



Continued Upward Trend in Non-Tuberculous Mycobacteria Isolation over 13 Years in a Tertiary Care Hospital in Korea

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Purpose: Despite decreased prevalence of tuberculosis, the incidence of the diseases associated with nontuberculous mycobacteria (NTM) has been increasing in South Korea and around the world. The present retrospective study was conducted to determine longitudinal changes in the epidemiology and distribution of NTM over 13 years at a tertiary care hospital in Korea.

Materials and Methods: We retrospectively analyzed data on *Mycobacterium* species over 13 years (January 2007 to December 2019) by utilizing the laboratory information system. *Mycobacterium* species were identified using biochemical tests and PCR-restriction fragment length polymorphism and Mycobacteria GenoBlot assays.

Results: After excluding duplicates from the initial pool of 17996 mycobacterial isolates, 7674 strains were analyzed and 2984 (38.9%) NTM were isolated. The proportion of NTM continuously increased over the 13-year period, from 17.0% in 2007 to 57.5% in 2019. Among the NTM isolates, the most common species were *Mycobacterium intracellulare* (50.6%), *M. avium* (18.3%), *M. fortuitum* complex (4.9%), *M. abscessus* (4.5%), *M. goodii* (3.3%), *M. kansasii* (1.1%), *M. chelonae* (1.0%), and *M. massiliense* (0.9%). In patients over the age of 70 years, the proportion of NTM among the isolates increased from 26.6% in 2007 to 62.0% in 2019, and that of *M. intracellulare* isolates among the NTM increased from 13.9% (11/79) in 2007 to 37.4% (175/468) in 2019.

Conclusion: The number of NTM isolates continuously increased over the study period, and the increase in the proportion of *M. intracellulare* in patients aged over 70 years was notable.

Key Words: Nontuberculous mycobacteria, *Mycobacterium intracellulare*, *Mycobacterium avium*, epidemiology, retrospective studies

INTRODUCTION

Although the prevalence of tuberculosis (TB) has been decreasing,¹ the incidence of nontuberculous mycobacteria (NTM) diseases is increasing both worldwide and in South Korea.^{2,3} As NTM can be found in soil, dust, and water, including natural water sources such as lakes, rivers, and streams, as well as

in municipal water sources,⁴ isolated NTM were initially considered to be merely contaminants and non-pathogenic. However, the incidence of diseases caused by NTM has increased, along with an increase in the number of case reports and case series from diverse countries and regions, showing pronounced differences in the distribution of NTM clinical isolates in different regions and countries.⁵

The exact cause of the observed increase in NTM diseases is not well-understood; however, increased elderly population, decreased immune function, and exposure to environmental mycobacteria have been reported to be possible causative factors.^{3,6} Since immune function becomes compromised with age, the incidence of NTM infections is higher in elderly people than in younger populations.⁷ Immunocompromised individuals are also at high risk for NTM infections. The vast majority (95%) of Acquired Immune Deficiency Syndrome (AIDS)-related *Mycobacterium avium* complex (MAC) infections are

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due to *M. avium*, not *M. intracellulare*, and *M. avium* infections typically occur when the CD4 cell count is below $0.05 \times 10^9/L$.⁸⁻¹⁰ Therefore, compromised cellular immunity is likely to be a risk factor for infections due to *M. avium* but not due to *M. intracellulare*.¹⁰ Although both *M. avium* and *M. intracellulare* are MAC species and aquatic organisms, their niches are different: *M. avium* tends to grow in aqueous suspensions, whereas *M. intracellulare* forms biofilms.^{11,12} Han, et al.¹⁰ showed that in non-AIDS patients, *M. intracellulare* is more pathogenic, and *M. intracellulare* infections in women tends to increase with age beyond menopause (>50 years), regardless of any underlying disease. In addition, their study revealed a high prevalence of *M. intracellulare* in men with lung cancer, implying that prior lung injury caused by cancer risk factors, such as smoking, predisposes lung tissues to attachment by the microbe, subsequently leading to colonization and infection.

With this background in mind, our retrospective study was performed to determine longitudinal changes in the epidemiology and distribution of NTM species over 13 years at a tertiary care hospital located in the southwestern region of Gangwon-do in South Korea.

MATERIALS AND METHODS

Data collection

From January 2007 to December 2019, relevant data were collected through the laboratory information system at Wonju Severance Christian Hospital, and all data were decoded automatically. Collected specimens were classified as pulmonary or extrapulmonary. If the same mycobacterial species was identified in several lower-respiratory specimens [i.e., sputum and bronchoalveolar lavage (BAL)] from the same patient during follow-up, only one of the initially isolated mycobacterial strains was included in the analyzed set of isolates.

