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Trends in the Isolation Rates and Species Distribution of Mycobacteria from 2014 to 2021 at Referral Clinical Laboratories in South Korea

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Abstract

We aimed to investigate the proportions of MTB- and NTM-positive tests and the distribution patterns of species isolated by contracted testing agencies in South Korea. Respiratory specimens submitted to contracted testing agencies in South Korea for AFB culture from January 2014 to December 2021 were included (533,713 specimens in total). Trends based on MTB and NTM detection, patient sex and age, culture medium type, and testing year were analyzed. MTB and NTM positive detection increased in the patients. The average ages of MTB- and NTM-positive patients increased in those aged ≥61 years. For solid culture, the MTB detection rate decreased from 5.9% in 2014 to 3.3% in 2018 and increased to 4.7% in 2021; the NTM detection rate increased from 2.1% in 2014 to 3.4% in 2018 and 3.7% in 2021. For liquid culture, the MTB detection rate decreased from 8.3% in 2014 to 5.5% in 2018 and increased to 6.0% in 2021; the NTM detection rate increased from 3.5% in 2014 to 5.5% in 2018 and decreased to 5.3% in 2021. An isolation ratio reversal between MTB and NTM was observed in 2018. In this study, we provide information on mycobacterial isolation rates and species distributions using AFB culture test results from Korea's referral laboratories. Increased MTB- and NTM-isolation rates were observed in individuals aged ≥60 years, indicating the need for regular testing and focused management for them. Expanding liquid culture applications, which show higher positivity rates than solid culture methods, is necessary.

Keywords: *Liquid medium, Mycobacteria,* Mycobacterium tuberculosis, *Nontuberculous mycobacteria, Solid medium*

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1. INTRODUCTION

Tuberculosis (TB) is a global health concern, affecting 6 million male and 3.4 million female individuals, of which 1.2 million are children. Between 2020 and 2021, the annual incidence rate of new TB cases per 100,000 individuals increased by 3.6%. The World Health Organization has set a goal of eliminating TB by 2035, with targets of reducing TB mortality by 95%, TB incidence by 90%, and the proportion of households facing catastrophic costs incurred by TB by 100% [1,2].

Nontuberculous mycobacteria (NTM) are mainly found in water and the surrounding environment, and their isolation from clinical specimens does not necessarily indicate a disease; however, NTM can cause pulmonary diseases in both immunocompromised and healthy individuals. Currently, NTM infections are reported worldwide [2], with the incidence of pulmonary diseases caused by steadily increasing NTM [3]. In contrast, the incidence of pulmonary TB caused by *Mycobacterium tuberculosis* (MTB) is decreasing. In South Korea, the rates of MTB and NTM isolated from respiratory specimens are increasing; additionally, an increasing trend in NTM detection from 2016 to 2020 has been reported [4]. As MTB is transmitted through close social interactions [5], the ecological diversity of MTB has been underestimated [6]. The incidence of MTB infection in the Moravia and Silesia regions (Czech Republic) was 1.97 per 100,000 inhabitants, and the proportion of infected adult men (aged 15–64) was higher for MTB than that for NTM. However, roughly an equal number of MTB and NTM cases were recorded in men and women older than 65 years [7].

It is necessary to distinguish between MTB and NTM during testing. Acting as opportunistic pathogens, NTM can be easily aerosolized in humidified environments, such as public bathhouses [8], hospital showerheads [9], and household faucets and aerators, further causing pulmonary infections and hypersensitivity pneumonitis [10]. In a study at Odense University Hospital, 172 infected patients who visited the hospital with symptoms suggestive of MTB or NTM infection were tested; chronic pulmonary aspergillosis was not identified in patients infected with MTB, and the incidence rate in patients with NTM infection increased to 8.2%. Given that the clinical importance and treatment policies differ depending on the strain, accurate strain identification is important [11]. In this study, we aimed to investigate the rates of MTB and NTM isolation in culture tests performed by South Korean referral testing centers and the distribution patterns of different species.

