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

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

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

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Corresponding Author:
Jae Kyung Kim
Department of Biomedical Laboratory Science, College of Health and Welfare, Dankook University Cheonan, Republic of Korea
Email: nerowolf@naver.com

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 (Gramegna *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 1: Positivity rate (%) of *Mycobacteria* by culture medium from 2014-2021

	NTM		MTB	
	Liquid medium	Solid medium	Liquid medium	Solid medium
2014	3.5	2.1	8.3	5.9
2015	4.3	2.5	6.6	5.3
2016	5.2	3.5	6.8	4.9
2017	5.2	3.1	7.0	4.3
2018	5.5	3.4	5.5	3.3
2019	4.9	3.3	5.2	3.3
2020	4.7	3.0	5.3	4.3
2021	5.3	3.7	6.0	4.7
Overall	4.8	3.1	6.3	4.5

NTM, Non-Tuberculous *Mycobacteria*; MTB, *Mycobacterium Tuberculosis*

Table 2: Detection rate of Non-Tuberculous *Mycobacteria* (NTM) by strains from 2014-2021

	2014		2015		2016		2017		2018		2019		2020		2021		Total		Average age at infection
NTM species	Detected	%	Detected	%	Detected	%	Detected	%	Detected	%	Detected	%	Detected	%	Detected	%	Detected	%	
M. abscessus[b]	80	8.7	91	8.4	94	7.0	203	8.3	168	5.0	323	8.7	320	8.7	359	8.8	1,638	7.9	64.4
M. aubagnense							2	0.1	11	0.3							13	0.1	72.7
M. avium[a]	120	13.0	128	11.8	161	12.0	539	21.9	862	25.5	922	24.8	955	26.0	1,087	26.8	4,774	23.1	66.1
M. celatum	1	0.1	3	0.3			3	0.1	2	0.1	1	0.0	2	0.1	2	0.0	14	0.1	64.6
M. chelonae	12	1.3	20	1.8	29	2.2	34	1.4	47	1.4	70	1.9	40	1.1	31	0.8	283	1.4	65.3
M. flavescens	1	0.1															1	0.0	74.0
M. fortuitum complex	39	4.2	47	4.3	97	7.2	130	5.3	157	4.6	175	4.7	142	3.9	137	3.4	924	4.5	65.3
M. gordonae	36	3.9	48	4.4	62	4.6	62	2.5	77	2.3	109	2.9	113	3.1	97	2.4	604	2.9	64.4
M. intracellulare[a]	482	52.3	557	51.4	609	45.3	960	39.1	1,347	39.8	1,371	36.8	1,418	38.5	1,705	42.0	8,449	40.9	70.2
M. kansasii	29	3.1	37	3.4	97	7.2	87	3.5	88	2.6	100	2.7	91	2.5	75	1.8	604	2.9	57.7
M. massiliense[b]							80	3.3	201	5.9	174	4.7	174	4.7	161	4.0	790	3.8	65.6
M. mucogenicum							17	0.7	15	0.4	32	0.9	20	0.5	12	0.3	96	0.5	69.2
M. scrofulaceum							2	0.1	3	0.1	3	0.1	4	0.1	2	0.0	14	0.1	68.5
M. simiae/M.genavense						0.0					6	0.2	18	0.5	24	0.6	48	0.2	64.7
M. szulgai			2	0.2	3	0.2	10	0.4	10	0.3	2	0.1	2	0.1	1	0.0	30	0.1	61.2
M. terrae/																			
M. nonchromogenicum	4	0.4	4	0.4	15	1.1	7	0.3	4	0.1	10	0.3	13	0.4	6	0.1	63	0.3	69.5
M. ulcerans/M. marinum		0.0		0.0							1	0.0	2	0.1	4	0.1	7	0.0	63.6
Multiple NTM[c]	75	8.1	86	7.9	141	10.5	247	10.1	278	8.2	215	5.8	189	5.1	188	4.6	1,419	6.9	67.5
Mycobacterium species[d]	43	4.7	61	5.6	37	2.8	73	3.0	115	3.40	211	5.7	177	4.8	167	4.1	884	4.3	64.4
Total	922		1,084		1,345		2,456		3,385		3,725		3,680		4,058		20,655	100.0	

[a]*M. Avium* Complex (MAC), [b]*Mycobacterium abscessus* complex, [c]more than two types of infections, [d]cannot distinguish species

