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Hip Prosthesis Infection Due to *Mycobacterium wolinskyi*

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***Mycobacterium wolinskyi*, first described in 1999, is a rapidly growing mycobacterium related to the *Mycobacterium smegmatis* group. Only eight cases of infection due to this microorganism have been reported, including three cases of bone infection. Here, we present the first case of a joint prosthesis infection cured with the combination of surgery and prolonged antibiotic therapy. The microorganism was identified by biochemical tests and 16S rRNA and Hsp65 gene sequence analysis.**

CASE REPORT

In February 2003, a total hip arthroplasty was performed for primary osteoarthritis in an 83-year-old woman. There were no unfavorable postoperational sequels. Four months later, the patient presented at the emergency ward with acute pain in the hip that had been operated on; this pain had appeared 10 days earlier. The patient was apyretic (she was taking paracetamol, dextropropoxyphene, and diclofenac), and there was no inflammation of the scar. Laboratory data revealed a discrete elevation of the white blood cell count (12×10^9/liter) and a moderate increase in C-reactive protein (40 mg/liter; normally <5 mg/liter). An arthrography of the left hip yielded no liquid, and there was no sign of unsealing. A small amount of liquid was injected into the joint and aspired for culture, which proved to be sterile. The patient continued to have pain, but there were no laboratory data suggesting an inflammatory syndrome. In January 2004, the persistence of the pain and the suspicion of an acetabular radiolucent line on a radiogram led to a second arthrography. It produced 5 ml of a purulent liquid containing 250 cells/mm^3 with 82% neutrophils. Gram staining of the pus showed no microorganisms, but cultures in blood culture bottles (Hemoline DIPH-F; bioMérieux SA, Marcy l'Etoile, France) incubated aerobically were positive within 7 days for a gram-positive bacillus, subsequently identified as *Mycobacterium wolinskyi*. A control puncture of the joint was carried out at the end of February 2004, and cultures in the same medium and on trypticase soy agar were again positive within 5 days for *M. wolinskyi*. This infection with *M. wolinskyi* prompted surgical intervention to remove acetabular components and cement. Cultures of intraoperative samples were positive, as before. Antibiotic treatment was initiated on the day of surgery, combining moxifloxacin (400 mg/day), minocycline (200 mg/day), and amikacin (750 mg/day). Three weeks later, the spacer was removed and a total hip arthroplasty was performed. Cultures of intraoperative samples remained negative. Amikacin was given for 1 month, and the combination of moxifloxacin and minocycline was maintained for a total duration of 6 months. Until November 2004, control cultures of the joint puncture liquid remained sterile. The evolution of the patient was favorable, with no pain, signs of inflammation, or radiographic abnormalities at the 1-year follow-up.

Bacteriology. The growth rate of this acid-fast bacillus on Trypticase soy agar within less than 7 days suggested a rapidly growing mycobacterium (RGM) (3). Additionally, the organism formed smooth-type colonies, grew well between 30°C and 42°C, and had no pigmentation. The nitrate reduction test was positive, and growth was inhibited in the presence of ethambutol (2 mg/liter). Other phenotypic characteristics of the isolate are presented in Table 1. A member of the *Mycobacterium smegmatis* group was suspected. The in vitro antibiotic susceptibility of the isolate was tested using standard disk diffusion and Etest. The results (Table 2) were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (6).

The isolate was definitively identified after 16S rRNA gene amplification of a ca. 1-kb fragment with primers UL2 (5′-GAGAGTTTGATCCTGGCTCAG-3′) and UL1 (5′-TGCACA

TABLE 1. Phenotypic characteristics of the *Mycobacterium wolinskyi* isolate

Test	Result[a]
Late pigmentation (>10 days) on 7H10 agar	−
3-day arylsulfatase	−
Nitrate reduction	+
Utilization of carbon sources	
Mannitol	+
Inositol	+
Growth on 5% NaCl	+
Tobramycin susceptibility (10 μg disk)	R

[a] −, negative; +, positive; R, resistant.