2. EXPERIMENTS

We analyzed the test results from respiratory specimens that were submitted for acid-fast bacillus (AFB) culture within referral testing centers in Yongin-si, South Korea, from January 2014 to December 2021. Figure 1 shows that this study included a total of 317,755 AFB cultures on a solid medium (14,118 MTB and 9,873 NTM) and 215,958 AFB cultures in a liquid medium (13,313 MTB and 10,617 NTM), which were examined for growth. For the AFB cultures, specimens were decontaminated using concentrated 3% N-acetyl-L-cysteine–NaOH and inoculated both onto solid media (3% Ogawa medium; Shinyang Chemical, Seoul, Korea) and into liquid media (BD BBL mycobacterial growth indicator tubes; BD Biosciences, Franklin Lakes, NJ, USA). The cultures on solid media were incubated for 8 weeks, and those in liquid media were incubated for 6 weeks. When AFB grew within the culture, TB antigen testing (TB Ag MPT64; Abbott Laboratories, Chicago, IL, USA) and MTB/NTM real-time polymerase chain reactions (AdvanSure nucleic acid D kit; LG Cham, Cheongju, Korea) were performed to differentiate between MTB and NTM. All test results were analyzed as secondary data using SPSS (IBM, Version number 29.0, New York, USA) for statistical analysis based on MTB and NTM detection and the sex and age of the patients. This study was

approved by the Institutional Review Board of Dankook University (DKU 2022-12-001).

