



# Current Knowledge of Mycobacterium Other Than Tuberculosis (MOTT) in this Current Era: Definition, Taxonomy, and Diagnose

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## Abstract

Globally, Pulmonary Tuberculosis (PTB) remains a health concern, with an annual increase in cases. Indonesia is the second-highest contributor to PTB cases globally, below India, which also saw an increase in cases, particularly after the COVID-19 pandemic. Nontuberculous Mycobacteria (NTM) infections contribute to the increase in PTB cases through misdiagnosis and overlapping conditions. The occurrence of changes in the composition of NTM species in the Mycobacterium genus is the premise for updating the diagnosis of NTM with several supporting examination modalities. Clinical, radiological, and microbiological criteria have been established by the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) for the diagnosis of NTM. The relationship between these three criteria is essential as a guideline for distinguishing infections caused by Mycobacterium tuberculosis (Mtb) from those caused by NTM.

**Keywords:** NTM, MOTT, pulmonary tuberculosis

## INTRODUCTION

Pulmonary tuberculosis (PTB) is still a global health concern and one of the main infectious causes of mortality, second only to HIV/AIDS. According to the World Health Organization (WHO), in 2022, nearly 90 percent of the incidence of PTB increased significantly from 2019 to 2021, with Indonesia contributing the second-most PTB cases in the world.<sup>1</sup>

As one of the endemic countries, the handling of PTB cases in Indonesia is a priority of the government program to take

on case finding as the first step in preventing the spread of active PTB infection in the community. So that there is no underdiagnosis or overlapping between Mycobacterium tuberculosis (Mtb) and Nontuberculous Mycobacteria (NTM) infections, the rapidity and accuracy of the diagnosis of PTB requires consideration of the results of microscopic examination.<sup>1,2</sup>

Pulmonary tuberculosis is a contagious infectious disease microscopically induced by the Mtb complex. *M. tuberculosis*, *M. bovis*, *M.*

*caprae*, *M. africanum*, *M. microti*, *M. canneti* and *M. pinnipedii* make up the Mtb complex group.<sup>2</sup> Based on the National Tuberculosis Control Guidelines, PTB is typically caused by infection with *M. tuberculosis*, *M. africanum*, *M. bovis* and *M. leprae*. Several studies have been conducted to identify additional Mycobacterium species that can induce PTB infection, also known as Mycobacterium Other Than Tuberculosis (MOTT).<sup>2,3</sup>

In countries that are developing, determining and identifying MOTT infection is difficult due to the lack of clinical findings and laboratory diagnostic tools. Typically, the clinical manifestations of MOTT infection are non-specific. Moreover, radiological and clinical characteristics share similarities with PTB. MOTT-infected patients with positive culture results are frequently regarded as contamination and, in some instances, are designated as clinical PTB and given antituberculosis drugs (ATD) without progressive clinical evaluation.<sup>4</sup>

## TAXONOMY OF MYCOBACTERIUM

In 1896, which was Lehmann and Neumann in Germany introduced the genus Mycobacterium, which has been identified as class Actinomycetes, ordo Actinomycetes, family Mycobacteriaceae, genus Mycobacterium. In concert with the development of phenotyping techniques, the taxonomy of Mycobacterium has been modified by employing phylogenomic analysis. There are 190 species in the

genus Mycobacterium and the pathogens are Mtb and *M. leprae*.<sup>5</sup>

The MOTT terminology was superseded by the term NTM Lung Disease in the Tuberculosis Physician Manual Pulmonary in 2022. NTM refers to subspecies of Mycobacterium other than Mtb complex and *M. leprae*. The majority of the 150 species in the NTM classification are nonpathogenic.<sup>6</sup>

Infections caused by NTM are frequently associated with contamination processes in the surrounding environment, with unknown transmission routes. Based on a study conducted by Morimoto et al., NTM infection or reinfection in humans is caused by the same genotype and is transmitted through the air. In addition, the sampling procedure can alter the microscopic results of the Mycobacterium genus, according to the study.<sup>7</sup>

