

Nontuberculous Mycobacterial Lung Disease Caused by *Mycobacterium shinjukuense*: The First Reported Case in Korea

Seong Mi Moon, M.D.^{1,*}, Su-Young Kim, Ph.D.^{1,*}, Myung Jin Chung, M.D.², Seung Heon Lee, Ph.D.³, Sung Jae Shin, Ph.D.⁴ and Won-Jung Koh, M.D.¹

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, ²Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, ³Korean Institute of Tuberculosis, Osong, ⁴Department of Microbiology, Yonsei University College of Medicine, Seoul, Korea

Mycobacterium shinjukuense is a novel species of nontuberculous mycobacteria (NTM) that was first reported in Japan in 2011. It is a slow-growing NTM pathogen that can cause chronic pulmonary infections. There are only a few reported cases of *M. shinjukuense* infections, all of which are from Japan. We reported a case of chronic lung disease caused by *M. shinjukuense*. The organism was identified by 16S rRNA, *rpoB*, and *hsp65* gene sequencing. To the best of our knowledge, this was the first confirmed case of lung disease caused by *M. shinjukuense* outside of Japan.

Keywords: Bronchiectasis; *Mycobacterium*; Nontuberculous Mycobacteria

Introduction

The class of nontuberculous mycobacteria (NTM) is generally comprised of mycobacteria other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. The prevalence of lung diseases caused by NTM is increasing worldwide, including in Japan and South Korea¹⁻⁴. NTM are ubiquitous in the environment. Therefore, it is crucial to isolate and identify the causative organisms for diagnosis. There are some

specific diagnostic criteria that have already been proposed⁵.

Mycobacterium shinjukuense, a novel slow-growing NTM pathogen, was introduced as a new species in 2011⁶. There are only a few reported cases of *M. shinjukuense* that met the diagnostic criteria for NTM lung disease⁶⁻⁹. All of the prior cases were reported from Japan. We report a case of NTM lung disease caused by *M. shinjukuense*, which was identified using 16S rRNA, *rpoB*, and *hsp65* gene sequencing. To the best of our knowledge, this is the first case of *M. shinjukuense* lung disease in Korea.

Case Report

A 56-year-old Korean woman with a history of pulmonary tuberculosis (10 years prior to presentation) was referred to our hospital with a mild, but persistent productive cough for three months. Otherwise, the patient was a healthy, non-smoker. She has not lived in Japan.

A chest radiography revealed subtle crowding of the bronchovascular bundle in both lower lung zones. A computed tomography scan of her chest revealed several nodules, micro-nodules, and bronchiectasis with lung atelectasis in the right middle lobe and the lingular division of the left upper lobe (Figure 1). There was no organic growth on the initial

Address for correspondence: Won-Jung Koh, M.D.

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea

Phone: 82-2-3410-3429, **Fax:** 82-2-3410-3849

E-mail: wjkoh@skku.edu

*Seong Mi Moon and Su-Young Kim contributed equally to this work.

Received: May 26, 2015

Revised: Jun. 23, 2015

Accepted: Jun. 25, 2015

©It is identical to the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>).

Copyright © 2015

The Korean Academy of Tuberculosis and Respiratory Diseases.

All rights reserved.

microbiological study of three sputum specimens. However, cultures of two bronchial washing specimens demonstrated NTM growth.

Initially, a polymerase chain reaction (PCR)-reverse blot hybridization assay method based on *rpoB* gene (REBA Myco-ID; M&D Inc., Wonju, Korea) was used to identify the species. However, this method was unsuccessful, because there were no available reference sequences of the organism^{10,11}. Next, we performed sequencing analysis of the nearly complete 16S rRNA gene sequence and partial sequences of the *rpoB* and *hsp65*¹²⁻¹⁴. The 16S rRNA, *rpoB*, and *hsp65* sequences were

100% identical to those of the *M. shinjukuense* type strain GTC 2738 (GenBank accession Nos. NR112623, AB268504 and AB268505, respectively). Phylogenetic analysis was performed the *rpoB* sequences from the isolated SMC-shi-001 strain, and sequences of closely related species within the slow growing mycobacteria class. It revealed that the isolated strain belongs to *M. shinjukuense* (Figure 2). The GenBank accession numbers of 14 species' sequences were compared to that of SMC-shi-001. The sequences were obtained from the GenBank sequence database.

