**Mycobacterium branderi** from Both a Hand Infection and a Case of Pulmonary Disease

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Received 3 April 2000/Returned for modification 16 May 2000/Accepted 22 July 2000

**Mycobacterium branderi**, a potential human pathogen first characterized in 1995, has been isolated from respiratory tract specimens. We report here a case in which *M. branderi* was the only organism isolated upon culture from a hand infection. This isolate, along with a second isolate from a bronchial specimen, was subjected to conventional identification tests for mycobacterial species. Further analysis by high-performance liquid chromatography (HPLC) of mycolic acids and 16S rRNA gene sequencing was performed, and the antibiotic susceptibility profile was determined for both strains. Biochemical tests and the HPLC pattern were consistent with that of *M. branderi* and *M. celatum*, which are very similar. The 16S rRNA gene sequence of both strains corresponded to that of *M. branderi* and enabled us to confidently differentiate this organism from other closely related species such as *M. celatum*. This contributes to a further understanding of the status of this species as a potential human pathogen as well as illustrating the need for molecular diagnostics as a complementary method for the identification of rare mycobacterial species.

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**CASE REPORT**

In February 1998, a 52-year-old female with a 4-year history of dermatomyositis presented to the emergency department of the St-Boniface General Hospital, Winnipeg, Canada, with pain and swelling of the right fifth digit and wrist. Early in the course of her disease, she had been treated with azathioprine (Imuran) and chloroquine (Plaquenil). These medications had been discontinued due to side effects. She worked as a bank teller until 1996, and was presently unemployed, living on a farm. Autoimmune tenosynovitis was diagnosed, and she was started on prednisone (20 mg a day). In July of the same year, she developed an ulcer on the fifth digit, which was swabbed and cultured. There was no history of trauma. Gram stain, fungal stain, viral cultures, fungal cultures, and aerobic and anaerobic bacterial cultures were all negative. An X-ray of the hand was normal. The white blood cell count was 11.8 × 10^9/liter, with 89% neutrophils. She was initially started on metronidazole and cefazolin, but there was poor clinical response to this therapy. A month later, she developed ulcerative subcutaneous nodules, and on examination, the patient had evidence of tenosynovitis of the right fifth digit and wrist. Chronic induration of the wrist, and subcutaneous white nodules along the volar aspect of the forearm. There was non-tender axillary adenopathy. Surgical debridement of the right palm and wrist was performed. Histopathology of this tissue revealed caseating granulomas. Cultures of the drainage were negative for bacterial and fungal culture. Acid-fast bacillus smears were positive, and she was started on isoniazid, rifampin, ethambutol, and pyrazinamide for presumptive *M. tuberculosis* infection. It subsequently grew a pure culture of acid-fast nontuberculous mycobacteria. The provincial Mycobacteriology Laboratory at the Health Sciences Centre, Winnipeg, Canada, identified the isolate as *M. branderi*. Following definitive identification, the antituberculous drugs were discontinued and the patient received empiric antimicrobial therapy with clarithromycin (1,000 mg twice a day [b.i.d.]) and trimethoprim-sulfamethoxazole (TMP-SMX) (one double-strength tablet [b.i.d.]). Two weeks later, the patient continued to have yellow, odorless discharge from the incision sites on the flexor aspect of the right palm and the right wrist. There were persistent erythema and induration surrounding the draining sinuses on the wrist. Ciprofloxacin (750 mg b.i.d.) was added to the treatment regimen, and the clarithromycin dose was decreased to 500 mg b.i.d. Six weeks later, there had been a dramatic improvement in her right hand and wrist, with resolution of the draining sinuses in the right palm and a significant decrease in the open area in the right wrist. The induration was mostly resolved, although she continued to have some erythema surrounding a 2.5-by-4-cm ulcer in the right wrist area, which was gradually resolving. She received a total of 19 months of antibiotics.

In January of 1999, an additional case of *M. branderi* was detected from a 74-year-old female with shortness of breath together with back and chest pain who was referred to a respiratory clinic at the Health Sciences Centre, Winnipeg. Chest X-ray revealed middle lobe collapse and peripheral pneumonic infiltrates. Clinical symptoms persisted after empiric ciprofloxacin treatment for 2 weeks. Three sputum samples were submitted to the same laboratory where the *M. branderi* isolate from the first case were identified. One of the three submitted sputum cultures grew *M. avium* complex. A bronchoalveolar lavage specimen was also submitted, which was negative for routine bacteriology but grew a pure culture of acid-fast nontuberculous mycobacteria, subsequently identified as *M. branderi*. One year later, the patient remained symptomatic and the underlying cause of disease remained unclear.