Acid-fast bacillus staining and culturing for *Mycobacterium* identification

Specimens, with the exception of body fluids, were decontaminated using the N-acetyl-L-cysteine (NALC)-2% sodium hydroxide (NaOH) method. Decontaminated specimens were stained with auramine-rhodamine, and the results were confirmed by Ziehl-Neelsen staining. The presence of acid-fast bacilli (AFB) in a specimen was defined as follows: trace, 1-2 AFB per 300× field; 1+, 1-9 AFB per 100× field; 2+, 1-9 AFB per 10× field; 3+, 1-9 AFB per 1× field; and 4+, more than 9 AFB per 1× field. The NALC-NaOH-pretreated specimens were then inoculated onto Ogawa solid medium (Shinyang Chemical, Seoul, South Korea). Beginning on September 1, 2008, liquid medium was also used [mycobacteria growth indicator tubes (MGITs); Becton Dickinson Diagnostic Systems, Sparks, MD, USA]. After June 2012, Ogawa medium (Union Lab, Seoul, South Korea),

sourced from the Korean Institute of Tuberculosis (Osong, South Korea), was used. Inoculum maintained in Ogawa medium was incubated at 37°C and 5% CO₂ for 8 weeks, and inoculum maintained in MGITs was incubated in the BACTEC MGIT 960 system (BD Diagnostic Systems, Sparks, MD, USA) for 6 weeks. A 1 mL aliquot from each MGIT culture was tested, and those that tested positive were subjected to AFB staining for verification. Contaminated cultures were excluded.

Molecular identification of *Mycobacterium*

DNA purification protocols were medium-type specific. Samples from cultures maintained in Ogawa medium were obtained using an inoculation loop and suspended in 200 µL sterilized distilled water in a 1.5 mL microtube. This mixture was centrifuged at 13000×g for 3 min, and the supernatant was removed. In contrast, for cultures maintained in MGIT tubes, 1 mL sample was removed, placed in a 1.5 mL microtube, and centrifuged at 13000×g for 3 min. The supernatant was removed, and a 1 mL aliquot of distilled water was added to the pellet, vortexed thoroughly, and centrifuged again at 13000×g for 3 min. For both sample types, after the supernatant was removed, 100 µL extraction buffer was added, and the sample was heated at 100°C for 20 min and centrifuged at 13000×g for 3 min.

For mycobacterial identification, extracted DNA was analyzed using Myco-ID (M&D Inc., Wonju, South Korea), which is based on restriction fragment length polymorphism.¹³ However, for samples collected after December 2, 2014, the AdvanSure Mycobacteria GenoBlot Assay (LG Chem, Cheongju, South Korea) was used for mycobacterial identification. This assay can classify and identify *Mycobacterium tuberculosis* complex (MTBC) species, as well as 20 different NTM species. The reverse line blot hybridization assay was processed using an AdvanSure hybridizer (LG Chem), and patterns in the line strips were subjected to automated analysis using AdvanSure GenoLine Scan (LG Chem).¹⁴ To distinguish *M. abscessus* and *M. massiliense*, the presence of the erythromycin ribosome methyltransferase (*erm*) gene was confirmed using the ERM-plus real-time PCR kit (LG Chem; not a commercial product). MAC was defined as a group of mycobacteria comprising *M. avium*, *M. intracellulare*, and *M. avium-intracellulare*.

Statistical analysis

The statistical significance of the recovery rates of MTBC and NTM according to sex and age was analyzed using chi-square and Fisher's exact tests. All probability values were two-sided, and *p* values less than 0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics v. 25.0 (IBM Corp., Armonk, NY, USA).

Ethical approval

In accordance with government regulations, the need for ethical review by the Institutional Review Board (IRB) was waived, and the exemption was approved by the IRB of Wonju Sever-

ance Christian Hospital (approval no. CR320013).

RESULTS

After excluding duplicates, a final set of 7674 mycobacterial isolates (from a total of 17996) was identified. The most common mycobacterial species were MTBC (61.1%), *M. intracellulare* (19.7%), *M. avium* (7.1%), *M. fortuitum* complex (1.9%), *M. abscessus* (1.8%), *M. gordonae* (1.3%), *M. kansasii* (0.4%), *M. chelonae* (0.4%), and *M. massiliense* (0.3%). MAC accounted for 69.3% of the NTM isolates (Table 1).

