CASE REPORT

A 43-year-old man with a history of intellectual disability fractured his right tibia in April 2011. A tibial intramedullary nailing was performed without any early postoperative complications. Five months later, he developed a progressive pain in his right knee. In February 2012, physical examination revealed arthritis, without fever, and an unaltered general condition. It is important to note that the patient lived on a farm with his family and frequently had superficial wounds caused by repeated falls, especially on his legs. The initial laboratory tests showed a leukocyte count of 17,000 cells/μL, C-reactive protein at 172 mg/liter, and an erythrocyte sedimentation rate of 71 mm at the first hour. A bone Tc99m-MDP scintigraphy revealed a high osteoblastic reaction around the nail, suggesting tibial osteomyelitis. Right-tibial X-ray showed consolidation of the fracture. Surgery was performed in order to remove the nail. The macroscopic examination showed a large quantity of pus around the material. Gram staining was negative.

Forty-eight hours after surgery, a mycobacterial strain that could not be identified through conventional phenotypic biochemical methods was grown on blood agar and chocolate plates from two distinct specimens with a high count of CFU/ml. The colonies were on-streak. The strain was sent to our University Hospital microbiological laboratory. The identification was performed using matrix-assisted laser desorption-ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik Bremen, Germany), and it revealed Mycobacterium thermoresistibile. The spectra were compared with the Bruker mycobacterium library 1.0, which contains 173 spectra of 93 mycobacteria, including one of M. thermoresistibile. Identification was carried out at the species level, with a score of ≥2. The confirmation for this identification was performed by sequencing the 16S rRNA and heat shock protein 65 (hsp65) genes. PCR amplification of the 16S rRNA and hsp65 genes was performed using previously described primers (references 1 and 2, respectively). The PCR products obtained were sequenced using a DNA sequencing kit (CEQ 2000 dye terminator cycle sequencing quick start kit; Beckman Coulter, Inc.) according to the manufacturer’s instructions. The 16S rRNA and hsp65 sequences were compared to sequences deposited in the BLAST® database of GenBank and were 98% and 99% similar, respectively, to those of M. thermoresistibile.

Through antimicrobial susceptibility testing using the Etest method (bioMérieux, Marcy l’Etoile, France) and the breakpoints recommended by the Clinical and Laboratory Standards Institute for rapidly growing mycobacteria (3), susceptibility to imipenem (MIC = 0.032 mg/liter), amikacin (MIC = 0.125 mg/liter), clarithromycin (MIC = 0.023 mg/liter), doxycycline (MIC = 0.032 mg/liter), levofloxacin (MIC = 0.016 mg/liter), moxifloxacin (MIC = 0.004 mg/liter), trimethoprim-sulfamethoxazole (MIC = 0.004 mg/liter), and linezolid (MIC = 0.125 mg/liter) was shown.

The empirical treatment with 500 mg/day oral levofloxacin and 3 g/day pristinamycin (macrolide) introduced immediately after surgery was stopped after the organism was identified. A treatment including 1 g/day levofloxacin plus 1 g/day clarithromycin plus 3,200/800 mg/day trimethoprim-sulfamethoxazole was initiated. This regimen allowed local improvement despite a slow cicatrization. Two weeks later, a general rash was noticed. Allergy to trimethoprim-sulfamethoxazole was suspected, and the administration of this antibiotic was stopped. Levofloxacin and clarithromycin continued to be given for 22 weeks. Inflammatory parameters were normalized after 3 months. No relapse was observed 6 months after the end of the treatment.

