

First Case of *Segniliparus rotundus* Pneumonia in a Patient with Bronchiectasis[∇]

Won-Jung Koh,^{1†} Go-Eun Choi,^{2†} Seung-Heon Lee,³ Young Kil Park,³
Nam Yong Lee,⁴ and Sung Jae Shin^{5*}

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea¹; Departments of Laboratory Medicine, School of Medicine, Pusan National University, Busan, South Korea²; Korean Institute of Tuberculosis, Osong, South Korea³; Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea⁴; and Department of Microbiology and Research Institute for Medical Sciences, Infection Signaling Network Research Center, College of Medicine, Chungnam National University, Daejeon, South Korea⁵

Received 19 May 2011/Returned for modification 23 June 2011/Accepted 5 July 2011

We report the first case of *Segniliparus rotundus* pneumonia in an adult with non-cystic fibrosis bronchiectasis. All isolates were identified as *S. rotundus* by 16S rRNA gene sequencing and *rpoB* PCR-restriction analysis. Antibiotic therapy with clarithromycin and ciprofloxacin for 2 months improved the patient's condition and achieved successful sputum conversion.

CASE REPORT

A 43-year-old woman was referred to Samsung Medical Center for chronic cough and sputum lasting more than 3 months. The patient's medical history included a tuberculous pleurisy at the age of 22 years. She lived in an urban area and had no history of smoking, alcoholism, or immunosuppressive drug use.

A human immunodeficiency virus (HIV) antibody test was negative. A high-resolution computed tomography (HRCT) chest scan revealed severe bronchiectasis, multiple small nodules, and branching centrilobular nodules. These findings suggested bronchiolitis, especially in the right lung (Fig. 1). There was no evidence indicating a specific cause of the bronchiectasis, such as cystic fibrosis. Multiple sputum specimens were examined for mycobacteria. The specimens were decontaminated and concentrated with NaOH plus *N*-acetyl-L-cysteine. Acid-fast bacilli (AFB) were detected more than five times on both auramine-rhodamine fluorescent stain and Ziehl-Neelsen methods, and liquid cultures of these multiple sputum specimens were incubated for 9 to 11 days with a Bactec MGIT 960 system (BD Diagnostics, Sparks, MD).

To diagnose 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; Becton Dickinson) for 7 days at 37°C and genomic DNA was extracted from cultured bacteria. Initial species identification using a reverse line blot hybridization assay (REBA Myco-ID; M&D, Inc., Wonju, South Korea) was unsuccessful. Because this assay was designed to detect and identify *Mycobacterium tuberculosis* and 19 species of nontuberculous mycobacteria

(NTM) (7), we considered our samples to be outside this detection range. We conducted a PCR-restriction fragment length polymorphism analysis (PRA) of the *hsp65* and *rpoB* genes (Table 1) which has been used effectively for the simultaneous identification of many mycobacterial species (4, 8, 11). Digestion of the *hsp65* gene at 37°C with MspI (New England BioLabs, MA) produced a 527-bp segment of the amplified PCR product, displaying a restriction pattern of 250-, 105-, and 75-bp DNA fragments (Fig. 2). This restriction pattern was identical to previously reported patterns of *Segniliparus rotundus* and *S. rugosus* (1). To confirm these results, reference strains of *S. rotundus* CIP 108380^T and *S. rugosus* CIP 108378^T (Institut Pasteur, Paris, France) were included in subsequent experiments. PRA of the *hsp65* gene found no difference between a clinical isolate and the two reference strains. PRA of the *rpoB* gene was performed using MspI and HaeIII to further distinguish the two species. These restriction enzymes were selected from the *rpoB* genes of *S. rotundus* CIP 108380^T (10) and *S. rugosus* (GenBank accession no. GL622756). The *rpoB* PRA using MspI and HaeIII restriction showed a unique digestion pattern for *S. rotundus* that distinguished it from *S. rugosus* and *Mycobacterium* spp. (Fig. 2) (11). Furthermore, the *rpoB* PRA of the clinical isolate using these two enzymes displayed a pattern identical to that of *S. rotundus* CIP 108380^T.

To confirm the accuracy of this identification, sequencing analyses of *rpoB* (7), the 16S–23S rRNA internal transcribed spacer (ITS) sequence (5), and 16S rRNA were performed (9). The *rpoB* sequences showed 99% similarity, and the 16S–23S rRNA ITS and 16S rRNA sequences showed 100% similarity to that of the *S. rotundus* reference strain (GenBank accession no. CP001958 and FJ468343) (10).

