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Increasing Recovery of Nontuberculous Mycobacteria from Respiratory Specimens over a 10-Year Period in a Tertiary Referral Hospital in South Korea

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Background: The number of patients with pulmonary disease caused by nontuberculous mycobacteria (NTM) has been increasing worldwide. The aim of this study was to evaluate long-term trends in the NTM recovery rate from respiratory specimens over a 10-year period in a tertiary referral hospital in South Korea.
Methods: We retrospectively reviewed the records of mycobacterial cultures of respiratory specimens at Samsung Medical Center from January 2001 to December 2011.
Results: During the study period, 32,841 respiratory specimens from 10,563 patients were found to be culture-positive for mycobacteria. These included 12,619 (38%) *Mycobacterium tuberculosis* and 20,222 (62%) NTM isolates. The proportion of NTM among all positive mycobacterial cultures increased from 43% (548/1,283) in 2001 to 70% (3,341/4,800) in 2011 ($p<0.001$, test for trend). The recovery rate of NTM isolates from acid-fast bacilli smear-positive specimens increased from 9% (38/417) in 2001 to 64% (1,284/1,997) in 2011 ($p<0.001$, test for trend). The proportion of positive liquid cultures was higher for NTM than for *M. tuberculosis* ($p<0.001$). The most frequently isolated NTM were *Mycobacterium avium-intracellulare* complex (53%) and *Mycobacterium abscessus-massiliense* complex (25%).
Conclusion: The recovery rate of NTM from respiratory specimens in South Korea has increased steadily.

Keywords: Nontuberculous Mycobacteria; *Mycobacterium tuberculosis*; Epidemiology; Korea

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Introduction

Mycobacteria other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* are referred to as nontuberculous mycobacteria (NTM)[1,2]. The incidence of lung disease caused by NTM in human immunodeficiency virus-negative patients has been increasing worldwide[3-8]. In addition, distribution of the NTM variants is not uniform, and there is a marked geographic variability in both the prevalence of NTM lung disease and the mycobacterial species responsible[9,10].

In South Korea, an intermediate tuberculosis (TB)-burden country, TB remains a serious public health problem. In countries with a high TB prevalence, patients with acid-fast bacilli (AFB)-positive sputum based on direct microscopic examination are considered positive for pulmonary TB and are treated empirically with anti-tuberculous drugs. This results in incorrect diagnoses or the unnecessary treatment of many patients with NTM[11-13]. NTM isolation in South Korea has increased, and differentiating between pulmonary TB and NTM lung disease is an important issue in clinical practice[12,14,15].

The aim of this study was to evaluate long-term trends in the recovery rate of NTM from respiratory specimens over 10-year period in a tertiary referral hospital in South Korea.

Materials and Methods

1. Study subjects

We retrospectively reviewed records from the Mycobacteriology Laboratory of Samsung Medical Center during the 11-year period from January 2001 to December 2011. Samsung Medical Center is a tertiary referral hospital in Seoul, South Korea, with 1,299 beds until 2007. In January 2008, 652 beds were added, giving a total of 1,951 beds. All identified NTM isolates were considered significant, with the exception of *Mycobacterium gordonae*, a well-known environmental contaminant[16-18]. Respiratory isolates from sputum, tracheal aspirate or bronchial washing specimens were included in this study and NTM isolates from non-respiratory specimens were excluded. The Institutional Review Board at Samsung Medical Center approved the study protocol. Informed consent was waived because of the retrospective nature of the study.

2. Specimen processing

Clinical specimens were stained using the Ziehl-Neelsen method according to the guidelines provided by the American Thoracic Society[19]. A positive smear was defined as containing >1 AFB per 100 high-power fields. Respiratory specimens were decontaminated using the 2% N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method. Solid media for mycobacterial culture were used. The processed specimens were plated onto 3% Ogawa solid media (Shinyang, Seoul, Korea) between 2001 and 2008[20]. A liquid culture system, a mycobacterial growth indicator tube (MGIT 960 system; Becton Dickinson, Sparks, MD, USA), was introduced from January 2009, and both solid and liquid media were incubated for 6 weeks between 2009 and 2011[21]. All positive cultures were subjected to AFB staining to confirm the presence of AFB and exclude contamination. In addition, positive liquid cultures were confirmed based on the presence of cord formation and MPT64 antigen tests (SD BIOLINE TB Ag MPT64 Rapid; Standard Diagnostics, Yongin, Korea). If these tests were negative, conventional polymerase chain reaction (PCR) using the MTB-ID V3 kit (M&D, Wonju, Korea) was performed to differentiate between *M. tuberculosis* and NTM. Positive cultures in solid media were also confirmed by conventional PCR. To identify NTM species, A PCR-restriction fragment length polymorphism method based on the *rpoB* gene was used to identify the NTM to the species level[18].

