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A Case of Chronic *Gordonia otitidis* Lung Infection Initially Regarded as Nontuberculous Mycobacterial Lung Disease

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The genus *Gordonia* is one of the mycolic acid-containing aerobic actinomycetes. This genus has 38 named species that are widespread in the natural environment; however, *Gordonia* species rarely cause human infections. A 76-year-old woman presented with cough and sputum for over 1 year and was suspected of having nontuberculous mycobacterial (NTM) lung disease. An NTM isolate from the sputum was initially identified as *Mycobacterium lentiflavum* or *Mycobacterium genavense* by genotypic identification targeting internal transcribed spacer (ITS). However, the isolate was finally confirmed as *Gordonia otitidis* by sequencing of 16S rRNA, *gyrB* and *secA1* genes. In patients with suspected NTM lung disease, the etiologic agent might be an organism other than NTM such as *G. otitidis* but still be identified as NTM without sequencing of 16S rRNA or other genes. Especially in case that a possible NTM isolate is identified as *M. lentiflavum* or *M. genavense* by the genotypic method targeting ITS, additional genotypic tests such as sequencing of 16S rRNA and other genes would be necessary for more reliable identification. **(Ann Clin Microbiol 2017;20:13-16)**

Key Words: Actinomycetales, *Gordonia*, Nontuberculous mycobacteria, Respiratory tract infections

INTRODUCTION

The genus *Gordonia* is one of the mycolic acid-containing aerobic actinomycetes. *Gordonia* species are widespread in the natural environment and rarely cause human infections [1,2]. *Gordonia otitidis* has been isolated from ear discharge, pleural fluid and blood [1]; however, it has not been associated with lung infection. We report a case of chronic lung infection with *G. otitidis* which was initially regarded as nontuberculous mycobacterial lung disease.

CASE REPORT

A 76-year-old woman presented with cough and sputum over 1 year. The patient had received supportive therapy for cough and sputum before she visited outpatient clinic in our university hospital, but the symptoms persisted. She had no history of smoking or pulmonary disease. She had received anti-hypertensive medications for 15 years. She was not immunocompromised and had no medical device. Chest computed tomography revealed multifocal bronchiectasis and centrilobular nodules with linear branching appearance at both lower lobes and right middle lobe suggesting nontuberculous mycobacteria (NTM) infection. She had no systemic symptoms including fever or chills. This patient was clinically considered NTM lung disease with classic symptoms and radiographic finding.

Sputum specimens were examined for acid-fast bacilli (AFB) by smear stain and culture. No specimen for gram stain or culture of ordinary bacteria was obtained. AFB stains were negative, but an AFB isolate was recovered after 23 days of incubation. The isolate was positive for AFB stain and negative for MPT64 antigen. Additionally, the isolate was identified as

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NTM by a real-time PCR (AdvanSure PCR, LG Life Sciences, Seoul, Korea) targeting internal transcribed spacer (ITS) and IS6110. Furthermore, the isolate was identified as *Mycobacterium lentiflavum* or *Mycobacterium genavense* by a PCR and reverse hybridization assay targeting ITS (AdvanSure Mycobacteria GenoBlot Assay, LG Life Sciences). To firmly establish species identification of the possible NTM isolate before starting definitive antimicrobial therapy, the 16S rRNA gene was sequenced after extracting DNA from the positive culture broth. In contrast to the results of the assays targeting ITS, the 16S rRNA sequence of the isolate showed 100% similarity with that of *Gordonia otitidis* strain IFM 10148 (GenBank accession no. AB122027) and ≥0.4% difference with other bacterial species; the isolate shared only 92.4% and 91.9% of the sequence with *M. lentiflavum* and *M. genavense*, respectively. The species of the isolate was thus identified as *G. otitidis* by 16S rRNA analysis.

