Clinical Significance of Nontuberculous Mycobacteria Isolated From Respiratory Specimens in Korea*

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Study objectives: Precise epidemiologic data regarding nontuberculous mycobacterial (NTM) lung infection in many Asian countries have been relatively unavailable. In order to determine the clinical significance of NTM isolated from respiratory specimens, we reviewed medical records from all patients from whom NTM isolates were recovered within a 2-year period.

Materials and methods: We identified all NTM isolates from respiratory specimens at the Samsung Medical Center (Seoul, South Korea) obtained from January 2002 to December 2003. We then reviewed the clinical and radiologic characteristics of the patients from whom NTM was isolated. Patients were classified as having either definite, probable, or unlikely NTM lung disease, as defined by the guidelines of both the American Thoracic Society and the British Thoracic Society.

Results: During the study period, 1,548 NTM isolates were recovered from 794 patients. Of these 794 patients, 131 patients (17%) were determined to have definite NTM lung disease, and 64 patients (8%) were designated as probable disease candidates. The most commonly involved organisms in the definite and probable NTM lung disease cases were Mycobacterium avium complex (n = 94, 48%) and Mycobacterium abscessus (n = 64, 33%). In 195 patients with NTM lung disease, 82 patients (42%) manifested the upper lobe cavitary form, 101 patients (52%) exhibited the nodular bronchiectatic form, and 12 patients (6%) exhibited the unclassifiable form.

Conclusions: About one fourth of the patients in whom NTM was isolated from respiratory specimens were found to have clinically significant NTM lung infections. The spectrum of organisms responsible for the NTM lung disease in these Korean patients differed from those reported in other regions of the world. However, the estimates of clinical significance in this study may be underestimates due to the retrospective analysis. Some of the patients might have “true” NTM lung disease that could be diagnosed with continued evaluation and follow-up.

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Key words: atypical mycobacteria; epidemiology; Korea; lung diseases; Mycobacterium abscessus; Mycobacterium avium complex

Abbreviations: AFB = acid-fast bacilli; NTM = nontuberculous mycobacteria; PCR = polymerase chain reaction

Mycobacteria other than Mycobacterium tuberculosis complex and Mycobacterium leprae are referred to, collectively, as nontuberculous mycobacteria (NTM).1–3 Human disease as the result of NTM infection is classified into four distinct clinical syndromes: pulmonary disease, lymphadenitis, cutaneous disease, and disseminated disease. Among these, chronic pulmonary disease is the most common

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localized clinical condition.\textsuperscript{1,2} Reports\textsuperscript{1,2} have demonstrated that disease attributable to NTM is on the rise, and NTM is responsible for an increasing proportion of mycobacterial disease in many developed countries. In addition, the distribution of the NTM variant is not uniform, and there appears to be marked geographic variability both with regard to the prevalence of NTM lung disease and with regard to the mycobacterial species responsible for it.\textsuperscript{1–4}

\textit{M. tuberculosis} is an obligate human pathogen with which no environmental reservoir has been associated. NTM, conversely, are ubiquitous organisms, and are frequently isolated from environmental sources, including surface water, tap water, and soil.\textsuperscript{1–3} Accordingly, 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. Both the American Thoracic Society and the British Thoracic Society have issued guidelines regarding the diagnosis of NTM lung disease.\textsuperscript{1,2} Both sets of guidelines highlight the difficulty inherent in differentiating patients with clinical lung disease caused by NTM from those in whom the isolation of clinical specimens raises a clinical suspicion of disease.

In South Korea, tuberculosis remains a serious public health problem, although its prevalence has been greatly reduced in recent years.\textsuperscript{5} In countries with classically high tuberculosis prevalence rates, like South Korea, those patients with acid-fast bacilli (AFB)-positive sputum on direct microscopic examination, or those displaying chest radiographic findings that suggest active tuberculosis, have generally been presumed to have pulmonary tuberculosis and were treated empirically with antituberculous drugs. Therefore, incorrect diagnoses of pulmonary tuberculosis led to the inappropriate or unnecessary treatment of many patients from whom NTM was isolated.\textsuperscript{6} Furthermore, when NTM are cultured and identified in respiratory specimens, NTM lung disease still requires differentiation from contamination or colonization. Thus, the detection of AFB in respiratory specimens, or the isolation of NTM species, may pose a diagnostic problem for the clinician. In recent years, this issue has become even more relevant, as the frequency with which NTM is detected in sputum or bronchial washings, as well as the number of patients with clinical disease caused by NTM, continues to increase rapidly in South Korea.\textsuperscript{7–12} The purpose of the present study was to determine the frequency with which various species of NTM isolates were cultured in a single institution over a given 2-year period, and to evaluate the clinical significance of isolated NTM species recovered from respiratory specimens.

