Isolation Rate and Species Distribution of Non-Tuberculous Mycobacteria From 2014-2021

1Tae Soung Kim, 2Ga Yeon Kim, 3Young Ki Lee and 1Jae Kyung Kim

1Department of Clinical Pathology, Graduate School of Dankook University, Cheonan, Republic of Korea
2Department of Public Health, Graduate School of Dankook University, Cheonan, Republic of Korea
3Department of Biomedical Laboratory Science, College of Health and Welfare, Dankook University Cheonan, Republic of Korea

Abstract: In Korea, the incidence of Mycobacterium tuberculosis infections has decreased; however, the incidence of lung diseases attributed to Non-Tuberculous Mycobacteria (NTM) is increasing. This study aimed to elucidate the isolation rate and distribution of NTM recently isolated at a Korean test center. Respiratory samples for acid-fast bacilli culture collected between January 2014 and December 2021 at a test center in Yongin-si were targeted. Acid-fast bacilli solid and liquid media culture and NTM identification tests were conducted. In NTM cultures, the positivity rate was increased in solid (3.7%) and liquid (5.3%) media in 2021, compared to that observed in 2014. NTM identification tests increased from 922 in 2014 to 3,385 in 2018 and 4,058 in 2021. The average NTM infection age increased from 64.7 years in 2014 to 68.5 years in 2021. NTM infections among those aged ≥61 years were substantially increased. The most frequently detected NTM species was Mycobacterium intracellulare (40.9%), followed by Mycobacterium avium (23.1%), with Mycobacterium avium complex considered dominant. The results suggest that NTM management in older individuals is necessary. We also confirmed the age and species-specific isolation rates and distribution of NTM, which can be helpful for NTM identification and NTM drug susceptibility testing. Moreover, it will be able to guide antibacterial agent selection and treatment.

Keywords: Mycobacterium avium Complex, Mycobacterium intracellulare, Mycobacterium tuberculosis, Non-Tuberculous Mycobacteria

Introduction

With the improving domestic environment and economy, the prevalence of Mycobacterium Tuberculosis (MTB) infections is decreasing, whereas that of Non-Tuberculous Mycobacteria (NTM) infections is increasing (Kim et al., 2021; Ahn et al., 2021). Therefore, NTM identification is of growing clinical importance. Among NTM, Mycobacterium Avium Complex (MAC) was the most prevalent from 2016-2020 (Kim et al., 2023). In areas with a high prevalence of Tuberculosis (TB), such as Korea, MAC lung disease and methods for its prevention should be considered. Attention should be paid to pneumonic disease groups with a high proportion of MAC (MAC/M. intracellulare, M. avium) (Bom et al., 2021; Kwon et al., 2007).

The clinical pattern of NTM infection is similar to that of MTB; thus, patients could be suspected of having TB and referred to hospitals (Khandelwal et al., 2022). In recent years, the incidence of persistent NTM lung infections has steadily increased, which is mainly due to an aging population with underlying lung disease and an expansion in the patient population receiving immunosuppressants (Grammegna et al., 2022). In Korea, with improvements in the environment, the incidence of MTB has decreased; however, the incidence of lung diseases attributed to NTM is increasing. This corresponds to the global increase in reported NTM infection cases (Kim et al., 2021; WHO, 2022).

NTM are capable of widespread infections and are opportunistic waterborne pathogens that can easily aerosolize in home shower heads, faucets, and hot tubs, causing respiratory infection (Falkinham, 2016). In patients with NTM, M. avium (49.3%) is the most commonly identified strain, followed by M. intracellulare (32.0%) and M. abscessus (12.7%) (Park et al., 2021). In
Korea, the incidence of MTB infections has decreased; however, the incidence of lung diseases attributed to NTM is increasing. This study aimed to investigate the isolation rate and distribution pattern of NTM recently isolated in domestic testing facilities to aid in the treatment and management of NTM infections and NTM drug susceptibility testing.