The examination of NTM growth in cultures reveals two distinct processes. First, species can develop rapidly in cultures lasting 7 to 10 days. *M. abscessus complex*, *M. chelonae*, *M. fortuitum*, and *M. mucogenicum* are the different varieties of Mycobacterium species. The second process is slow growth with a culture duration of >14 days. Included in this category of Mycobacterium species are *M. avium complex* (MAC), *M. chimaera* and *M. kansasii*. *M. avium* and *M. intercellulare sp* are MAC group members.<sup>8</sup>

The identification approach of NTM is utilized for accurate diagnosis and treatment. *M. gordonae*, for instance, is a species that rarely causes clinical manifestations and does not necessitate

treatment despite its detection via culture examination. Other species, such as MAC, *M. kansasii*, and *M. abscessus* can induce pulmonary infections and are pathogenic. As described in Table 1, the infection process of NTM involves the lymphatic

system, skin and soft tissues.<sup>6,9,10</sup> There are significant microscopical differences between Mtb and NTM, such as the positivity value on the Acid-Fast Bacilli (AFB) examination.<sup>9</sup>

Table 1. Description and Predilection of Pathogenic NTM<sup>6,10,11</sup>

Organism	Predilection	Description
<i>M. avium complex (MAC)</i>	<ul style="list-style-type: none"> <li>• Lung</li> <li>• Lymphatics</li> </ul>	<ul style="list-style-type: none"> <li>• Existing in all environments.</li> <li>• Lady Windermere Syndrome is prevalent in HIV-infected and elderly individuals, particularly women.</li> </ul>
<i>M. xenopi</i>	Lung	Frequently observed in GERD patients
<i>M. abscessus complex</i>	Lung	<ul style="list-style-type: none"> <li>• Pathogenic.</li> <li>• Resistant to macrolide antibiotics.</li> </ul>
<i>M. kansasii</i>	Lung	<ul style="list-style-type: none"> <li>• Usually found in water pipes.</li> <li>• Adults and the elderly with a history of COPD are frequently infected.</li> </ul>
<i>M. fortuitum</i>	<ul style="list-style-type: none"> <li>• Lung</li> <li>• Skin</li> <li>• Soft tissue</li> </ul>	Typically nonpathogenic, but can cause nosocomial infections.
<i>M. marinum</i>	<ul style="list-style-type: none"> <li>• Skin</li> <li>• Soft tissue</li> </ul>	<ul style="list-style-type: none"> <li>• Forms granulomas in fish tank or pool water (Fish Fancier's Finger).</li> <li>• Capable of surviving and reproducing at temperatures between 27° and 37°C.</li> </ul>
<i>M. ulcerans</i>	<ul style="list-style-type: none"> <li>• Skin</li> <li>• Infections in incisions caused by mycolactone toxin trauma, that include nodule-like, asymptomatic skin efflorescence and ulcers</li> </ul>	<ul style="list-style-type: none"> <li>• Prevalent in subtropical and tropical zones.</li> <li>• Increases throughout the rainy season and flooding.</li> <li>• Children and young adults are frequently affected.</li> </ul>
<i>M. decipiens</i>	<ul style="list-style-type: none"> <li>• Skin lesions</li> <li>• Lymph nodes</li> </ul>	Resulting in synovitis and lymphadenitis
<i>M. shigaense</i>	<ul style="list-style-type: none"> <li>• Skin</li> <li>• Lung</li> <li>• Lymph nodes</li> </ul>	Possible outcomes include lung infection, cutaneous infection, and widespread infection.