For the analysis of phenotypes, the positive culture broth was inoculated onto a blood agar plate (BAP) and a chocolate agar, and incubated for 72 hrs at 35°C with 5% CO_2. Non-hemolytic, light yellow-colored colonies grew on BAP, and rough, wrinkled colonies that are light yellow grew on chocolate agar. The isolate was gram positive branching rod with beaded appearance, acid fast and catalase positive. Also, the ability to hydrolyze urea was noted. There were no characteristics significant enough to differentiate the isolate from NTM, actinomycetes or *Nocardia* spp.

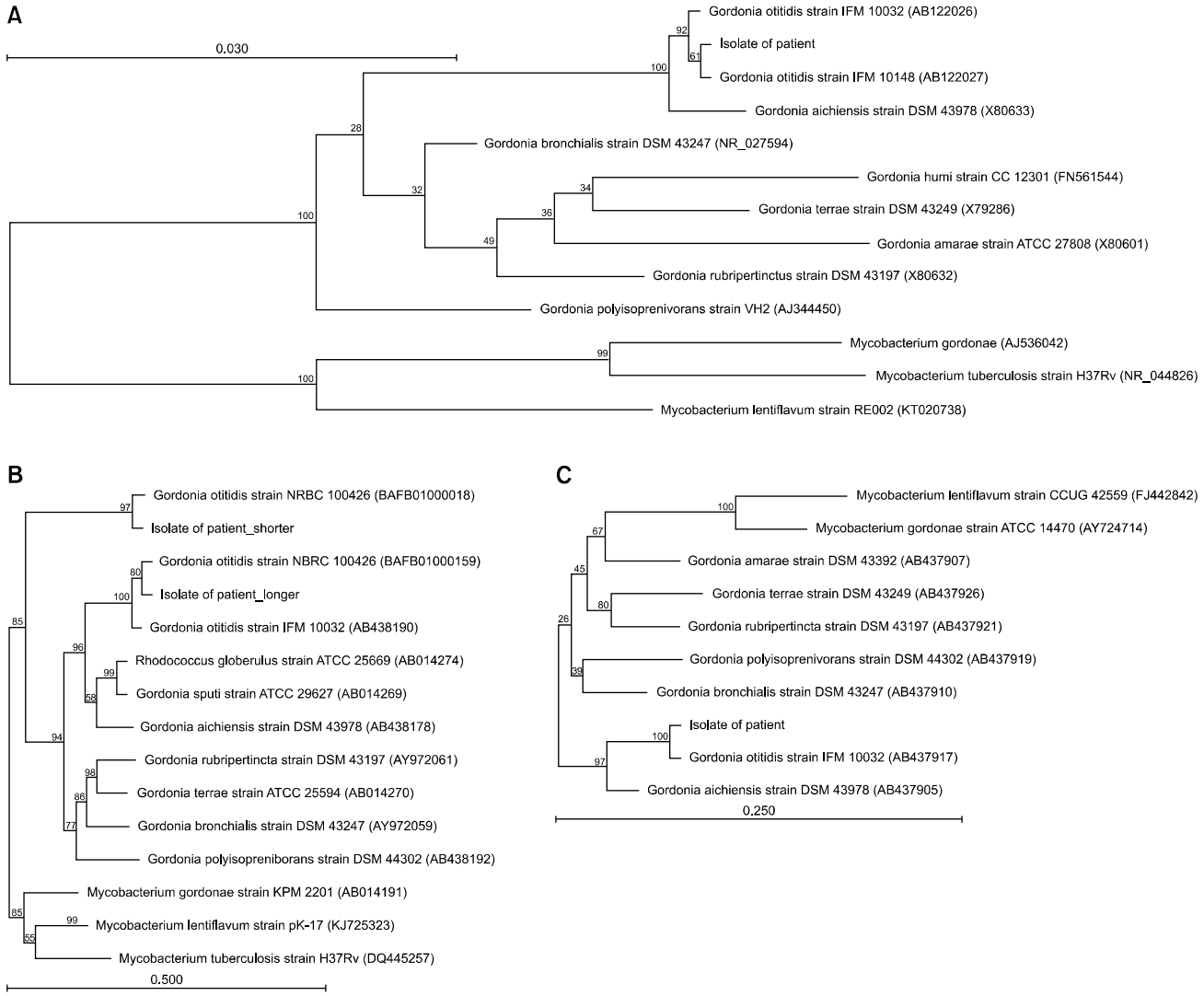

Fig. 1. Phylogenetic trees based on 16S rRNA (A), *gyrB* (B), and *secA1* (C) gene sequences showing the phylogenetic positions of the isolate from the present patient and related *Gordonia* and *Mycobacterium* species. The tree was constructed by the neighbor-joining method. Bootstrap values (from 1000 replications) are shown at branch points. The scale bar length of 0.01 indicates 1% sequence distance.

To resolve the discrepancy between the results of the above-mentioned assays targeting ITS and 16S rRNA genes, the isolate was analyzed by additional sequencing of *gyrB* and *secA1* genes according to a previous study with modifications [1]. PCR amplification of *gyrB* was performed with degenerate primers UP-1F (5′-GAG GTC GTC ATG ACC CAG CTG CAY GCN GGN GGN AAR TTY GA-3′) and UP-2R-modi (5′-AGC AGC GTC GAG ATG TGC TGG CCR TCN ACR TCN GCR TCN GTC A-3′). Thermal cycling was performed as follows: 94°C for 3 min; 35 cycles of 94°C for 40 s, 63°C for 40 s, and 72°C for 1 min; 72°C for 10 min. Two separated PCR products, the longer and the shorter, which were approximately 1,200 bp of the *gyrB* fragments were obtained by electrophoresis on 1% agarose gels at 100 V for 90 min. These