**Materials and Methods**

Respiratory samples for Acid-Fast Bacilli (AFB) culture collected between January 2014 and December 2021 at a referral testing center (Yongin-si in Korea) were targeted. Considering that testing for NTM is performed nationwide. The AFB solid and liquid media culture tests were performed in 317,755 (NTM, 9,873) solid media and 215,958 (NTM, 10,617) liquid media samples. In addition, 17,918 cases confirmed as NTM and age-confirmed in the AFB culture test and 19,146 cases whose age was confirmed in the NTM strain identification test were analyzed.

For AFB cultures, depending on the culture conditions of the medium manufacturer, specimens were decontaminated using 3% N-acetyl-L-cysteine-NaOH and inoculated onto both solid media (3% Ogawa medium, Shinyang Chemical, Seoul, Korea) and in liquid media (BD BBL Mycobacterial growth indicator tubes, Becton Dickinson and Co., Sparks, MD, USA). The solid media cultures were incubated for 8 weeks (36±1°C) and the liquid media cultures were incubated for 6 weeks (36.5±1.5°C). MTB and NTM were discriminated by performing TB antigen (TB Ag MPT64, Abbott, Yongin, Korea) and MTB/NTM real-time polymerase chain reaction (AdvanSure Nucleic Acid Kit/LG Cham, Cheongju, Korea) tests when the AFB multiplied. NTM were identified using a line probe assay (MolecuTech REBA Myco-ID, YD diagnostics, Yongin city, Korea). A hybridization reaction is performed between a single product strand amplified by biotin-labeled primers and a specific probe of the rpoB gene region attached to the membrane.

The test results of different samples from the same patient were not considered for analysis. However, considering that testing for NTM is performed nationwide, NTM cases were analyzed as secondary data using IBM SPSS Statistics (version 29.0; IBM Corp., Armonk, NY, USA) using arithmetic statistics by species, year, culture medium, and age.

This study was approved by the Institutional Review Board (IRB) of Dankook University (IRB No. DKU 2022-12-001) and was conducted by the principles of the declaration of Helsinki. Medical data for statistical examinations were used retrospectively. Patient data were anonymized and no patient personal information was used; hence, the requirement of obtaining informed consent was waived by the IRB of Dankook University.

**Results**

**Distribution of NTM by Age**

In this study, the most frequently detected NTM were *M. intracellulare* (40.9%), followed by *M. avium* (23.1%) and *M. abscessus* (7.9%). The average age of infected individuals was 70.2, 66.1, and 64.4 years for *M. intracellulare*, *M. avium*, and *M. abscessus*, respectively. A greater positivity rate increase occurred in those aged ≥71 years. Among NTM, MAC was the predominant group of *Mycobacteria* (Figs. 1-2).

**Distribution of Mycobacteria by Medium**

NTM of infected persons, from 2014-2021, was analyzed in liquid and solid culture media. The positivity rate in liquid and solid culture media for MTB cultivation decreased from 2014-2021. The positivity rate of NTM increased from 3.5% in 2014 to 5.3% in 2021 in liquid media and from 2.1% in 2014 to 3.7% in 2021 in solid media (Table 1).

**Distribution of Mycobacteria by Year**

In this study, NTM identification tests increased from 922 in 2014 to 3,385 in 2018 and 4,058 in 2021, of which *M. intracellulare* was most frequently detected.

