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Pulmonary Infection Caused by *Mycobacterium neoaurum*: The First Case in Korea

Chang-Ki Kim, M.D.[1], Soo In Choi, M.D.[2], Byung Ryul Jeon, M.D.[2], Yong-Wha Lee, M.D.[2], You Kyoung Lee, M.D.[2], and Hee Bong Shin, M.D.[2]

Department of Laboratory Medicine[1], Korean Institute of Tuberculosis, Osong; Department of Laboratory Medicine and Genetics[2], Soonchunhyang University College of Medicine, Cheonan, Korea

Mycobacterium neoaurum is rapidly growing mycobacteria that can cause human infections. It commonly causes bloodstream infections in immunocompromised hosts, and unlike other mycobacteria species, it rarely causes pulmonary infections. We confirmed the first pulmonary infection case in Korea caused by *M. neoaurum* using full-length 16S rRNA gene sequencing.

INTRODUCTION

Rapidly growing mycobacteria are generally defined as nontuberculous mycobacteria (NTM), which grow within a week on culture media [1]. Currently, there are more than 130 known species of NTM, 70 of which are rapidly growing species, including *Mycobacterium neoaurum* [2].

M. neoaurum is commonly associated with catheter-related infections in immunocompromised hosts [3, 4], meningoencephalitis [5], and urinary tract infections [6]. Pulmonary infections caused by *M. neoaurum* are very rare; there has been only one reported case in the literature [7].

M. neoaurum is not easily identified by using phenotypic laboratory methods and additional tests may be required to confirm the diagnosis [2]. We report a case of pulmonary infection caused by *M. neoaurum*, which was identified by using a molecular method. To the best of our knowledge, this is the first report of an *M. neoaurum* infection in Korea.

CASE REPORT

A 25-yr-old woman visited the Department of Pulmonology and Allergy Clinic at a university hospital to evaluate her cough and whitish sputum that began a month ago. She didn't have any history of chronic illnesses and was not on any medication. To test for common conditions, such as asthma and bronchitis, the physician arranged for allergy and induction tests, and all of the results were negative. Her initial blood cell counts were as follows: hemoglobin, 15.3 g/dL; white blood cells, 8.64×10^9/L (neutrophils, 66.1%; lymphocytes, 20.5%; monocytes, 10.2%; eosinophils, 2.4%; and basophils, 0.8%); and platelets, 353×10^9/L. There was no prominent change in the routine chemistry tests, except for mildly elevated hepatic enzyme levels (AST, 43 IU/L; and ALT, 65 IU/L). An acid-fast bacillus (AFB) smear based on auramine fluorescent stain showed trace result (1-2 AFB/300 fields) according to the Centers for Disease Control and Prevention (CDC) report system. High resolution computed

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tomography (HRCT) scans were arranged to further investigate the presence of pulmonary mycobacteria infection. A HRCT scan displayed a well-marginated centrilobular nodule with branching in the left upper lobe, apicoposterior segment, indicating the presence of pulmonary tuberculosis in the left upper lobe. The physician performed bronchoscopy, which revealed no specific endobronchial lesions, but cultures of bronchial washing specimens for AFB were positive. An endobronchial washing specimen culture was negative for other bacterial or fungal pathogens. On the basis of these results, the patient was thought to have a mycobacterial lung infection and was given rifampicin, ethambutol, and pyrazinamide.

AFB was identified by an auramine fluorescent stain and further confirmed by Ziehl-Neelsen-stained smears from colonies grown on Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, MD, USA) liquid medium. On blood agar plates, we observed yellow colonies within 4 days after inoculation and incubation at 42°C. The results of the nitrate reduction test were negative. Cultural characteristics indicated that the organism belonged to a group of rapidly growing mycobacteria.

The American Thoracic Society (ATS) has suggested diagnostic criteria of nontuberculous mycobacterial lung disease, which require clinical, radiographic, and microbiologic evidence [8]. In our case, the pulmonary symptoms of the patient and HRCT scan findings suffice clinical and radiologic criteria. In terms of the microbiologic criteria, we obtained positive culture results for NTM from at least one bronchial washing specimen without any evidence of an M. tuberculosis complex infection.