*M. branderi* is a newly described species of mycobacterium (12), and its role as a human pathogen is not well defined. The previously described isolates of *M. branderi* were respiratory...
tract isolates obtained from nine patients, some of whom had
cavitary mycobacteriosis of the lungs (12). Repeat samples
presented M. branderi as the only cultivable organism, suggest-
ing its potential pathogenic role in humans (12).
Clinical specimens consisting of hand drainage from the first
patient and bronchoalveolar lavage fluid from the second pa-
tient were submitted for mycobacteriology culture. Middle-
brook 12B liquid medium (Becton-Dickinson, Sparks, Md.)
grew acid-fast bacilli that were subcultured onto Middlebrook
7H10 agar. Each specimen grew a pure culture of
M. branderi. On Middlebrook 7H10 agar medium, each clinical isolate of
M. branderi showed two colony types: one white and the other
opaque. Both colony types were nonchromogenic, raised,
smooth edged, and domed. The Kinyoun acid fast stain dem-
strated pleomorphic, beaded, slightly curved acid-fast bacilli.
The species isolated was later confirmed to be
M. branderi (45°C) and was first described in 1992 by Brander et al. as
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Biochemical testing of the specimens was performed by con-
ventional methods as previously described (11, 13, 16) and
gave identical results for the two organisms isolated (Table 1).
Both showed growth from 25 to 42°C and were nonchromo-
genomic. Niacin, nitrate reductase, Tween 80 hydrolysis, urease,
tellurite reduction, and iron uptake tests were all negative; as
well, there was no acid production from mannitol, sorbitol, and
inositol. Both organisms could not utilize sodium citrate as
a sole source of carbon. Both organisms were positive for heat-
stable catalase, arylsulfatase activity, and pyrazinamidase.

Mycolic acid analysis by high-performance liquid chroma-
tography (HPLC) was performed according to the standard-
ized method (5). The HPLC analysis of mycolic acids of both
isolates produced a chromatographic pattern very similar to
the patterns produced by M. celatum (6), with the same reten-
tion times, but with thicker-looking peaks, different peak
height ratios, and less separation within the peaks of the second
cluster. Both species have HPLC chromatographic pat-
terns occurring as double clusters not unlike those of M. xenopi
and the M. avium complex. The patterns of both isolates also
showed additional small peaks at the front of the second clus-
ter.

Sequence-based species identification using the 16S rRNA
gene (14) was performed for both specimens. With the first
specimen, both colony types were sequenced to confirm culture
purity. Sequencing reactions were performed with the ABI
PRISM BigDye Terminator Cycle Sequencing Ready Reaction
kit (PE Biosystems, Foster City, Calif.) and run on an ABI
PRISM 310 Genetic Analyzer (PE Biosystems) according to
the manufacturer’s instructions. Resulting sequences were as-
sembled and analyzed using Lasergene software (DNASTAR,
Inc. Madison, Wis.), resulting in a 1,458-bp fragment of the
16S rRNA gene, equivalent to positions 28 to 1490 of the
Escherichia coli 16S rRNA gene. The sequences of both organ-
isms isolated, which were identical, showed highest percent
similarity (99.7%) to that of M. branderi ATCC 51789 (Gen-
Bank accession no. X82234).

BACTEC 12B radiometric broth macrodilution sensitivity
testing was performed on the clinical isolates according to the
method used for M. avium complex strains (8, 15). The follow-

Discussion. Nontuberculosis mycobacterium (NTM) species
are becoming increasingly important in the clinical setting,
causing nosocomial outbreaks or pseudo-outbreaks; pulmo-
nary disease; lymphadenitis; skin, soft tissue, or skeletal infec-
tions; and AIDS-related and -nonrelated disseminated infec-
tions, among others (1). Although it is generally believed that
the environment is the source of most NTM infections, their
pathogenesis remains irresolute and a continuous provision of
studies regarding NTM-related infections is required for fur-
ther knowledge and understanding. This particular study de-
scribes two cases implicating M. branderi.

M. branderi was first described in 1992 by Brander et al. as
part of the Helsinki group (2), consisting of 14 pure isolates
later confirmed to be M. branderi and M. celatum (12). On the
basis of biochemical and lipid characteristics and 16S ribo-
somal sequencing, the nine M. branderi organisms were as-
guarded a unique species. M. branderi is initially separated from
similar slow-growing species by biochemical test results includ-
ing growth at 45°C, negative Tween 80 hydrolysis, and positive
14-day arylsulfatase test (2). Based on 16S rRNA gene se-
quences, M. branderi is distinct from, but most closely related to,
M. celatum (12).