Materials and Methods

\textbf{Study Subjects}

All consecutive isolates from a mycobacterial laboratory at the Samsung Medical Center (a 1,250-bed tertiary referral hospital in Seoul, South Korea) during the 2-year period from January 2002 to December 2003 were collected and analyzed. All identified NTM isolates were considered to be significant, with the exception of \textit{Mycobacterium gordonae}, a well-known environmental contaminant.\textsuperscript{3,13,14} Patients with culture findings positive for \textit{M. gordonae} were included in the study only if multiple isolates were recovered. NTM isolates recovered from nonrespiratory specimens, including urine and joint fluid, were excluded from this study.

\textbf{AFB Smears and Cultures}

Clinical specimens were stained using the Ziehl-Neelsen method according to the guidelines provided by the American Thoracic Society.\textsuperscript{15} The results of smear microscopy were reported semiquantitatively. A positive smear was defined as one with > 1 AFB per 100 high-power fields.

Sputum or bronchial washing specimens were decontaminated using the N-acetyl-L-cysteine 2% NaOH method. The processed specimens were plated onto 3% Ogawa medium. The inoculated tubes were incubated at 37°C and then inspected weekly for 8 weeks. All AFB isolates were assessed to distinguish between \textit{M. tuberculosis} and NTM, according to growth rates, colonial morphologies, and pigmentation, and by with a commercial DNA probe (Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test; Gen-Probe; San Diego, CA).\textsuperscript{15} During the study period, no changes were made in the laboratory methods utilized in the preparation of smears, the reading of smears, or the processing or culturing of specimens.

\textbf{Identification of NTM Species}

NTM species identification was performed using a polymerase chain reaction (PCR)-restriction fragment length polymorphism method, based on the \textit{rpoB} gene.\textsuperscript{16} After the extraction of genomic DNA from an isolate, we performed PCR amplification of the \textit{rpoB} region using a primer set consisting of 5’TCAAGGAGAACCGCTACCA-3’ (RPO5’\textsuperscript{16}) and 5’-GAGATGTGAT-CAGGCTCTGC-3’ (RPO3’). After successful amplification of the 360-base pair PCR products was confirmed, the PCR products were digested with restriction enzyme, including \textit{MspI} (Boehringer Mannheim Biochemicals, Mannheim, Germany) and \textit{HaeIII} enzyme (Takara Shuzo; Shiga, Japan). The samples were then loaded onto a 4% Metaphor agarose gel (FMC BioProducts; Rockland, ME). Subsequently, enzyme-digested fragments were visualized under ultraviolet light after staining with ethidium bromide. According to the sizes of the restricted fragments, the identification of NTM species proceeded on the basis of the algorithm.\textsuperscript{16}

\textbf{Diagnostic Criteria for NTM Lung Disease}

In 1997, the American Thoracic Society issued a revised set of diagnostic criteria for NTM lung disease.\textsuperscript{3} In 2000, the British Thoracic Society also published its guidelines for the management of NTM lung disease.\textsuperscript{5} According to the British Thoracic Society guidelines, NTM lung disease is indicated when positive cultures develop from specimens of sputum obtained at least 7 days apart (positive culture twice) in a patient whose chest radiograph suggests mycobacterial infection and who may or may
not manifest symptoms or signs. The American Thoracic Society guidelines, which include diagnostic criteria that are more strict than those of the British Thoracic Society statement, are as follows: in symptomatic patients with infiltrate, nodular, or cavitary disease, or a high-resolution CT scan showing multifocal bronchiectasis and/or multiple small nodules, the following apply: (1) three positive sputum/bronchial wash cultures with negative AFB smears, or two positive cultures and one positive AFB smear in the last 12 months; (2) if only one bronchial wash is available, then a positive culture with a 2+ to 4+ AFB smear or a 2+ to 4+ growth on solid media; (3) if sputum or bronchial wash prove nondiagnostic, then a transbronchial or lung biopsy that yields NTM, or a biopsy exhibiting mycobacterial histopathologic features and one or more spu, or bronchial washings, are positive for NTM, even if only in low numbers.

