Original Article

Regional differences of nontuberculous mycobacteria species in Ulsan, Korea

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Background: In Korea recently, nontuberculous mycobacteria (NTM) have been more frequently isolated in respiratory specimens, while *Mycobacterium tuberculosis* (MTB) isolations have decreased. The major NTM lung disease species in Korea are *M. intracellulare*, *M. avium*, and *M. abscessus*, whereas *M. kansasii* is a rare species. This retrospective study was performed to determine if there are region-specific characteristics of lung disease-causing NTM species in Ulsan, a highly industrialized city in Korea.

Methods: Between January 2010 and July 2013, the results of all acid-fast bacilli (AFB) cultures of respiratory specimens performed at Ulsan University Hospital (Ulsan, Korea) were collected. NTM were identified and regional differences of NTM species were compared.

Results: AFB cultures were performed on 33,567 respiratory specimens, obtained from 10,208 patients, during the study period. Further, 10% of the specimens (3,287/33,567) were AFB culture-positive [MTB, 2,288/3,287 (70%); NTM 999/3,287 (30%)]. The proportion of NTM isolations gradually increased between 2010 and 2013, at 25% and 38%, respectively. The most common NTM species was *M. intracellulare* (356/999, 36%), followed by *M. kansasii* (295/999, 30%), *M. avium* (161/999, 16%), *M. abscessus* (117/999, 12%) and *M. fortuitum* (39/999, 4%). This trend was maintained throughout the study period.

Conclusions: In Ulsan, NTM isolation from respiratory specimens is increasing, consistent with previous studies performed in Korea. The distribution of respiratory NTM species, however, differed from previous studies that were performed in other regions of Korea: *M. kansasii* was the second most common NTM species in Ulsan. In Ulsan, there is a regional difference in the NTM species isolated.

Keywords: Nontuberculous mycobacteria (NTM); *M. kansasii*; Ulsan

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Background

In Korea, nontuberculous mycobacteria (NTM) are frequently identified in acid-fast bacilli (AFB) respiratory cultures. Indeed, NTM isolation rates have been reported to be equal to or exceed those of *Mycobacterium tuberculosis* (MTB) (1-5). With the increasing prevalence of NTM isolations in respiratory specimens, the occurrence of NTM lung disease, which is the most common disease caused by NTM, is increasing in Korea (5).

The NTM species that cause lung disease vary between countries and regions (6-8). In the United States and Japan, *M. avium* complex (*M. intracellular* and *M. avium*) and *M. kansasii* are the major causes of NTM lung disease. In the United Kingdom, *M. kansasii* is the most prevalent species in England and Wales. By contrast, *M. malmoense* is most common in Scotland, and *M. xenopi* in southeast England. In Korea, *M. intracellulare*, *M. avium*, and

M. abscessus have been reported to be the major causative species of NTM lung disease (2-4,9). In contrast, *M. kansasii* is thought to be a rare cause of NTM lung disease in Korea.

This retrospective study was performed to determine if there are region-specific characteristics of lung disease-causing NTM species in Ulsan, a highly industrialized city in Korea.

Methods

Study population

At Ulsan University Hospital, a 1,000-bed referral hospital in Ulsan South Korea, the results of AFB cultures of respiratory specimens, collected between January 2010 and July 2013, were retrospectively analyzed. Respiratory specimens included sputa and bronchoscopic specimens, which included bronchial washing fluids and bronchoalveolar lavage fluids. This study was approved by the Institutional Review Board and Ethics Committee of Ulsan University Hospital.

Acid fast bacilli culture and identification

Respiratory specimens were cultured in both solid and liquid media: 3% Ogawa solid egg-based medium (Asan Pharmaceutical, Seoul, Korea) and Mycobacteria Growth Indicator Tube liquid medium (Becton Dickinson, Sparks, MD, USA). If an AFB culture was positive in either liquid or solid medium, high performance liquid chromatography was performed to identify the mycobacterial species (10).

Diagnostic criteria for NTM lung disease

The diagnosis of NTM lung disease was performed according to the 2007 American Thoracic Society (ATS)/Infectious Diseases Society Of America (IDSA) guidelines (11).

