

## Spread of Nontuberculous Mycobacteria From 1993 to 2006 in Koreans

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In Korea, the prevalence of nontuberculous mycobacterial (NTM) pulmonary disease has risen, observed primarily in immunocompetent patients with or without preexisting lung disease. The purpose of this study was to determine the frequency of various species of NTM isolates from respiratory specimens in a single institution over a 14-year period in Korea. All samples referred to our reference laboratory over a 14-year period in Korea were analyzed. From 1993 to 2000 our laboratory used conventional NTM identification methods, and from 2001 we adapted PCR-restriction

fragment length polymorphism analysis (PRA). A total of 17,915 isolates were collected from 1993 to 2006. The most frequently isolated organisms were *M. avium complex* ( $n = 11,705$ , 65%), *M. abscessus* ( $n = 2,076$ , 11.59%), *M. fortuitum complex* ( $n = 1,279$ , 7.14%), *M. chelonae complex* ( $n = 1,134$ , 6.33%), *M. kansasii* ( $n = 762$ , 4.25%), *M. szulgai* ( $n = 139$ , 0.78%), *M. celatum* ( $n = 87$ , 0.49%), *M. scrofulaceum* ( $n = 18$ , 0.10%) and *M. marium* ( $n = 11$ , 0.06%). J. Clin. Lab. Anal. 22:415–420, 2008. © 2008 Wiley-Liss, Inc.

**Key words:** identification; nontuberculous mycobacteria (NTM); PCR-restriction fragment length polymorphism (PRA)

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### INTRODUCTION

The genus *Mycobacterium* has more than 100 species (1). Particularly, nontuberculous mycobacteria (NTM) are ubiquitous and have the ability to colonize and cause serious infection. Several recent publications have reported that disease caused by NTM is on the rise (2–4), and that NTM is responsible for an increasing proportion of mycobacterial disease in many developed countries (5–7). In addition, the distribution of NTM species and their relative contributions to clinical disease are heterogeneous (5,8–9).

In Korea, the prevalence of NTM pulmonary disease has increased owing to the growing numbers of NTM infected immunocompetent patients with or without preexisting lung disease (10–13). Future projections suggest that patients with NTM pulmonary disease will be as common as those with pulmonary tuberculosis in routine clinical settings in Korea.

*M. tuberculosis* is an obligate human pathogen with which no environmental reservoir has been associated. NTM, conversely, are ubiquitous, and are frequently isolated from environmental sources, including surface water, tap water, and soil (5,8). In South Korea, tuberculosis remains a serious public health problem, although its prevalence has been greatly reduced in recent years (14). In countries with a high TB burden,

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like South Korea, those patients with acid-fast bacilli (AFB)-positive sputum on direct microscopic examination, or those displaying chest radiographic findings suggestive of active tuberculosis, have generally been presumed to have pulmonary tuberculosis, receiving treatment with antituberculous drugs. An incorrect diagnosis of pulmonary tuberculosis has led to the inappropriate treatment of many patients from whom NTM was isolated (15). To further complicate matters, the diagnosis of disease owing to NTM can be challenging, as colonization and contamination of laboratory cultures by NTM must be ruled out (16).

Thus, the detection of AFB in respiratory specimens and/or the isolation of NTM species can pose diagnostic problems for the clinician. In recent years, this issue has become even more relevant, as the frequency of NTM detection in sputum and bronchial washings—as well as the number of patients with clinical disease caused by NTM—have risen in South Korea (6,17–20).

Given these clinical challenges, further knowledge of epidemiologic trends of NTM prevalence in Korea is needed. The purpose of this study was to determine the frequency with which various species of NTM isolates from respiratory specimens were cultured in a single institution over a given 14-year period in Korea.

## MATERIALS AND METHODS

### Collection and Preparation of the Samples

All samples referred to our reference laboratory from various hospital and clinics in Korea, during the 14 years from 1993 to 2006 were analyzed. Samples analyzed were cultured isolates (89.6%), sputum (8.4%), and others (2%, bronchial wash, urine, tissue, pleural fluid, pericardium fluid, etc.). Aside from cultured isolates, all clinical specimens were decontaminated using the *N*-acetyl-L-cysteine 2% NaOH method and stained using the Ziehl–Neelsen method according to the guidelines provided by the American Thoracic Society (21). Then the treated specimens were plated onto Löwenstein–Jensen (L-J) solid medium and incubated at 37°C for conventional biochemical testing. All cultured colonies were evaluated according to the growth rates, colonial morphologies, and pigmentation and by with the basis of conventional techniques that included microbiological characteristics and biochemical tests (22).

