

The First Korean Case of Nontuberculous Mycobacterial Lung Disease Caused by *Mycobacterium abscessus* Subspecies *bolletii* in a Patient with Bronchiectasis

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We report the first Korean case of lung diseases caused by *Mycobacterium abscessus* subsp. *bolletii* in a previously healthy male, except for a previous history of pulmonary tuberculosis and bronchiectasis. All serial isolates are identified as *M. abscessus* subsp. *bolletii* by multi-locus sequence analysis based on the *hsp65*, *rpoB*, and 16S rRNA fragments. At the genetic level, the isolate has the *erm*(41) gene with a T28 sequevar, associated with clarithromycin resistance, and no *rrl* mutation. The isolate is resistant to clarithromycin. Although the symptoms and radiographic findings have improved after combination of antibiotics, the follow-up sputum cultures are persistently positive.

Keywords: Bronchiectasis; Mycobacterium Infections, Nontuberculous; Nontuberculous Mycobacteria; Sequence Analysis, DNA

Introduction

Rapidly growing mycobacteria (RGM) are relatively common pathogens of lung disease caused by nontuberculous

mycobacteria (NTM) in Korea¹. Of the RGM species, *Mycobacterium abscessus* complex is most commonly associated with NTM lung disease. Although subspeciation of *M. abscessus* complex is still controversial, *M. abscessus* complex is currently divided into three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii*².

In Korea, *M. abscessus* subsp. *bolletii* is identified in only 1.3–1.6% of clinical strains of *M. abscessus* complex³. Although a case with disseminated infection of *M. abscessus* subsp. *bolletii* was reported⁴, there has been no report of NTM lung disease caused by *M. abscessus* subsp. *bolletii* in Korea. Here, we describe a case of chronic pulmonary disease caused by *M. abscessus* subsp. *bolletii*.

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Case Report

A 66-year-old male was referred to our hospital for management of probable NTM lung disease. He was an ex-smoker and had a history of previous treatment for pulmonary tuber-

culosis 10 years prior. Six months before his visit to our hospital, the patient reported chronic cough and sputum. He was initially diagnosed with acid-fast bacilli (AFB) smear-positive pulmonary tuberculosis and received isoniazid, rifampin,

ethambutol, and pyrazinamide for 6 months. However, AFB smears were persistently positive, and NTM were cultured from his sputum specimens. He was then referred to our hospital.

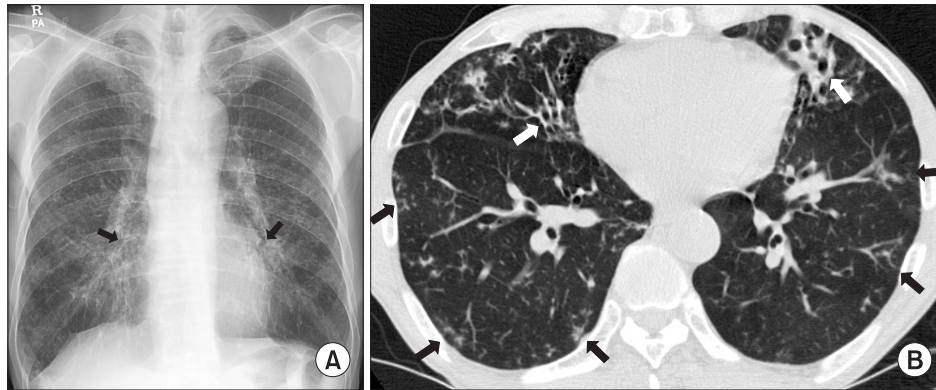


Figure 1. A 66-year-old male with bronchiectasis and nontuberculous mycobacterial lung disease caused by *Mycobacterium abscessus* subsp. *bolletii*. (A) Chest radiography revealed bilateral multifocal tram-track signs (black arrows), suggesting bronchiectasis. (B) A transverse chest computed tomography scan (3.0-mm-section thickness) revealed bilateral bronchiectasis (white arrows) in the middle right lobe and the lingular segment of the upper left lobe as well as multiple tree-in-bud appearances (black arrows), suggesting bronchiolitis in both lungs.