Mycobacterium thermoresistibile is a nontuberculous mycobacterium (NTM) rarely reported in human infections. To the best of our knowledge, since its first description in 1966 by Tsukamura (4), only 6 cases of human infections have been published. Here, we report a case of osteomyelitis caused by this pathogen, associated with an orthopedic nail. There are two possible hypotheses for the source of infection. First, the infection may be of nosocomial origin, caused by this rapid growing NTM (5), since it occurred 5 months after the centromedullary nailing. The literature states that rapidly growing mycobacteria, especially M. chelonae, M. fortuitum, and M. abscessus, can potentially be health care as-
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sex/a</th>
<th>Age (yr)</th>
<th>Underlying condition</th>
<th>Infection site</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>W/72</td>
<td>60</td>
<td>Diabetes mellitus</td>
<td>Knee prosthesis-related osteomyelitis</td>
<td>Yes, 2 mo after surgery</td>
<td>Culture in mycobacterium-specific medium Antibiotherapy</td>
<td>40 wk</td>
<td>Yes, removal of prosthesis Recovery 7 mo after beginning of therapy</td>
</tr>
<tr>
<td>2</td>
<td>W/11001</td>
<td>50</td>
<td>None</td>
<td>Lung infection</td>
<td>No</td>
<td>Culture in Middlebrook 7H11 medium, at 25, 37 and 45°C in 5% CO2; biochemical tests</td>
<td>Yes</td>
<td>RIF/LZD/STR</td>
</tr>
<tr>
<td>3</td>
<td>M/11001</td>
<td>64</td>
<td>Hypogammaglobulinemia</td>
<td>Lung and sinus infection</td>
<td>No</td>
<td>Culture in Middlebrook 7H11 medium and in LJM at 25, 35, 45, and 52°C in 7% CO2; biochemical tests</td>
<td>Yes</td>
<td>RIF/EMB/STR</td>
</tr>
<tr>
<td>4</td>
<td>M/11001</td>
<td>41</td>
<td>Transplant recipient, diabetes mellitus</td>
<td>Skin infection</td>
<td>Yes, 3 mo after surgery</td>
<td>Culture at 42 and 50°C; biochemical tests; HPLC</td>
<td>?</td>
<td>RIF/EMB/STR</td>
</tr>
<tr>
<td>5</td>
<td>W/41</td>
<td>41</td>
<td>None</td>
<td>Mammaplasty implant infection</td>
<td>Yes, 3 mo after surgery</td>
<td>Culture in LJM at 37°C and in Middlebrook 7H10 medium at 42 and 52°C; biochemical tests; HPLC</td>
<td>Yes</td>
<td>RIF/EMB</td>
</tr>
<tr>
<td>6</td>
<td>W/11001</td>
<td>35</td>
<td>None</td>
<td>Skin infection</td>
<td>No</td>
<td>Culture in LJM at 37°C; biochemical tests; HPLC; coinfection with M. fortuitum</td>
<td>Yes</td>
<td>LVX/DOX</td>
</tr>
<tr>
<td>7</td>
<td>M/43</td>
<td>43</td>
<td>Intellectual disability</td>
<td>Tibial-nail-related osteomyelitis</td>
<td>Yes, 5 mo after surgery</td>
<td>Culture in blood agar; MALDI-TOF MS; 16SrRNA and hsp65 sequencing</td>
<td>No</td>
<td>LVX/CLR/SXT 24 wk</td>
</tr>
</tbody>
</table>
associated (6). However, in contrast to other NTM, *M. thermoresistibile* has not been isolated in water samples (7). No similar cases of infection associated with foreign devices have been reported in the institution. Osteomyelitis remains a rare event among NTM infections. Another hypothesis is that a traumatic inoculation with *M. thermoresistibile* present in the soil (4) was followed by a local infection of the knee and the device.

Using the Medline database, 6 human infections were found, as summarized in Table 1. Three of the 6 human infections reported in the literature occurred 3 months after surgery. In 2 of these cases, the surgery was associated with implants, as reported in our case. The remaining cases of infection involved the skin or lungs. We also found one case in a patient suffering from chronic obstructive pulmonary disease, where *M. thermoresistibile* was found in expectoration and was responsible for colonization, since it did not meet the criteria of the American Thoracic Society (ATS) for infection (8). The majority of patients (4/6) were immunocompromised as described in Table 1, which is often observed in infections due to the presence of other NTM (9).

We found a positive culture on blood agar, as frequently obtained with rapidly growing mycobacteria. In our case, *M. thermoresistibile* was rapidly identified with MALDI-TOF MS. The conventional methods used for mycobacterial identification are expensive and time consuming (e.g., conventional biochemical tests and molecular tools). MALDI-TOF MS is currently used for routine identification of a large diversity of bacterial species in medical microbiological laboratories (10). Recently, this technique has also been used to rapidly identify the most clinically relevant mycobacteria and may now represent an interesting alternative in identifying mycobacteria (11). The identification of *M. thermoresistibile* was performed by biochemical tests and/or high-performance liquid chromatography in all previous cases listed in Table 1. This current case is the first in the literature in English to report a human infection with *M. thermoresistibile* reliably identified by using MALDI-TOF MS and 16S rRNA and *hsp65* sequencing. The Etest was used for susceptibility testing, since a good correlation with the reference agar dilution method is required (14).

Previously only one case of *M. thermoresistibile* infection related to an orthopedic device had been reported (Table 1) (13). The treatment included the removal of the prosthesis and long-term antibiotics, as in our case (13). While no guidelines existed for the treatment of *M. thermoresistibile* infections, our choice of antibiotics was based on susceptibility testing and on ATS guidelines for NTM infections (14). We followed the recommendations for the treatment of *M. fortuitum* bone infections, for which a 6-month regimen, including at least two agents with in vitro activity, is required (14).

*M. thermoresistibile* may cause health care-associated infections, particularly in the presence of implants. MALDI-TOF MS may allow for the identification of this bacterium and other NTM while they are not being targeted in the first-line bacteriological tests.

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**REFERENCES**