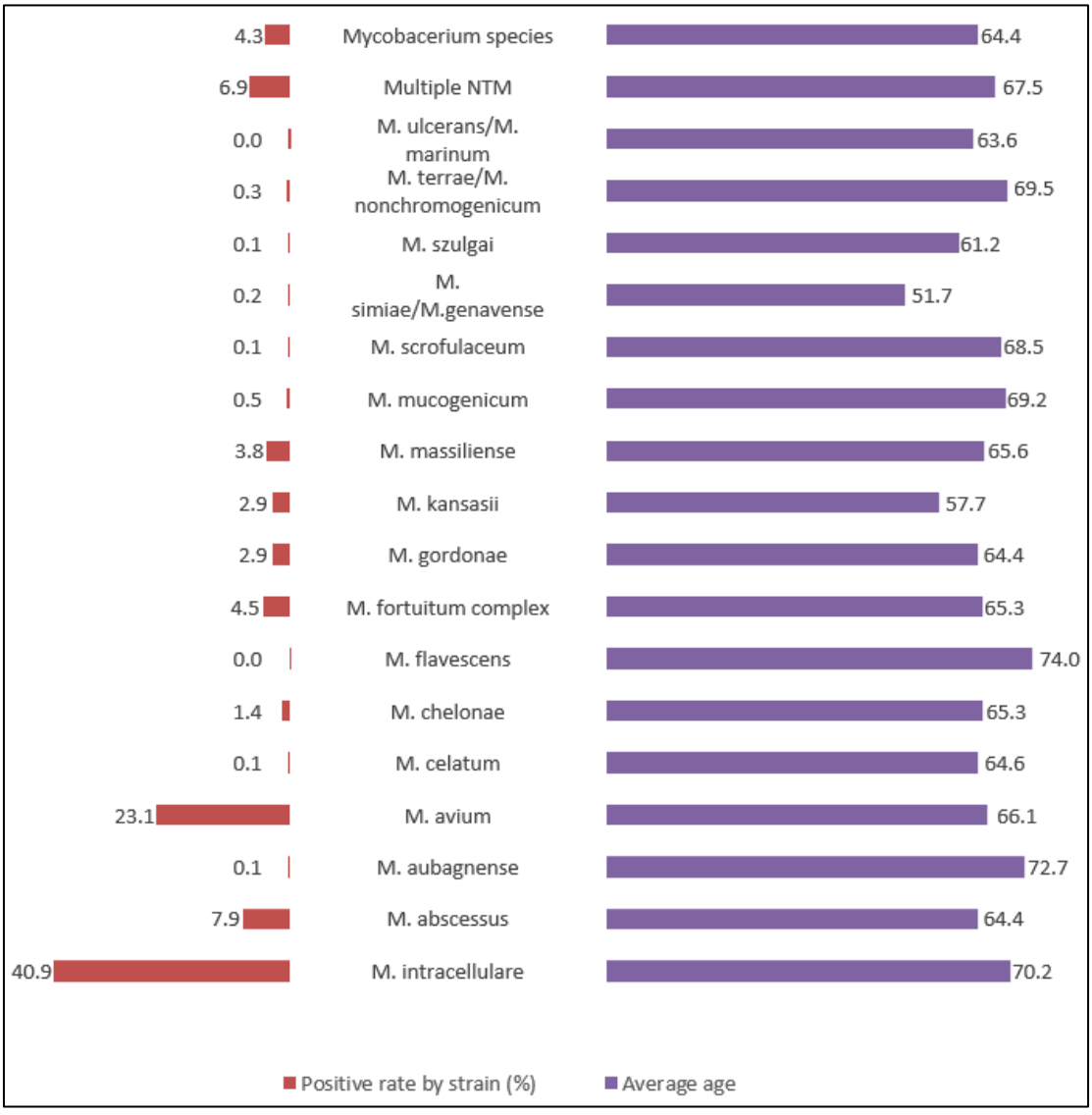

Fig. 1: Comparison of *Mycobacteria* positivity rates from 2014-2021. NTM, Non-Tuberculous *Mycobacteria*