Drug susceptibility testing was performed at the Korean Institute of Tuberculosis by using a broth microdilution method in the same manner as used for rapidly growing mycobacteria (RGM), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (3, 12). MICs were determined after incubation at 30°C for 3 days.

* Corresponding author. Mailing address: Department of Microbiology, College of Medicine, Chungnam National University, 6 Munwha-dong, Jung-gu, Daejeon 301-747, South Korea. Phone: 82-42-580-8246. Fax: 82-42-585-3686. E-mail: sjshin@cnu.ac.kr.

† These authors contributed equally to this work.

∇ Published ahead of print on 13 July 2011.

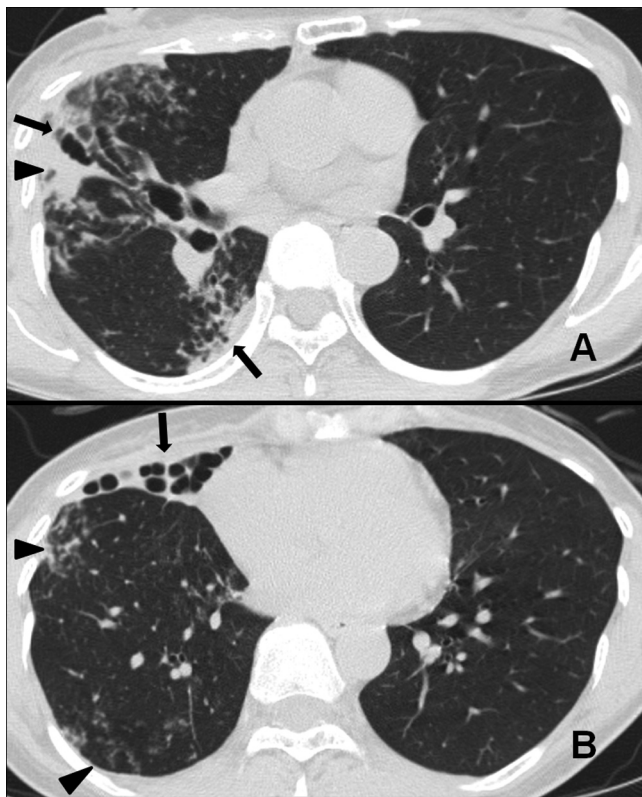


FIG. 1. A 43-year-old woman with *Segniliparus rotundus* pneumonia. (A) A high-resolution computed tomography (HRCT) chest scan at the level of the proximal lower lobar bronchus shows extensive bronchiectasis and lobular consolidation. (B) An HRCT scan obtained at the lung base shows severe bronchiectasis in the right middle lobe. Multiple small nodules and branching centrilobular nodules form a tree-in-bud pattern in the right lower lobe.

The *Segniliparus* species reference strains and the clinical isolate were resistant to amikacin, sulfamethoxazole, tobramycin, and ethambutol. The clinical isolate was more resistant than *S. rotundus* CIP 108380^T to cefoxitin, doxycycline, and imipenem. The clinical isolate and *S. rotundus* CIP

108380^T were susceptible to clarithromycin, ciprofloxacin, and moxifloxacin (Table 2).

The patient was treated with oral clarithromycin (1,000 mg/day) and ciprofloxacin (1,000 mg/day) for 2 months. The treatment outcome was favorable; the patient's symptoms resolved completely, radiographic findings improved, and sputum smear and culture were negative after 2 months of antibiotic therapy.

Segniliparus is a novel genus consisting of two species (*S. rotundus* and *S. rugosus*) that was first named in 2005 (1). Both reference strains of *Segniliparus* were isolated from human sputum (1, 10), and the characterization study demonstrated that they shared some phenotypical characteristics with rapidly growing mycobacteria (1).

In this article, we have described a case of *S. rotundus* pneumonia in a patient with non-cystic fibrosis bronchiectasis. To our knowledge, this is the first reported case of *S. rotundus* pneumonia. *S. rugosus* in patients with cystic fibrosis in the United States and Australia has recently been reported (2, 6). These cases suggest that *Segniliparus* species may be emerging respiratory pathogens that can cause pneumonia in patients with bronchiectasis.