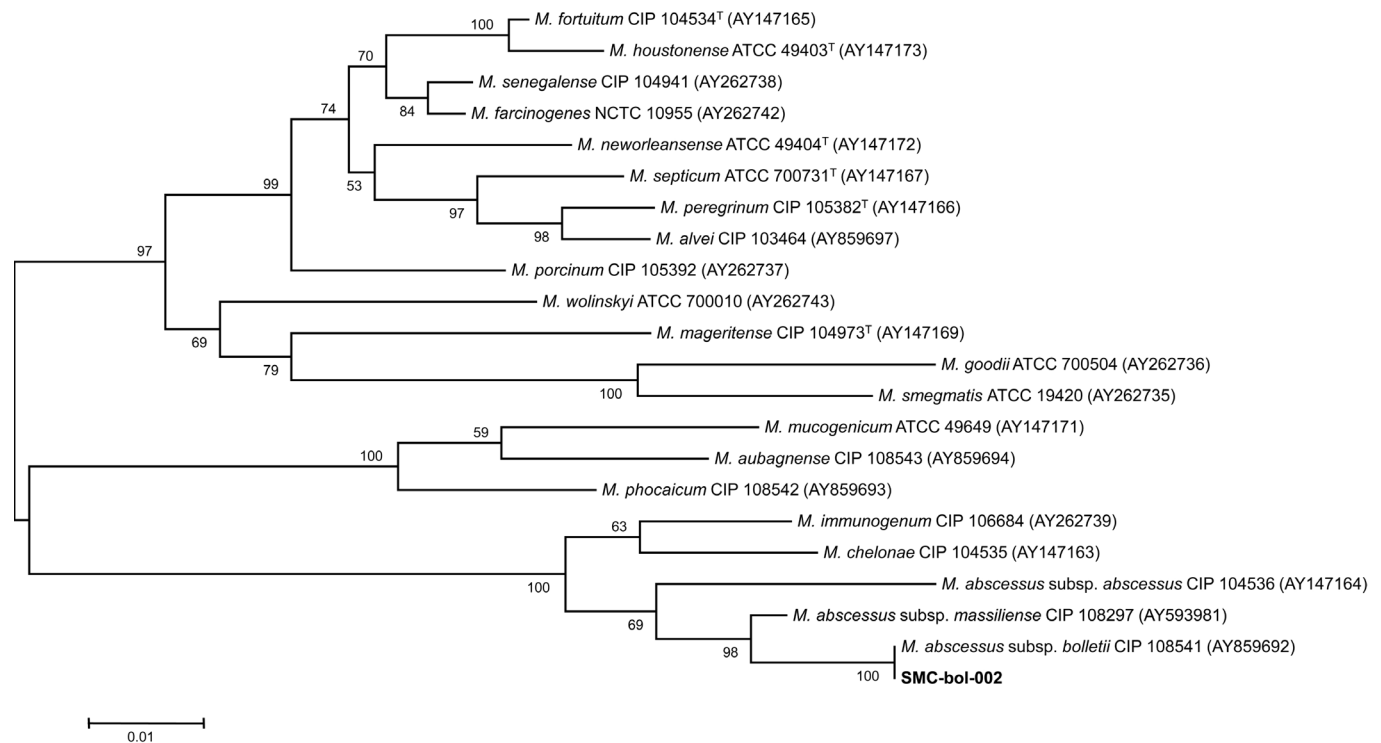


Figure 2. The phylogenetic position of isolate SMC-bol-002 and other species belong to the rapidly growing mycobacteria are based on *rpoB* sequences. This tree was constructed by using the neighbor-joining method and visualized with MEGA 5.0. The percentages at the nodes represent bootstrap levels which are supported by 1,000 re-sampled datasets. Scale bars indicate evolutionary distances in base substitutions per site. *M.*: *Mycobacterium*.

Table 1. Antimicrobial drugs and MIC breakpoints

| Drugs | MIC ($\mu\text{g/mL}$) for category | | | MIC of the isolate ($\mu\text{g/mL}$) |
|----------------|---------------------------------------|--------------|------------|---|
| | Susceptible | Intermediate | Resistant | |
| Amikacin | ≤ 16 | 32 | ≥ 64 | 8 |
| Cefoxitin | ≤ 16 | 32–64 | ≥ 128 | 32 |
| Imipenem | ≤ 4 | 8–16 | ≥ 32 | 32 |
| Clarithromycin | ≤ 2 | 4 | ≥ 8 | 8 |
| Ciprofloxacin | ≤ 1 | 2 | ≥ 4 | 16 |
| Moxifloxacin | ≤ 1 | 2 | ≥ 4 | 8 |
| Doxycycline | ≤ 1 | 2–4 | ≥ 8 | >32 |

Drug susceptibility testing was performed by using a broth microdilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI)⁷.

MIC: minimum inhibitory concentration.

Physical examination showed that the patient was 172.3 cm tall and weighed 56.1 kg. Laboratory tests results were unremarkable. A human immunodeficiency virus antibody test was negative. The chest radiograph and computed tomography (CT) scan revealed multifocal bronchiectasis and bronchiolitis in both lungs that suggested the nodular bronchiectatic form of NTM lung disease (Figure 1).