Comparison of the major NTM species in regions of Korea

To date, there have been four detailed surveys of respiratory NTM species in Korea (2,4,9); surveys were performed at the university hospital in Seoul [Seoul Samsung Medical Center (Seoul-SMC) and Seoul National University Hospital (Seoul-SNU)] (5,9), 1 was performed at the university hospital in Jeju (4), and 1 was performed at the referral hospital in Masan (2). To determine the regional differences in NTM species in Korea, the data from the previously published papers were compared to the data obtained in this study. The raw data from the study at Seoul National University (Seoul-SNU) (5) were obtained directly from the corresponding author, as there was insufficient data in the published paper. The number of patients with respiratory NTM and the number patients with NTM lung disease were compared separately. There were data limitations: data from the Jeju study was only available for patients with respiratory NTM and date from the Seoul-SNU study was only available for patients with NTM lung disease.

Statistical analysis

Statistical analyses were performed using the software R (a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria; Version 3.0.2). Independent *t*-test and Kruskal-Wallis test were used to compare means. And, Fisher's exact test and Cochran-Armitage trend test were used to compare frequencies. A P value of less than 0.05 was considered to be significant.

Results

Numbers of patients and respiratory specimens

A total 33,567 respiratory specimens from 10,208 patients were collected for AFB culture during the study period.

Mycobacterial isolations from respiratory specimens

Over the 4-year collection period, 3,287 (10%) AFB culture-positive mycobacteria were isolated from 33,567 specimens. Of the samples in which mycobacteria were isolated, 70% were positive for MTB (2,288/3,287), while the remaining 30% were positive for NTM (999/3,287).

Change in proportions of NTM and MTB isolations from respiratory specimens

The proportion of NTM isolations increased, from 25% to 38% in 2010 and 2013, respectively. Over the same time period, the proportion of MTB isolations significantly decreased (*Figure 1*; P<0.001, Cochran-Armitage trend test).

Identification of NTM species isolated from respiratory specimens

In Ulsan, the most common NTM species was *M. intracellulare* (356/999, 36%), followed by *M. kansasii* (295/999, 30%), *M. avium* (161/999, 16%), *M. abscessus* (117/999, 12%), *M. fortuitum* (39/999, 4%), *M. szulgai* (23/999, 2%),

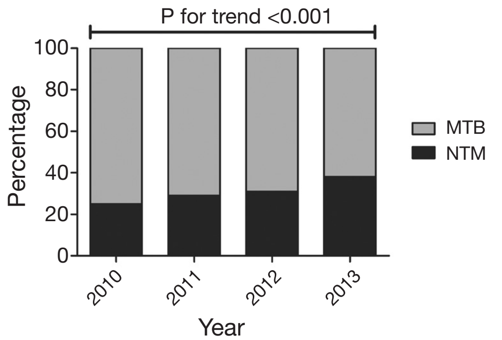

Figure 1 The proportion of nontuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* (MTB) isolations in Ulsan, Korea between January 2010 and July 2013.

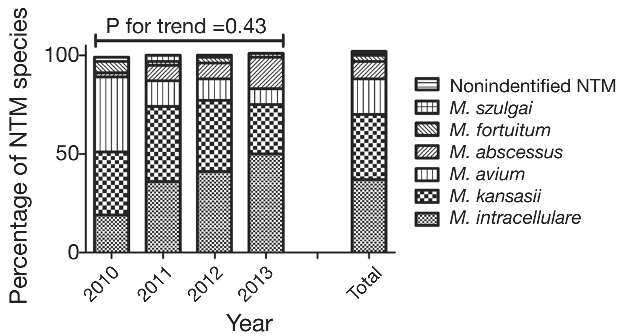

Figure 2 The proportion of nontuberculous mycobacteria (NTM) species in Ulsan, Korea between January 2010 and July 2013.

M. chelonae (3/999, 0.3%), *M. simiae* (1/999, 0.1%), and unidentified NTM (4/999, 0.4%). During the study period, *M. kansasii* was consistently the second most common NTM species (*Figure 2*; P=0.43, Cochran-Armitage trend test).

Pulmonary tuberculosis, respiratory NTM, and NTM lung disease frequency in patients

A total of 12% of patients (1,171/10,208 patients) were identified to be AFB culture-positive with mycobacteria isolated from the specimens. Of the mycobacteria infected patients, 65% (765/1,171) were diagnosed with pulmonary tuberculosis and 35% (410/1,171) were diagnosed with respiratory NTM. A total of 245 of the 410 patients diagnosed with respiratory NTM [21% (245/1,171) of all AFB culture-positive patients] were diagnosed with NTM lung disease.