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TABLE 2. Antimicrobial susceptibility of the *Mycobacterium wolinskyi* isolate

Drug	MIC (μg/ml)	Interpretation[a]
Amikacin	2	S
Ofloxacin	0.7	S
Ciprofloxacin	0.25	S
Moxifloxacin	0.12	S
Minocycline	0.09	S
Linezolid	0.7	S
Imipenem	3	S
Cefoxitin	24	I
Tobramycin	24	R
Clarithromycin	16	R

[a] S, susceptible; I, intermediate susceptibility; R, resistant.

CAGGCCACAAGGGA-3′) and sequencing of a ca. 300-bp fragment with primers UL2 and UL3 (5′-CCCACTGCTGCC TCCCGTAG-3′), as previously described (4). Sequence analysis was performed using a GenBank BLAST search (National Center for Biotechnology, Bethesda, MD). Identity of 100% over the entire 300-nucleotide fragment was found with *M. wolinskyi* (database entry AY457083). This identification was confirmed by sequence analysis of the *hsp65* gene using primers HSP 65F (5′-CCAACGATGGTGTGTCCAT-3′) and HSP65R (5′-CTTGTCGAACCGCATACCC-3′).

Discussion. Among the RGM, *M. wolinskyi* belongs to the *M. smegmatis* group, which includes *M. smegmatis* sensu stricto and two new species described in 1999: *M. goodii* and *M. wolinskyi* (2). Biochemically, the *M. smegmatis* group can be distinguished from the *M. fortuitum* group and the *M. chelonae-M. abscessus* group with the 3-day arylsulfatase test. All members of the *M. fortuitum* group and the *M. chelonae-M. abscessus* group exhibit arylsulfatase activity after 3 days, as opposed to members of the *M. smegmatis* group (3). The *M. smegmatis* group is susceptible to ethambutol but not clarithromycin, a trait which could also help to distinguish it from other RGM.

M. wolinskyi does not produce any pigment (2, 3), which is the case for approximately 95% of isolates of *M. smegmatis* sensu stricto and 78% of the *M. goodii* isolates. *M. wolinskyi* is the only nonpigmented species of RGM that is susceptible to ethambutol. The three species of the *M. smegmatis* group can also be separated on the basis of tobramycin susceptibility. *M. smegmatis* sensu stricto is susceptible to tobramycin (MIC $\leq$ 1 μg/ml), while *M. goodii* is of intermediate susceptibility (2 to 8 μg/ml) and *M. wolinskyi* is resistant (>8 μg/ml) (3).

As is apparent from this case report, molecular identification, including sequence analysis of the16S rRNA, Hsp65 (3), RpoB, SodA, and RecA (1) genes, offers rapid and accurate identification of RGM, which is not always possible with traditional phenotypic and biochemical tests. With a combined analysis of several gene sequences, it has been recently shown that *M. wolinskyi* is phylogenetically clearly distinct from *M. smegmatis* and *M. goodii* (1). This approach should be useful to classify novel RGM species.

RGM are infrequent causes of bone infections (3). To our knowledge, eight infections due to *M. wolinskyi* have been reported in the literature (2, 3, 5), with clinical histories reported for seven of them. There were three cases of bone infection: one of osteomyelitis of the sternum following cardiac surgery, one of osteomyelitis of the foot, and one of osteomyelitis of the elbow following open fracture (2). The other cases included two of community-acquired cellulitis after local trauma, one of surgical wound infection, and one of an infected arteriovenous shunt in a patient on hemodialysis.

The case presented here is the first reported infection of a joint prosthesis due to *M. wolinskyi*. RGM are environmental bacteria and can contaminate water, but the portal of entry of the infection in our patient is unclear. No outbreak was noted during this period in our hospital. We cannot fully exclude the possibility that the bacterium was introduced during the first arthrography, but we do not consider this very likely, since the patient reported pain and since a moderate inflammatory syndrome with increased C-reactive protein and white blood cell count was present before the arthrography was performed.

Surgery combined with antibiotic treatment led to the cure of the hip infection reported here. Generally, cures of osteomyelitis due to RGM have been accomplished by surgical drainage and removal of the prosthesis combined with antibiotic therapy for a minimum of 6 months (3, 5), based on in vitro susceptibilities of the isolate. It appears from this case that new fluoroquinolones, such as moxifloxacin, are promising for the treatment of this type of infection.

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