The frequency of isolation of *Mycobacterium* species according to specimen type was analyzed. *M. gordonae* (98.0%), *M.*

massiliense (96.2%), and *M. kansasii* (93.9%) were mostly found in the sputum/BAL. MTBC (3.9%) and *M. chelonae* (3.4%) were commonly detected in the pleural fluid. *M. chelonae* (6.9%), *M. fortuitum* complex (6.2%), *M. abscessus* (3.7%), and MTBC (2.4%) were frequently observed in wounds. The isolation frequency of each species according to specimen type is shown in Table 2.

The proportion of male patients in this study was 57.7%. In the group aged 40–59 years, the proportion of NTM in samples from female patients was significantly greater than that in samples from male patients ($p < 0.001$). However, in the group aged 70–89 years, the recovery rate of NTM in samples from male patients was significantly greater than that in samples from female patients ($p < 0.001$) (Table 3).

Of the four NTM species with more than 100 isolates, *M. intracellulare*, *M. avium*, and *M. fortuitum* complex were more frequently isolated from males, while *M. abscessus* was more frequently isolated from females. For *M. intracellulare*, the number of isolates increased after 50 years of age: the proportion of total NTM was 46.3% (203/438) for patients in their 50–59 years, 45.6% (272/597) for patients aged 60–69 years, 53.4% (573/1074) for patients aged 70–79 years, and 54.6% (315/577) for patients aged 80–89 years (Table 4).

The proportion of NTM among all mycobacteria showed an upward trend over the 13-year study period and increased from 17.0% in 2007 to 57.5% in 2019 (Fig. 1). Moreover, in patients aged over 70 years, the proportion of *M. intracellulare* isolates among all mycobacterial isolates continuously increased from 13.9% (11/79) in 2007 to 37.4% (175/468) in 2019 (Fig. 2).

Table 1. Number and Proportion (%) of Mycobacterial Isolates

<i>Mycobacterium</i> species	Isolates (n=7674)	Isolates/total	Isolates among NTM
<i>Mycobacterium tuberculosis</i> complex	4690	61.1	
<i>Mycobacterium avium</i> complex*	2067	26.9	69.3
<i>Mycobacterium avium</i> *	546	7.1	18.3
<i>Mycobacterium intracellulare</i> *	1509	19.7	50.6
<i>Mycobacterium avium-intracellulare</i>	12	0.2	0.4
<i>Mycobacterium fortuitum</i> complex*	145	1.9	4.9
<i>Mycobacterium abscessus</i> *	135	1.8	4.5
<i>Mycobacterium gordonae</i> *	99	1.3	3.3
<i>Mycobacterium kansasii</i> *	33	0.4	1.1
<i>Mycobacterium chelonae</i> *	29	0.4	1.0
<i>Mycobacterium massiliense</i>	26	0.3	0.9
<i>Mycobacterium terrae</i> complex*	24	0.3	0.8
<i>Mycobacterium lentiflavum</i> / <i>M. genavense</i> *	18	0.2	0.6
<i>Mycobacterium szulgai</i> *	5	0.1	0.2
<i>Mycobacterium flavescens</i> *	4	0.1	0.1
<i>Mycobacterium celatum</i> *	4	0.1	0.1
<i>Mycobacterium mucogenicum</i>	4	0.1	0.1
<i>Mycobacterium scrofulaceum</i> *	4	0.1	0.1
<i>Mycobacterium nonchromogenicum</i>	4	0.1	0.1
<i>Mycobacterium aubagnense</i>	3	0.04	0.1
<i>Mycobacterium phocaicum</i>	3	0.04	0.1
<i>Mycobacterium mucilaginosus</i>	2	0.03	0.1
<i>Mycobacterium malmoeense</i> *	1	0.01	0.03
<i>Mycobacterium austroafricanum</i>	1	0.01	0.03
<i>Mycobacterium gastri</i> *	1	0.01	0.03
<i>Mycobacterium phlei</i>	1	0.01	0.03
<i>Mycobacterium vaccae</i> *	1	0.01	0.03
<i>Mycobacterium</i> species	370	4.8	12.4

NTM, nontuberculous mycobacteria.

Data are presented as n and %.

*Species that could be identified using the AdvanSure Mycobacteria GenoBlot Assay (LG Chem.).