In the patients diagnosed with respiratory NTM (N=410), the causative mycobacterial species were *M. intracellulare* (169/410, 41%), *M. kansasii* (102/410, 25%), *M. avium* (61/410, 15%), *M. abscessus* (33/410, 8%), *M. fortuitum* (24/410, 6%), *M. szulgai* (15/410, 4%), *M. chelonae* (3/410, 0.7%), *M. simiae* (1/410, 0.2%), and unidentified NTM (2/410, 0.5%). In the patients diagnosed with NTM lung disease (N=245), the causative mycobacterial species were *M. intracellulare* (93/245, 38%), *M. kansasii* (80/245, 33%), *M. avium* (39/245, 16%), *M. abscessus* (22/245, 9%), *M. fortuitum* (7/245, 3%), *M. szulgai* (3/245, 1%), and unidentified NTM (1/245, 0.4%).

Patients with NTM lung disease were older than those with pulmonary tuberculosis (mean age 62 and 55 years, respectively; P<0.001). There was a predominance of men in both the pulmonary tuberculosis and NTM lung disease patient populations. However, there were sex differences in the causative mycobacterial species isolated from the NTM lung disease patients. Patients with lung disease caused by *M. kansasii* were predominantly male, while patients with lung disease caused by the other NTM species were predominantly female (P<0.001; *Table 1*).

Regional differences in NTM species in patients with respiratory NTM and NTM lung disease

When the results of previous Korean NTM distribution studies were compared with the results obtained in this study, we found that in patients with respiratory NTM, the major causative species differed (*Figure 3A*). In the Masan study (2), the causative mycobacterial species were *M. intracellulare* (28/57, 49%), *M. abscessus* (12/57, 21%), *M. fortuitum* (7/57, 12%), *M. chelonae* (4/57, 7%), and *M. avium* (3/57, 5%). In the Jeju study (4), the causative mycobacterial species were *M. intracellulare* (27/62, 44%), *M. avium* (14/62, 23%), *M. abscessus* (8/62, 13%), *M. kansasii* (5/62, 8%), and *M. fortuitum* (4/62, 7%). In the Seoul-SMC study (9), the causative mycobacterial species were *M. fortuitum* (217/794, 27%), *M. abscessus* (141/794, 18%), *M. avium* (111/794, 14%), *M. intracellulare* (108/794, 14%), M. gordonae (84/794, 11%), *M. terrae* (48/794, 6%), *M. szulgai* (32/794, 4%), *M. chelonae* (25/794, 3%), and *M. kansasii* (14/794, 2%). In contrast, in Ulsan the proportion of respiratory NTM patients infected with *M. kansasii* was significantly higher (P<0.001; *Figure 3B*).

Similarly, when the distributions of NTM lung disease causative mycobacterial species were compared, the major causative species differed between the Korean regions

Table 1 Patients with pulmonary tuberculosis (*Mycobacterium tuberculosis*) and NTM lung disease in Ulsan, Korea between January 2010 and July 2013

	Patients (number)	Male (%)	P value	Age, years (mean)	P value
M. tuberculosis	765.0	56	0.825	55.2±19.6	<0.001
NTM lung disease	245.0	55		61.6±14.3	
NTM lung disease by species			<0.001		0.001
M. intracellulare	93.0	41		66.6±13.1	
M. avium	39.0	41		58.1±13.6	
M. kansasii	80.0	85		59.4±13.9	
M. abscessus	22.0	32		59.1±16.8	
M. fortuitum	7.0	29		58.9±16.2	
M. szulgai	3.0	100		62.7±15.4	
Unidentified NTM	1.0	0		38.0	

NTM, nontuberculous mycobacteria.