3. Statistical analysis

Data are expressed as numbers and percentage. Categorical variables were analyzed using Pearson's χ^2 test or Fisher's exact test. A p-value of <0.05 was considered to indicate statistical significance. All statistical tests were performed using PASW version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

1. Annual numbers of mycobacterial isolates and patients

From 2001 to 2011, the number of samples requested for mycobacterial culture increased steadily from 10,931 in 2001 to 22,966 in 2011. During this period, 32,841 respiratory specimens from 10,563 patients were mycobacteria-positive. These included 12,619 (38%) *M. tuberculosis* and 20,222 (62%) NTM isolates.

The changes in the recovery rates of *M. tuberculosis* and NTM among the requested specimens differed during the study period. The annual rate of NTM isolation from requested specimens increased from 5% in 2001 to 15% in 2011 (p<0.0001, test for trend), while *M. tuberculosis* isolation remained stable, being 6% in both 2001 and 2011 (p=0.710, test for trend) (Figure 1). The annual number of NTM isolates increased rapidly from 548 in 2001 to 3,341 in 2011, while the annual number of *M. tuberculosis* isolates was 735 in 2001

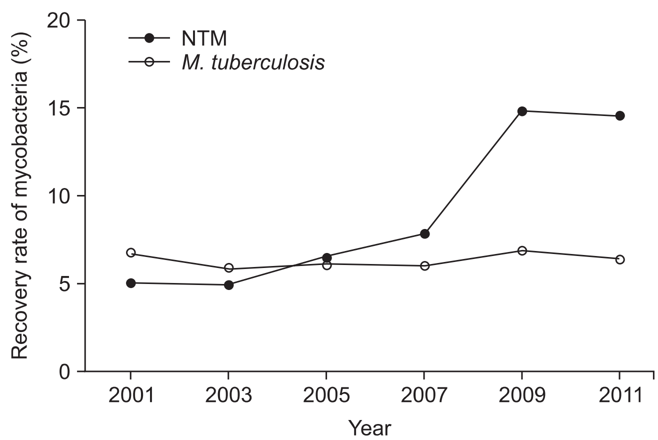

Figure 1. Changes in the rate of recovery of mycobacteria from respiratory specimens. The annual percentage of nontuberculous mycobacteria (NTM) isolated (filled circles) from requested specimens increased, while that of *Mycobacterium tuberculosis* (open circles) remained stable.

Table 1. The number of mycobacterial isolates of *Mycobacterium tuberculosis* and NTM

	2001	2003	2005	2007	2009	2011
No. of requested specimens	10,931	19,381	17,406	17,980	23,729	22,966
Positive culture specimens, n (%)	1,283 (12)	2,093 (11)	2,187 (12)	2,482 (14)	5,143 (22)	4,800 (21)
M. tuberculosis	735 (7)	1,139 (6)	1,059 (6)	1,076 (6)	1,635 (7)	1,459 (6)
NTM	548 (5)	954 (5)	1,128 (6)	1,406 (8)	3,508 (15)	3,341 (15)

NTM: nontuberculous mycobacteria.

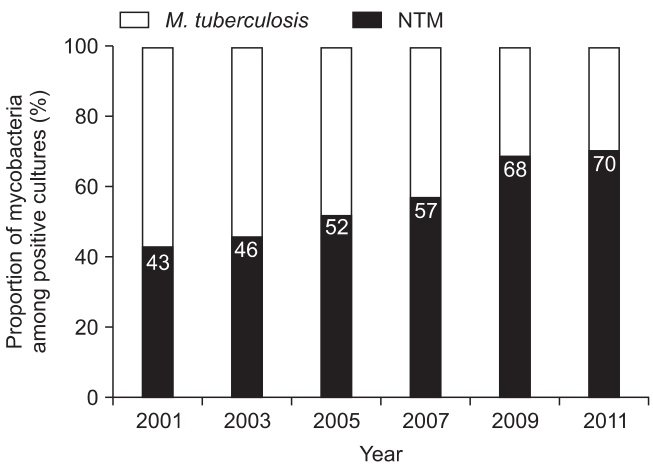

Figure 2. Changes in the proportions of *Mycobacterium tuberculosis* and nontuberculous mycobacteria (NTM) of all positive mycobacterial cultures during the study period. The proportion of NTM increased significantly.