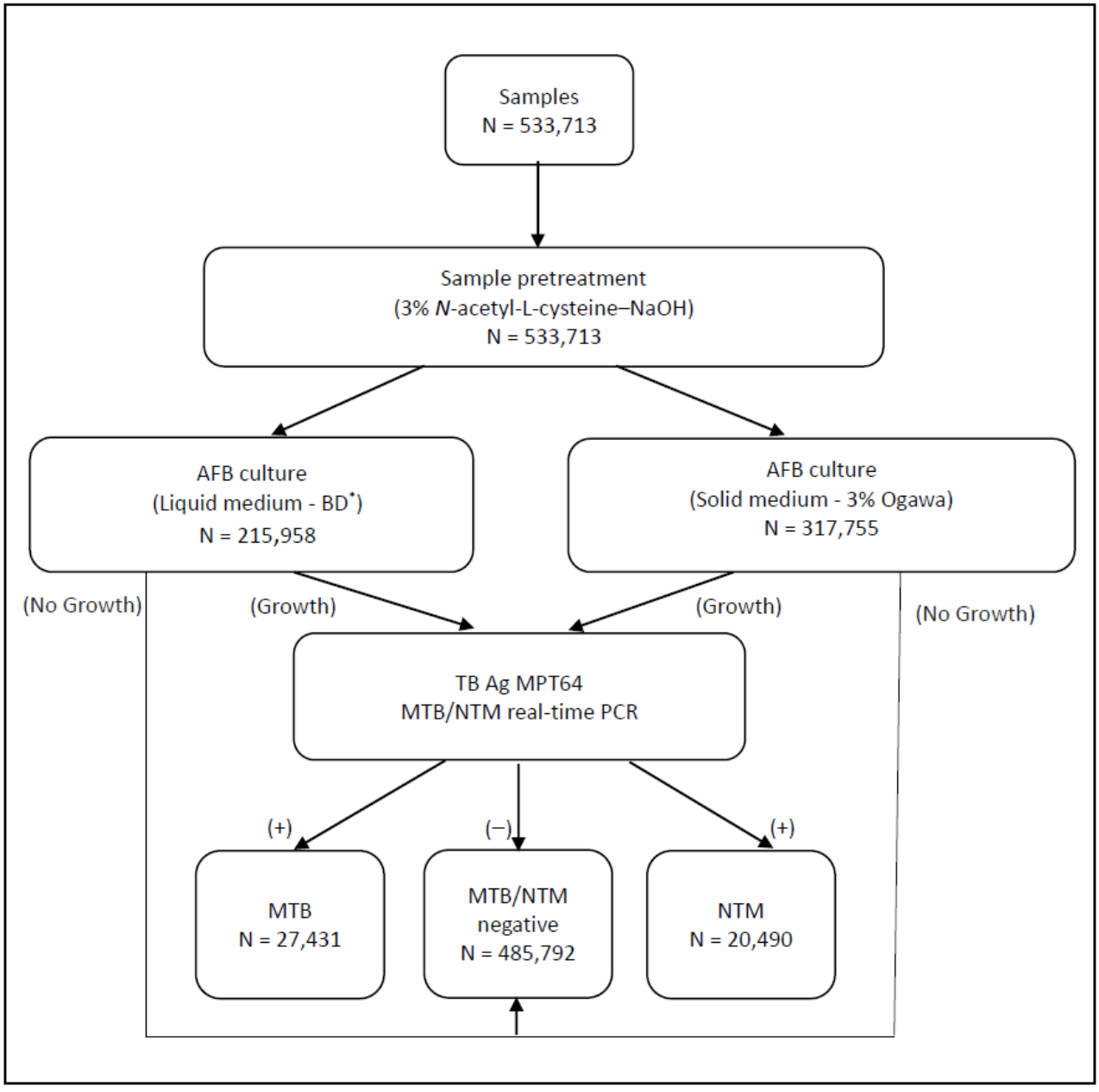

Figure 1. Flowchart of the study methodology

3. RESULTS

3.1 Distribution of NTM and MTB According to Age and Sex

Table 1 shows increased incidence of MTB and NTM in both men and women. The number of men who tested positive for MTB increased from 1,617 in 2014 to 2,278 in 2021, and that of women who tested positive for MTB increased from 822 in 2014 to 1,370 in 2021. The number of men who tested positive for NTM increased from 543 in 2014 to 1,642 in 2021, and that of women who tested positive for NTM

increased from 410 in 2014 to 1,357 in 2021. The positivity rates for MTB were 3.6% for men and 1.8% for women in 2014, and 3.2% for men and 1.9% for women in 2021. The positivity rates for NTM were 1.2% for men and 0.9% for women in 2014, and 2.3% for men and 1.9% for women in 2021. The mean ages of patients positive for MTB and NTM also increased to 68.5 and 66.1 years, respectively, in 2021. Figure 2 shows trends of increasing incidence in those over 61 years of age.

Table 1. Distribution of mycobacteria in men and women from 2014 to 2021

Year	Total number of tests	Men				Women			
		MTB	Positive rate (%)	NTM	Positive rate (%)	MTB	Positive rate (%)	NTM	Positive rate (%)
2014	45,521	1,617	3.6	543	1.2	822	1.8	410	0.9
2015	56,695	1,530	2.7	769	1.4	1,077	1.9	656	1.2
2016	67,561	1,859	2.8	1,057	1.6	1,136	1.7	988	1.5
2017	67,617	2,004	3.0	1,156	1.7	1,239	1.8	1,131	1.7
2018	74,163	1,779	2.4	1,437	1.9	1,047	1.4	1,348	1.8
2019	70,079	1,594	2.3	1,280	1.8	1,074	1.5	1,309	1.9
2020	81,115	2,436	3.0	1,447	1.8	1,313	1.6	1,532	1.9
2021	70,962	2,278	3.2	1,642	2.3	1,370	1.9	1,357	1.9
Average	66,714	1,887	2.9	1,166	1.7	1,135	1.7	1,091	1.6

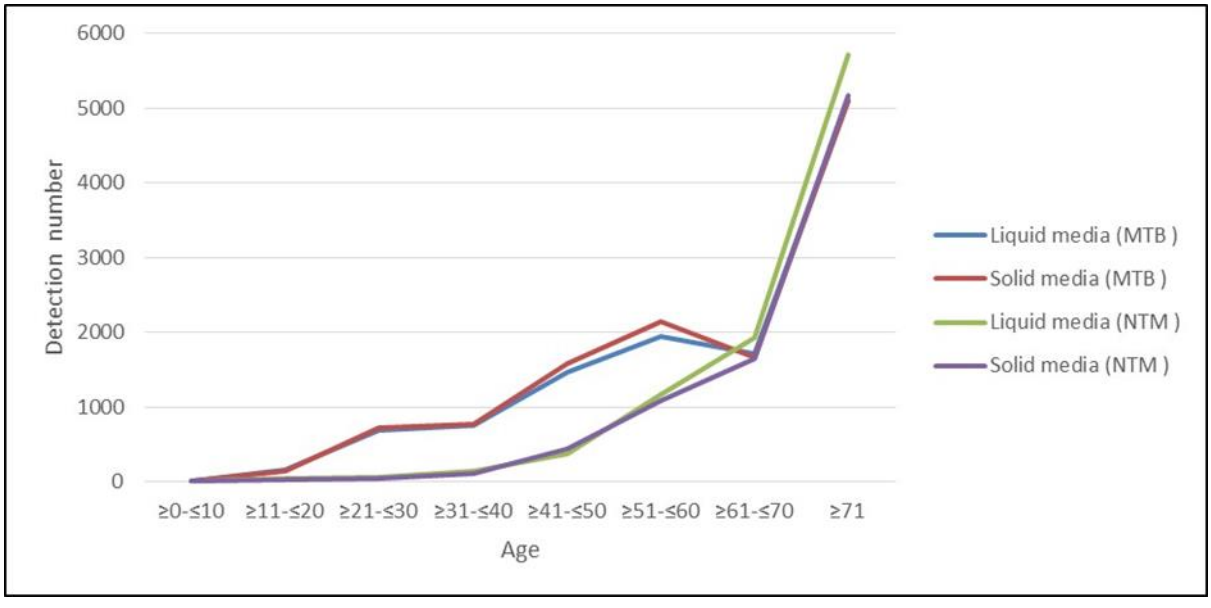

Figure 2. MTB and NTM detection in solid and liquid media according to patient age (2014–2021)