DISCUSSION

There are several factors that need to be considered when estimating and comparing the prevalence of NTM pulmonary disease (NTM-PD). Unlike TB, which is a notifiable disease in most regions due to management policies, NTM-PD is not a notifiable disease. Its diagnosis depends on the integration of clinical, radiological, and microbiological findings, as summarized in the American Thoracic Society/Infectious Diseases Society of America 2007 criteria, which have become the accepted definition for this disease.¹⁵ In the genus *Mycobacterium*, there are more than 200 species and 13 subspecies.¹⁶ Many NTM species show similar phenotypic characteristics¹⁷ and high DNA homology¹⁸; therefore, they have been sorted into groups or complexes, each consisting of two to seven species, although some species remain ungrouped. The prevalence of NTM-PD and the proportions of different NTM species may vary based on the following: country,³ study period,¹⁹ and methods employed for isolation^{20,21} and identification.¹⁹ The AdvanSure Mycobacteria GenoBlot assay (LG Chem) used in this study has been reported to be comparable to the GenoType Mycobacterium CM/AS assay (Hain Life-science, Nehren, Germany) in terms of performance and may be useful as a routine method for NTM identi-

Table 2. Number and Proportion (%) of *Mycobacterium* Isolates according to Specimen Type

<i>Mycobacterium</i> species	Pulmonary system			Extrapulmonary						Other [†] n=89 (1.2)
	Sputum/BAL n=6873 (89.6)	Pleural F n=240 (3.1)	Wound* n=186 (2.4)	Catheter n=118 (1.5)	Urine n=54 (0.7)	Peritoneal F n=44 (0.6)	CSF n=37 (0.5)	Synovial F n=18 (0.2)	Pericardial F n=15 (0.2)	
<i>M. tuberculosis</i> complex	4124 (87.9)	182 (3.9)	111 (2.4)	86 (1.8)	43 (0.9)	22 (0.5)	31 (0.7)	8 (0.2)	13 (0.3)	70 (1.5)
<i>M. avium</i> complex	1920 (92.9)	34 (1.6)	41 (2.0)	26 (1.3)	9 (0.4)	14 (0.7)	5 (0.2)	6 (0.3)	1 (0)	11 (0.5)
<i>M. avium</i>	537 (98.4)	2 (0.4)	4 (0.7)	1 (0.2)	0 (0)	1 (0.2)	0 (0)	1 (0.2)	0 (0)	0 (0)
<i>M. intracellulare</i>	1372 (90.9)	31 (2.1)	37 (2.5)	25 (1.7)	9 (0.6)	13 (0.9)	5 (0.3)	5 (0.3)	1 (0.1)	11 (0.7)
<i>M. avium-intracellulare</i>	11 (91.7)	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. fortuitum</i> complex	131 (90.3)	0 (0)	9 (6.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	4 (2.8)
<i>M. abscessus</i>	126 (93.3)	2 (1.5)	5 (3.7)	0 (0)	0 (0)	1 (0.7)	0 (0)	1 (0.7)	0 (0)	0 (0)
<i>M. goodii</i>	97 (98.0)	0 (0)	1 (1.0)	1 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. kansasii</i>	31 (93.9)	0 (0)	1 (3.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	0 (0)
<i>M. chelonae</i>	26 (89.7)	1 (3.4)	2 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. massiliense</i>	25 (96.2)	0 (0)	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. terrae</i> complex	21 (87.5)	0 (0)	3 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. lentiflavum/M. genavense</i>	18 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. szulgai</i>	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. flavescens</i>	0 (0)	1 (25.0)	0 (0)	2 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25.0)
<i>M. celatum</i>	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. mucogenicum</i>	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. scrofulaceum</i>	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. nonchromogenicum</i>	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. aubagnense</i>	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. phocaicum</i>	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. mucilagenosus</i>	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. malmoense</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
<i>M. austroafricanum</i>	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. gastri</i>	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. phlei</i>	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. vaccae</i>	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>M. species</i>	322 (87.0)	20 (5.4)	11 (3.0)	3 (0.8)	2 (0.5)	7 (1.9)	1 (0.3)	1 (0.3)	1 (0.3)	2 (0.5)

BAL, bronchoalveolar lavage; F, fluid; CSF, cerebrospinal fluid.

Data are presented as n (%).

*This included abscess and deep wound aspirations, which are regarded as dirty wounds, [†]This included organ biopsy, gastrointestinal fluid, drain fluid, other fluid, blood, surgical specimens, and stool samples.