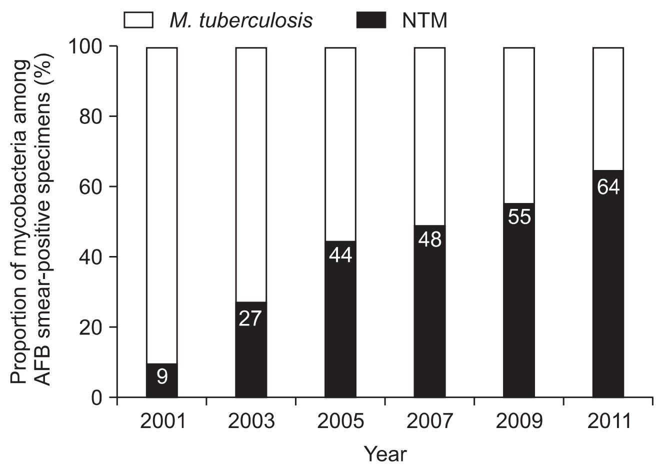

Figure 3. Changes in the proportion of *Mycobacterium tuberculosis* and nontuberculous mycobacteria (NTM) of acid-fast bacilli smear-positive respiratory specimens. The proportion of NTM increased rapidly during the study period. AFB: acid-fast bacilli.

which increased steadily to 1,459 in 2011 (Table 1).

2. Proportion of NTM among mycobacterial isolates

The proportion of *M. tuberculosis* and NTM among all positive mycobacterial cultures changed significantly. The proportion of NTM among all positive mycobacterial cultures increased from 43% (548/1,283) in 2001 to 70% (3,341/4,800) in 2011 ($p<0.001$, test for trend) (Figure 2).

3. Proportion of NTM among AFB smear-positive specimens

Of the 12,876 AFB smear-positive respiratory specimens, *M. tuberculosis* was recovered from 6,611 (51%) and NTM from 6,265 (49%) during the study period. The proportion of NTM isolates increased from 9% (38/417) in 2001 to 64% (1,284/1,997) in 2011 ($p<0.001$, test for trend) (Figure 3).

4. Influence of liquid culture on NTM recovery

To evaluate the influence of the liquid culture system on the rapid increase in NTM isolation, we analyzed the recovery rate of mycobacteria from liquid and solid media from 2009 to 2011. During this 3-year period, both solid and liquid media were used simultaneously to isolate mycobacteria from 69,219 respiratory specimens. Of these, 14,685 (21%) specimens were positive for *M. tuberculosis* or NTM. Among all positive cultures, 27% of *M. tuberculosis* and 38% of NTM were recovered in only liquid media ($p<0.001$). In liquid media, the proportion of AFB smear-negative specimens positive for NTM (51%) was higher than for *M. tuberculosis* (38%) ($p<0.001$) (Table 2).

5. Prevalent NTM species from respiratory specimens

During the study period, the most frequently isolated NTM organisms were *Mycobacterium avium-intracellulare* complex (53%), *Mycobacterium abscessus-massiliense* complex (25%), *Mycobacterium fortuitum* (6%), and *Mycobacterium kansasii* (1%). The proportion of *M. avium-intracellulare* complex increased steadily (Figure 4).

Table 2. Mycobacteria recovery rate after introduction of the liquid culture system (2009–2011)

	No. (%) of cultures positive for		
	M. tuberculosis	NTM	All mycobacteria
All specimens (n=14,685)			
Liquid and solid media	3,146 (69)	5,561 (55)	8,707 (59)
Liquid medium only	1,247 (27)	3.853 (38)	5,100 (35)
Solid medium only	159 (4)	719 (7)	878 (6)
AFB smear-positive specimens (n=5,510)			
Liquid and solid media	1,792 (82)	2,745 (83)	4,537 (82)
Liquid medium only	360 (16)	405 (12)	765 (14)
Solid medium only	48 (2)	160 (5)	208 (4)
AFB smear-negative specimens (n=9,175)			
Liquid and solid media	1,354 (57)	2,816 (41)	4,170 (46)
Liquid medium only	887 (38)	3,448 (51)	4,335 (47)
Solid medium only	111 (5)	559 (8)	670 (7)

NTM: nontuberculous mycobacteria; AFB: acid-fast bacilli.

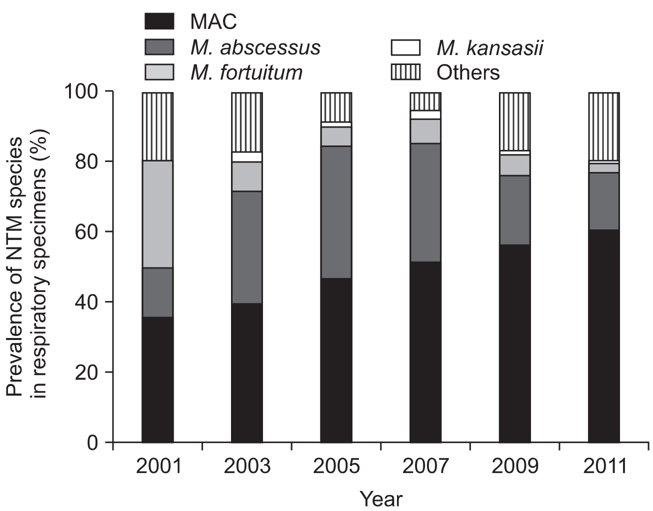

Figure 4. Prevalence of nontuberculous mycobacteria (NTM) species in respiratory specimens. *Mycobacterium avium-intracellulare* complex (MAC) and *Mycobacterium abscessus-massiliense* complex accounted for the majority of the isolated NTM species.