<table>
<thead>
<tr>
<th>Year</th>
<th>Liquid medium</th>
<th>Solid medium</th>
<th>Liquid medium</th>
<th>Solid medium</th>
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NTM, Non-Tuberculous *Mycobacteria*; MTB, *Mycobacterium Tuberculosis*
Table 2: Detection rate of Non-Tuberculous Mycobacteria (NTM) by strains from 2014-2021

<table>
<thead>
<tr>
<th>NTM species</th>
<th>2014 Detected %</th>
<th>2015 Detected %</th>
<th>2016 Detected %</th>
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<th>2018 Detected %</th>
<th>2019 Detected %</th>
<th>2020 Detected %</th>
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<td>88</td>
<td>2.6</td>
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*MAC: Mycobacterium abscessus complex, *more than two types of infections, *cannot distinguish species

Fig. 1: Comparison of Mycobacteria positivity rates from 2014-2021. NTM, Non-Tuberculous Mycobacteria

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4.3 Mycobacterium species
6.9 Multiple NTM
6.0 M. ulcerans/M. marinum
0.3 M. terrae/M. nonchromogenicum
0.1 M. szulgai
0.2 M. simiae/M. genavense
0.1 M. scrofulaceum
0.5 M. mucogenicum
0.3 M. massilense
0.5 M. kansasii
0.5 M. gordonae
4.5 M. fortuitum complex
0.0 M. flavescens
1.4 M. chelonei
0.1 M. celatum
23.1 M. avium
0.1 M. auragnum
0.1 M. abscessus
7.9 M. intracellular
40.9 M. intracellular

Positive rate by strain (%) | Average age

323
isolated NTM (71.0%) (Okoi et al., 2022). The positivity rates of the most frequently detected NTM in this study were 40.9% for *M. intracellulare*, 23.1% for *M. avium*, and 7.9% for *M. abscessus*. This differed from the rates reported by Koh et al. (2006), in which *M. abscessus* was dominant; however, the previous study performed a smaller number of tests, which is different from this study. The mean ages for the onset of *M. intracellulare*, *M. avium*, and *M. abscessus* infections were 70.2, 66.1, and 64.4 years, respectively, which was consistent with previous findings.

The incidence of MAC infection in humans is increasing, with the lungs being the most common site of infection (Park et al., 2021; Kaczmarskwska et al., 2022). MAC is the most commonly isolated NTM worldwide; however, the prevalence of other strains varies by country and region (Hoefsloot et al., 2013). NTM MAC was most frequently isolated from individuals aged ≥61 years, whereas the increase in the positivity rate was greater in those aged ≥71 years than in those aged ≥61 years. As the age of individuals infected with NTM increases, the importance of managing lung diseases attributed to NTM is expected to grow. Furthermore, the average age of infected individuals with NTM in 2014 was 64.7 years, increasing to 68.5 years in 2021. Although more single NTM infections than multiple infections were observed in the same person, the average age of the infected individuals was higher in those with multiple infections (Ahn et al., 2021). In a previous study (Ahn et al., 2021), the number of NTM isolates increased continuously during the study period and the *M. intracellulare* ratio significantly increased in patients ≥70 years old, showing a similar pattern to the present study.

According to a study in 2021, the NTM isolation rate was 3.89% in broth and 229 (39.8%) of 575 isolates were NTM in *Mycobacterial Growth Indicator Tubes* (MGIT; liquid media (BD BBL Mycobacterial growth indicator tubes, Becton Dickinson and Co., Sparks, MD, USA). The liquid media cultures were incubated for 6 Weeks (36.5±1.5°C) (Okoi et al., 2022). In this study, from 2014-2021, NTM was subjected to analysis in culture media. The positivity rate of NTM increased from 3.5% in 2014 to 5.3% in 2021 in liquid media and from 2.1% in 2014 to 3.7% in 2021 in solid media. Therefore, as the positivity rate of NTM increased in both solid and liquid culture media, it appears imperative to utilize both methods.

