CASE REPORT

Infection due to *Mycobacterium thermoresistibile*: report of a case associated with orthopedic device and review

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Running title: *Mycobacterium thermoresistibile* osteomyelitis

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Abstract:

*Mycobacterium thermoresistibile* is a rapidly growing environmental non-tuberculous mycobacterium, seldom reported in human infections. Here we describe a rare case of tibial nail-related osteomyelitis due to *Mycobacterium thermoresistibile*. We also reviewed literature about the infections caused by this pathogen.
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. Initial laboratory tests showed a leukocyte count, 17 G/L, C-reactive protein, 172 mg/L and erythrocyte sedimentation rate, 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. A 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 UFC/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*. 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 ≥ 2. The confirmation for this identification was performed by sequencing 16S ribosomal RNA (16S rRNA) and heat shock protein 65 (*hsp65*) genes. PCR amplification of the 16S rRNA and
hsp65 encoding genes was performed using the previously described primers (respectively (1) and (2)). PCR products obtained were sequenced using a DNA sequencing kit (Quick Start kit, CEQ 2000 Dye Terminator Cycle Sequencing, Beckman Coulter, Inc), according to the manufacturer’s instructions. The 16S r RNA and hsp65 sequences were compared to sequences deposited in the BLAST database of GenBank® and were 98% and 99% similar to that of M. thermoresistibile, respectively.

Through antimicrobial susceptibility testing using the E-test 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/L), amikacin (MIC = 0.125 mg/L), clarithromycin (MIC = 0.023 mg/L), doxycycline (MIC = 0.032 mg/L), levofloxacin (MIC = 0.016 mg/L), moxifloxacin (MIC = 0.004 mg/L), trimethoprim-sulfamethoxazole (MIC = 0.004 mg/L) and linezolid (MIC = 0.125 mg/L), was shown.

The empiric treatment with oral levofloxacin 500mg/day and pristinamycin (macrolide) 3g/day introduced immediately after surgery was stopped after the organism was identified. A treatment including levofloxacin 1g/day plus clarithromycin 1g/day plus trimethoprim-sulfamethoxazole 3200mg/800mg/day was initiated. This regimen allowed local improvement despite a slow cicatrisation. Two weeks later a general rash was noticed. Allergy to trimethoprim-sulfamethoxazole was suspected and this antibiotic stopped being administered. 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 non-tuberculous mycobacterium (NTM) rarely reported in human infections. To the best of our knowledge, since its first description in 1966
by Tsukamura et al. (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. Firstly, the infection may be of nosocomial origin, caused by this rapid growing NTM (5), since it occurred 5 months after the centromedullary nailing. Literature states that rapidly growing mycobacteria, especially M. chelonae, M. fortuitum and M. abscessus can potentially be healthcare–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 out of 6 human infections reported in literature occurred three 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. Conventional methods used for mycobacterial identification are expensive and time consuming (e.g. conventional biochemical tests, 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). 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 English literature to report a human infection with *M. thermoresistibile* reliably identified, using MALDI-TOF MS, 16S rRNA and *hsp65* sequencing. The E-test was used for susceptibility testing, since a good correlation with the reference agar dilution method was shown (12).

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 extended 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 healthcare-associated infections, particularly in the presence of implants. MALDI-TOF MS may allow for the identification of this bacterium and other NTM while not being targeted in the first line bacteriological tests.

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References


<table>
<thead>
<tr>
<th>Cases (ref)</th>
<th>Sex/age (years)</th>
<th>Underlying condition</th>
<th>Infection site</th>
<th>Health-care associated infection</th>
<th>Diagnosis</th>
<th>Culture in Mycobacteria specific media</th>
<th>Antimicrobial therapy</th>
<th>Duration</th>
<th>Surgery</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(13) W/72</td>
<td>Diabetes mellitus</td>
<td>Knee prosthesis-related osteomyelitis</td>
<td>Yes, 2 months after surgery</td>
<td>Culture in LJMJ at 35°C and in VersaTREK® liquid media; biochemical tests and HPLC</td>
<td>Yes</td>
<td>MOX + Lzd 6w, then Lzd replaced with DOX</td>
<td>40 ws</td>
<td>Yes, removal of prosthesis</td>
<td>Recovery 7 months after therapy beginning</td>
<td></td>
</tr>
<tr>
<td>(15) W/50?</td>
<td>None</td>
<td>Lungs ± sinus 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 + EMB + STR</td>
<td>?</td>
<td>No</td>
<td>Afebrile after 1 w</td>
<td></td>
</tr>
<tr>
<td>(16) M/64</td>
<td>Hypogammaglobulinaemia</td>
<td>Lungs ± sinus infection</td>
<td>No</td>
<td>Culture in Middlebrook 7H11 medium and in LJMJ, at 25, 35, 45, and 52°C in 7% CO2; biochemical tests</td>
<td>Yes</td>
<td>RIF 600mg/d + EMB 15mg/kg/d + STR 1g 2/w 1 month then RIF + EMB</td>
<td>?</td>
<td>No</td>
<td>Recovery</td>
<td></td>
</tr>
<tr>
<td>(17) M/41</td>
<td>Transplant recipient, diabetes mellitus</td>
<td>Skin infection</td>
<td>Yes, 3 months after surgery</td>
<td>Culture at 42°C and 50°C; biochemical tests and HPLC</td>
<td>?</td>
<td>RIF 600mg/d + EMB 1200mg/d</td>
<td>1 year</td>
<td>Yes, debridement</td>
<td>Recovery</td>
<td></td>
</tr>
<tr>
<td>(18) W/41</td>
<td>None</td>
<td>Mammoplasty implant infection</td>
<td>Yes, 3 months after surgery</td>
<td>Culture in LJMJ at 37°C, and in Middlebrook 7H10 medium at 42°C and 52°C; biochemical tests and HPLC</td>
<td>Yes</td>
<td>RIF 600mg/d + EMB 1600mg/d then 1000mg/d</td>
<td>?</td>
<td>Yes, removal of implant</td>
<td>Recovery 16 months after therapy beginning</td>
<td></td>
</tr>
<tr>
<td>(19) W/?</td>
<td>None</td>
<td>Skin infection</td>
<td>No</td>
<td>Culture in LJMJ at 37°C; biochemical tests and HPLC</td>
<td>Yes</td>
<td>LVX 1000mg/d + DOX 200mg/d</td>
<td>12 ws</td>
<td>No</td>
<td>Recovery 9 months after therapy beginning</td>
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**Our case: M/43 Intellectual disability**

| Tibial nail-related osteomyelitis | Yes, 5 months after surgery | Culture in blood agar MALDI-TOF MS 16S rRNA and hsp65 | No | LVX 1000mg/d + CLR 1000mg/d + SXT 3200/800mg/d 2ws; then LVX + CLR | 24 ws | Yes, removal of material | Recovery 6 months after therapy beginning |

Reference: ref; woman, W; man, M; Löwenstein Jensen medium, LJMJ; high performance liquid chromatography, HPLC; day, d.; week, w.; moxifloxacin, MOX; linezolid, LZD; doxycycline, DOX; rifampicin, RIF; ethambutol, EMB; streptomycin, STR; levofloxacin, LVX; clarithromycin, CLR; trimethoprim-sulfamethoxazole, SXT.