice daily, oral moxifloxacin 400 mg daily, oral co-trimoxazole 960 mg twice daily	Minimum of 12 months after culture conversion

Species	Drug regimen	Duration
M. abscessus Constitutive macrolide-resistant cases	*Initial phase*:	
	≥1 month iv amikacin 15 mg/kg daily or 3× per week and iv tigecycline 50 mg twice daily + where tolerated iv imipenem 1 g twice daily	
	Continuation phase:	
	Nebulised amikacin and 2–4 of the following antibiotics guided by drug susceptibility results + patient tolerance: oral clofazimine 50–100 mg daily, oral linezolid 600 mg daily or twice daily (with pyridoxine 50 mg daily), oral minocycline 100 mg twice daily, oral moxifloxacin 400 mg daily, oral co-trimoxazole 960 mg twice daily	Minimum of 12 months after culture conversion

Note: iv = intravenous.

Table 3. Recommended treatment regimen for *M. kansasii, M. malmoense, M. xenopi and M. abscessus* pulmonary diseases [138].

after the emergence of chloramphenicol-resistant MAC [142]. If microbiologic, clinical or radiographic improvements are not shown after 6 months of appropriate therapy or achieved conversion of sputum to AFB culture negative after 12 months of appropriate therapy, patients are considered as treatment failures [22].

In addition to antibiotics, for patients with MAC lung infection, adjunctive therapies may also be given. Patients whose disease is predominantly localized to one lung, poor response to drug therapy, the development of macrolide-resistant MAC disease or the presence of significant disease-related complications such as hemoptysis might be considered for surgery. Although adjuvant pulmonary resection is complicated, it provides high level of treatment success rate in selected patients [143, 144]. Successful treatment of disseminated MAC in persons with AIDS is based on treatment of both the mycobacterial infection and the HIV infection. Both clarithromycin and azithromycin have been shown to be effective in combination regimens for the treatment of disseminated MAC. But, treatment of these cases may be complicated by adverse drug effects [22, 145].

Recommended treatment regimen for *M. kansasii, M. malmoense, M. xenopi and M. abscessus* is described in **Table 3**. The treatment for *M. abscessus* pulmonary disease should comprise an initial phase antibiotic regimen followed by a continuation phase antibiotic regimen. However, individuals with a history of treatment intolerance or treatment failure should be managed in collaboration with a physician experienced in managing NTMPD.

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