examined previously (*Figure 4A*). In the Masan study (2), the NTM lung disease causative mycobacterial species were *M. intracellulare* (19/26, 73%), *M. abscessus* (5/26, 19%), *M. fortuitum* (1/26, 4%), and M. chelonae (1/26, 4%). In the Seoul-SMC study (9), the NTM lung disease causative mycobacterial species were *M. abscessus* (64/195, 33%), *M. intracellulare* (56/195, 29%), *M. avium* (38/195, 19%), *M. fortuitum* (21/195, 11%), *M. kansasii* (7/195, 4%), *M. chelonae* (6/195, 3%), and *M. szulgai* (2/195, 1%). In the Seoul-SNU study (5), the major causative mycobacterial species were *M. avium* (212/585, 36%), *M. abscessus* (163/585, 28%), *M. intracellulare* (156/585, 27%), *M. kansasii* (18/585, 3%), *M. gordonae* (7/585, 1%), and *M. terrae* (4/585, 0.7%). In our study of Ulsan patients, the proportion of cases in which *M. kansasii* was the NTM lung disease causative species was significantly higher (P<0.001; *Figure 4B*).

Discussion

Similar to what has been observed previously in other regions of Korea, we showed that NTM isolation is increasing in Ulsan. However, the distribution of NTM species was different from previous studies: *M. kansasii* was the second most common NTM species in Ulsan; whereas in other regions of Korea, *M. kansasii* is rarely reported. Thus, we concluded that there is a regional difference in the NTM species isolated in Ulsan, Korea.

Consistent with previous studies, we found that in Ulsan, the proportion of NTM isolations has been increasing. Specifically, between 2010 and 2013, the proportion of NTM isolations increased from 25% to 38%. Over the same time period, the proportion of MTB isolations significantly decreased. In a previous study in Seoul, it was found that the proportion of NTM isolations increased from 22.2% in 2002 to 45.9% in 2008 (5); in a previous study in Jeju, the proportion of NTM isolations increased from 7% in 2003 to 19% in 2011 (4). The increase in NTM isolation appears to be happening throughout Korea, and the rate of NTM isolations seems to be proportionally correlated with the degree of urbanization, as Seoul is the most populated city in Korea and Jeju is a rural resort town in Korea.

Worldwide, NTM isolations have been increasing, a phenomenon that can be partially explained (3). First, improved laboratory diagnostic methods have enhanced recovery of mycobacteria (12). Second, exposure to NTM via aerosolized water is considered to be a cause of increased NTM infection in modern people (8). Finally, host factors including old age and chronic obstructive pulmonary disease are risk factors for the development of NTM disease (13). Further studies are required to fully elucidate the reason for the worldwide rapid increase in NTM isolations.

Depending on countries and/or regions, the distributions of lung disease-causing NTM species are different (6-8). In Korea, according to previous studies, *M. intracellulare*, *M. avium*, and *M. abscessus* are the major NTM lung disease species (2-4,9), in contrast, *M. kansasii* is a rare NTM lung disease species (3). In the current study, we showed that in Ulsan the distribution of NTM species differed when compared with other regions of Korea. *M. kansasii* was identified in 25% of patients with respiratory NTM and in

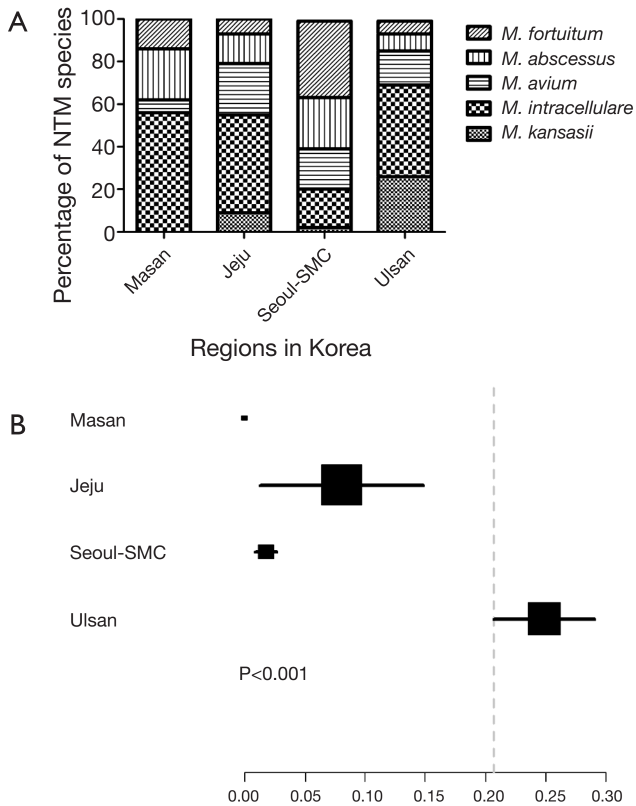

Figure 3 Nontuberculous mycobacteria (NTM) species in patients with respiratory NTM: (A) the distribution of NTM species in regions of Korea; (B) the ratios of *Mycobacterium kansasii* isolation by region of Korea. Seoul-SMC, Seoul Samsung Medical Center.

