

## Case Report

## Successfully treated spondylodiscitis due to a previously unreported mycobacterium

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Received 20 July 2005  
Accepted 31 August 2005

A non-tuberculous mycobacterium was isolated, following a vertebral needle aspiration, from the blood of a patient with severe spondylodiscitis. The strain turned out to be different from any known mycobacterial species and was quite drug-susceptible *in vitro*. The patient improved markedly following treatment with meropenem, clarithromycin and amikacin.

## Introduction

The rapidly growing non-tuberculous mycobacteria were considered for many years to be minor members of the genus *Mycobacterium*, with only a few species recognized, and those often incorrectly grouped together in the *Mycobacterium fortuitum*–*Mycobacterium chelonae* complex (Wolinsky, 1979; Wayne & Sramek, 1992). In recent years, however, a quite different scenario has emerged mainly thanks to the investigations of Wallace and co-workers (Brown-Elliott & Wallace, 2002). Not only has the number of rapidly growing species identified increased considerably, but the prevalence of clinically significant infections due to such organisms has also become clear. The present case joins the large number of mycobacterial pathologies involving joints and bone (Brown-Elliott & Wallace, 2002).

## Case report

A 61 year old male suffering from cirrhosis was hospitalized because of severe backache. Radiographic and tomographic pictures revealed clear signs of infection, with massive destruction of the 3rd and 4th dorsal vertebrae (Fig. 1). A needle biopsy yielded purulent material which was cultured for common bacteria without isolation of significant pathogens (rare colonies of coagulase-negative staphylococcus). Culture for mycobacteria was not done. The day after the intervention, because of a sudden rise in the patient's temperature, three blood cultures were made for aerobic and anaerobic bacteria. An empirical treatment with

meropenem (1 g i.v. q8h), undertaken at the same time, turned out to be effective, with rapid disappearance of the fever. The isolation of an unidentified mycobacterium from all three blood cultures for aerobic bacteria led to the addition to the treatment of clarithromycin (500 mg i.v. bid) and amikacin (1 g i.v. die), to which the strain was susceptible *in vitro*. The patient was discharged after 56 days with prescription of protracting the treatment for 4 months. At a 15-week check-up, the patient did not report any fever relapse, while the backache had improved substantially.

## Microbiological investigation

In the case described here, a previously unreported rapidly growing mycobacterium was responsible for spondylodiscitis in an elderly man. Although neither the biopsy specimen



Fig. 1. Tomographic scan revealing massive destruction of vertebral bodies.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene and 16S–23S ITS sequences of strain FI-05038 are DQ067465 and DQ185131.

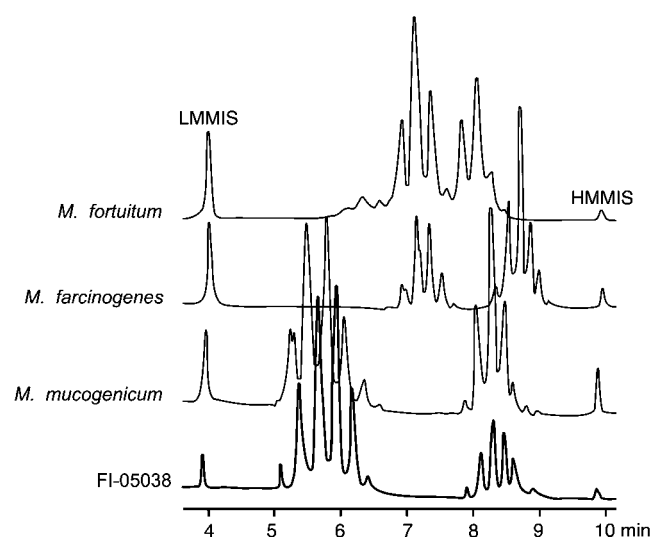
	88	171	440
FI-05038	GGTGCTC	CCGAATAGGACCGCATGCTTCATGGTGTG	TCAATAGGGACGAAGCGCAAGTGACGGTACCTGTAGAAGAAGGACC
<i>M. farcinogenes</i>	...A...	...G.....A.GC.....	.....A.....
<i>M. fortuitum</i> ATCC 49403	...A...	...G.....A.GC.....	.....A.....
<i>M. fortuitum</i> ATCC 49404	...A...	.....GCT.....G...	.....A.....
<i>M. fortuitum</i> CIP 104534	...A...	.....T...A.GC.....	.....A.....
<i>M. mucogenicum</i> ATCC 49649	...A...	.....A.GC.....	...CC.....G.....C...
<i>M. mucogenicum</i> ATCC 49650	...A...	.....A.GC.....	.....A.....C...
<i>M. mucogenicum</i> N519C <sup>a</sup>	...A...	.....A.GC.....	...CC.....T.....G.....C...
<i>M. mucogenicum</i> N241 <sup>a</sup>	...A...	.....A.GC.....	...CC.....N.....A.....C...
<i>M. mucogenicum</i> N252 <sup>a</sup>	...A...	.....A.GC.....	...CC.....A.....C...
<i>M. mucogenicum</i> N281 <sup>a</sup>	...A...	.....A.GC.....	...CN.....N.....G.....C...
<i>M. mucogenicum</i> N242 <sup>a</sup>	...A...	.....A.GC.....	.....TG.....A.....C...
<i>M. mucogenicum</i> 3.1 <sup>b</sup>	...A...	.....T...A.GCT.....G...	...CC.....G.....C...
<i>M. senegalense</i>	...A...	...G.....A.GC.....	.....A.....