3.2 MTB and NTM Isolation Rates According to the Culture Medium

Table 2 shows that the positivity rate of MTB detection on solid media decreased from 5.9% in 2014 to 3.3% in 2018 and then increased to 4.7% in 2021. Moreover, the positivity rate of NTM detection increased

from 2.1% in 2014 to 3.4% in 2018 and further increased to 3.7% in 2021. In liquid media, the positivity rate of MTB detection decreased from 8.3% in 2014 to 5.5% in 2018 and then slightly increased (to 6.0%) in 2021. Moreover, the positivity rate of NTM detection increased from 3.5% in 2014 to 5.5% in 2018 and then slightly decreased (to 5.3%) in 2021.

Table 2. Distribution of mycobacteria according to culture medium from 2014 to 2021

Year	Liquid medium					Solid medium					Overall* (%)	
		MTB		NTM			MTB		NTM		MTB	NTM
	Total number of tests	Number of positive tests	Positive rate (%)	Number of positive tests	Positive rate (%)	Total number of tests	Number of positive tests	Positive rate (%)	Number of positive tests	Positive rate (%)		
2014	15,210	1,269	8.3	526	3.5	30,311	1,794	5.9	622	2.1	72.7	27.3
2015	21,548	1,424	6.6	919	4.3	35,147	1,861	5.3	894	2.5	64.4	35.6
2016	26,280	1,796	6.8	1,365	5.2	41,281	2,029	4.9	1,448	3.5	57.6	42.4
2017	27,148	1,888	7.0	1,408	5.2	40,469	1,748	4.3	1,243	3.1	57.8	42.2
2018	30,330	1,676	5.5	1,680	5.5	43,833	1,465	3.3	1,471	3.4	49.9	50.1
2019	28,190	1,456	5.2	1,386	4.9	41,889	1,393	3.3	1,371	3.3	50.8	49.2
2020	35,727	1,909	5.3	1,665	4.7	45,388	1,967	4.3	1,376	3	56.0	44.0
2021	31,525	1,895	6.0	1,668	5.3	39,437	1,861	4.7	1,448	3.7	54.7	45.3
Total (Average)	215,958	13,313	(6.3)	10,617	(4.8)	317,755	14,118	(4.5)	9,873	(3.1)	(58.0)	(42.0)

Overall*, overall proportion among mycobacterial culture-positive isolates

3.3 Distribution of NTM and MTB According to The Isolation Year

Table 2 shows that the overall proportion of mycobacterial culture-positive isolates of MTB decreased from 72.7% in 2014 to 54.7% in 2021, whereas that of NTM increased from 27.3% in 2014 to 45.3% in 2021, showing a reversal of the overall proportion among mycobacterial culture positive isolates in 2018. Figure 3 shows that the average age of patients infected with NTM increased from 64.7 years in 2014 to 68.5 years in 2021, and that of patients infected with MTB increased from 58.8 years in 2014 to 66.1 years in 2021.

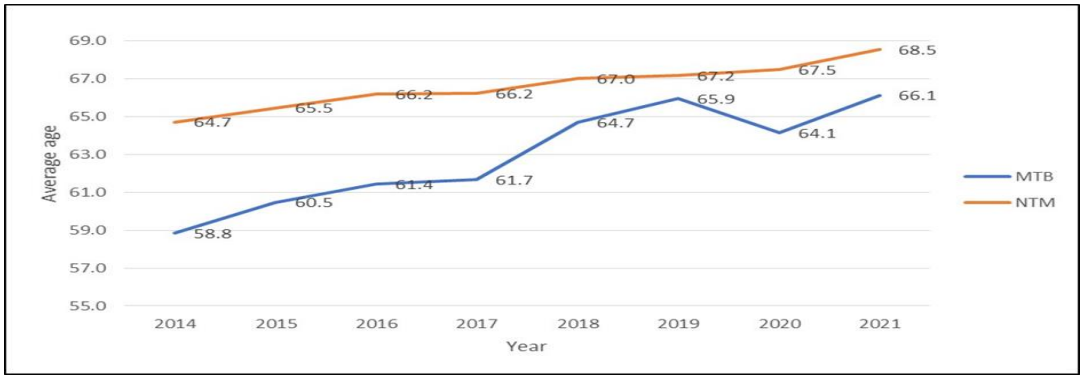

Figure 3. Average age of patients infected with MTB or NTM (8-year period, 2014–2021)