The accurate identification of bacteria is important for the evaluation of the clinical implications of emerging pathogens in respiratory infections. However, *Segniliparaceae* may be confused with nonchromogenic rapidly growing mycobacteria in microscopic examination, due to the acid-fast staining properties of these species. In our case, molecular diagnostic methods allowed the accurate identification of *S. rotundus* and its differentiation from mycobacteria and *S. rugosus*, especially in the sequencing analyses of 16S rRNA genes and *rpoB* PRA using *MspI* and *HaeIII*.

Few studies of *S. rotundus* infection have been published, and reliable information about antibiotic therapy is limited. The *S. rotundus* reference strain and isolate were susceptible to several oral antibiotics, including clarithromycin, ciprofloxacin, and moxifloxacin. In contrast, the *S. rugosus* reference strain was highly resistant to these antibiotics. These results were consistent with those of previous studies (2, 6). Our patient

TABLE 1. Genes and oligonucleotide primers used and percent similarity in sequencing analysis with *Segniliparus rotundus* reference strain (GenBank accession no. CP001958)

Gene	Primer sequence (5'-3') ^c	Product size (bp)	Similarity (%)	Reference
<i>hsp65</i>	(F) GAG GGC GTC ATC ACC GTC GAG G (R) CGG CGA TGG CGT CGG AGT CAC C	527	NA ^d	4
<i>rpoB</i> ^a	(F) TCA AGG AGA AGC GCT ACG A (R) GGA TGT TGA TCA GGG TCT GC	360	99	7
16S-23S ITS ^b	(F) TTGTACACACCGCCCGTCA (R) TCTCGATGCCAAGGCATCCACC	487	100	10
16S rRNA	(F) GAGTTTGATCCTGGCTCAGGA (R) AAGGAGGTGATCCAGCCGCA	560	100	9

^a The gene used in both molecular methods of PRA and sequence analysis.

^b 16S-23S rRNA internal transcribed spacer of mycobacteria.

^c F, forward; R, reverse.

^d NA, not applicable, due to no information.

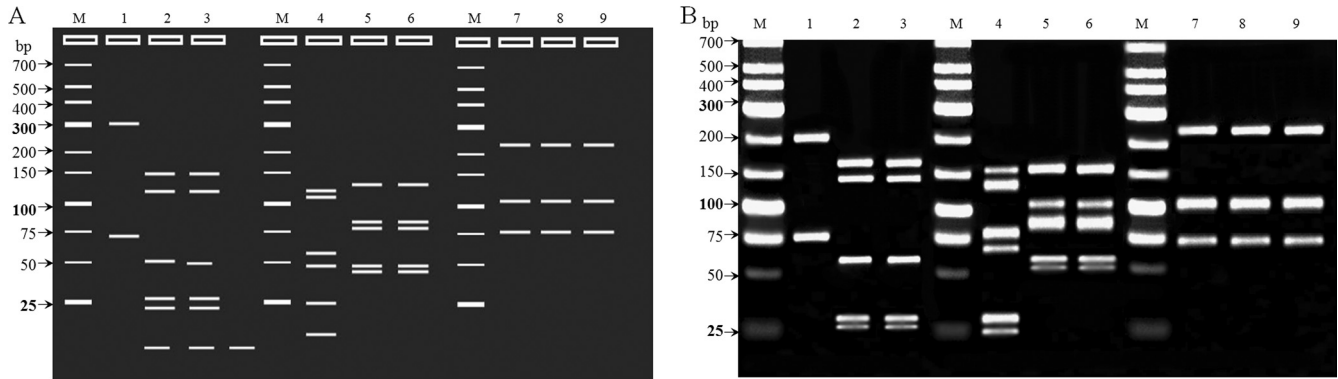


FIG. 2. PCR restriction-enzyme polymorphism analysis (PRA). (A) Simulation of PRA of the *hsp65* and *rpoB* genes. (B) PRA electrophoresis results. M, size marker; lanes 1 to 3, *hsp65* amplicons digested by *MspI* from *S. rugosus* CIP 108378^T, *S. rotundus* CIP 108380^T, and the clinical isolate of this case, respectively; lines 4 to 6, *rpoB* amplicons digested by *MspI* from *S. rugosus* CIP 108378^T, *S. rotundus* CIP 108380^T, and the clinical isolate of this case, respectively; lines 7 to 9, *rpoB* amplicons digested by *HaeIII* from *S. rugosus* CIP 108378^T, *S. rotundus* CIP 108380^T, and the clinical isolate of this case, respectively.

showed symptomatic, radiographic, and microbiological improvements after 2 months of treatment with clarithromycin and ciprofloxacin. Further studies should seek to establish the optimal antibiotic therapy for *S. rotundus* infection.