### Species Identification

From 1993 to 2000 our laboratory used conventional NTM identification methods. Standard traditional methods of NTM speciation relies upon the phenotypic characteristics of biochemical testing, pigment

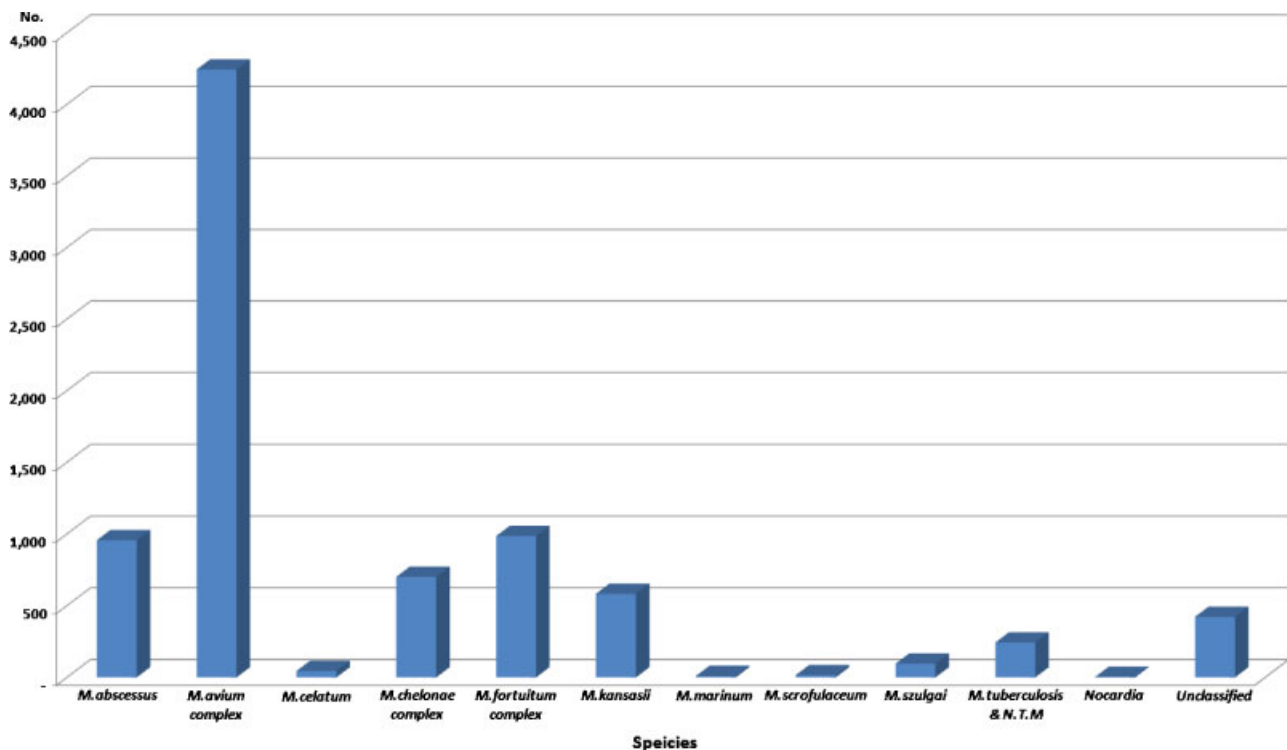


Fig. 1. Total NTM frequencies of the patients for 14 years (1993~2006).

production, growth characteristics, and colonial morphology had been performed until 2000 (23–25). Nowadays, molecular methods present many advantages over conventional methods of NTM identification. The results are obtained rapidly, are reliable and reproducible, and even mixed or contaminated cultures can be examined. Therefore, as of 2001, the Korean Institute of Tuberculosis has been using PCR-restriction fragment length polymorphism (PRA) methods using the novel region of the *rpoB* gene (26–28). After the extraction of genomic DNA from an isolates, we perform PCR amplification of the *rpoB* region using a primer set consisting of 5'-TCAAGGAGAAGCGCTACGA-3' (RPO 5') and 5'-GGATGTTGATCAGGGTCTGC-3' (RPO 3'). After successful amplification of the 360-base pair PCR products is confirmed, the PCR products are digested with restriction enzyme, including *BstEII* (Fermentas Life Science, MBI, Lithuania) and *KpnI* enzyme (Fermentas Life Science, MBI, Lithuania). The samples are then loaded onto a 4% Metaphore agarose gel (FMC BioProducts; Rockland, ME). Subsequently, enzyme-digested fragments are visualized under ultraviolet light after staining with ethidium bromide. According to the sizes of the restricted fragments, the NTM species can be identified on the basis of established algorithms (27–28).