According to a recent study on AFB, the isolation rate of NTM among positive AFB isolated from the entire respiratory tract increased from 38.0% in 2010 to 72.4% in 2019 and the isolation rate of TB increased from 62.0% in 2010 to 42.4% in 2016 (Baek, 2020). The rate of TB infection was high in males and females, older adults, and especially males aged ≥85 years. Males and females aged ≥65 years accounted for 36.8 and 28.9% of tuberculosis

![Image](60x362 to 283x513)

**Fig. 2:** Distribution of Non-Tuberculous *Mycobacteria* (NTM) strains by age of infected persons from 2014-2021

![Image](60x555 to 283x725)

**Fig. 3:** Comparison of average age of *Mycobacteria* infected persons from 2014-2021. NTM, Non-Tuberculous *Mycobacteria*; single NTM, infection with one species; multiple NTM, infection with more than one species

The average age of infected individuals was 66.1 years for MTB, 66.3 years for single NTM, and 71.0 years for multiple NTM infections. The average age of multiple NTM infections was high and continued to increase (Table 2, Fig. 3).

**Discussion**

Among 299 patients with sputum-positive samples in AFB smears and cultures, *M. tuberculosis* was isolated from 229 (76.6%) cases, and MAC (*M. avium* and *M. intracellulare*) and *M. abscessus* accounted for 54.3% and 37.1% of cases among 70 isolated NTM cases (23.4%), respectively (Koh et al., 2006). According to a recent study, among patients diagnosed with NTM infection, MAC (66.8%) and *M. abscessus* (17.6%) were the most commonly detected species (Tsai et al., 2022). In a West African country, MAC was also the most frequently
infection cases, respectively (Lu et al., 2022). The MTB isolation rate decreased and the NTM isolation rate gradually increased, demonstrating an increasing trend from 39.9% in 2016 to 57.2% in 2020 (Kim et al., 2023). In this study, NTM identification tests increased from 922 in 2014 to 3,385 in 2018 and 4,058 in 2021, which is consistent with the results of previous studies.

In the United States, the incidence and prevalence of NTM lung disease was 67.6% in women aged 69 years (Winthrop et al., 2020). In Indonesia, 21.3% of cultures were positive for Mycobacteria, of which 85% were MTB and 15% were NTM; 72% of NTM-positive patients were aged ≥35 years (Saptawati et al., 2019). Old age is a major determinant of disease severity and host susceptibility to a number of lung infections, including NTM. However, the age-related mechanism has not been elucidated (Rhoades et al., 2022). In this study, the average age of infected individuals increased from 64.7 years in 2014 to 67.0 years in 2018 and 68.5 years in 2021. Furthermore, the average age at infection in 2021 was 66.1 years for MTB, 66.3 years for single NTM, and 71.0 years for multiple NTM infections. Therefore, the average age at infection for multiple NTM is high and continues to increase.

In this study, the analysis of the test results by sex, age, and year of NTM may not have been truly representative when the presence of duplicate patients or repeat tests was not ruled out. However, considering that testing for NTM is performed nationwide, the results confirmed the isolation rate and bacterial species distribution by age and species. The findings may be helpful for future NTM identification or antimycobacterial susceptibility testing, as well as for continuous management of NTM infections, particularly among older individuals. Additionally, if selective treatment and management of NTM, which has a high prevalence, becomes possible, it will positively impact public health.

Conclusion

The most frequently detected NTM species was M. intracellulare, followed by M. avium, with complex considered dominant. NTM infections among those aged ≥61 years were substantial. NTM management in older individuals is necessary and these results confirmed the age and species-specific isolation rates and distribution of NTM, which can facilitate NTM identification and NTM drug susceptibility testing. Additionally, further research is required to design strategies for managing disease recurrence to reduce the risk of these diseases in the future.

Acknowledgment

We acknowledge the support of the employees of our testing center in Yongin, South Korea, for helping us to access tuberculosis test data. The data used in this study were obtained from Seoul clinical laboratories (https://www.sclab.co.kr); owing to the company’s policy, we are unable to make the data publicly available.

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Author’s Contributions

Tae Soung Kim and Ga Yeon Kim: Made substantial contributions to and conception and design of the study. These authors contributed equally to this study.

Young Ki Lee and Jae Kyung Kim: Made substantial contributions and acquisition and analysis of the data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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