After the isolate was identified, we planned to start antibiotics because of persistent productive cough and progression of multiple nodules on the chest radiography during 6 months of follow-up. However, the patient was reluctant to receive long-term antibiotic therapy and refused further follow-up.

Discussion

NTM species are well known for causing chronic pulmonary disease. Some of the most commonly isolated species are the *Mycobacterium avium* complex, *Mycobacterium abscessus* complex, and *Mycobacterium kansasii*⁵. Other NTM species have also been implicated in lung disease. More than 150 NTM species have been discovered, and the number is still increasing as mycobacteriology methods continue to improve¹⁵. *M. shinjukuense* is a novel species of *Mycobacterium* that was first reported by Saito et al.⁶ in Japan in 2011. *M. shinjukuense* was described a slow growing, non-chromogenic *Mycobacterium* species. Compared to other mycobacteria, *M. shinjukuense* has unique gene sequences. Although it is phylogenetically related to *M. tuberculosis*, *Mycobacterium ulcerans*, and *Mycobacterium marinum*, it demonstrated <70% reassociation in DNA-DNA hybridization⁶.

It is essential to accurately identify isolated NTM species in



Figure 1. A 57-year-old female with a chronic cough productive with sputum. Axial computed tomography (CT) imaging shows several nodules, micro-nodules, and bronchiectasis with lung atelectasis in the right middle lobe. It also reveals bronchiectasis with lung atelectasis in the lingular division of the left upper lobe. These are typical CT findings of the nodular bronchiectatic form commonly seen in nontuberculous mycobacterial disease.

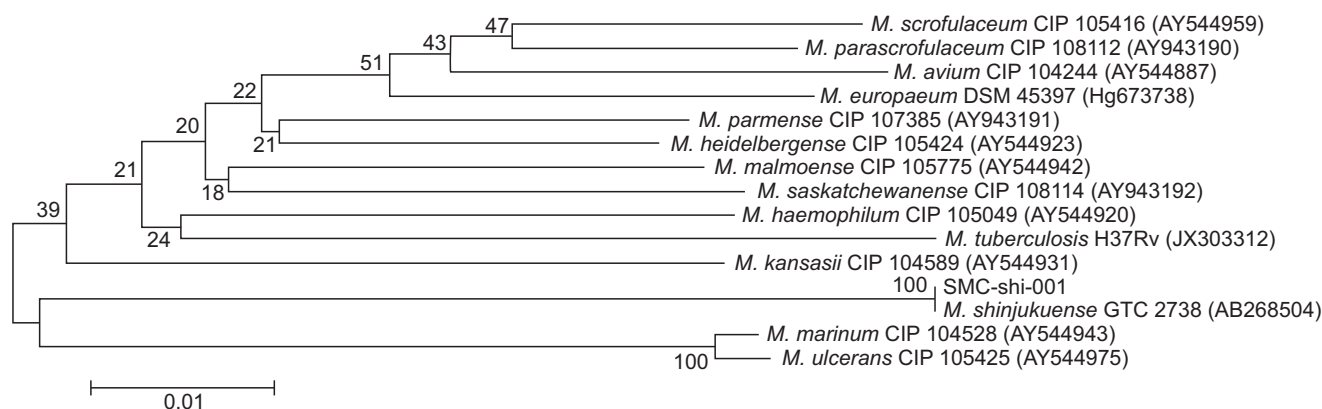


Figure 2. The phylogenetic position of isolated SMC-shi-001 strain from the patient in this report and other species belonging to the slow growing mycobacteria, based on the *rpoB* sequence. This tree is constructed using the neighbor-joining method. The percentages indicated at nodes represent bootstrap levels supported by 1,000 re-sampled datasets. Scale bars indicate evolutionary distance in base substitutions per site. *M.*, *Mycobacterium*.

order to select an appropriate antimicrobial regimen. However, commercial kit for the identification of NTM species cannot identify *M. shinjukuense*. These kits use techniques such as PCR-restriction fragment length of polymorphism analysis or REBA Myco-ID based on the *rpoB* gene. However, they cannot detect newly defined or infrequent species, such as *M. shinjukuense*. In this case, *M. shinjukuense* was not initially identified with a commercially available kit, but was with gene sequencing of the 16S rRNA, *rpoB* and *hsp65* genes. To the best of our knowledge, this is the first case of lung disease caused by *M. shinjukuense* occurring outside of Japan.