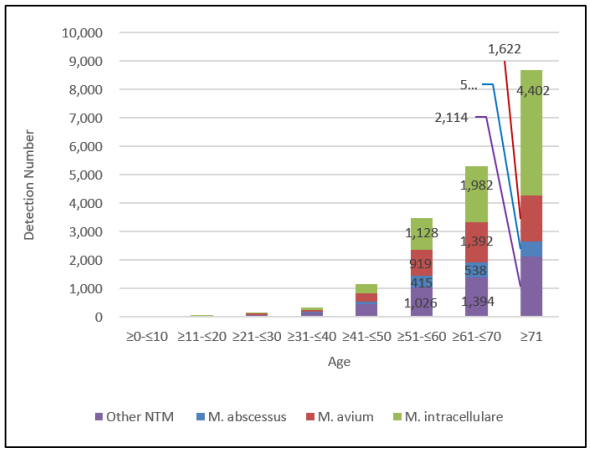

Fig. 2: Distribution of Non-Tuberculous *Mycobacteria* (NTM) strains by age of infected persons from 2014-2021

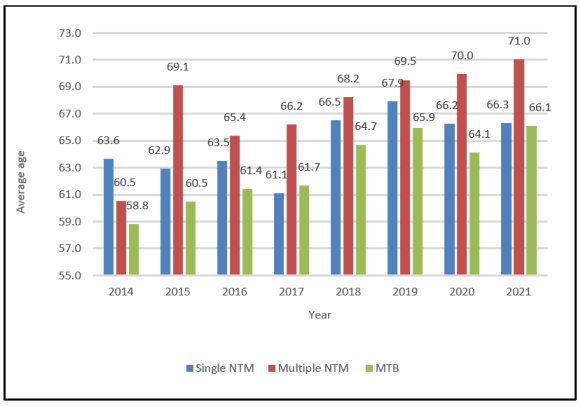

Fig. 3: Comparison of average age of *Mycobacteria* infected persons from 2014-2021. NTM, Non-Tuberculous *Mycobacteria*; single NTM, infection with one species; multiple NTM, infection with more than one species

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 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; Kaczmarkowska *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

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.

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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.

References

Ahn, K., Kim, Y. K., Hwang, G. Y., Cho, H., & Uh, Y. (2021). Continued upward trend in non-tuberculous *Mycobacteria* isolation over 13 years in a tertiary care hospital in Korea. *Yonsei Medical Journal*, *62*(10), 903. 10.3349/ymj.2021.62.10.903

Baek, H. G. (2020). Study on the prevalence of lung disease of *non-tuberculosis Mycobacterium* isolated from respiratory specimens in Gwangju Second Hospital over the last 10 years. *Korean Journal of Clinical Laboratory Science*, *52*(4), 349-355. https://doi.org/10.15324/kjcls.2020.52.4.349

Bom, L., Lee, N. Y., & Kim, D. J. (2021). Changes in the isolation rates and distribution patterns of non-tuberculous *Mycobacteria* at the university hospital of Korea. *Journal of the Korea Academia-Industrial Cooperation Society*, 22 (11): 219-227. https://doi.org/10.5762/KAIS.2021.22.11.219

Falkinham, J. O. (2016). Current epidemiologic trends of the Nontuberculous *Mycobacteria* (NTM). *Current Environmental Health Reports*, *3*, 161-167. https://doi.org/10.1007/s40572-016-0086-z

Gramegna, A., Lombardi, A., Lorè, N. I., Amati, F., Barone, I., Azzarà, C., ... & Blasi, F. (2022). Innate and adaptive lymphocytes in non-tuberculous *Mycobacteria* lung disease: A review. *Frontiers in Immunology*, *13*, 927049. https://doi.org/10.3389/fimmu.2022.927049

Hoefsloot, W., Van Ingen, J., Andrejak, C., Ängeby, K., Bauriaud, R., Bemer, P., ... & Wagner, D. (2013). The geographic diversity of nontuberculous *Mycobacteria* isolated from pulmonary samples: An NTM-NET collaborative study. *European Respiratory Journal*, *42*(6), 1604-1613. https://doi.org/10.1183/09031936.00149212

Kaczmarkowska, A., Didkowska, A., Kwiecień, E., Stefańska, I., Rzewuska, M., & Anusz, K. (2022). The *Mycobacterium avium* complex-an underestimated threat to humans and animals. *Annals of Agricultural and Environmental Medicine*, *29*(1). https://doi.org/10.26444/aaem/136398

Khandelwal, S., Malhotra, B., Dubey, W., & Bhargava, S. (2022). Characterization of Non-tuberculous *Mycobacteria* by Line Probe Assay Isolated from Pulmonary *tuberculosis* Suspected Patients at Rajasthan. *Journal of Pure and Applied Microbiology*, *16*(2). https://doi.org/10.22207/JPAM.16.2.17