Evaluation of Clinical Significance of Isolated NTM

The medical records of all study patients were reviewed, including the following information: age, sex, respiratory symptoms, smoking history, underlying pulmonary and nonpulmonary illnesses, history of antituberculous therapy, history of immunosuppressive drug use, results of anti-HIV antibody, AFB smear status, number of positive isolates, and available lung biopsy results. Simple chest radiography and available chest CT images were reviewed by two independent chest radiologists (T.S.K., K.S.L.), both of whom were unaware of any clinical information, except for the fact that positive NTM isolates were detected in the respiratory specimens. Radiographic findings were evaluated with regard to the presence or absence of nodules, consolidation, cavity lesions, and bronchiectasis.

Patients were classified as having definite, probable, or unlikely NTM lung disease, based on the above criteria. Definite NTM lung disease was diagnosed if the patient fulfilled the clinical, radiologic, and microbiological criteria of the American Thoracic Society. Probable NTM lung disease was diagnosed if the patient fulfilled the British Thoracic Society criteria but did not meet the American Thoracic Society criteria; such as the case with two positive cultures with negative AFB smear sputum specimens in the last 12 months, although the patient exhibited respiratory symptoms and abnormal chest radiography or CT findings. NTM lung disease was judged unlikely if the patient fulfilled none of the criteria for either definite or probable NTM lung disease. The patients with definite or probable NTM lung disease were regarded to have clinically significant NTM lung infection.

After evaluating the clinical significance of isolated NTM, the clinically significant NTM lung infections were classified into three variants, according to radiographic and CT findings. The upper lobe cavitary form was defined as the presence of cavitary opacities in the upper lobes, either with or without findings of underlying chronic obstructive lung disease in the remaining lungs (Fig 1). The nodular bronchiectatic form was defined as the presence of bronchiectasis and multiple nodules on the chest CT scan, regardless of the presence of small cavities in the lungs (Fig 2, 3). When a patient’s disease belonged to neither the upper lobe cavitary nor the nodular bronchiectatic form, it was considered unclassifiable. In the unclassifiable form, multifocal lobular or segmental consolidations usually comprised the predominant finding.

RESULTS

Frequency of NTM Isolates

From 2002 to 2003, cultures of 1,548 respiratory specimens from 794 patients were found to be positive for NTM. The source of the isolate was sputum (n = 1,383, 89%), bronchial wash (n = 139, 9%), or lung tissue (n = 26, 2%). The mean age of the 794 patients was 56 ± 15 years (± SD). In 1,548 NTM isolates, the most frequently isolated organisms were Mycobacterium avium complex (n = 491, 32%), Mycobacterium abscessus (n = 442, 29%), and Mycobacterium fortuitum complex (n = 268, 17%) [Table 1].

The number of NTM isolates in a given individual was different between specific NTM species. Thirty-four percent (75 of 219 patients) from whom M avium complex was isolated and 35% (50 of 141 patients) from whom M abscessus was isolated had more than two positive cultures findings. In the patients from whom M fortuitum complex was isolated; however, only 15% (33 of 217 patients) had more than two positive culture findings, and 85% (184 of 217 patients) had only one positive M fortuitum complex culture finding. Therefore, among the 794 patients, the three most frequently isolated species were, in decreasing order, M avium complex (n = 219, 28%), M fortuitum complex (n = 217, 27%), and M abscessus (n = 141, 18%) [Table 1].

Clinical Significance of NTM Isolates

Patients were then grouped into three disease categories based on their likelihood of NTM disease
according to the definitions cited above. Of these 794 patients, 131 patients (17%) received a diagnosis of definite NTM lung disease, 64 patients (8%) had probable disease, and 599 patients (75%) had unlikely disease. Thus, approximately one fourth (195 of 794 patients) of those with NTM isolates were classified as having clinically significant NTM lung infections.