**Fig. 2.** Alignment of regions of the 16S rRNA gene sequence able to differentiate strain FI-05038 from *M. farcinogenes*, *M. fortuitum* (three different sequevars), *M. mucogenicum* (all sequevars available in GenBank) and *M. senegalense*. Base pair positions are indicated according to the *Escherichia coli* sequence. *a*, Sequences taken from Hall *et al.* (2003); *b*, from unpublished GenBank accession number AY627003.

nor the blood of the patient were cultured for mycobacteria, the infection could be diagnosed thanks to the ability of the strain to grow in three blood-culture bottles specific for aerobic organisms (Bactec Plus Anaerobic/F; Becton Dickinson) taken at 30 min intervals when the patient was feverish. All bottles were scored positive by the automatic instrumentation Bactec 9240 (Becton Dickinson) within 2 to 4 days of incubation, and smooth, white colonies, which turned out to belong to a Gram-uncertain rod (strain FI-05038), grew on a blood agar subculture in 2 days at 37 °C. Because of the failure to obtain a confident identification with conventional phenotypic methods, the sequence of the first third of the 16S rRNA gene of the organism was determined (Cloud *et al.*, 2002). On the basis of this sequence (GenBank accession no. DQ067465), the strain was shown to belong to the genus *Mycobacterium* but differed from any known species. The most closely related taxa were *Mycobacterium farcinogenes*, *M. fortuitum*, *Mycobacterium mucogenicum*, *Mycobacterium porcinum* and *Mycobacterium senegalense*, all differing by 6 bp in a 466 bp region of the 16S rRNA gene sequence (Fig. 2). The partial sequence of the 16S–23S internal transcribed spacer (ITS) (GenBank accession no. DQ185131) was also different from that of any other organism, with the closest one (*M. mucogenicum*) differing by 34 bp out of 360 bp investigated.

A Ziehl–Neelsen smear confirmed that the organism was acid-fast. Phenotypic investigations revealed that the strain was non-photochromogenic and produced smooth colonies in 2 days at 25–37 °C; it was furthermore able to grow on MacConkey agar without crystal violet but was inhibited by cephalothin (Wallace *et al.*, 1993) and on Lowenstein–Jensen agar with 5% NaCl. Among biochemical tests, Tween 80 hydrolysis, 3-day arylsulfatase and urease were positive, while nitrate reduction was negative. Catalase activity was low (less than 45 mm foam). HPLC analysis revealed a pattern of cell-wall mycolic acids roughly compatible with that of *M. mucogenicum* but clearly different from those of the other species most closely related at the genetic level (Fig. 3).

Susceptibility testing performed using the agar elution method (Brown *et al.*, 1992) revealed effectiveness of amikacin ( $\leq 6 \mu\text{g ml}^{-1}$ ), cefoxitin ( $\leq 30 \mu\text{g ml}^{-1}$ ), ciprofloxacin ( $\leq 2 \mu\text{g ml}^{-1}$ ), clarithromycin ( $\leq 2 \mu\text{g ml}^{-1}$ ), doxycycline ( $\leq 6 \mu\text{g ml}^{-1}$ ), imipenem ( $\leq 8 \mu\text{g ml}^{-1}$ ) and tobramycin ( $\leq 8 \mu\text{g ml}^{-1}$ ), with the strain being resistant to trimethoprim/sulfamethoxazole ( $\geq 30 \mu\text{g ml}^{-1}$ ) only. Imipenem is usually the only carbapenem tested against rapidly growing mycobacteria; at clinicians' request, we also added meropenem and ertapenem, and both turned out to be active ( $\leq 8 \mu\text{g ml}^{-1}$ ).



**Fig. 3.** Representative HPLC pattern of cell-wall mycolic acids of strain FI-05038 in comparison with those of *M. mucogenicum*, *M. fortuitum* and *M. farcinogenes*. LMMIS, Low-molecular-mass internal standard; HMMIS, high-molecular-mass internal standard. The patterns of *M. porcinum* and *M. senegalense* have not been reported as overlapping substantially with that of *M. fortuitum*.

## Discussion

We think, in this case, that the presence of the strain in the blood was accidental, having been very likely released from the vertebral lesion as a consequence of the trauma of the needle aspiration. The present isolation, obtained from a sterile site, unquestionably fulfils the American Thoracic Society criteria for clinical significance (American Thoracic Society, 1997).

The growth of several mycobacteria in non-dedicated blood-culture bottles (Jacomio *et al.*, 1998) or on blood agar plates (Drancourt *et al.*, 2003) has already been reported. In this case, this less known feature, along with a number of lucky coincidences, allowed a severe bone infection to be diagnosed and cured. Once again, the too-often disregarded usefulness of extending the microbiological investigation of biopsy samples to mycobacteria has been confirmed.

Once we had excluded the possibility that the test strain belonged to the species *M. farcinogenes*, *M. fortuitum*, *M. porcinum* and *M. senegalense* on the basis of the phenotypic and genotypic differences, the possibility remains that it is a previously unreported variant of *M. mucogenicum*. The agreement of most phenotypic features (Springer *et al.*, 1995; Wallace *et al.*, 1993) supports such a hypothesis. On the other hand, 1.3 and 10.1 % mismatches in the investigated regions of the 16S rRNA gene and 16S–23S ITS, respectively, and the conflicting results of cephalothin tests (susceptibility to this antibiotic is very consistent in *M. mucogenicum*; Springer *et al.*, 1995) favour the suggestion that it belongs to an as-yet unrecognized species. We feel that our current knowledge is not sufficient to make such a decision; furthermore, according to our convictions (Tortoli, 2003), the description of a novel species should not be based on a single strain. At the same time, we are confident that this report will stimulate others to characterize similar strains, increasing our knowledge of this organism.

The case reported here supports a large literature base (Brown-Elliott & Wallace, 2002) that contradicts the widespread conviction that rapidly growing non-tuberculous mycobacteria almost always play the role of a contaminant in clinical specimens.

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