## RESULTS

A total of 17,915 isolates belonging to 10,242 patients were collected from 1993 to 2006. The mean age of the

patients was 63.4-years old (SD 15.6); 4,491(43.8%) of these patients were men and 5,751 (56.2%) were women. From 1993 to 2000, cultures of 5,656 respiratory specimens from 2,434 patients were requested for NTM identification. And from 2001 to 2006, 12,259 isolates from 7,812 patients were identified. Before using the PRA assay *M. abscessus* could not be identified at the species level, and *M. avium* and *M. intracellulare* were simply grouped as *M. avium complex*. In addition, *M. chelonae* and *M. gordonae* were clustered as *M. chelone complex* until 2,000. Among the 17,915 isolates, the most frequently isolated organisms were *M. avium complex* ( $n=11,705$ , 65%), *M. abscessus* ( $n=2,076$ , 11.59%), *M. fortuitum complex* ( $n=1,279$ , 7.14%), *M. chelonae complex* ( $n=1,134$ , 6.33%), *M. kansasii* ( $n=762$ , 4.25%), *M. szulgai* ( $n=139$ , 0.78%), *M. celatum* ( $n=87$ , 0.49%), *M. scrofulaceum* ( $n=18$ , 0.10%), and *M. marinum* ( $n=11$ , 0.06%) (Table 1). Based on the number of patients in whom species were observed, the order of the prevalent species was *M. avium complex* ( $n=5,964$ , 41%), *M. fortuitum complex* ( $n=986$ , 9.63%), *M. abscessus* ( $n=957$ , 9.34%), *M. chelonae complex* ( $n=913$ , 8.91%), *M. kansasii* ( $n=584$ , 5.70%), *M. szulgai* ( $n=96$ , 0.94%), *M. celatum* ( $n=45$ , 0.43%), *M. scrofulaceum* ( $n=16$ , 0.16%), and *M. marinum* ( $n=10$ , 0.10%) (Fig. 1, Table 1).

From 2001 to 2006 data according to patients, total number of *M. intracellulare* ( $n=3,047$ , 71.8%) was consistently more common than *M. avium* ( $n=1,197$ , 28.2%) within *M. avium complex* samples ( $n=4,244$ , 100%). Similarly, within *M. chelonae complex* ( $n=702$ ,

TABLE 1. Frequently Isolated NTMs According to the Patients and Identification Numbers

Species	1993–2006		1993–2006		1993–2000		1993–2000		2001–2006		2001–2006	
	Identification		Patients		Identification		Patients		Identification		Patients	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
<i>M. avium complex</i>	11,705	65	5,964	41	4,731	84	1,720	71	6,974	56.89	4,244	54.33
( <i>M. avium</i> )	1,777	9.92	1,197	11.69	0	0	0	0	1,777	14.50	1,197	15.32
( <i>M. intracellulare</i> )	5,197	29.01	3,047	29.75	0	0	0	0	5,197	42.39	3,047	39.00
<i>M. fortuitum complex</i>	1,279	7.14	986	9.63	287	5.07	246	10.11	992	8.09	740	9.47
<i>M. abscessus</i>	2,076	11.59	957	9.34	0	0	0	0	2,076	16.93	957	12.25
<i>M. chelonae complex</i>	1,134	6.33	913	8.91	332	6	215	8.83	802	6.54	702	8.99
( <i>M. chelonae</i> )	123	0.69	102	1.00	0	0	0	0	123	1.00	102	1.31
( <i>M. gordonae</i> )	679	3.79	600	5.86	0	0	0	0	679	5.55	600	7.68
<i>M. kansasii</i>	762	4.25	584	5.70	131	2	105	4.31	631	5.15	479	6.13
Unclassified	450	2.51	423	4.13	13	0.23	13	0.53	437	3.56	410	5.25
<i>M. tuberculosis</i> & NTM	250	1.40	244	2.38	76	1	74	3.04	174	1.42	170	2.18
<i>M. szulgai</i>	139	0.78	96	0.94	54	0.95	37	1.52	85	0.69	59	0.76
<i>M. celatum</i>	87	0.49	45	0.43	15	0.27	9	0.37	72	11.00	36	10.29
<i>M. scrofulaceum</i>	18	0.10	16	0.16	13	0	11	0.45	5	0.04	5	0.06
<i>M. marinum</i>	11	0.06	10	0.10	4	0.07	4	0.16	7	0.06	6	0.08
<i>Nocardia</i>	4	0.02	4	0.04	0	0	0	0	4	0.03	4	0.05
Total	17,915	100.00	10,242	100.00	5,656	100	2,434	100.00	12,259	100.00	7,812	100.00