Table 3. Number and Proportion (%) of MTBC and NTM Isolates according to Age and Sex of Patients

Age (yr)	MTBC isolates			NTM isolates			Total	p value*
	Female	Male	Subtotal	Female	Male	Subtotal		
0–9	4 (23.5)	13 (76.5)	17 (81.0)	0 (0)	4 (100)	4 (19.0)	21 (0.3)	0.546
10–19	31 (36.5)	54 (63.5)	85 (89.5)	2 (20.0)	8 (80.0)	10 (10.5)	95 (1.2)	0.486
20–29	109 (46.4)	126 (53.6)	235 (93.3)	6 (35.3)	11 (64.7)	17 (6.7)	252 (3.3)	0.375
30–39	149 (44.9)	183 (55.1)	332 (85.1)	32 (55.2)	26 (44.8)	58 (14.9)	390 (5.1)	0.147
40–49	148 (31.8)	317 (68.2)	465 (74.6)	92 (58.2)	66 (41.8)	158 (25.4)	623 (8.1)	<0.001
50–59	158 (22.9)	533 (77.1)	691 (61.2)	225 (51.4)	213 (48.6)	438 (38.8)	1129 (14.7)	<0.001
60–69	252 (34.9)	470 (65.1)	722 (54.7)	233 (39.0)	364 (61.0)	597 (45.3)	1319 (17.2)	0.122
70–79	625 (47.9)	680 (52.1)	1305 (54.9)	410 (38.2)	664 (61.8)	1074 (45.1)	2379 (31.0)	<0.001
80–89	444 (59.4)	304 (40.6)	748 (56.5)	241 (41.8)	336 (58.2)	577 (43.5)	1325 (17.3)	<0.001
≥90	55 (61.1)	35 (38.9)	90 (63.8)	29 (56.9)	22 (43.1)	51 (36.2)	141 (1.8)	0.621
Total	1975 (42.1)	2715 (57.9)	4690 (61.1)	1270 (42.6)	1714 (57.4)	2984 (38.9)	7674 (100)	

MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria.

Data are presented as n (%).

*p value indicates significant difference between the number of female and male patients in each age group.

Table 4. Number and Proportion (%) of Four *Mycobacterium* Species according to Age and Sex of Patients

Age (yr)	<i>Mycobacterium intracellulare</i>			<i>Mycobacterium avium</i>			<i>Mycobacterium fortuitum</i> complex			<i>Mycobacterium abscessus</i>		
	Female	Male	Subtotal	Female	Male	Subtotal	Female	Male	Subtotal	Female	Male	Subtotal
0–9	0 (0)	2 (100)	2 (0.1)	0 (0)	1 (100)	1 (0.2)	0 (0)	1 (100)	1 (0.7)	0 (0)	0 (0)	0 (0)
10–19	1 (11.1)	8 (88.9)	9 (0.6)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)
20–29	4 (50.0)	4 (50.0)	8 (0.5)	2 (50.0)	2 (50.0)	4 (0.7)	0 (0)	1 (100)	1 (0.7)	0 (0)	2 (100)	2 (1.5)
30–39	14 (48.3)	15 (51.7)	29 (1.9)	9 (75.0)	3 (25.0)	12 (2.2)	3 (60.0)	2 (40.0)	5 (3.4)	3 (100)	0 (0)	3 (2.2)
40–49	38 (52.8)	34 (47.2)	72 (4.8)	31 (77.5)	9 (22.5)	40 (7.3)	3 (37.5)	5 (62.5)	8 (5.5)	8 (100)	0 (0)	8 (5.9)
50–59	101 (49.8)	102 (50.2)	203 (13.5)	68 (68.7)	31 (31.3)	99 (18.1)	2 (22.2)	7 (77.8)	9 (6.2)	20 (64.5)	11 (35.5)	31 (23.0)
60–69	119 (43.8)	153 (56.3)	272 (18.0)	57 (42.9)	76 (57.1)	133 (24.4)	11 (27.5)	29 (72.5)	40 (27.6)	11 (40.7)	16 (59.3)	27 (20.0)
70–79	229 (40.0)	344 (60.0)	573 (38.0)	67 (41.1)	96 (58.9)	163 (29.9)	22 (40.7)	32 (59.3)	54 (37.2)	21 (50.0)	21 (50.0)	42 (31.1)
80–89	149 (47.3)	166 (52.7)	315 (20.9)	26 (31.3)	57 (68.7)	83 (15.2)	5 (19.2)	21 (80.8)	26 (17.9)	8 (38.1)	13 (61.9)	21 (15.6)
≥90	13 (50.0)	13 (50.0)	26 (1.7)	7 (63.6)	4 (36.4)	11 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (0.7)
Total	668 (44.3)	841 (55.7)	1509 (100)	267 (48.9)	279 (51.1)	546 (100)	47 (32.4)	98 (67.6)	145 (100)	71 (52.6)	64 (47.4)	135 (100)

Data are presented as n (%).