## DIAGNOSIS

The diagnosis of NTM infection remains difficult for all medical professionals. According to the ATS/IDSA, the interaction between clinical, radiological, and microbiological criteria can serve as a diagnostic strategy. As radiologic indicators, X-ray examination and High-Resolution Computed Tomography (HRCT) are utilized. While microbiological criteria can be determined through Bronchial Alveolar Lavage (BAL) or sputum examination at two distinct times, BAL examination is preferred.<sup>12</sup>

### Clinical Criteria

Based on its location, the symptoms of NTM infection are divided into Pulmonary NTM and Extrapulmonary NTM. Pulmonary NTM manifestations differ considerably and depend on the severity of infection and other underlying diseases. Pulmonary NTM infection shares clinical similarities with PTB infection, including hemoptysis, shortness of breath, lethargy, and weight loss. However, other symptoms such as fever and nighttime perspiration are uncommon in Pulmonary NTM infection.<sup>6</sup>

The clinical criteria for Pulmonary NTM consist of:<sup>6</sup>

1. NTM due to other underlying diseases
  - a. Bronchiectasis, cystic fibrosis, COPD, and pulmonary tuberculosis are the underlying diseases.
  - b. These characteristics are frequently infected by the pathogens MAC, *M. abscessus* and *M. kansasii*.

2. NTM without other underlying disease
  - a. The majority of clinical manifestations occur in nonsmoking women >50 years without underlying pulmonary disease.
  - b. Progressive cough and persistent shortness of breath are the presenting symptoms.

Based on study results conducted by Lee et al, the prevalence of pulmonary NTM was 58.8% and that of extrapulmonary NTM was 41.2%. The highest incidence of extrapulmonary NTM 35.3%, was found in lymph nodes, followed by skin, lymph nodes and soft tissue.<sup>12</sup> The clinical manifestations of Extrapulmonary NTM infection are frequently found in patients with HIV/AIDS, a history of immunosuppressant drugs, cancer, and organ transplantation.<sup>13</sup>

### Radiologic Criteria

The radiologic examination is one of the modalities of diagnosis of Pulmonary NTM infection. Pulmonary NTM radiologic characteristics are also highly variable and difficult to distinguish from PTB. The recommended radiologic tests are X-ray and HRCT.<sup>6,14,15</sup>

Radiologic criteria are two types, there are:<sup>6</sup>

1. Features of Fibrocavities
  - a. The radiologic characteristics are comparable to post-infection PTB.
  - b. Most of the lesions are located in the superior lobe.
  - c. Frequent complications include progressive fibrosis, atelectasis, and traction bronchiectasis.

- d. Other characteristics include heterogeneous linear and nodular opacities with or without calcification.
2. Features of Nodular Bronchiectasis
- There is multifocal cylindrical bronchiectasis and 1-3 mm centrilobular nodules described as "Tree in bud opacities." Frequently observed in patients with NTM and no underlying disease.
  - It is frequently discovered in the middle lobe of the right lung.
  - It is known as the "Lady Windermere Syndrome" and is more prevalent in elderly women.
- The species of Mycobacterium that causes pulmonary NTM infection may exhibit certain radiological characteristics,

but these characteristics are not considered pathognomonic. Some HRCT images of Mycobacterium species are presented below.

Based on the research conducted by Lee et al, patients with the underlying disease have a significant amount of nodules and opacities measuring >2 cm in diameter, with or without cavities described as "ill-defined nodules". Statistically, there was no significant difference between immunosuppressants and immunocompromised, according to the study's findings, which indicated that the immunocompromised control group differed only in the appearance of poorly defined nodules.<sup>16</sup>

