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First Case of *Mycobacterium longobardum* Infection

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Mycobacterium longobardum is a slow-growing, nontuberculous mycobacterium that was first characterized from the *M. terrae* complex in 2012. We report a case of *M. longobardum* induced chronic osteomyelitis. A 71-yr-old man presented with inflammation in the left elbow and he underwent a surgery under the suspicion of tuberculous osteomyelitis. The pathologic tissue culture grew *M. longobardum* which was identified by analysis of the 65-kDa heat shock protein and full-length 16S rRNA genes. The patient was cured with the medication of clarithromycin and ethambutol without further complications. To the best of our knowledge, this is the first report of a *M. longobardum* infection worldwide.

Key Words: *Mycobacterium longobardum*, Nontuberculous mycobacteria, Osteomyelitis

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INTRODUCTION

Mycobacterium longobardum is a novel nontuberculous mycobacterium (NTM) that was first characterized as a separate species in 2012 [1]. It was previously included in the *M. terrae* complex, a classification drafted in the 1970s that included *M. nonchromogenicum*, *M. terrae*, and *M. triviale*. Four new species were then added to this group: *M. hiberniae*, *M. arupense*, *M. kumamotonense*, and *M. senuense* [2-5]. Despite the fact that the *M. terrae* complex is ubiquitous in the environment and is generally considered a nonpathogenic NTM [6], *M. terrae* complex infections affecting the upper extremities and lungs have been continuously reported [7-9]. We report the recovery and identification of *M. longobardum* from surgically resected tissue of a patient with chronic osteomyelitis.

CASE REPORT

A 71-yr-old man presented with symptoms of pain, redness, and swelling in the left elbow on May 7, 2012. The patient had been previously diagnosed with left elbow bursitis and had undergone surgery to resect the infected tissue at a university hospital in January 2011. At that time, a pathology review of the excised tissue confirmed the diagnosis of tuberculous bursitis with chronic granulomatous inflammation, without isolation of *Mycobacterium tuberculosis* (MTB). The patient received the antituberculosis drugs of isoniazid, rifampicin, ethambutol, and pyrazinamide for seven months.

The patient had a history of being treated for hypertension and diabetes. Physical examination at presentation revealed surgical scarring and tenderness around the left elbow. The patient's electrolytes, liver function, and complete blood counts were normal. His blood pressure was 107/76 mmHg and his two-hour postprandial glucose level was 106 mg/dL. Magnetic resonance imaging (MRI) of the left elbow revealed localized bone marrow signal alterations at the left ulnar olecranon and substantial effusion with marked synovial hypertrophy in the radiocapitellar joint.

The patient was assessed as having tuberculous osteomyelitis

according to the history and MRI results. The left elbow was incised and drained using sterilized devices on May 8, 2012. Histological examination of the resected tissue showed necrotizing granulomatous inflammation (Fig. 1). No organisms were detected on Gram or Ziehl-Neelsen staining, and the direct culture of the tissue sample for bacteria was sterile. In addition, there was no evidence of malignancy. The biopsied tissue was cultured for mycobacteria, and mycobacterial growth was observed on Löwenstein-Jensen medium 26 days later. The isolate was acid-fast bacillus and colonies grew rough and unpigmented both in the dark and after light exposure. The mycobacterium was identified as NTM using the Seeplex MTB/NTM ACE Detection Kit (Seegene Inc., Seoul, Korea).

The 16S rRNA gene was sequenced for the NTM identification. The primers and PCR setting used to amplify the target region, the first 500 bp of the 16S rRNA gene, were as previously described [10, 11]. Sequences were analyzed using an ABI PRISM 3730 series DNA Analyzer (Applied Biosystems, Foster City, CA, USA). For the first 500 bp of the 16S rRNA gene sequence, the isolate showed a 98.6% identity match with GenBank sequence JN571166.1 (*M. longobardum*), a 97.7% identity match with GenBank sequence FJ268583.1 (*M. senuense*), and a 97.5% identity match with GenBank sequences NR_043905.1 (*M. senuense*) and GQ184162.1 (*M. terrae*). However, the Clinical and Laboratory Standards Institute guidelines indicate that a *Mycobacterium* sp. that has a 95-99% nucleotide identity with 16S rRNA gene sequences of other species cannot be definitively identified by 16S rRNA gene sequencing [12].