Etiology of NTM Lung Disease

The implicated NTM organisms are shown in Table 2. The most common organism isolated was \textit{M avium} complex, which accounted for 94 of the cases (48%) of clinically significant NTM lung infection (\textit{Mycobacterium intracellulare} in 56 patients and \textit{M avium} in 38 patients). \textit{M abscessus} accounted for 64 cases (33%). All together, \textit{M avium} complex and \textit{M abscessus} were implicated in 81% of the cases of both definite and probable NTM lung disease.

Clinical and Radiographic Characteristics of Patients With NTM Lung Disease

The mean age of the 195 patients with clinically significant NTM lung infections was 63 years (range,
patients (47%). Fifty-seven patients exhibited unilateral cavities, and 35 patients exhibited bilateral cavities. These cavities were most frequently observed in the upper lobes. Bronchiectasis was detected in 129 patients (66%). Consolidation was detected in 60 patients (31%) and was normally combined with cavitary lesions in the upper lobes. Eighty-two of 195 patients (42%; 62 men and 20 women) had the upper lobe cavitary form (Fig 1), 101 patients (52%; 20 men and 81 women) had the nodular bronchiectatic form (Fig 2, 3), and 12 patients (6%; 6 men and 6 women) had unclassifiable variants.

### Discussion

NTMs were first observed shortly after the discovery by Koch of the tubercle bacillus and were believed to represent environmental contamination or colonization; it was not until the 1950s that NTM were identified as potential pathogens. Since that time, these organisms have been implicated in an increasingly large proportion of pulmonary infections throughout the world, in both immunocompetent and immunocompromised hosts. The reasons for this increasing rate of NTM lung disease are unclear, but may result, at least in part, from an increase in the number of immunocompromised patients due to factors such as HIV infection, advances in science and technology with regard to the isolation and identification of NTM, and a decline in the incidence of tuberculosis. It has been speculated that infection with tuberculosis and bacillus Calmette-Guerin may provide cross-immunity protection against NTM infection.

There is also a marked geographic variability both in the prevalence of disease and in the mycobacterial species that are responsible for disease. In the United States, NTM lung disease is most commonly attributable to M avium complex, with M kansasii being second. In the United Kingdom, M kansasii is the pathogen most commonly associated with NTM lung disease in England and Wales, while Mycobacterium malmoense is the most common in Scotland. M xenopi predominates in Southeast England. In Japan, the most common cause of NTM pulmonary disease is M avium complex, followed by M kansasii. However, precise epidemiologic data regarding NTM disease in many Asian countries have remained largely unavailable until now.

This is the first study to document the clinical significance of NTM isolation from respiratory specimens in South Korea. The major findings of this study included the following: (1) a substantial proportion (25%) of the NTMs isolated from respiratory...
specimens were clinically significant; (2) *M avium* complex was the most common NTM isolated from clinical respiratory specimens, and also represented the most common pathogen in NTM lung disease in South Korea; and (3) contrary to the case in other many countries, *M abscessus* was the second most commonly isolated pathogen, and *M kansasii* was relatively uncommon in South Korea.

*M avium* complex has been reported to account for up to 60 to 80% of etiologic organisms in many parts of the world, including the United States and Japan.\(^4,17,21\) In this study, we determined that *M avium* complex was also the organism most commonly (48%) responsible for NTM lung disease in South Korea.

Rapidly growing mycobacteria, such as *M abscessus* (formerly *M chelonae* subspecies *abscessus*), have been reported to account for only approximately 10% of NTM lung disease etiology in the United States\(^4,17\) and < 5% in Japan.\(^4,21,22\) Interestingly, the second most common cause of NTM lung disease in the present study in South Korea was *M abscessus*.

Unlike other NTMs, *M kansasii* has never been isolated in soil or natural water supplies but has been recovered consistently from tap water in cities in which *M kansasii* is endemic. In Japan, as previously mentioned, the incidence of pulmonary disease caused by *M kansasii* has increased and has become the second most common (approximately 20%) cause of NTM pulmonary disease, next to *M avium* complex disease, since the late 1970s.\(^21,23\) *M kansasii* lung disease was once especially prevalent along the south coast of the Pacific Ocean in Japan, which is the principle industrial area.\(^23\) However, isolation of *M kansasii* appears to be rather uncommon in other Asian countries, including Hong Kong, Taiwan, and South Korea,\(^7,24,25\) all of which are now well-industrialized countries.

