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First Case of Pulmonary *Mycobacterium parascrofulaceum* Infection in a Patient With Bronchiectasis in Korea

Kyoung-Bo Kim, M.D.[1], Sung-Gyun Park, M.D.[1], Jae-Seok Park, M.D.[2], Wonmok Lee, M.D.[1], Jung-Sook Ha, M.D.[1], Nam-Hee Ryoo, M.D.[1], Dong-Seok Jeon, M.D.[1], and Jae-Ryong Kim, M.D.[1]

Departments of Laboratory Medicine[1] and Internal Medicine[2], Keimyung University School of Medicine, Daegu, Korea

Dear Editor

Mycobacterium parascrofulaceum is a member of slow-growing scotochromogenic nontuberculous mycobacteria (NTM) that can cause opportunistic infections in immunocompromised patients [1]. Only a few clinical infections with this strain have been reported to date [1-4]. We describe a case of *M. parascrofulaceum* pulmonary infection in a patient with bronchiectasis. To our knowledge, this is the first case of *M. parascrofulaceum* infection in Korea.

A 65-yr-old man was referred to our hospital because of recurrent hemoptysis and prulent sputum for two days. He had a history of stomach cancer that had been treated by using total gastrectomy with Billroth II anastomosis and chemotherapy seven years ago; at that time, he was diagnosed as having bronchiectasis. Our laboratory tests revealed peripheral blood leukocyte counts of 6.48×10^9/L with 81.2% neutrophils. Chest computed tomography revealed no interval changes in the preexisting bronchiectasis of the lower left lobe of the lung. Nonetheless, new peribronchial infiltration and centrilobular nodules were found in the middle right lobe and lower left lobe. Acid-fast bacillus (AFB) staining, qualitative real-time PCR for *Mycobacterium tuberculosis* (COBAS TaqMan MTB Test; Roche, Basel, Switzerland), and nested PCR for NTM (MTB & NTM PCR Kit; BioSewoom Inc., Seoul, Korea) in the expectorated-sputum specimens all tested negative. Sputum cultures in liquid media were performed in triplicate by using the Mycobacteria Growth Indicator Tube System (MGIT, Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and positive results were obtained from two cultures. For species identification, a PCR-based reverse line blot hybridization assay for the ITS gene (AdvanSure Mycobacteria GenoBlot Assay; LG Life Sciences, Seoul, Korea) was performed on both culture isolates, revealing poor discrimination of species (*Mycobacterium lentiflavum*/*Mycobacterium genavense*).

For definitive species identification, sequencing analysis of the 16S rRNA, *hsp65*, and *rpoB* genes was performed. Primers used for PCR are shown in Table 1 [5-7]. 16S rRNA sequences obtained were searched against publicly available databases by using the basic local alignment search tool (BLAST) [8] and EzTaxon [9]. When the sequences were submitted to BLAST, both culture isolates revealed 99.74% similarity (767/769 bp) with *M. parascrofulaceum* HSC68. The next closest match was *M. parascrofulaceum* ATCC BAA-614 with similarity of 99.61% (776/779 bp). In EzTaxon, the isolates exhibited 99.36% similarity (773/778 bp) with *M. parascrofulaceum* ATCC BAA-614 followed by *M. eu-*

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Corresponding author: Wonmok Lee
Departments of Laboratory Medicine, Keimyung University School of Medicine, 56 Dalseong-ro, Jung-gu, Daegu 700-712, Korea
Tel: +82-53-250-7733, Fax: +82-53-250-7275
E-mail: lwm1034@gmail.com

Table 1. Primers and amplicon sizes for PCR analysis of the 16s rRNA, *hsp65*, and *rpoB* genes

Gene	Primer name	Direction	Primer sequence	Product size (bp)	Reference
16s rRNA	4F	Forward	5′-TTGGAGAGTTTGATCCTGGCTC-3′	786	[5]
	801R	Reverse	5′-GGCGTGGACTTCCAGGGTATCT-3′		
hsp65	HSPF3	Forward	5′-ATCGCCAAGGAGATCGAGCT-3′	634	[6]
	HSPR4	Reverse	5′-AAGGTGCCGCGGATCTTGTT-3′		
rpoB	MycoF	Forward	5′-GGCAAGGTCACCCCGAAGGG-3′	730	[7]
	MycoR	Reverse	5′-AGCGGCTGCTGGGTGATCATC-3′		