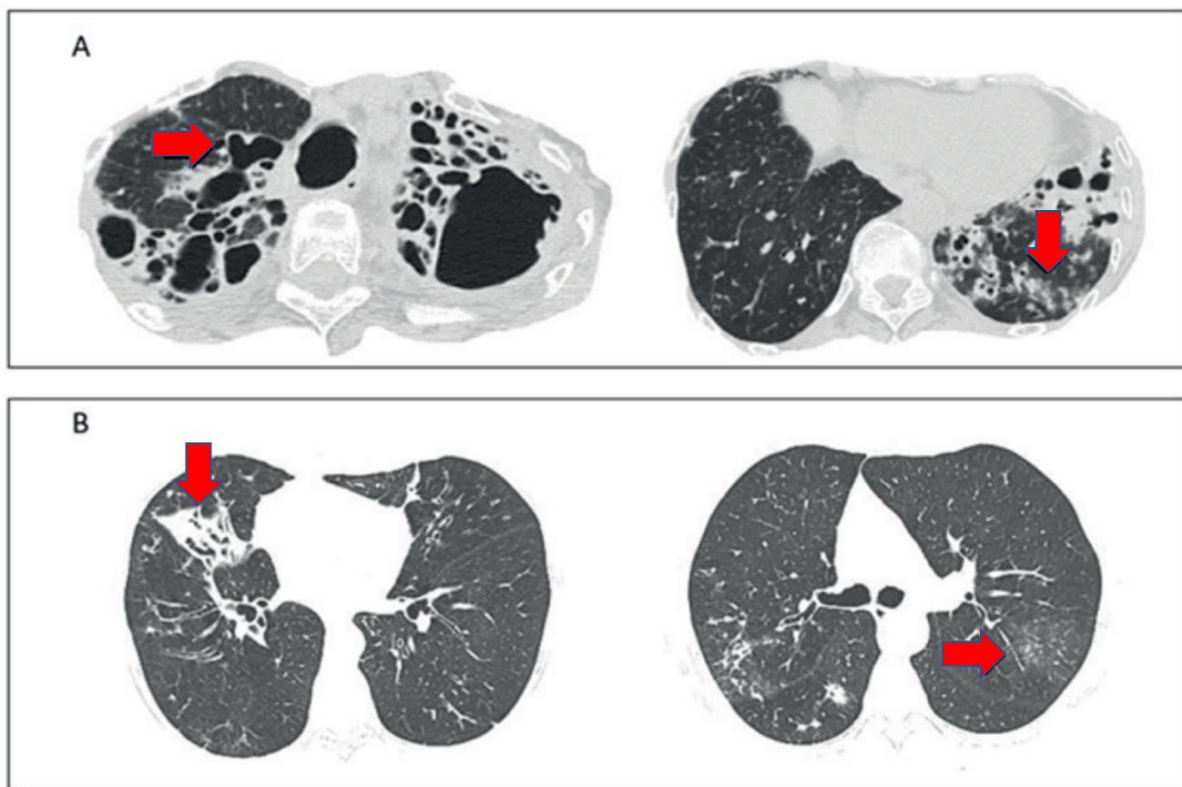


Figure 1. (A) CT scan of an immunocompromised patient infected with *M. asiaticum*; extensive varicose bronchiectasis and parietal bronchial hypertrophy. Centrilobular opacity with characteristics of ground glass. (B) CT scan of a patient infected with *M. avium-intercellulare*; the middle lobe underwent consolidation. Middle lobe and lingual region dominant bronchiectasis with characteristics of ground glass, centrilobular opacity<sup>17</sup>