TABLE 2. Annual NTM Frequencies of the Patients

Species	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
<i>M. avium</i> complex	217	191	191	147	207	264	263	240	441	479	536	959	919	910	5,964
( <i>M. avium</i> )	0	0	0	0	0	0	0	0	81	94	135	281	304	302	1,197
( <i>M. intracellulare</i> )	0	0	0	0	0	0	0	0	360	385	401	678	615	608	3,047
<i>M. fortuitum</i> complex	42	16	25	14	16	28	48	57	66	133	158	136	139	108	986
<i>M. abscessus</i>	0	0	0	0	0	0	0	0	85	115	159	240	194	164	957
<i>M. chelonae</i> complex	22	15	15	14	25	40	42	42	66	148	165	140	97	82	913
( <i>M. chelonae</i> )	0	0	0	0	0	0	0	0	6	15	32	23	21	5	102
( <i>M. gordonae</i> )	0	0	0	0	0	0	0	0	60	133	133	117	76	77	596
<i>M. kansasii</i>	6	9	10	11	7	19	16	27	44	50	69	114	100	102	584
Unclassified	0	1	2	0	2	0	5	3	8	60	72	75	88	107	423
<i>M. tuberculosis</i> & NTM	0	0	0	0	0	0	0	0	41	39	49	39	36	40	244
<i>M. szulgai</i>	3	3	0	1	4	3	8	15	5	14	10	11	11	8	96
<i>M. celatum</i>	0	0	0	0	0	0	0	0	4	5	8	14	10	4	45
<i>M. scrofulaceum</i>	1	3	0	1	2	4	0	0	0	2	0	0	3	0	16
<i>M. marinum</i>	0	0	0	0	2	1	0	1	1	0	1	1	2	1	10
<i>Nocardia</i>	0	0	0	0	0	0	0	0	0	0	2	0	1	1	4
Total	291	238	243	188	265	359	382	385	761	1,045	1,229	1,729	1,600	1,527	10,242

100%), the number of *M. gordonae* ( $n = 600$ , 85.5%) was always more commonly observed than *M. chelonae* ( $n = 102$ , 14.5%) (Table 2).

Based on annual figures (Table 2), *M. fortuitum* complex ( $n = 1,279$ , 7.14%), *M. chelonae* complex ( $n = 1,134$ , 6.33%), and *M. kansasii* ( $n = 762$ , 4.25%) have increased steadily since 2001 ( $P$ -value by Cochran-Armitage trend test: *M. fortuitum* complex;  $P = 0.0370$ , *M. chelonae* complex;  $P = 0.0106$  and *M. kansasii*;  $P < 0.001$ ) (Fig. 2).

## DISCUSSION

This study has several limitations. First, we do not have the clinical data to determine the proportion of isolates associated with clinical disease. Both the American Thoracic Society and the British Thoracic Society have issued guidelines regarding the diagnosis of NTM lung disease. Both sets of guidelines highlight the difficulty of differentiating patients with clinical lung disease caused by NTM from those in whom NTM is nonpathogenic. The isolation of NTM species from a respiratory sample is insufficient evidence for the presence of NTM lung disease, the diagnosis of which must rely on clinical, radiographic, and bacteriologic criteria. Second, we cannot determine whether the rise in NTM isolates is owing to increased frequency of testing or whether these trends represent a true epidemiologic trend.

However, our data represent one of the largest collections of nationally representative isolates spanning more than a decade (2,29–31). Furthermore, our results show that the prevalence of NTM among clinical isolates is growing and that the relative frequency of certain NTM species is dynamic over time. The major

rise in the frequency of NTM isolation and the identification, which occurred around the time of change is significantly dependent upon the improvement of the laboratory techniques. In particular, until we adapted the PRA method in 2001, identifying, what was known as, the *M. chelonae* subsp. *abscessus* (32) was incapable; but now, with the PRA method, the species can be classified as *M. abscessus*. Although our samples is not strictly speaking population based, it likely is a true representation of the epidemiology of NTM in Korea, unless referral patterns to the laboratory have changed significantly, something that we do not believe has occurred.

There is marked geographic variability in the prevalence of mycobacterial species that are responsible for disease (33). In the United States and Japan, *M. avium* complex and *M. kansasii* are the most common species (8,34), whereas in England and Scotland, the most commonly observed species are *M. kansasii* and *M. malmoense*, respectively (6). Until now, prevalence data for NTM species in Asian countries have not been reported (9).