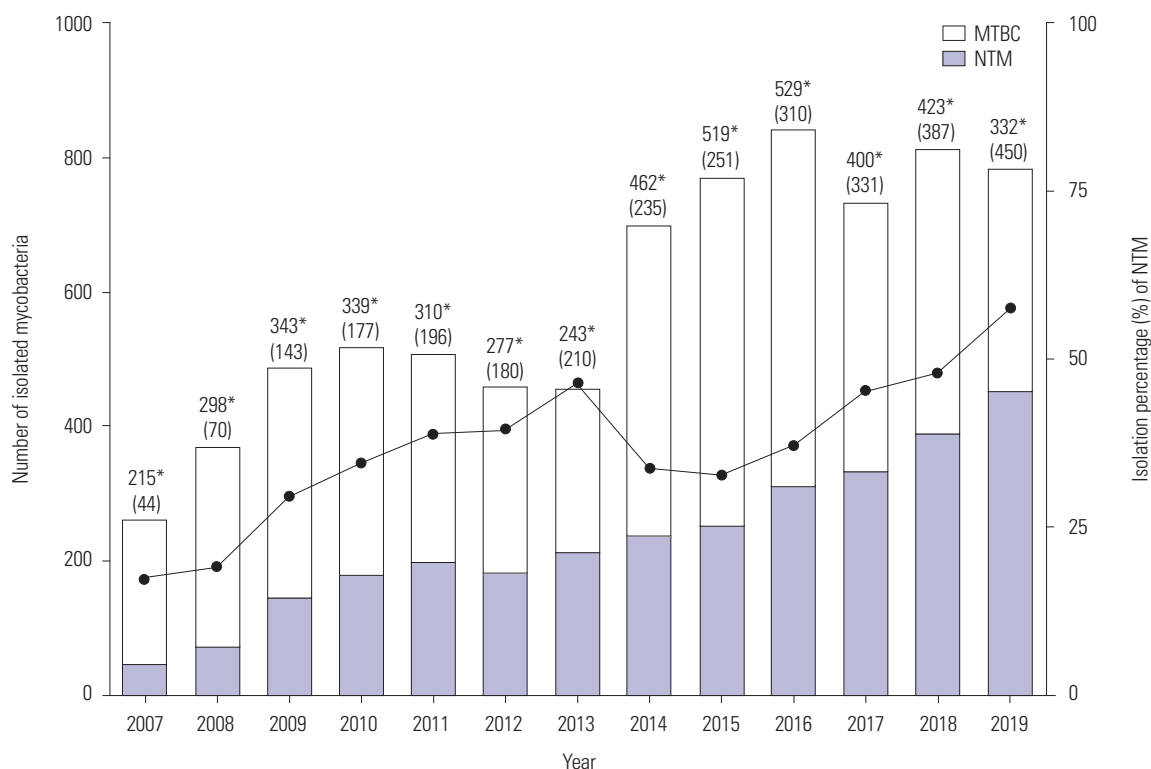
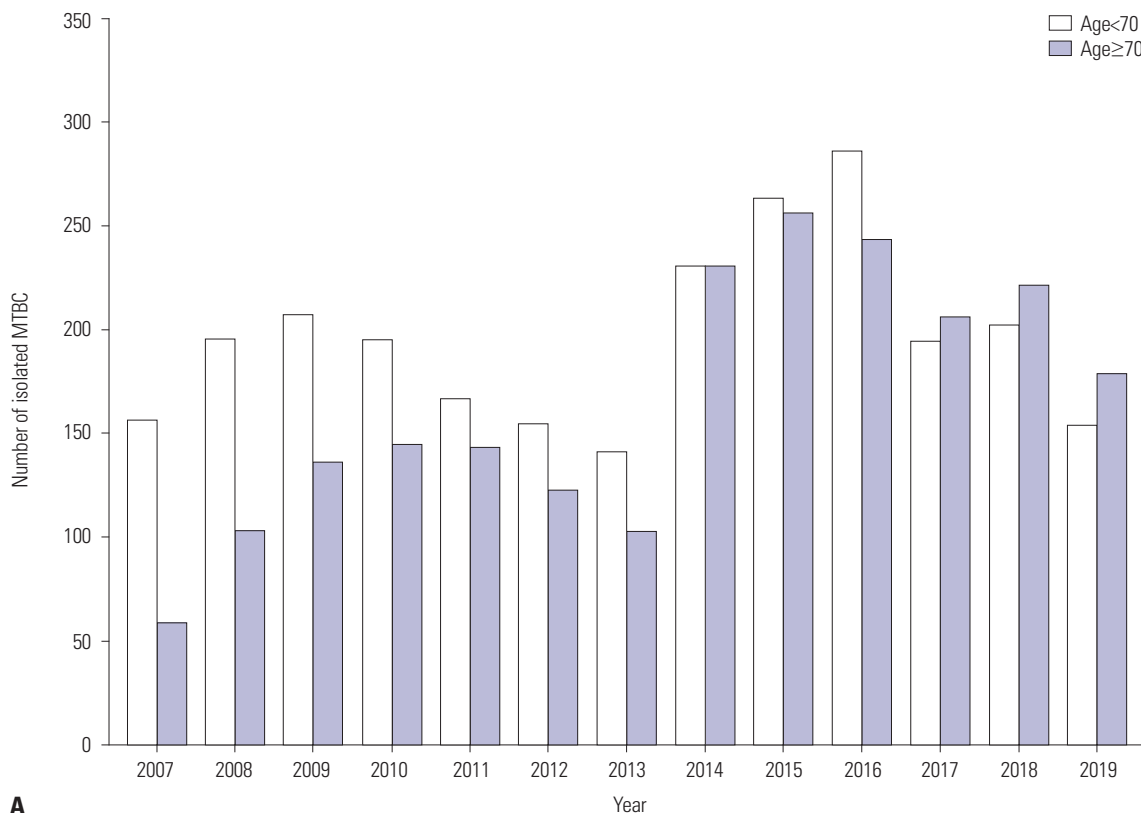