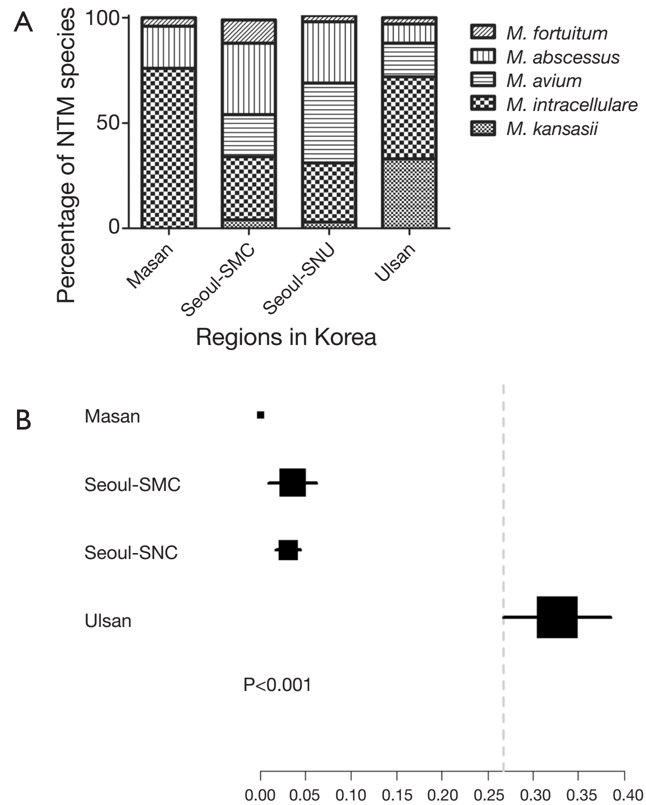

Figure 4 Nontuberculous mycobacteria (NTM) species in patients with NTM lung disease: (A) the distribution of NTM species in regions of Korea; (B) the ratios of *Mycobacterium kansasii* isolation by region of Korea. Seoul-SMC, Seoul Samsung Medical Center; Seoul-SNU, Seoul National University Hospital.

33% of patients with NTM lung disease. Thus, there is a significant regional difference in the distribution of NTM species within Korea.

The high proportion of *M. kansasii* isolations in Ulsan is not well understood. Unlike other NTM, *M. kansasii* is not found in soil or natural water supplies, but it has been recovered from tap water in cities where *M. kansasii* is endemic (11,14). In addition, *M. kansasii* is more likely to be isolated in urban or industrialized regions (14,15). However, urbanization and/or industrialization are not critical factors for M. kansasii infection, as there have been reports of rare *M. kansasii*—distribution in highly urbanized or industrialized cities (2,5,9). It is possible that there are environmental and occupational risk factors that are associated with *M. kansasii* infection. Thus, further studies to elucidate factors that are associated with *M. kansasii* infection are required.

There are limitations to this study. First, this study was a retrospective, single-center study. There may sources of bias that have not been identified or controlled for in this study. As our hospital is the only referral medical center in Ulsan, the results are likely indicative of the extent of respiratory NTM isolations in Ulsan. Second, our research identified the high proportion of *M. kansasii* species isolations in Ulsan, although we were unable to explain this observation. Therefore, further research is required.

Conclusions

In conclusion, we showed that in Ulsan respiratory NTM infection and NTM lung disease is increasing, similar to what has been observed worldwide and in other regions of Korea. However, the distribution of NTM species differed from previous studies in Korea: *M. kansasii*, a rarely reported organism in other areas of Korea, was extremely common and was the second most commonly observed NTM species following *M. intracellulare*. Therefore, there is a regional difference in the distribution of NTM species in Ulsan, Korea.

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