The major finding of this study has been to summarize the most common NTM isolates from clinical specimens in South Korea. Like the United States and many other reported countries, *M. avium* complex was the most common species; but contrary to the other countries, *M. fortuitum* complex was the second most common, followed by *M. abscessus* and *M. chelonae* complex. Among the rapidly growing mycobacteria, *M. fortuitum* complex and *M. abscessus* have not been reported as predominant species among NTM isolates in other countries. In Japan and many other countries, *M. kansasii* is endemic, but in Korea, it ranks only fifth in prevalence among all of the isolated NTMs. However,

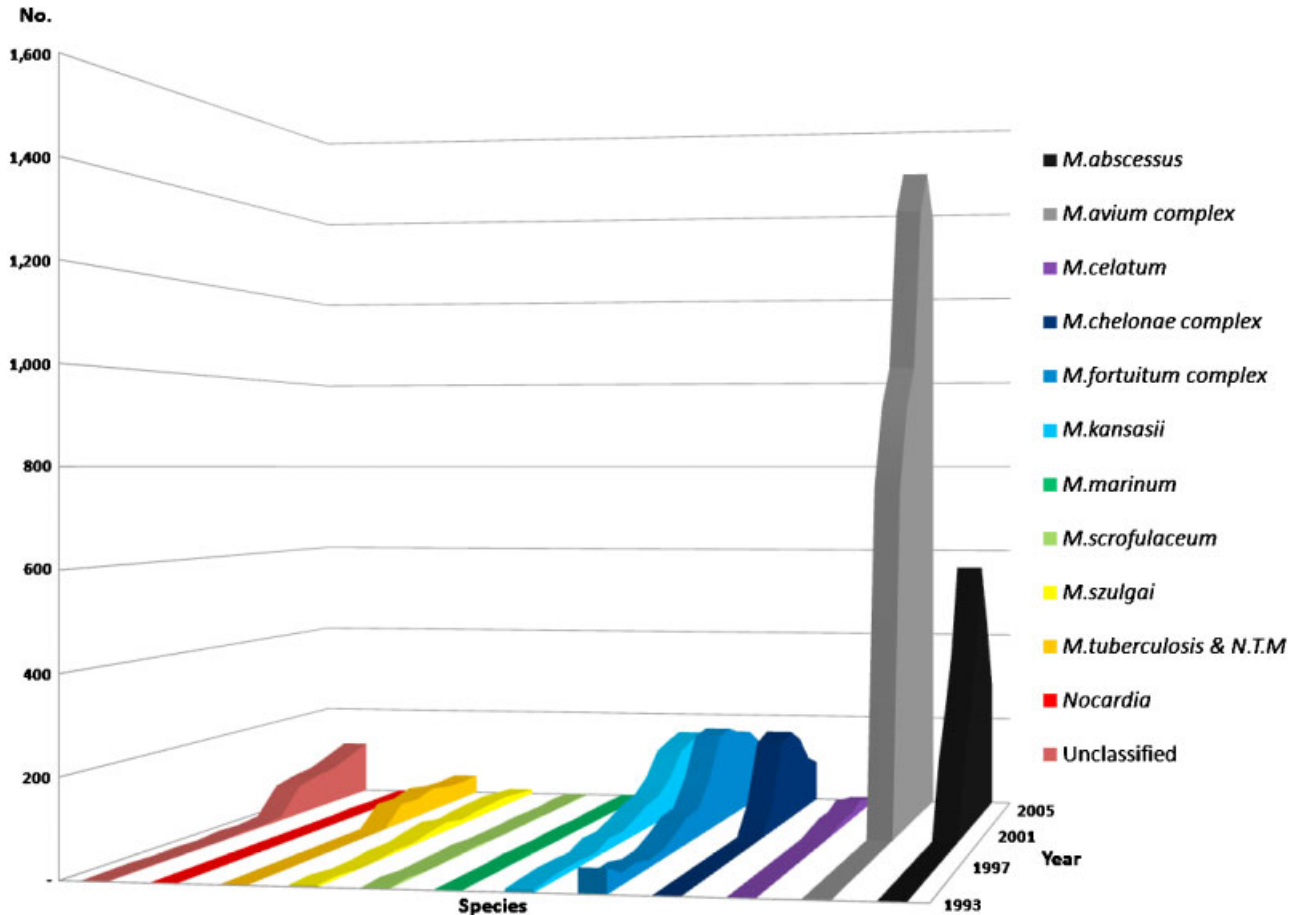


Fig. 2. Annual NTM frequencies of the patients.

*M. kansasii* is steadily increasing with significant trends in Korea.

In conclusion, the most common isolates of NTM patients in South Korea were *M. avium complex* and *M. fortuitum complex*. The most commonly encountered NTM species existing in Korean patients are different from those reported in other countries.

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