Tortoli et al. [1] reported that 65-kDa heat shock protein (*hsp*65) and full 16S rRNA gene sequencing are useful for the identification of *M. longobardum*. For accurate identification, the *hsp*65 and additional 16S rRNA genes of the isolate were analyzed. Using primers amplifying 468-1,053 bp (F1 5′-TGC CAG CAG CCG CGG TAA-3′, R1 5′-CGG GAC TTA ACC CAA CAT CT-3′) and 1,010-1,500 bp (F2 5′-TGG CTG TCG TCA GCT CGT-3′, R2 5′-AAG GAG GTG ATC CAA CCG CA-3′) of the 16S rRNA gene, PCR was performed as described for sequencing of the first 500 bp of the 16S rRNA gene sequence. For the full 16S rRNA gene sequence, the isolate showed a 99.2% identity match with GenBank sequence JN571166.1 (*M. longobardum*), a 98.6% identity match with GenBank sequence HM770865.1 (*M. terrae*), and a 98.4% identity match with GenBank sequence GU084182.2 (*M. heraklionense*). The 16S rRNA gene phylogenetic tree of the mycobacterium isolated in this case was most closely related to JN571166.1 (*M. longobardum*) and was classified as *M. longobardum* (Fig. 2). The primers and PCR settings used to amplify the *hsp*65 gene were as described previously [13]. Moreover, *hsp*65 sequencing of the isolate showed a 99.4% match with sequences JN571166.1 (*M. longobardum*), EF601223.1 (*M. terrae*), and JX154097.1 (*M. terrae*). We concluded that the isolate was *M. longobardum*.

After identifying *M. longobardum*, the patient was prescribed clarithromycin and ethambutol empirically. The antimicrobial susceptibility test (AST) using the broth microdilution method revealed that the isolate was susceptible to clarithromycin, ethambutol, moxifloxacin, and sulfamethoxazole, but resistant to amikacin, ciprofloxacin, and rifampicin. The therapy was maintained, and the patient was cured without further complications.

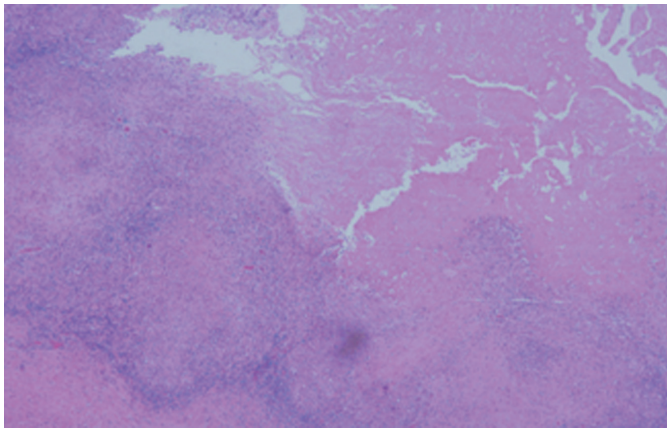

Fig. 1. Pathological specimens of left elbow soft tissue from the patient with *Mycobacterium longobardum* infection. Necrotizing granulomatous inflammation with cystic abscess formation was observed (H&E, ×40).

DISCUSSION

The patient had chronic osteomyelitis due to a *M. longobardum* infection, which is known to grow rough and unpigmented colonies both in the dark and after light exposure, test positive for nitrate reductase and arylsulfatase, and test negative for niacin accumulation, Tween 80 hydrolysis, tellurite reduction, urease, and β-glucosidase, as assessed by the analysis of seven isolates stored frozen at -80°C [1]. However, owing to a lack of clinical reports, there is no information about the pathogenicity or clinical features of *M. longobardum*. We can deduce the clinical features of *M. longobardum* from those of the *M. terrae* complex including *M. longobardum*, which is known to have low pathogenicity and frequently infects the upper extremities [6, 7].