Discussion

The recovery rate of NTM among requested specimens and the annual number of NTM isolates increased steadily over a 10-year period at our institution, particularly after 2009 when the liquid culture system was introduced, while the number of *M. tuberculosis* isolates remained. NTM were isolated more frequently than *M. tuberculosis* in the latter part of the study period. Moreover, NTM was isolated from more than 50% of AFB smear-positive respiratory specimens in recent years, which was traditionally considered diagnostic criterion of pulmonary TB in countries with a high TB burden.

The incidence and prevalence of NTM lung disease has been increasing worldwide, including in South Korea[14,15,18,22]. There are several explanations for the increased recovery of NTM. First, improved laboratory techniques have likely enhanced recovery of mycobacteria. For example, the introduction of liquid culture methods allowed for sensitive detection of both *M. tuberculosis* and NTM. The increased recovery rate was more prominent for NTM than *M. tuberculosis*, especially in AFB smear-negative respiratory specimens, when liquid culture methods were combined with solid medium for mycobacterial culture, as reported previously[23]. However, the increase in NTM isolates and patients with NTM lung disease could not be fully explained by the introduction of liquid culture methods at our institution. The annual number of NTM isolates and the number of patients with NTM isolates were more than those of *M. tuberculosis* from 2005 or 2006 when the liquid culture system had not been in use at our institution. Thus, there could be a true increase in the number of NTM isolates.

Both the environmental sources and host factors could be reasons for rapid increase of recovery of NTM in Korea. It is generally accepted that environmental sources, especially municipal water systems, are the reservoir for most human infections caused by NTM. Aerosolized water exposure within modern populations (e.g., showers, hot tubs, etc.) could be an important reservoir for NTM lung disease[9]. Given that some host factors such as old age and chronic obstructive pulmonary disease is risk factors of NTM lung disease[24,25], the increase of the number of aging population and the prevalence of chronic obstructive pulmonary disease could also contribute to the increase of NTM isolates in Korea.

NTM has been isolated from more than 50% of AFB smear-positive respiratory specimens in recent years. Although the definitive diagnosis of pulmonary TB normally requires isolation of *M. tuberculosis* from respiratory specimens, TB detection is based primarily on the microscopic examination of sputum for AFB[19]. At our institution, the recovery rate of NTM from AFB smear-positive sputum specimens between 1998 and 2001 was 9%[12]. Therefore, early differentiation between pulmonary TB and NTM lung disease in patients with AFB smear-positive specimens is very important[26,27]. There is considerable overlap in the clinical and radiographic characteristics of pulmonary TB and NTM lung disease[26]. Nucleic acid amplification tests for laboratory diagnosis of pulmonary TB could be used to exclude TB in patients with AFB smear-positive samples[28].

There is marked geographic variability in the distribution of the NTM species[9,10]. In many countries, *M. avium-intracellulare* complex is the most commonly isolated NTM organism, followed by *M. kansasii*[1,6]. In this study, *M. avium-intracellulare* complex and *M. abscessus-massiliense* complex accounted for the majority of isolated NTM species, while *M. kansasii* was relatively uncommon. This is in agreement with previous reports from South Korea[15,18,22,29].

Our study has several limitations. First, it was a retrospective survey at a single tertiary referral hospital. Thus, our data could be biased by the demographic and clinical characteristics of the patients visiting our hospital. Second, the clinical significance of the isolated NTM was not evaluated, because we could not obtain the clinical and radiological data of the patients with NTM. Third, this study analyzed the number of culture-positive mycobacterial isolates rather than the number of patients with positive mycobacterial culture. Therefore, our data does not permit us to estimate the true change of disease prevalence during the study period.

In conclusion, the recovery rate of NTM from respiratory specimens at our institution has been increasing steadily over a 10-year period, particularly after 2009 when the liquid culture system was introduced. NTM are now isolated from more than 50% of AFB smear-positive respiratory specimens. Therefore, early differentiation between pulmonary TB and NTM lung disease in patients with AFB smear-positive specimens in South Korea is critical.

Acknowledgements

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