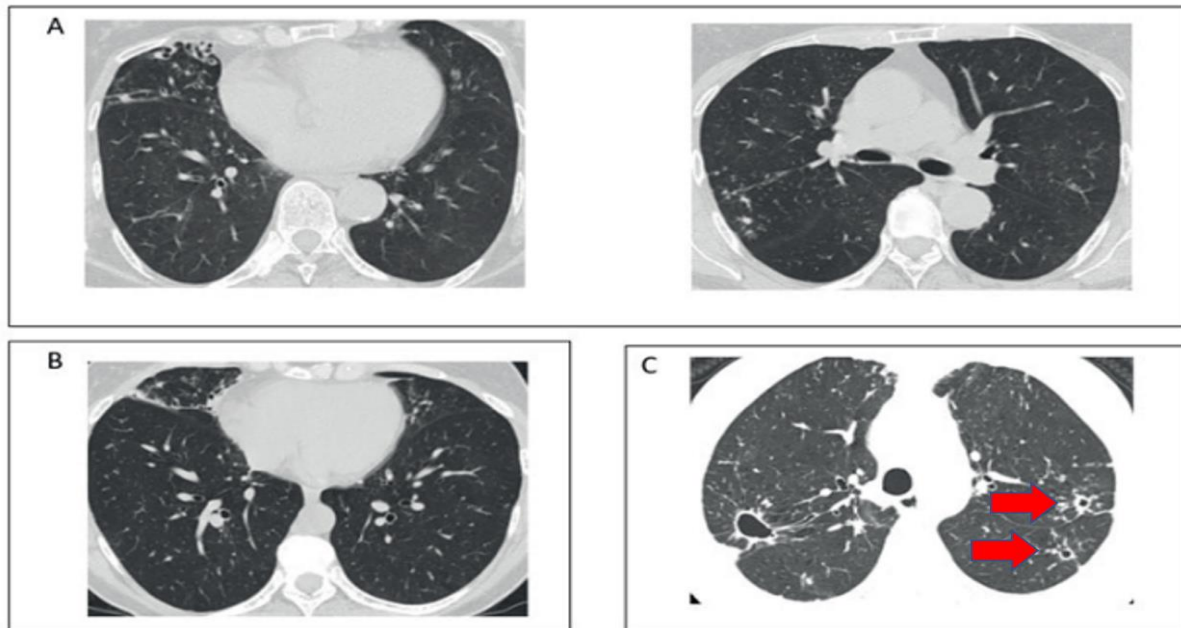


Figure 2. (A) A CT scan of a patient infected with *M. fortuitum*; middle lobe bronchiectasis with micronodules in the right upper lobe. (B) A CT scan of a patient infected with *M. Intercedulare*: middle lobe and lingula bronchiectasis with micronodules. (C) Cavities with solid walls in the upper lobe, micronodules in the centrilobular and "tree in the bud" in the left upper lobe<sup>17</sup>

Fowler et al conducted a cohort study in which culture-positive NTM patients were compared with culture-negative NTM patients and HRCT examination and scoring was performed. The research revealed that there was no significant difference between the two groups' scores.<sup>15</sup>

Based on a study by Bonnet et al, 2/3 of Pulmonary NTM patients show fibrocavities with bronchiectasis of as much as 60% and cavities as high as 40%. These findings suggest that former PTB patients who will develop a post-inflammatory bronchiectasis picture will face similar radiological challenges. From the above studies, it can be concluded that there is no specific picture for Pulmonary NTM infection, but the results can explain that the predilection of lesions that appear on radiology is in the middle lobe and lingula location.<sup>18,19</sup>

### Microbiological Criteria

The majority of Mycobacterium included in the NTM group are nonpathogenic, so sputum examination is continued in the microbiological examination. Patients with clinical non-productive cough may be recommended bronchoscopy as an alternative to radiological images of nodular bronchiectasis for sample collection, as it is considered to have high sensitivity. Sputum induction can be given N-acetylcysteine but has the potential to result in contamination of the results thus affecting the viability of Mycobacterium.<sup>8,20</sup>

Based on the Clinical Laboratory Standards Institute, chlorhexidine can be used as another alternative in sputum induction without disturbing Mycobacterium viability. A study conducted at the Department of Microbiology, Faculty of Medicine, University of Indonesia also

indicates that the use of N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) for sputum induction has a high contamination rate of Mycobacterium culture examination results, resulting in a lower rate of Mycobacterium positivity.<sup>6,21,22</sup>

### 1. Acid Fast Bacilli (AFB)

The morphology of Mycobacterium species is "Acid-fast" and mycolic acid is a long-chain fatty acid (C60 - C90) that is linked to arabinogalactan via glycolipid bonds and to peptidoglycan via phosphodiester bridges. So that Mtb can withstand acid alcohols and carbol fuchsin, the cell wall is composed of mycobacterial sulfolipids and polysaccharides such as arabinogalactan and arabinomannan.<sup>23</sup> In NTM, the sensitivity of BTA examination is considered to be low, leading to the use of

additional examination modalities. Some AFB stains can be seen in Table 2.