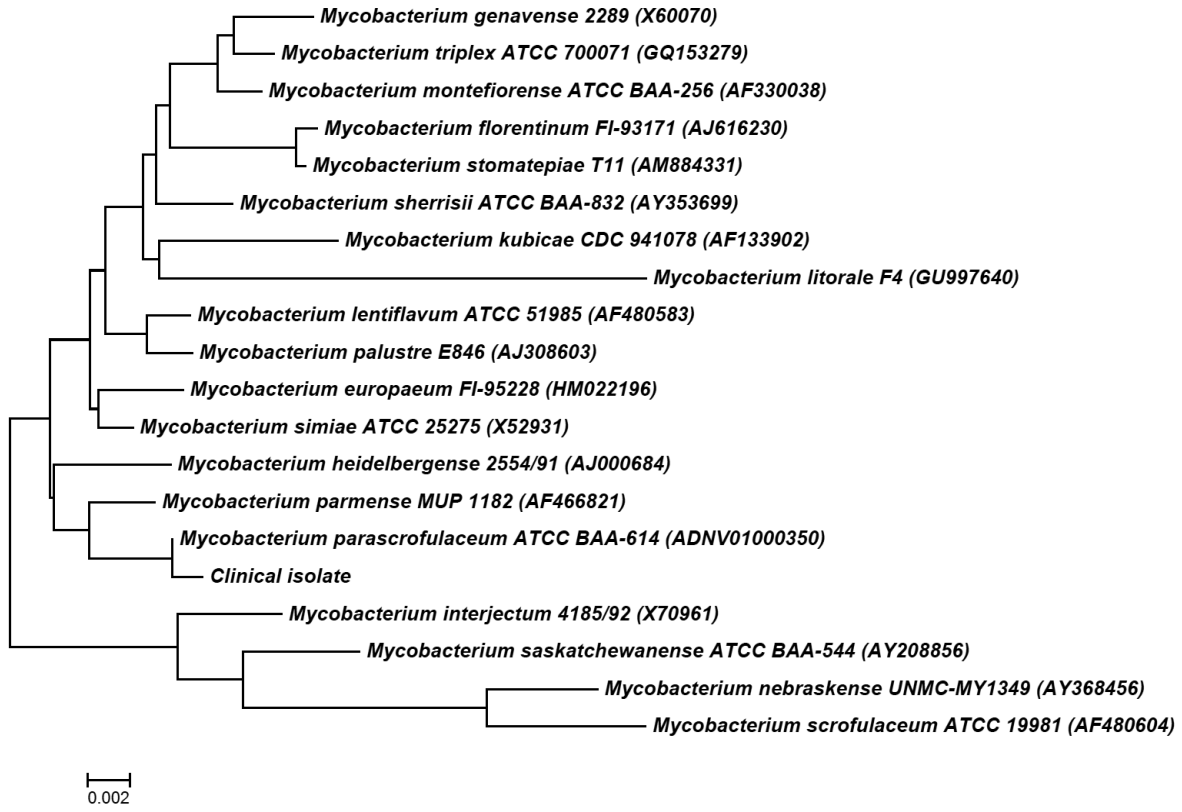

Fig. 1. The neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of our *Mycobacterium parascrofulaceum* isolate and 19 similar microorganisms. The scale bar corresponds to 0.2% sequence divergence.

ropaeum FI-95228 with similarity of 98.94% (750/758 bp). In the BLAST search, sequences of *hsp65* and *rpoB* genes revealed 99.83% (602/603 bp) and 99.32% (740/745 bp) similarity with *M. parascrofulaceum*, respectively. The other best matches were *Mycobacterium* sp. MOTT-01 (99.83%, 602/603 bp) and *Mycobacterium* sp. MOTT-27 (99.06%, 739/746 bp), respectively. A phylogenetic tree, which was constructed using the neighbor-joining method by means of the MEGA software, version 5.05 (http://www.megasoftware.net), identified this isolate as *M. parascrofulaceum* (Fig. 1). Although there was a possibility of colonization of the respiratory tract, the patient was diagnosed as having *M. parascrofulaceum* pulmonary infection according to his newly developed radiological images. At diagnosis, he had only a mild cough with no progression according to chest radiography. Therefore, he did not receive long-term antibiotic therapy. Additional sputum cultures one year after the initial sputum analysis yielded negative results. Currently, he exhibits no symptomatic aggravation and regularly visits our hospital (and does not take medication). Therefore, it is possible that this isolate, *M. parascrofulaceum*, caused a transient infection rather than a progressive disease or colonization. We need long-term follow-up and repeat sputum analysis to confirm either the progressive disease or

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transient infection.

There are >150 species comprising the genus *Mycobacterium*, with a significant increase in the number of newly identified species recently. The latter phenomenon can be attributed in part to development of molecular techniques enabling accurate identification via detection of differences in the 16S rRNA gene [10]. *M. parascrofulaceum* is one of the species discovered recently after sequence-based identification and was previously known as the "MCRO 33" group of mycobacteria (GenBank accession No. AF152559). Discrimination of *M. parascrofulaceum* from other NTM is difficult because it shows phenotypic characteristics similar to those of *Mycobacterium scrofulaceum* and is genotypically close to *Mycobacterium simiae* [1]. Direct 16S rRNA gene sequence analysis is a powerful tool for confirming this strain; additional sequence analysis of the *hsp65*, *rpoB*, and ITS genes may be useful for unequivocal identification [10].

As the genetic diversity of NTM continues to be elucidated and new species are discovered, more accurate and specific diagnostic tests will be necessary for NTM identification. Multigene sequence analysis may be needed to discriminate NTM species accurately.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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