To further identify the mycobacteria, reverse hybridization assays (GenoType Mycobacterium CM/AS, Hain Lifescience, Nehren, Germany) were performed. The test results were consistent with *Mycobacterium celatum*, but cultural characteristics and clinical features were inconsistent with this species. Therefore, full-length 16S rRNA gene sequencing was performed by Macrogen (Seoul, Korea) using the following detailed methods:

Using bacterial colonies grown on blood agar plates, bacterial genomic DNA samples were extracted using InstaGene Matrix (BIO-RAD, Hercules, CA, USA). The following primers were used for PCR: forward primer 27F, 5'-AGAGTTTGATCMTGGCT-CAG-3'; and backward primer 1492R, 5'-TACGGYTACCTTGT-TACGACTT-3'. This primer pair amplified a 1,492-bp fragment of the 16S rRNA gene of the *Escherichia coli* 16S rRNA gene. The PCR reaction was performed with 20 ng of genomic DNA as a template in a 30 μL reaction mixture using EF-Taq-DNA polymerase (SolGent, Daejeon, Korea). The following PCR program was used: activation of Taq polymerase at 95°C for 2 min;

35 cycles at 95°C, 55°C, and 72°C for 1 min; and a 10-min step at 72°C.

The amplification products were purified via a multiscreen filter plate (Millipore Corp., Billerica, MA, USA). Sequencing reactions were performed using the PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95°C for 5 min, placed on ice for 5 min, and then analyzed using an ABI Prism 3730XL DNA analyzer (Applied Biosystems).

After sequencing, the raw sequence file (.abi) was processed using Lasergene SeqMan Pro 7.1 (DNASTAR, Madison, WI, USA) to get trimmed nucleotide sequences. We compared the obtained sequences with those deposited in GenBank (http://www.ncbi.nlm.nih.gov). The sequence of the 16S rRNA gene was consistent with that of *M. neoaurum* ATCC25796 (FJ172307.1) based on having a sequence similarity of 99.8%.

A phylogenetic tree was generated using the Neighbor-Joining method, which is based on nucleotide sequences of the clinical isolate with those of other Mycobacteria reference strain sequences prepared from the National Center for Biotechnology Information (NCBI) database using Mega 5.0 software [9] (Fig. 1).

The drug susceptibility test was performed at the Korean Institute of Tuberculosis using first-line and second-line drugs, and the minimal inhibitory concentrations (MIC) were determined using the broth microdilution method in Muller-Hinton media. MICs were interpreted based on the broth microdilution interpretive criteria for rapidly growing mycobacteria [10] (Table 1).

After 1 month of rifampicin, ethambutol, and pyrazinamide treatment, the patient was placed on a clarithromycin regimen for 4 months, and her symptoms improved.

DISCUSSION

NTM are ubiquitous organisms that are found in the environment. Pulmonary infections are the most common manifestation of NTM infections, and they are often caused by M. avium complex (MAC). Currently, the overall incidence of NTM infections in Korea is increasing [11].

M. neoaurum was first isolated from soil and reported in 1972 [12]. Since then, there have been 25 cases of human infections, ranging from sepsis to skin infections. Literature reviews have noted that *M. neoaurum*-related cases were mostly catheter- or line-related sepsis instead of meningoencephalitis, skin infections [13, 14], or pulmonary infections [7].

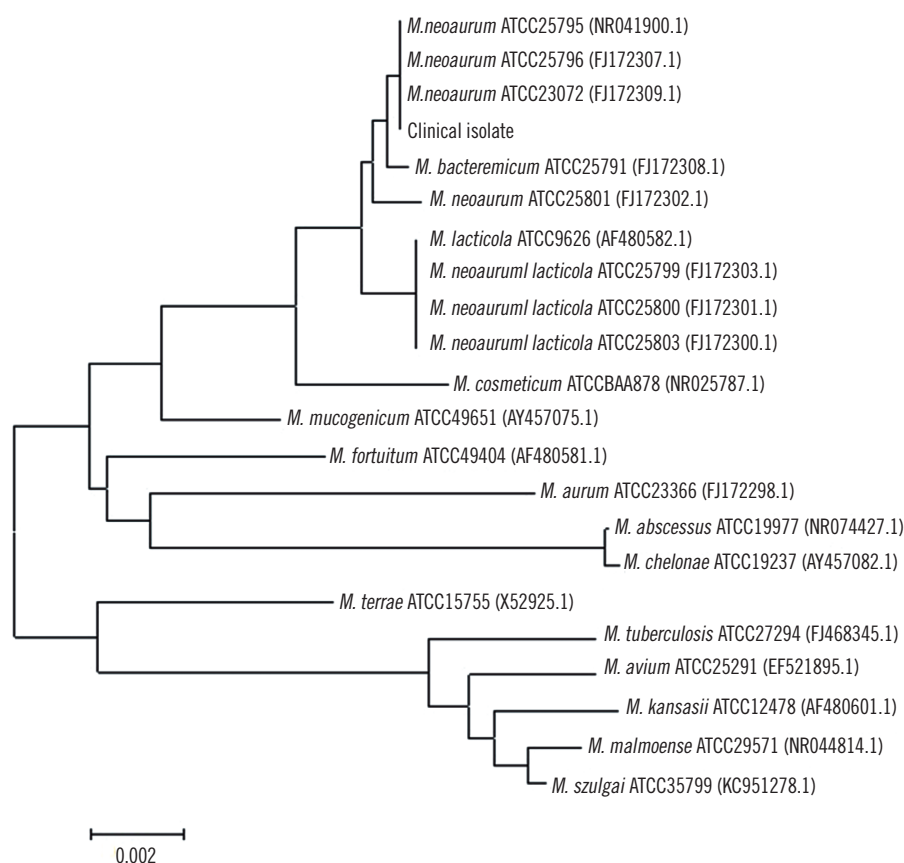Initially, the clinical isolate described in our case was misiden-