According to previous reports, *M. shinjukuense* has been isolated from sputum and bronchial lavage fluid from Japanese patients with bronchiectasis and/or cavitary lesions⁶⁻⁹. None of these patients was immunocompromised. There are only two case reports that address the antibiotic treatment of *M. shinjukuense*. In one case, the clinical efficacy of combination therapy including clarithromycin, rifampin, and ethambutol was addressed⁷. In another, the efficacy of the standard anti-tuberculosis drugs including isoniazid, rifampin, and ethambutol was explored^{8,9}. We could not evaluate treatment efficacy; however, because the patient was reluctant to undergo long-term antibiotic treatment.

In conclusion, this is the first report of NTM lung disease caused by *M. shinjukuense* outside of Japan. *M. shinjukuense* should be considered as a potential causative pathogen in cases of NTM lung disease. Its diagnosis can be verified by performing multilocus sequence analysis of *rpoB*, *hsp65*, and 16S rRNA fragments from clinical isolates.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (A120647) and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2060552).

References

1. Kendall BA, Winthrop KL. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. *Semin Respir Crit Care Med* 2013;34:87-94.
2. Morimoto K, Iwai K, Uchimura K, Okumura M, Yoshiyama T, Yoshimori K, et al. A steady increase in nontuberculous mycobacteriosis mortality and estimated prevalence in Japan. *Ann Am Thorac Soc* 2014;11:1-8.
3. Koh WJ, Chang B, Jeong BH, Jeon K, Kim SY, Lee NY, et al. Increasing recovery of nontuberculous mycobacteria from respiratory specimens over a 10-year period in a tertiary referral hospital in South Korea. *Tuberc Respir Dis* 2013;75:199-204.
4. Kwon YS, Koh WJ. Diagnosis of pulmonary tuberculosis and nontuberculous mycobacterial lung disease in Korea. *Tuberc Respir Dis* 2014;77:1-5.
5. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175:367-416.
6. Saito H, Iwamoto T, Ohkusu K, Otsuka Y, Akiyama Y, Sato S, et al. *Mycobacterium shinjukuense* sp. nov., a slowly growing, non-chromogenic species isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2011;61:1927-32.
7. Futatsugi K, Nishio K, Aida S, Okabayashi K, Nakano Y, Ryuzaki M, et al. Case report: a case of pulmonary infectious disease due to *Mycobacterium shinjukuense*. *Nihon Naika Gakkai Zasshi* 2011;100:3637-9.
8. Watanabe K, Shinkai M, Yamaguchi N, Shinoda M, Hara Y, Ishigatsubo Y, et al. *Mycobacterium shinjukuense* lung disease that was successfully treated with antituberculous drugs. *Intern Med* 2013;52:2653-5.
9. Oshima K, Yokouchi H, Minemura H, Saito J, Tanino Y, Munakata M. Pulmonary infection caused by *Mycobacterium shinjukuense*. *Ann Am Thorac Soc* 2015;12:958-9.
10. Wang HY, Bang H, Kim S, Koh WJ, Lee H. Identification of *Mycobacterium* species in direct respiratory specimens using reverse blot hybridisation assay. *Int J Tuberc Lung Dis* 2014;18:1114-20.
11. Wang HY, Kim H, Kim S, Kim DK, Cho SN, Lee H. Performance of a real-time PCR assay for the rapid identification of *Mycobacterium* species. *J Microbiol* 2015;53:38-46.
12. Turenne CY, Tschetter L, Wolfe J, Kabani A. Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous *Mycobacterium* species. *J Clin Microbiol* 2001;39:3637-48.
13. Telenti A, Marchesi F, Balz M, Bally F, Bottger EC, Bodmer T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol* 1993;31:175-8.
14. Devulder G, Perouse de Montclos M, Flandrois JP. A multi-gene approach to phylogenetic analysis using the genus *Mycobacterium* as a model. *Int J Syst Evol Microbiol* 2005;55(Pt 1):293-302.
15. Somoskovi A, Salfinger M. Nontuberculous mycobacteria in respiratory infections: advances in diagnosis and identification. *Clin Lab Med* 2014;34:271-95.