

First Case of Pulmonary Disease Caused by a *Mycobacterium avium* Complex Strain of Presumed Veterinary Origin in an Adult Human Patient

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We report the first case of pulmonary disease caused by a strain of *Mycobacterium avium* complex of presumed veterinary origin in an elderly patient. All serial isolates were identified by multilocus sequence analysis based on *rpoB*, *hsp65*, and 16S rRNA fragments. Disease persisted despite macrolide-based combination antibiotic therapy.

CASE REPORT

A 77-year-old man was referred to our hospital due to progression of lung disease caused by nontuberculous mycobacteria (NTM). Fourteen months prior, he was diagnosed with *Mycobacterium avium* lung disease and treated with clarithromycin, ethambutol, and rifampin. Drug susceptibility testing (DST) was not performed. Despite >12 months of antibiotic therapy, the patient failed to show any clinical improvement.

He was a nonsmoker and a retired police officer. He had no history of plenty of time around farm animals. His leukocyte count was 10,160/ μ l, with a differential of 71% neutrophils. The erythrocyte sedimentation rate was 114 mm/h, and his C-reactive protein (CRP) level was increased to 10.54 mg/dl. A human immunodeficiency virus antibody was negative. A chest radiograph and computed tomography (CT) scan revealed a large cavitory lesion in the right upper lobe and multiple consolidations in both lungs (Fig. 1).

Numerous (4+) acid-fast bacilli (AFB) were detected with both auramine-rhodamine fluorescent staining and the Ziehl-Neelsen staining method according to standard guidelines (1). A 4+ AFB smear was defined as >9 AFB per field (1). NTM were isolated more than five times from sputum specimens in a liquid culture system (Bactec MGIT 960 system; BD Diagnostics, Sparks, MD). To identify the etiological agent, bacteria grown in the MGIT 960 culture system were initially propagated in 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% (vol/vol) oleic acid-albumin-dextrose-catalase (OADC; BD Diagnostics) for 14 days at 37°C and subcultured in egg-based 3% Ogawa solid medium (Shinyang, Seoul, South Korea), and genomic DNA was extracted from the cultured bacteria. Isolate SMC1 was initially identified as *M. avium* using a reverse line blot hybridization assay (REBA Myco-ID; M&D, Inc., Wonju, South Korea) based on the *rpoB* gene (2).

To confirm the accuracy of this identification, sequencing analyses of *rpoB*, *hsp65*, and 16S rRNA were performed (3–5). The *hsp65* and 16S rRNA sequences were 100% identical to those of the *M. avium* strain (GenBank accession no. CP000479 and GQ153272, respectively). The *rpoB* sequences showed 99.7% similarity, with only a 2-base mismatch, to that of the *M. avium* subsp. *avium* strain (GenBank accession no.

GQ153306). Additionally, the *rpoB* sequences showed 99.6% similarity, with only a 3-base mismatch, to those of the *M. avium* subsp. *hominissuis* strain (GenBank accession no. EF521911), *M. avium* subsp. *paratuberculosis* strain (GenBank accession no. EF521906), and *M. avium* subsp. *silvaticum* strain (GenBank accession no. JN935808). Interestingly, the *rpoB* sequences were 100% similar to those of the *Mycobacterium* spp. belonging to the *M. avium* complex (MAC) of veterinary origin (GenBank accession no. JF327744 and JF327745).

A phylogenetic tree, based on *rpoB* gene sequences from the isolate SMC1 and from those of closely related species within the MAC, is shown in Fig. 2. The sequences were compared with those obtained from the GenBank sequence database. The *rpoB* gene sequences of the 18 strains were aligned to those of the type strains of closely related mycobacteria by using CLUSTAL_X software (6). The topological results and tree were inferred via the neighbor-joining method, visualized by using the MEGA 5.0 software package (7), and evaluated by bootstrap analyses based on 1,000 resamplings. Phylogenetic analysis based on partial *rpoB* sequences showed that this strain was a novel MAC isolate of veterinary origin (Fig. 2).

Drug susceptibility testing was performed by using a broth microdilution method according to the guidelines (8), which revealed that SMC1 was resistant to clarithromycin (MIC, >64 μ g/ml) and linezolid (MIC, 64 μ g/ml) and was susceptible to moxifloxacin (MIC, 1 μ g/ml). SMC1 had an expected point mutation of A→C at position 2059 in the 23S rRNA gene, which is known as the major mechanism of acquired macrolide resistance (9).

The patient was treated with antibiotics, including isoniazid, rifampin, ethambutol, and streptomycin, in our hospital. Despite this combination antibiotic therapy for more than 12 months, the patient's symptoms and radiographic findings

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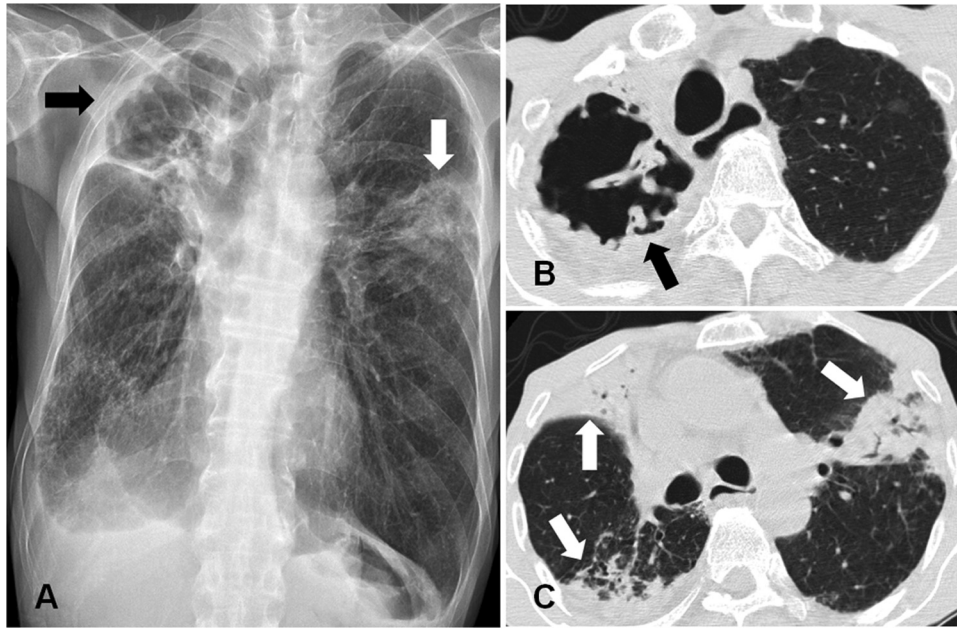