### 2. Molecular Method

Molecular methods based on (1) Phenotyping and biochemical characteristics, (2) Genomics, (3) DNA Probe Assay, (4) Species-specific PCR Detection of Mycobacterium, and (5) Whole Genome Sequencing can be used to identify Mycobacterium species.<sup>24</sup>

In-vitro amplification of Deoxyribo Nucleic Acid (DNA) or Ribo Nucleic Acid (RNA) chains is used to evaluate the phenotype of NTM. This method is the basis for conventional Polymerase Chain Reaction (PCR), real-time PCR, PCR restriction fragment length polymorphism analysis, oligonucleotide array and sequencing.<sup>20,25</sup>

Table 4. Type of Sputum Staining<sup>21</sup>

Type of Staining	Description
Ziehl Neelsen	<ul style="list-style-type: none"> <li>All specimen</li> <li>Result: bright red with blue background</li> <li>For all <i>Mycobacterium</i></li> </ul>
Kinyoun	<ul style="list-style-type: none"> <li>Requires a systopin or a solid culture medium</li> <li>Result: bright red</li> <li>Species that can be detected: <i>M. fortuitum</i>, <i>M. chelonae</i>, <i>M. abscessus</i>, MAC</li> </ul>
Rapid Modified Auramine O Fluorescent	<ul style="list-style-type: none"> <li>All specimen</li> <li>Result: orange-yellow or light green with a dark background</li> <li>No coloring occurred on Mycobacterium-rapid grow</li> </ul>
Fite (Gomori-methenamine Silver Stain)	<ul style="list-style-type: none"> <li>The specimen was taken from the soft tissue</li> <li>Result: Pink with blue background</li> <li><i>M. Leprae</i> detected</li> </ul>
Periodic Acid Schiff (PAS) Stain	<ul style="list-style-type: none"> <li>The specimen was taken from the soft tissue</li> <li>Result: Pink</li> <li><i>M. kansasii</i>, MAC, <i>M. ulcerans</i>, <i>M. Chelonei</i></li> </ul>

There are a few methods that can be used, such as:

#### a. PCR

Gene-Xpert MTB/RIF was developed by the WHO to detect Mtb and Rifampicin resistance via the Nucleic Acid Amplification Technique (NAAT). This test is an example of real-time PCR, which gives results within 2 hours.<sup>26</sup> Positive AFB and Gene-Xpert test results can be used as evidence for an NTM infection suspicion. These two tests can enhance the diagnosis, with a sensitivity of 87% for distinguishing Mtb from MAC.<sup>6</sup>

The Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) recommend the use of Gene-Xpert with a positive AFB result as the initial step in the diagnosis of NTM:

Table 5. Diagnosis of Mycobacterium based on AFB and Gene-Xpert<sup>4</sup>

Sputum AFB	Sputum Gene-Xpert	Interpretation
Positive	Positive	Mtb
Positive	Negative	Suspect of NTM
Negative	Positive	Mtb
Negative	Negative	Suspect of non infection

#### b. DNA Probe Assay

*Accu-probe* (Gen-Probe) was the first DNA probe developed to identify Mycobacterium. This Mycobacterium identification test has limitations, as it can only detect *Mtb complex*, *M. avium complex*, *M. kansasii*, and *M. gordonae*.<sup>24</sup>

Line Probe Assay (LPA) was created from the DNA Probe assay of the first generation. LPA examination is comprised of two types: The first, Inno LiPA

Mycobacterium V2 which detects and identifies the genus Mycobacterium and 16 other Mycobacterium species based on 16S-23S rRNA, including Mtb complex, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, *M. xenopi*, *M. chelonae*, *M. gordonae*, *M. fortuitum complex*, *M. malmoense*, *M. genavense*, *M. simiae*, *M. smegmatis*, *M. haemophilum*, *M. marinum*/*M. ulcerans* dan *M. celatum*.<sup>24</sup>

The second type of LPA test is the GenoType Mycobacterium assay, which is used to distinguish between Mtb based on *gyrB gene polymorphism*. GenoType Mycobacterium CM (*Common Mycobacteria*) stimulates the Mtb molecule and 24 NTM species, whereas GenoType Mycobacterium AS (additional species) stimulates the identification of 19 NTM species.<sup>24</sup>