The patient's sputum was positive for AFB staining, and NTM were isolated more than three times in a liquid culture system (Bactec MGIT 960 system; BD Diagnostics, Sparks, MD, USA). To identify the etiological agent, bacteria were initially propagated in 7H9 broth (Difco Laboratories, Detroit, MI, USA) supplemented with 10% (vol/vol) oleic acid-albumin-dextrose-catalase (BD Diagnostics) for 7 days at 37°C, sub-cultured in egg-based 3% Ogawa solid media (Shinyang, Seoul, Korea), and genomic DNA was extracted from the cultured bacteria. Initial species identification using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method based on the *rpoB* gene (Myc-ID; M&D Inc., Wonju, Korea) was *M. abscessus* complex⁵.

To speciate the isolates, sequencing analysis of the nearly complete 16S rRNA gene, partial *hsp65*, and *rpoB* sequences were performed using GenBank (<http://blast.ncbi.nlm.nih.gov/>) with the BLAST algorithm⁶. The 16S rRNA sequences showed 100% identity with *M. abscessus* subsp. *abscessus* (GenBank accession no. NR074427), *M. abscessus* subsp. *massiliense* (GenBank accession no. NR074421), *M. chelonae* (GenBank accession no. AY457082), and *M. abscessus* subsp. *bolletii* (GenBank accession no. NR043236). The *hsp65* and *rpoB* sequences were 100% identical to those of the *M. abscessus* subsp. *bolletii* type strain (GenBank accession nos. FJ607778 and AY859692). Phylogenetic analysis based on the *rpoB* sequences from the isolate SMC-bol-002 and from those of closely related species within the RGM showed that this strain belonged to *M. abscessus* subsp. *bolletii* (Figure 2).

Drug susceptibility testing was performed using a broth

microdilution method (Table 1)⁷. At the genetic level, the isolate was genotyped for *erm*(41) polymorphism and for *rrl* mutations; these are known as the two main mechanisms of macrolide resistance⁸. The isolate had the *erm*(41) gene with a T28 sequevar and no *rrl* mutation acquisition.

The patient received oral clarithromycin and moxifloxacin, along with an initial 4-week course of intravenous amikacin and cefoxitin. During 4 weeks of initial antibiotic treatment, his symptoms and CT lesions were improved and sputum culture results were negative after 2, 3, and 4 weeks after treatment. Although he took oral clarithromycin and moxifloxacin for a total duration of 24 months, follow-up sputum cultures were persistently positive after 2 months after antibiotic treatment.

Discussion

This is the first reported case of *M. abscessus* subsp. *bolletii* lung disease in Korea. Previous reports showed that patients with *M. abscessus* subsp. *bolletii* lung disease had mild compromised respiratory function, as was seen in the current report^{9,10}. The reported number of patients with *M. abscessus* subsp. *bolletii* lung disease was less than 10 before this case^{9,10}. The limited number of reports makes it difficult to characterize patients with *M. abscessus* subsp. *bolletii* lung disease.

The *M. abscessus* complex has undergone many taxonomic classification changes since its first description in 1953¹¹. The dramatic change in mycobacterial taxonomy came with the availability and reliability of DNA sequencing, including 16S rRNA, *rpoB*, and *hsp65*. Adekambi et al.¹² suggested that the *M. abscessus* complex should be divided into three species, *M. abscessus* sensu stricto, *M. massiliense*, and *M. bolletii*, according to the results of *rpoB* sequencing. Recent evidence based on comparative analysis of whole genome sequences has suggested that *M. abscessus* can be divided into three sub-

species: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*¹³. It is important to distinguish the three subspecies of *M. abscessus* complex, because of their differences in susceptibility to clarithromycin³. Commonly, *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* are inducible resistant, susceptible, and resistant to clarithromycin, respectively³.

The combination of macrolide, cefoxitin (or imipenem), and amikacin has been recommended as the first line antimicrobial drug treatment for *M. abscessus* lung disease¹⁴. However, the appropriate duration of intravenous antibiotic therapy or combination of oral antibiotics is not known. We used intravenous antibiotics for 4 weeks as previously reported cases of *M. abscessus* lung disease¹⁵, because of the phenotypic similarities between *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*. Although drug susceptibility testing showed *in vitro* resistance, clarithromycin and moxifloxacin were prescribed during outpatient treatment following the previous report^{3,15}. We finally achieved improvement in his respiratory symptoms and prevented progression of radiological findings, although we were unable to achieve negative conversion of sputum AFB culture.

In summary, we report the first case of *M. abscessus* subsp. *bolletii* lung disease in Korea. *M. abscessus* subsp. *bolletii* lung disease should be considered when the pathogen is identified as *M. abscessus* complex by a non-sequencing method, such as PCR-RFLP, and has resistance to clarithromycin. In such cases, further molecular techniques may be needed to accurately identify the specific subspecies.

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