two PCR products were separately extracted from the gel, and each was directly sequenced using sequencing primers sF1 (5′-GAG GTC GTC ATG ACC CAG CTG CA-3′) and sR1296-modi (5′-AGC AGC GTC GAG ATG TGC TGG CC-3′). The nucleotide sequence was analyzed with the use of a BLAST search of GenBank database (BLASTN, version 2.5.0+). The longer nucleotide sequence showed 99.3% similarity to *G. otitidis* NRBC 100426^T (GenBank accession no. BAFB01000159), which is the same as strain IFM 10032^T). Also, the shorter one showed 96.6% similarity to *G. otitidis* NRBC 100426^T (GenBank accession no. BAFB01000018). Although the two PCR products had different sizes and sequence divergence, the sequences were consistent with the two *gyrB* paralogues of *G. otitidis*. Similarly, *secA1* gene was amplified by primers secA1-F (5′-GTA AAA CGA CGG CCA GGA CAG YGA GTG GAT GGG YCG SGT GCA CCG-3′) and secA1-R (5′-CAG GAA ACA GCT ATG ACG CGG ACG ATG TAG TCC TTG TC-3′). Thermal cycling was performed as follows: 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; 72°C for 10 min. Amplified fragment (approximately 500 bp) was directly sequenced with the same primers. A BLAST search of the GenBank database showed that the *secA1* gene sequence of the isolate from the patient was 100% homologous with that of *G. otitidis* strain IFM 10032^T (GenBank accession no. AB437917). Phylogenetic trees for 16S rRNA, *gyrB* and *secA1* genes (Fig. 1) were constructed by the neighbor-joining method and drawn using CLC Workbench software 7 (CLC Bio, Aarhus, Denmark). Based on these results, the final genotypic identification was confirmed as *G. otitidis* and not NTM.

The patient received oral cefpodoxime and levofloxacin for 10 months. However, the symptoms waxed and waned, and chest radiographs did not change significantly.