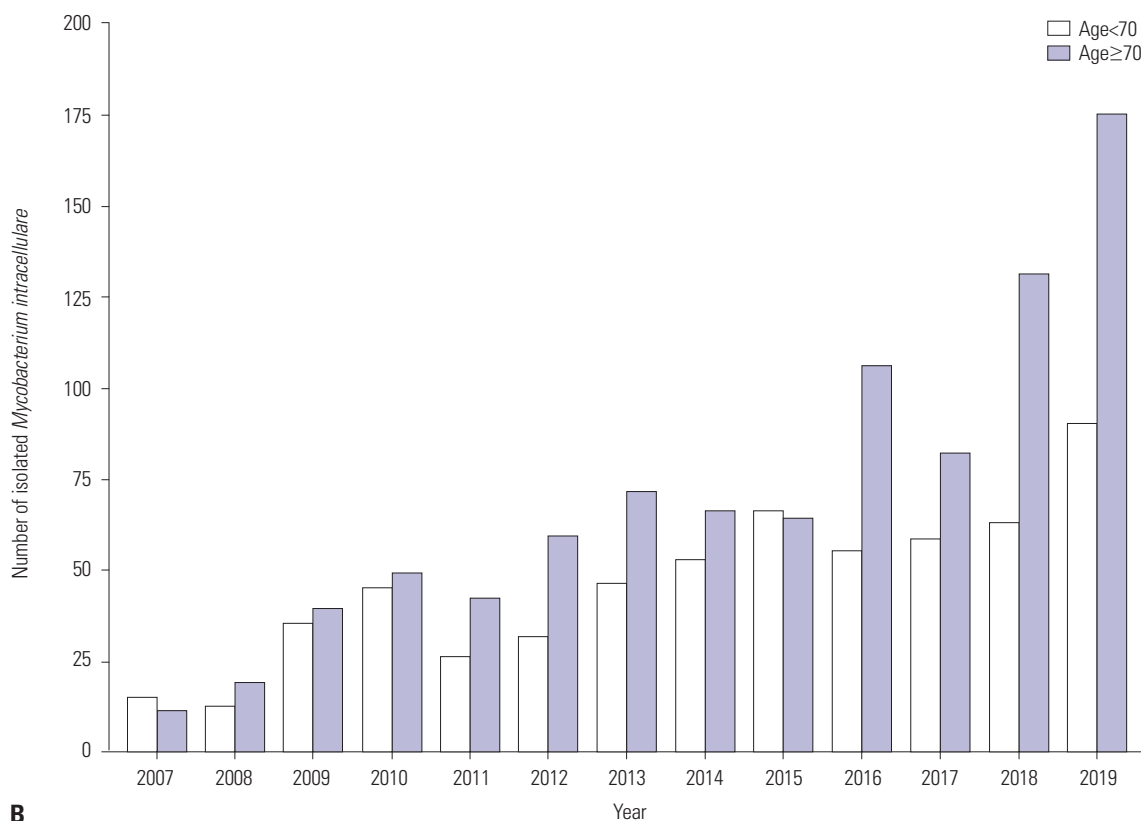
Fig. 1. Continued upward trend in the proportion of NTM isolates over the 13-year study period. Filled circles (●) denote the percentage of NTM isolates each year, while asterisks (*) represent the number of MTBC isolates each year. The number in brackets is the number of NTM isolates. MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria.