Accurate identification is important for the treatment of *M. terrae* complex infection. The present patient was misdiagnosed and initially treated for extrapulmonary tuberculosis. Although

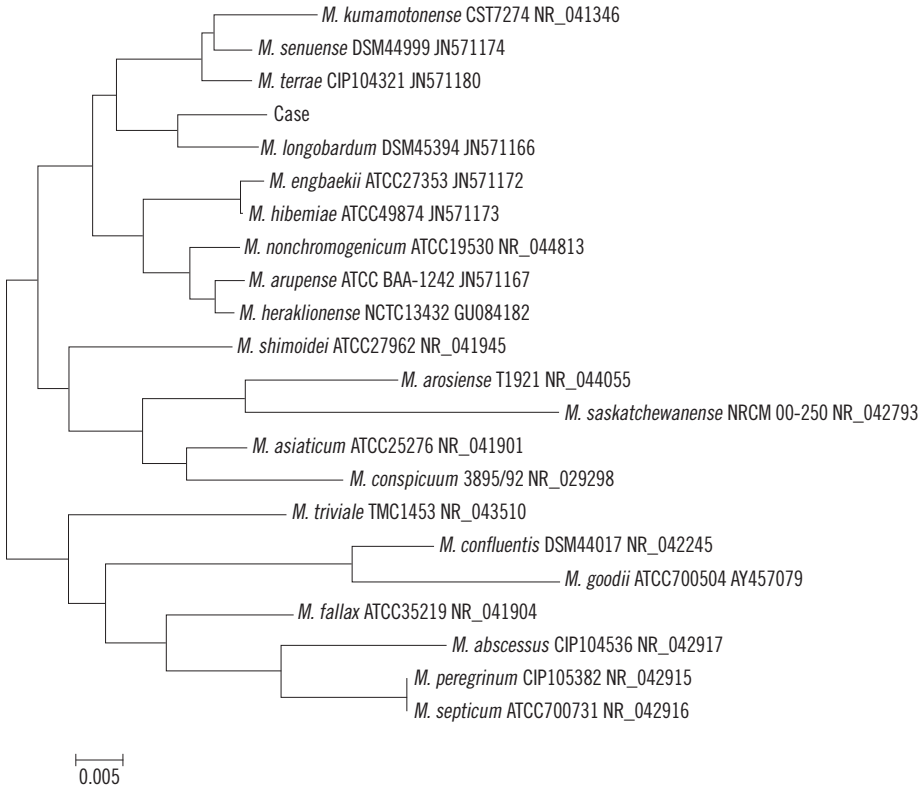

Fig. 2. Phylogenetic tree based on 16S rRNA sequences constructed using the neighbor-joining method. Nucleotide sequences were used to align the reference panels provided by the National Center for Biotechnology Information Reference Sequence (RefSeq) database. This isolate was identified as *Mycobacterium longobardum*.

he underwent surgery to resect the infected tissue, he experienced recurrence. A substantial number of patients infected with *M. terrae* also show persistent disease resulting in repeated debridement, tendon extirpation, or amputation due to misdiagnosis and inadequate antibiotic therapy [7].

The isolate was correctly identified after analysis of the *hsp*65 and full-length sequences of the 16S rRNA gene of *M. longobardum*, which are unique from those of other species [1]. For specific identification of most mycobacteria, information about the 5' end of the 16S rRNA gene is sufficient [11]. However, caution should be exercised when interpreting findings using public database references because species may share identical 16S rRNA gene sequences or have intraspecies heterogeneity [11, 14] and insufficient sequence data have been used to compare isolate sequences like those in this newly characterized species. The AST pattern of our isolate was similar to that in the report by Tortoli et al. [1] with the exception of moxifloxacin resistance, in which the AST pattern of *M. longobardum* isolates showed that this species is susceptible to clarithromycin and sulfamethoxazole and resistant to ciprofloxacin, moxifloxacin, linezolid, and streptomycin. According to the AST pattern, a multidrug antibiotic regimen including a macrolide and surgical debridement may still be a useful initial approach to manage patients with *M. longobardum*, similar to those with the *M. terrae* complex [7].

This is the first reported case of *M. longobardum* infection causing chronic osteomyelitis. Proper molecular identification led to a more accurate classification of this organism and an appropriate course of treatment.

Authors' Disclosures of Potential Conflicts of Interest

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

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