Clinicians and physicians should be aware that AFB bacteria other than *Mycobacterium* spp. in respiratory infections may be *S. rotundus*, and further studies should investigate the significance and clinical importance of *Segniliparus* species.

TABLE 2. Antimicrobial susceptibility patterns for *S. rotundus* and both reference strains of *Segniliparus* species

Antibiotic	MIC (μg/ml) ^a		
	Clinical isolate of <i>S. rotundus</i> identified in this report	<i>S. rotundus</i> CIP 108380 ^T	<i>S. rugosus</i> CIP 108378 ^T
Amikacin	>128	>128	>128
Cefoxitin	>256	2	8
Ciprofloxacin	1	≤0.125	>16
Clarithromycin	2	4	64
Doxycycline	>32	2	>32
Imipenem	16	≤0.5	4
Moxifloxacin	1	≤0.125	≤0.125
Rifampin	>16	≤0.125	>16
Sulfamethoxazole	>128	16	>128
Tobramycin	>32	>32	>32
Ethambutol	>32	>32	>32

^a MICs were interpreted using criteria for mycobacteria and aerobic actinomycetes.

This work was supported by National Research Foundation of Korea (NRF) grant no. 2011-0006228 funded by the Korean government (MEST).

REFERENCES

- Butler, W. R., et al. 2005. Novel mycolic acid-containing bacteria in the family *Segniliparaceae* fam. nov., including the genus *Segniliparus* gen. nov., with descriptions of *Segniliparus rotundus* sp. nov. and *Segniliparus rugosus* sp. nov. *Int. J. Syst. Evol. Microbiol.* **55**:1615–1624.
- Butler, W. R., et al. 2007. First isolations of *Segniliparus rugosus* from patients with cystic fibrosis. *J. Clin. Microbiol.* **45**:3449–3452.
- CLSI. 2011. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes. M24–A2. CLSI, Wayne, PA.
- Devallois, A., K. S. Goh, and N. Rastogi. 1997. Rapid identification of mycobacteria to species level by PCR-restriction fragment length polymorphism analysis of the *hsp65* gene and proposition of an algorithm to differentiate 34 mycobacterial species. *J. Clin. Microbiol.* **35**:2969–2973.
- Frothingham, R., and K. H. Wilson. 1993. Sequence-based differentiation of strains in the *Mycobacterium avium* complex. *J. Bacteriol.* **175**:2818–2825.
- Hansen, T., et al. 2009. *Segniliparus rugosus* infection, Australia. *Emerg. Infect. Dis.* **15**:611–613.
- Lee, H., H. E. Bang, G. H. Bai, and S. N. Cho. 2003. Novel polymorphic region of the *rpoB* gene containing *Mycobacterium* species-specific sequences and its use in identification of mycobacteria. *J. Clin. Microbiol.* **41**:2213–2218.
- Lee, H., H. J. Park, S. N. Cho, G. H. Bai, and S. J. Kim. 2000. Species identification of mycobacteria by PCR-restriction fragment length polymorphism of the *rpoB* gene. *J. Clin. Microbiol.* **38**:2966–2971.
- Ninet, B., et al. 1996. Two different 16S rRNA genes in a mycobacterial strain. *J. Clin. Microbiol.* **34**:2531–2536.
- Sikorski, J., et al. 2010. Complete genome sequence of *Segniliparus rotundus* type strain (CDC 1076). *Stand. Genomic Sci.* **2**:203–211.
- Whang, J., et al. 2011. PCR-restriction fragment length polymorphism of the *rpoB* gene for identification of *Mycobacterium avium* subsp. *paratuberculosis* and differentiation of *Mycobacterium avium* subspecies. *Diagn. Microbiol. Infect. Dis.* **70**:65–71.
- Woods, G. L. 2000. Susceptibility testing for mycobacteria. *Clin. Infect. Dis.* **31**:1209–1215.