FIG 1 A 77-year-old man with *Mycobacterium avium* complex strain infection of presumed veterinary origin. (A) Chest radiograph shows a large cavity in the right upper lobe (black arrow) and a segmented consolidation in the left upper lobe (white arrow). (B) Chest computed tomography (CT) scan shows a large cavity in the right upper lobe (black arrow). (C) Chest CT scan shows multiple segmented consolidations in both lungs (white arrows).

worsened. Sputum AFB staining and culture examinations were persistently positive.

Among NTM, MAC is a common cause of disease (10, 11). *M. avium* is ubiquitous in nature and an opportunistic pathogen for both animals and humans (12). *M. avium* is subdivided into four

subspecies: *avium*, *hominissuis*, *paratuberculosis*, *silvaticum* (13). *M. avium* subsp. *avium* is known to be a bird-type strain that causes a contagious disease among birds, while *M. avium* subsp. *hominissuis* can infect humans, pigs, cattle, and other animals (13). *M. avium* subsp. *avium* has been detected in human patients, pigs, and other animals; however, these appear to be rare cases (14).

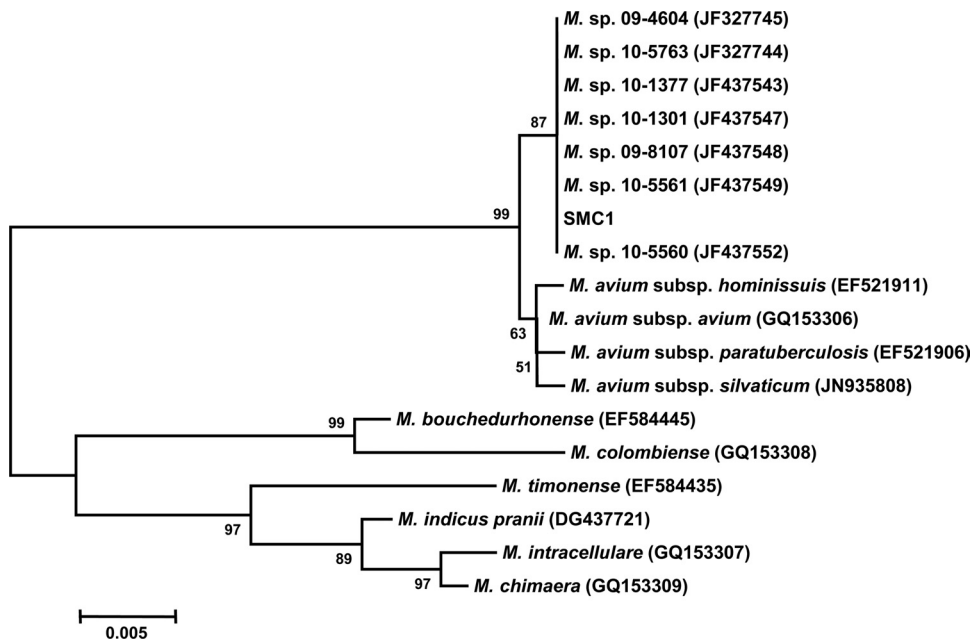


FIG 2 The phylogenetic position of isolate SMC1 and other species belonging to the *Mycobacterium avium* complex based on partial *rpoB* gene sequences. This tree was constructed using the neighbor-joining method. The percentages indicated at nodes represent bootstrap levels supported by 1,000 resampled data sets. Scale bars indicate evolutionary distance in base substitutions per site.

The *rpoB* gene was sequenced using Myco-F/Myco-R primers, which amplify a 711-bp fragment, the hypervariable region of the *rpoB* gene, and permits one-step identification of MAC isolates at the species or subspecies level, as well as the detection of potentially novel MAC species otherwise unable to be determined by 16S rRNA gene sequence analysis (15). The isolate SMC1 was clustered among novel MAC isolates from veterinary hosts, including elk, cats, cattle, and pigs, although it did not demonstrate 100% identity with the *rpoB* sequence for any of the clades containing *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis*, or *M. avium* subsp. *silvaticum*. The insertion element profiles of these *Mycobacterium* spp. of veterinary origin were negative for IS900, IS901, and DT1 and positive for IS1245, which is similar to the results for *M. avium* subsp. *hominissuis* (16). The taxonomic position of these isolates within the MAC was not defined. SMC1 is an undefined *Mycobacterium* spp. of veterinary origin but is genetically close to MAC. We report the first case of MAC strain infection, of presumed veterinary origin, in an elderly adult patient.

The high genetic relatedness among *M. avium* subsp. *hominissuis* isolated from pigs and humans was reported in the Netherlands, Sweden, Germany, and Finland (17–20). Like these strains, isolate SMC1 may infect humans infrequently. Direct transmission of NTM between animals and humans has not yet been proven. It remains to be determined whether humans are infected directly from animals or if they are infected from common environmental sources.

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