#### c. Loop-Mediated Isothermal Amplification (LAMP)

The LAMP method was created by amplifying DNA polymerase to recognize 6 DNA sequencing. LAMP is a simple, rapid, and cost-effective molecular method with stringent requirements.<sup>27</sup> Positive interpretation in Mtb infection with positive *rpoB* and specific IS6110, but negative interpretation in NTM infection with positive *rpoB* and negative IS6110.<sup>28</sup>

### 3. Culture Test

The culture test is the gold standard for the diagnosis and evaluation of NTM infections. There are 2 sample media for Mycobacterium culture, namely (1) Liquid culture media called Mycobacteria Growth Indicator Tubes (MGIT) with a culture time

of 2 weeks, (2) Solid culture media, namely Lowenstein Jensen (LJ) or egg-based medium containing green malachite to inhibit the growth of other contaminating organisms. Even though neither of these tests has a sensitivity of one hundred percent, they are used together.<sup>6,15,29,30</sup>

Rapidly Growing Mycobacteria (RGM) is a new culture medium for major pathogens, including *M. chelonae*, *M. abscessus* and *M. fortuitum* introduced by Friedmann. RGM media culture is the optimal medium for Rapidly Growing Mycobacteria, while MGIT is optimal for Slowly Growing Mycobacteria and requires a considerable amount of time for pathogen identification.<sup>31,32</sup>

#### 4. Histology Test

The examination of tissue samples is not recommended in the diagnosis of NTM if clinical and microbiological criteria already indicate the presence of NTM. Unlike extrapulmonary NTM, tissue samples are recommended for diagnosis of intrapulmonary NTM. Granulomatous inflammation with or without tissue necrosis, and the presence of NTM organisms, is pathognomonic of NTM on histologic examination. However, granulomatous inflammation is not specific to NTM, so histology examination must be complemented by additional diagnostic methods.<sup>21</sup>

At least one culture examination yielded a positive result for NTM growth. On tissue biopsy, neither granulomatous inflammatory tissue nor NTM organisms were detected in immunocompromised

patients. In this patient population, histologic characteristics included foamy-histiocytes containing mycobacteria, poorly formed granulomas, or without inflammatory tissue.<sup>21,22</sup>

#### 5. Antimicrobial Sensitivity Test

*Clinical Laboratory Standards Institute* (CLSI) and ATS/IDSA has published criteria for sensitivity testing as the gold standard test for determining microbial susceptibility through culture and microdilution culture growth. In general, NTM sensitivity testing is performed on patients with clinically significant NTM isolates.<sup>21</sup>

In addition, CLSI recommends that CLSI examine NTM species that are infrequently pathogenic, such as *M. gordonae*, *M. mucogenicum* atau *M. terrae*. The initial screening sensitivity test is the rifampicin sensitivity test. This is followed by other species, such as *M. kansasii*, if rifampicin resistance is detected. In the macrolide group, it is recommended to choose alternative species. MAC are organisms able to lead to macrolide resistance.<sup>21</sup>

So, it can be concluded that the microbiological criteria include:<sup>30</sup>

- a. Positive sputum culture from two different sputum samples. If the result is negative, the sputum test and culture must be repeated; or
- b. Positive culture of BAL or Bronchial toilet; or
- c. *Transbronchial* or Lung biopsy with Mycobacterial histology (Granulomatous Inflammation or AFB) and a positive NTM culture; or

d. Biopsy shows mycobacterial histology (Inflammatory Granulomatus or AFB) and at least one positive sputum or BAL culture for NTM.

## CONCLUSION

Accurate diagnosis of NTM is a challenge for countries endemic to PTB so that pathogen eradication efforts can be well implemented. Mycobacterium infections other than Mtb complex and *M. leprae* were formerly known as MOTT. The switch from MOTT to NTM terminology and the NTM taxonomy can be used as a clinical reference to distinguish between Mtb and NTM infections. Understanding the taxonomy of Mycobacterium is crucial for determining the subsequent diagnostic step. The selection of diagnostic modalities with high sensitivity and specificity is a primary concern for medical professionals. In expediting diagnostics, examination duration and cost-efficiency are also considered.

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