Fig. 1. Phylogenetic relationships of clinical isolate with related Mycobacterium species on the basis of 16S rRNA gene sequences by neighbor-joining method. Scale bar represents 2 nucleotide substitutions per 1,000 nucleotides.

Table 1. Comparison of the antimicrobial drug susceptibility of this clinical isolate with those from other studies

Antimicrobial agents	Susceptibility of our case isolate			% Susceptibility in other studies*
	Dilutional range (µg/mL)	MIC (µg/mL)	Interpretation	
Amikacin	1-128	≤1	Susceptible	100
Cefoxitin	2-256	32	Intermediate	100
Ciprofloxacin	0.125-16	≤0.125	Susceptible	100
Clarithromycin	0.5-64	2	Susceptible	80
Doxycycline	0.25-32	≤0.125	Susceptible	100
Imipenem	0.5-64	2	Susceptible	100
Moxifloxacin	0.125-16	≤0.125	Susceptible	100
Rifampicin	0.125-16	16	-	-
Trimethoprim/Sulfa-methoxazole	0.25/4.75-32/608	16/304	Resistant	100
Tobramycin	0.25-32	4	Intermediate	100
Ethambutol	0.25-32	>32	-	-
Linezolid	2-64	≤2	-	100

*Adapted from reference [7, 18].
Abbreviation: MIC, minimal inhibitory concentration.

tified as *M. celatum* by the reverse hybridization assay. However, on the basis of sequencing the full-length 16S rRNA gene, the isolate was re-identified as *M. neoaurum*.

For accurate *Mycobacterium* spp. identification, sequencing

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multiple housekeeping genes is invaluable. However, we believe 16S rRNA sequencing with phylogenetic analysis would be enough to identify *M. neoaurum* in this case for several reasons. First, we performed full-length 16S rRNA gene sequencing (1,492 bp), which was used to confirm its identity in roughly a half of the previously reported cases [4, 7, 15, 16]. Second, *M. neoaurum* has a unique 16S rRNA gene sequence that can be used for identification [2]. Finally, and most of all, our phylogenetic analysis revealed that the clinical isolate forms a tight cluster with *M. neoaurum* ATCC23072, ATCC25795, and ATCC25796 (Fig. 1), which were all confirmed to be *M. neoaurum* in other studies [17]. The misidentification of *M. neoaurum* can usually occur due to its similarity to *M. lacticola* group. However, this clinical isolate was sufficiently discriminated among other rapidly growing mycobacteria [18].

Antimicrobial susceptibility test could be an adjunctive test for identification of rapidly growing mycobacteria. But susceptibility test result for cefoxitin, tobramycin, and trimethoprime/sulfamethoxazole of this isolate didn't coincide with other studies, as *M. neoaurum* is known as pan-susceptible to antimicrobial drugs [2, 7, 18] (Table 1). We are not sure whether this finding has clinical significance, but further study will reveal much more information.

Contrary to other reported cases of *M. neoaurum*, we described a pulmonary infection in a fully immunocompetent patient. Any patient with an atypical mycobacteria pulmonary infection should be investigated further for a correct identification of NTM.

This is the first reported case of pulmonary infection with *M. neoaurum* in Korea, and it emphasizes the importance of proper molecular identification for the diagnosis and treatment of NTM.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

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