Abdominal Abscess Caused by Mycobacterium llatzerense

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Mycobacterium llatzerense was cultured from a subdiaphragmatic abscess. To our knowledge, this is the first report of isolation of this rapidly growing mycobacterium from a human. Growth characteristics and antimicrobial susceptibilities different from those previously reported for environmental isolates were observed.

CASE REPORT

A 57-year-old morbidly obese woman was admitted to the Hospital of the University of Pennsylvania for drainage of a left-sided subdiaphragmatic abscess that resulted after multiple gastric surgeries for morbid obesity and gastroesophageal reflux disease over the course of several years. The main findings at laparotomy included a leaking Roux-en-Y anastomosis, a large subdiaphragmatic abscess, and a splenic infarct. The leak was repaired and the abscess drained. Approximately 50 ml of pus from the abscess was obtained and sent for bacterial, mycobacterial, and fungal culture studies.

Gram staining of the abscess drainage showed few Gram-positive cocci, and a fluorochrome stain was negative for acid-fast bacilli. After 1 day of incubation, a few Candida albicans organisms were isolated from inhibitory mold agar (BD, Sparks, MD) at 27°C and moderate numbers of Enterococcus spp. from blood agar (BD) at 37°C. The patient was administered linezolid (600 mg every 12 h) for 14 days. Six days after the initial incubation, fewer than 10 colonies of a rapidly growing mycobacterium (isolate 9-9768) were detected on Middlebrook 7H11 nonselective plates (BD). The Bactec MGIT (BD) broth flagged positive after 10 days, and upon subculture, colonies were detected 5 days later. The 9-9768 mycobacterial colonies were dry and nonpigmented and grew at both 30°C and 37°C on Löwenstein-Jensen slants and Middlebrook 7H11 nonselective and selective plates.

Mycobacterium 9-9768 was identified as M. llatzerense by both hsp65 (1) and gyrB gene PCR and sequence analysis. The gene sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) under accession numbers KC737844 and KC737845, respectively. Because the published growth temperature phenotype of M. llatzerense did not match the growth phenotype of the clinical isolate, further molecular identification procedures were undertaken in Spain. A multilocus sequence analysis (MLSA) study that included 16S rRNA, internal transcribed spacer 1 (ITS1), gyrB, hsp65, recA, rpoB, and sodA genes was performed as described previously (2). The amplified products were purified with Multiscreen HTS PCR 96-well filter plates (Millipore). Sequence reactions were carried out using ABI Prism BigDye Terminator version 3.1, and the sequences were read with an automatic sequence analyzer (3130 genetic analyzer; Applied Biosystems). All sequences were deposited in the EMBL database under accession numbers HG810917 to HG810920.

The closest type strain in the phylogenetic analysis for all the genes tested was M. llatzerense. The sequence of the ITS1 region was not included in the MLSA because some Mycobacterium type strains contain more than one operon. For five of the six genes studied, sequence similarities with M. llatzerense MG13 were higher than 98.7% (99.3% for 16S rRNA, 98.7% for hsp65, 99.2% for recA, 99.7% for rpoB, and 100% for sodA genes); the gyrB gene showed 87.2% similarity. A concatenated sequence analysis showed overall similarity of 98.3%. Concatenated gene sequences were aligned and evolutionary distances calculated as previously described (3) (Fig. 1). Furthermore, total genomic DNA was isolated (3) and DNA-DNA hybridizations were performed in duplicate using a nonradioactive method as described by Ziemke et al. (4). Reference DNA of M. llatzerense strains MG14 and 9-9768 was doubly labeled with digoxigenin (DIG)-11-dUTP and biotin-16-dUTP using a nick translation kit (Boehringer, Mannheim, Germany). Labeled DNA was hybridized with DNA of strains MG13, MG14 (3), 9-9768, and M. bolletii CCUG 50184T. The DNA-DNA relatedness of strain 9-9768 to MG13 and MG14 was higher than 70%, confirming that 9-9768 is a M. llatzerense species.

Antibiotic susceptibility testing was performed using a Sensititre Rapidly Growing Mycobacteria plate (TREK Diagnostics, Columbus, OH) according to the manufacturer’s instructions (5). Interpretation of the antimicrobial agent MICs was performed according to Table 8 in Clinical and Laboratory Standards Institute (CLSI) document M24-A2 (6) except for the following: moxifloxacin breakpoints were from M100-S19 (Table 2G in the same document), and trimethoprim-sulfamethoxazole breakpoints were from M100-S19 (Table 2C) (7). The breakpoints for doxycycline were used for minocycline per Barbara Elliott at the University of Texas (personal communication, 2010).

The susceptibility profile of this isolate was similar to the profile of environmental isolates tested previously by Etest (3) in that it was susceptible to amikacin, ciprofloxacin, clarithromycin, imipenem, and linezolid (Table 1). It differed from those environmental isolates by being resistant to minocycline and intermediate to tobramycin (the previously reported isolates were susceptible to both).

The abdominal wound healed slowly, and the patient was...
transferred to a long-term acute-care hospital for wound care. She was eventually lost to follow-up.

In this report, we describe what we believe is the first case of isolation of *M. llatzerense* from a human. This rapidly growing mycobacterium was initially found in hemodialysis water in a hospital in Mallorca, Spain (3), and was then described in tap and shower water in two homes in the Netherlands (8), in the Parisian urban tap water production and distribution system (9), and in tap water from a skilled nursing facility in Pennsylvania (10). There has also been one case of invasive pneumonia attributed to *M. llatzerense*, identified by PCR from paraffin-embedded lung tissue, but in which no organism was recovered (11). The most likely pathogenesis of the patient’s intra-abdominal abscess is that swallowed tap water containing the mycobacterium entered the peritoneal space from the leaking Roux-en-Y anastomosis, along with gastric microorganisms.

Of note, the *M. llatzerense* 9-9768 isolate reported here showed growth characteristics different from those of isolates recovered from environmental sources. Our strain was able to grow at 30°C and 37°C whereas previously reported strains grew only at 22°C and 30°C (3). Limited data are available to help clinicians select antimicrobial therapy against *M. llatzerense*; this is the first human isolate for which susceptibilities have been obtained. This isolate was resistant to minocycline and intermediate to tobramycin, in contrast to the environmental isolates, which were susceptible to both. Loss of patient follow-up precludes us from determining the patient’s response to antibiotic therapy.

**Nucleotide sequence accession numbers.** Gene sequences de-
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