4. DISCUSSION

We observed an increase in the number of male and female patients infected with MTB from 2014 to 2021. As of 2021, male patients infected with MTB outnumbered female patients infected with MTB in China [12]. Moreover, most patients infected with MTB were 80 years of age or older [13]. Among patients with active TB, women tended to be slightly younger than men, and both male and female patients with active TB were 75 years of age or older [14]. In a study of the Moravia and Silesia regions (Czech Republic), NTM-associated pulmonary disease was more frequent in women than in men [7]. In a region of West Africa, women had a higher prevalence of NTM-associated disease compared to the youngest age group studied [15]. In Italy, the majority of male patients with TB are older than 65 years of age [16]. A study conducted in Uruguay showed that the incidence of NTM disease was higher in individuals aged 60 years or older [17]. In this study, the number of male patients with NTM increased from 543 in 2014 to 1,642 in 2021, while that of female patients increased from 410 in 2014 to 1,357 in 2021. The average age of patients infected with MTB or NTM in 2021 was 68.5 and 66.1 years, respectively, indicating an increasing trend in patients aged 61 years or older. Therefore, either the inclusion of interferon-gamma release assay testing in health screenings for the older adult population or the consideration of other policies is required.

According to a study performed in 2021, the isolation rates of MTB and NTM were higher in liquid media than in solid media [10]. In addition, numerous NTM were isolated from mycobacterial growth indicator tube cultures [15]. Other studies have shown that AFB cultures are more sensitive in detecting mycobacteria than AFB smear staining methods [18,19]. In this study, the positivity rate of MTB detection decreased from 5.9% in 2014 to 4.7% in 2021 on solid media, whereas that of NTM detection increased from 2.1% in 2014 to 3.7% in 2021. In liquid media, the positivity rate of MTB detection decreased from 8.3% in 2014 to 6.0% in 2021, whereas that of NTM detection increased from 3.5% in 2014 to 5.3% in 2021. According to recent research on mycobacteria isolated from respiratory specimens at Gwangju Second Hospital in South Korea, the rate of MTB isolation among all positive cases decreased in 2016 compared to that in 2010, whereas the rate of NTM isolation increased in 2016 compared to that in 2010 and further continued to increase in 2019 [20]. Other studies have also shown that the rate of MTB isolation decreased while the rate of NTM isolation gradually increased, with the NTM isolation rate increasing in 2020 compared to that in 2016, although a decrease in the NTM isolation rate was observed between 2019 and 2020 [4]. In other studies, the NTM isolation rate increased compared to the MTB isolation rate from 2014 to 2019; however, in 2018, the NTM isolation rate was similar to that of MTB, but higher than that of MTB in 2019, indicating a reversal of the isolation rate in 2018 [21]. In another study, the proportion of NTM increased nearly five-fold from 2006 to 2018 [17]. In addition, the proportion of NTM compared to that of MTB has been steadily increasing in both Korea and China [3,12]. In the present study, the overall MTB proportion among mycobacterial culture-positive isolates decreased from 72.7% in 2014 to 57.6% in 2016 and 54.7% in 2021, whereas the overall NTM proportion among mycobacterial culture-positive isolates increased from 27.3% in 2014 to 42.4% in 2016 and 45.3% in 2021, indicating a reversal of the overall proportion among mycobacterial culture-positive isolates in 2018. Examinations or screening for NTM should be considered as a TB control measure, as the overall NTM proportion among mycobacterial culture-positive isolates continues to increase. We should also consider preventive education [22].

5. CONCLUSION

Using the test results of respiratory specimens submitted for AFB culture at consignment testing centers in

Yongin-si, South Korea, from January 2014 to December 2021, we analyzed the distribution of NTM and MTB according to age and sex, and the distribution of MTB according to culture medium. All results were analyzed based on the MTB and NTM isolation rates and the distribution of NTM and MTB according to the year of isolation. The analysis is limited because it was conducted using only the results of samples that were requested for analysis by the inspection agency. However, the study is meaningful as we analyzed the results of the isolation and species distribution of mycobacteria from outsourced testing agencies in South Korea. We found that the isolation rates of both MTB and NTM infections are increasing, and regular screening and proactive management are necessary for individuals aged >60 years. Our findings thus indicate the need for promoting and expanding the use of liquid cultures, which show a higher detection rate than solid cultures.

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