DISCUSSION

The genus *Gordonia* is one of the mycolic acid-containing aerobic actinomycetes. It has 38 validly named species (http://www.bacterio.net) which are widespread in the natural environment [2]. *Gordonia* species have been known to rarely cause human infections; however, they are being recognized increasingly as human pathogens and detected by clinical microbiology laboratories. Infections caused by *Gordonia* spp. are usually related with medical devices in both immunocompromised and immunocompetent patients [3,4]. Members of the genus *Gordonia* are catalase-positive, gram-positive to gram-variable, slightly acid-fast and non-motile.

G. otitidis was first discovered in 2000 from ear discharge of a young female patient with external otitis (strain IFM 10032^T= NBRC 100426^T), and in 2002 from pleural fluid of an elderly male with bronchitis (strain IFM 10148) [5]. Disseminated cases with septic emboli in lungs or endocarditis were also reported in pediatric patients, and all were related with central venous catheter [4]. However, this patient had neither systemic symptoms nor central venous catheter.

G. otitidis produces short elementary branching hyphae that disintegrate into rod- and cocci-like elements. Colonies are white, changing with time to apricot or pale orange with rough and irregular margins [5]. Due to acid-fast nature of the organism, infections caused by this genus can lead to easy confusion with *Mycobacterium* spp., without 16S rRNA gene sequencing to differentiate them. Sequencing of *gyrB* and *secA1* genes can be of additional help to discriminate between the species because the rate of molecular evolution of these genes is reportedly greater than that of 16S rRNA [1,6].

A timely identification of mycolic acid-containing bacteria to the species level can be important for choosing effective therapy [4,7]. In patients with suspected NTM lung disease, the etiologic agent might be an organism other than NTM such as *G. otitidis* but still be identified as NTM without sequencing analysis of 16S rRNA or other genes. Especially in case that a possible NTM isolate is identified as *M. lentiflavum* or *M. genavense* by the genotypic method targeting ITS, additional genotypic tests such as sequencing of 16S rRNA and other genes would be necessary for more reliable identification. In our case, molecular diagnostic methods using the sequencing analyses of 16S rRNA, *gyrB* and *secA1* genes allowed the accurate identification of *G.*

otitidis and differentiation between mycobacteria and *G. otitidis*.

This is the first reported case of pulmonary infection with *G. otitidis*, and it emphasizes the importance of proper molecular identification for the diagnosis and treatment of *Gordonia* species.

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=국문초록=

최초에 비결핵성 미코박테리아 폐질환으로 의심되었던 *Gordonia otitidis*에 의한 만성 폐감염 1예

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Gordonia 속은 미콜산을 함유하는 산소성 방선균의 하나이며 이 속에는 자연 환경에 널리 분포하는 38개의 종이 있다. 그러나 *Gordonia* 균종이 인체 감염을 일으키는 경우는 드물다. 76세 여자 환자가 1년 넘게 지속된 기침과 가래로 내원하여 비결핵성항산균 폐질환으로 의심되었다. 가래에서 배양된 비결핵성항산균 균주는 internal transcribed spacer (ITS)를 이용한 유전형적 방법에 의해 처음에는 *Mycobacterium lentiflavum* 또는 *Mycobacterium genavense*로 동정되었다. 그러나 해당 균주는 16S rRNA, *gyrB*, *secA1* 유전자들의 염기서열 분석에 의해 최종적으로는 *Gordonia otitidis*로 동정되었다. 비결핵성항산균 폐질환이 의심되는 환자에서 원인균이 비결핵성항산균이 아닌 *G. otitidis*와 같은 다른 균종일 수 있으며, 16S rRNA나 다른 유전자의 염기서열 분석 없이는 비결핵성항산균으로 잘못 동정될 수도 있다. 특히 비결핵성항산균의 가능성이 있는 균주가 ITS를 이용한 유전형적 방법에 의해 *M. lentiflavum* 또는 *M. genavense*로 동정된 경우, 더 정확한 동정을 위해서는 16S rRNA 및 다른 유전자의 염기서열 분석과 같은 추가적인 유전형적 검사가 필요할 수 있다. [Ann Clin Microbiol 2017;20:13-16]

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