Kim, J., Ju, H. J., Koo, J., Lee, H., Park, H., Song, K., & Kim, J. (2021). Distribution and Antimicrobial Resistance of Non-Tuberculous *Mycobacteria* during 2015~ 2020: A Single-Center Study in Incheon, South Korea. *Korean Journal of Clinical Laboratory Science*, *53*(3), 225-232. https://doi.org/10.15324/kjcls.2021.53.3.225

Kim, K. J., Oh, S. H., Jeon, D., & Chang, C. L. (2023). Isolation and Antimicrobial Susceptibility of Nontuberculous *Mycobacteria* in a Tertiary Hospital in Korea, 2016-2020. *Tuberculosis and Respiratory Diseases*, *86*(1), 47. https://doi.org/10.4046/trd.2022.0115

Koh, W. J., Yu, C. M., Suh, G. Y., Chung, M. P., Kim, H., Kwon, O. J., ... & Lee, K. S. (2006). Pulmonary TB and NTM lung disease: Comparison of characteristics in patients with AFB smear-positive sputum. *The International Journal of Tuberculosis and Lung Disease*, *10*(9), 1001-1007. https://www.ingentaconnect.com/content/iuatld/ijtld/2006/00000010/00000009/art00011

Kwon, Y. S., Koh, W. J., Chung, M. P., Kwon, O. J., Lee, N. Y., Cho, E. Y., ... & Kim, B. T. (2007). Solitary pulmonary nodule due to *Mycobacterium intracellulare*: The first case in Korea. *Yonsei Medical Journal*, *48*(1), 127-130. DOI:10.3349/ymj.2007.48.1.127

Lu, Y., Cai, G., Liu, Y., He, F., & Aoyagi, K. (2022). Epidemiological features of *tuberculosis* infection in a rural prefecture of Japan from 2007-2018. *Scientific Reports*, *12*(1), 13511. https://doi.org/10.1038/s41598-022-17608-y

Okoi, C., Anderson, S. T., Mulwa, S., Worwui, A., Antonio, M., Gehre, F., & Adetifa, I. (2022). Pulmonary non-tuberculous *Mycobacteria* in colonisation and disease in The Gambia. *Scientific Reports*, *12*(1), 19523. https://doi.org/10.1038/s41598-022-22777-x

Park, Y., Kwak, S. H., Yong, S. H., Lee, S. H., Leem, A. Y., Kim, S. Y., ... & Kang, Y. A. (2021). The association between behavioral risk factors and nontuberculous *Mycobacterial* pulmonary disease. *Yonsei Medical Journal*, *62*(8), 702. https://doi.org/10.3349/ymj.2021.62.8.702

Rhoades, N., Davies, M. H., Kohama, S., Bermudez, L., Winthrop, K. L., Mattison, J., ... & Messaoudi, I. (2022). Rhesus Macaque Model of Pulmonary Aging and Non-tuberculous *Mycobacterium* Infection Reveals Age Related Mechanisms of Disease Severity. In *A97. Research Outside of the NTM Box* (pp. A2155-A2155). American Thoracic Society. https://doi.org/10.1164/ajrccm-conference.2022.205.1_MeetingAbstracts.A2155

Saptawati, L., Kusumo, H., & Suryawati, B. (2019). Prevalence of Non-Tuberculous *Mycobacteria* (NTM) in Surakarta, Indonesia: Higher Than Expected. *KnE Life Sciences*, 132-138. https://doi.org/10.18502/kls.v4i12.4166

Tsai, Y. V., Derrick, C., Yunusa, I., Weissman, S., Al-Hasan, M. N., Justo, J. A., & Bookstaver, P. B. (2022). Epidemiology, Outcomes and Tolerability of Protracted Treatment of Nontuberculous *Mycobacterial* Infections at a Community Teaching Hospital in the Southeastern United States. *Antibiotics*, *11*(12), 1720. https://doi.org/10.3390/antibiotics11121720

Winthrop, K. L., Marras, T. K., Adjemian, J., Zhang, H., Wang, P., & Zhang, Q. (2020). Incidence and prevalence of nontuberculous *Mycobacterial* lung disease in a large US managed care health plan, 2008-2015. *Annals of the American Thoracic Society*, *17*(2), 178-185. https://doi.org/10.1513/AnnalsATS.201804-236OC

WHO. (2022). Global *tuberculosis* report 2022. *World Health Organization.* https://www.who.int/publications/i/item/9789240061729