fication in clinical settings, especially in locations where MAC is the main cause of NTM infection.²² The NTM species most commonly isolated worldwide belong to the MAC, comprising 34%–61% of isolates, depending on the continent, with the highest proportions reported in North America and Oceania.²³ A nationwide survey in Japan showed that the distribution of the two major MAC species, *M. avium* and *M. intracellulare*, followed a clear gradient from the northern to southern regions.²⁴ The relative ratio of *M. avium* to *M. intracellulare* (*M. avium*/*M.*

intracellulare) in different parts of the world, in decreasing order, is North America (9.8)>South America (4.9)>Asia (2.8)>Europe (1.5)>South Africa (0.27)>Australia (0.25).⁵ In Korea, the prevalence of *M. avium* was high mainly in the capital city of Seoul,^{2,25–27} whereas the prevalence of *M. intracellulare* was high in southern cities, such as Busan, Yangsan, and Ulsan.^{28,29} Ambient temperature, heavy rainfall, flooding, and drought are likely to influence the prevalence of environmental microorganisms.³⁰ Although NTM are found in soil and water, the fac-



A



B

Fig. 2. The number of MTBC (A) and *Mycobacterium intracellulare* (B) isolates over the 13-year study period. The height of left and right bars are the number of isolated mycobacteria in patients aged less than 70 years and more than 70 years, respectively. MTBC, *Mycobacterium tuberculosis* complex.

tors that influence their transmission from the environment to humans are largely unknown.⁶ The environmental reservoirs reported to be predominantly associated with *M. avium* are water and soil, while those associated with *M. intracellulare* include dust and soil.⁶ *M. intracellulare* infection was shown to be more common in agricultural regions and was associated with shallow soil,³¹ and there was no significant association between the incidence of *M. intracellulare* and temperature or rainfall.³² Shallow soil depth, which is associated with poor crop yields, as well as the process of “deep ripping” to improve yields, have been hypothesized to result in aerosolization of mycobacteria from the soil.³² However, there may be other aspects of soil quality or other yet unknown environmental factors that favor *M. intracellulare* growth in these regions.

Han, et al.¹⁰ reported that *M. intracellulare* was frequently isolated from postmenopausal women with estrogen deficiency. However, in the present study, both *M. intracellulare* and *M. avium* were frequently isolated in men over 60 years of age. *M. intracellulare* was isolated from extrapulmonary specimens, such as wounds (7%), whereas *M. avium* was isolated only from respiratory specimens. In addition, 50% of NTM isolates from synovial fluid were *M. intracellulare*. Although the exact reason for the geographic diversity in distribution of mycobacterial species is unknown, the degree of human exposure to certain endemic NTM species is considered to be the most important factor. We postulated that, depending on environmental factors such as occupation, residence, or participation in activities associated with water and/or soil, a deficiency in cellular immunity may be a risk factor for infection when exposed to *M. avium*. Underlying lung disease or prior lung injury resulting from bronchiectasis and smoking may be a risk factor for *M. intracellulare* infection.

Further research is needed to identify the major exposure routes and environmental sources of NTM infections. This information would then enable the design of effective control strategies for diseases associated with NTM.

AUTHOR CONTRIBUTIONS

Conceptualization: Young Uh. **Data curation:** Young Uh. **Formal analysis:** Kwangjin Ahn and Young Uh. **Investigation:** Kwangjin Ahn and Young Uh. **Methodology:** Gyu Yel Hwang and Hyunmi Cho. **Project administration:** Young Uh. **Resources:** Kwangjin Ahn and Young Uh. **Software:** Kwangjin Ahn. **Supervision:** Young Uh. **Validation:** Young Keun Kim. **Visualization:** Kwangjin Ahn and Young Uh. **Writing—original draft:** Kwangjin Ahn. **Writing—review & editing:** all authors. **Approval of final manuscript:** all authors.

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