The clinical and radiographic characteristics of patients with NTM lung disease in the present study were comparable to those in many of the previously published reports.\(^8,9,26–34\) NTM lung disease has two distinct radiographic manifestations: an upper lobe cavitary form, and a nodular bronchiectatic form.\(^1\) The upper lobe cavitary form, which is the classic and most widely recognized presentation of NTM lung disease, is usually seen in middle-aged or elderly men who smoke or abuse alcohol. Underlying disorders commonly include COPD, previous tuberculosis, and silicosis.\(^1,26\) The characteristic radiographic findings associated with the upper lobe cavitary form include heterogeneous nodular and cavitary opacities, which are indistinguishable from those observed in conjunction with pulmonary tuberculosis.\(^1,26\) The nodular bronchiectatic form has only recently been characterized\(^1\) and occurs predominantly in nonsmoking middle-aged or elderly women who exhibit no underlying lung disease.\(^1,27–31\) The radiographic features of the nodular bronchiectatic form include bronchiectasis and multiple nodules, which tend to be most severe in the lingular segment and in the right middle lobe. In our study, 42% of patients with clinically significant NTM lung infection exhibited the upper lobe cavitary form, 52% exhibited the nodular bronchiectatic form, and 6% had the unclassifiable form. These figures were quite similar to previous reports from the United States and Japan.\(^22,35,36\)

The present study has several limitations that are generally inherent in retrospective analyses. Most importantly, continued investigation and follow-up duration were not sufficient to fully evaluate the clinical significance in some of the patients. Unlike tuberculosis, multiple positive culture findings are required before the diagnosis of NTM lung disease can be established. As the results of chest radiography in a patient with NTM disease can be normal at the time of presentation, the initial culture may be dismissed as colonization or contamination.\(^27\) A delay of several weeks before the initial mycobacterial culture becomes positive may also result in the patient being lost to follow-up, before a serial culture

### Table 2—Etiology of Clinically Significant NTM Lung Infection*

| Organisms            | Patients With Definite NTM Lung Disease (n = 131), No. | Patients With Probable NTM Lung Disease (n = 64), No. | Total (n = 195), No.(%)
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><em>M avium</em> complex</td>
<td>66</td>
<td>28</td>
<td>94 (48)</td>
</tr>
<tr>
<td><em>M intracellulare</em></td>
<td>42</td>
<td>14</td>
<td>56*</td>
</tr>
<tr>
<td><em>M avium</em></td>
<td>24</td>
<td>14</td>
<td>38*</td>
</tr>
<tr>
<td><em>M abscessus</em></td>
<td>52</td>
<td>12</td>
<td>64 (33)</td>
</tr>
<tr>
<td><em>M fortuitum</em> complex</td>
<td>4</td>
<td>17</td>
<td>21 (11)</td>
</tr>
<tr>
<td><em>M kansasii</em></td>
<td>5</td>
<td>2</td>
<td>7 (4)</td>
</tr>
<tr>
<td><em>M chelonae</em></td>
<td>2</td>
<td>4</td>
<td>6 (3)</td>
</tr>
<tr>
<td><em>M szulgai</em></td>
<td>1</td>
<td>1</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>M celatum</em></td>
<td>1</td>
<td>0</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

*Data are presented as No. of cases.

\(\text{Original Research}\)
can be performed. In addition, as organisms may be shed only intermittently, a clearer discussion of the significance of NTM infection can be established if many subsequent cultures are taken over several months.\textsuperscript{37} In addition, we used only solid media, rather than liquid media, for mycobacterial culture during the study period. It has been shown that the liquid culture system exhibited a higher recovery rate regarding the \textit{M avium} complex and other NTM.\textsuperscript{1} Therefore, some of the cases classified as unlikely NTM lung disease may have, in fact, been true NTM lung disease that was not detected during the follow-up period. This could be especially true in the patients with relatively virulent NTM species, such as \textit{M kansasii}, \textit{M abscessus}, or \textit{M szulgai}.

In conclusion, a substantial proportion (25\%) of patients from whom NTM isolates were recovered exhibited clinically significant NTM lung infections in South Korea. The most common etiologies of NTM lung disease included \textit{M avium} complex and \textit{M abscessus}. The spectrum of organisms responsible for the NTM lung disease in these Korean patients differed from those reported in other regions of the world.

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