For the two patient isolates described in this report, the
conventional biochemical test panel for mycobacterial species
identification was not conclusive due to the generally inert
nature of this organism and its similar biochemical profile with
other species. M. branderi resembles M. celatum, M. xenopi,
M. avium complex, and M. malmonese in growth characteristics
(2). M. branderi and M. xenopi show no enzymatic difference
(2), but M. branderi is differentiated on the basis of its smooth
and dome-shaped colonies on 7H10 agar, increased growth at
25°C, lack of pigmentation, and differing HPLC patterns of
fatty acids and alcohol composition (12). M. branderi is differ-
entiated from most of the M. avium complex by a positive
aryl sulfatase test (2) and from M. malmonese and M. shimodei

TABLE 1. Growth characteristics and biochemical testing results of
the two clinical isolates in comparison with M. branderi (12) and the
closely related species M. celatum (4)*

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Clinical isolates</th>
<th>M. branderi</th>
<th>M. celatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth temp</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>31°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42°C</td>
<td>+</td>
<td>+ (45°C)</td>
<td>+ (45°C)</td>
</tr>
<tr>
<td>Pigment production</td>
<td>Light</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase, &gt;45 mm foam</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>68°C catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arylsulfatase activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tween 80 hydrolysis</td>
<td>-</td>
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<tr>
<td>Urease activity</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tellurite reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5% NaCl tolerance</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Pyrazinamidase</td>
<td>Trace to 2+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Iron uptake</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Sodium citrate utilization</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Acid production from:</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Sorbitol</td>
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<tr>
<td>Inositol</td>
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</table>

* +, positive; -, negative.

a A pale yellow pigment may be observed in older cultures.
by a negative Tween 80 hydrolysis test (12). Occasionally, *M. branderi* may be differentiated from older cultures of *M. celatum* by a lack of pigment, although in general *M. celatum* is nonchromogenic. We have found that Tellurite reduction was negative for both clinical strains of *M. branderi*, whereas strains of *M. celatum* (*n* = 24) are positive for this test (4). This may serve as a tool to differentiate these two species biochemically. Further analysis by HPLC of mycolic acids and 16S rRNA sequencing was required for species differentiation between *M. branderi* and *M. celatum*.

The mycolate pattern of *M. branderi* is of the same type as that of *M. avium* complex and *M. xenopi*, as all contain alpha-, keto-, and carboxymycolates (2). The HPLC pattern of both clinical isolates demonstrated a double cluster profile matching those of the limited number of strains of *M. branderi* analyzed to date and very closely resembling the pattern of *M. celatum*. In contrast with the *M. celatum* pattern, *M. branderi* appears to have better-developed early peaks in the first cluster, less-separated peaks in the second cluster, and different ratios for the peak heights with thicker peaks (M. M. Floyd, personal communication). The pattern obtained with *M. branderi* ATCC 51798T has the same characteristics as the patterns of the two clinical isolates, with the exception of the smaller peaks in front of the second cluster being less evident. Furthermore, these smaller peaks may occasionally be detected in strains of *M. celatum*. The difference between the chromatographic patterns of *M. celatum* and *M. branderi* can be subtle, and more strains must be examined before these particular variations to the *M. celatum* patterns can be attributed only to *M. branderi* (W. R. Butler, personal communication).

At present, 16S rRNA gene sequencing remains the sole definitive means to differentiate between them. The 16S rRNA gene sequence of the clinical isolates were most closely matched with that of *M. branderi* ATCC 51789T (EMBL or GenBank accession no. X82234), with a 99.7% similarity, which included three mismatches and one ambiguity (or n). *M. branderi* ATCC 51789T and ATCC 51788 were sequenced in our laboratory and were found to have a 100% similarity to each other and with both isolates. The region of the 16S rRNA gene containing the differences between the GenBank-submitted sequence and those determined in our laboratory are indicated in Fig. 1. Despite this discrepancy, a significantly lower percent similarity, 95.3 to 95.5, was seen with the various *M. celatum* clusters designated type 1 (accession no. L08169), type 2 (accession no. L08170) (4), and type 3 (accession no. Z46664) (3), demonstrating the ability of 16S rRNA gene sequencing to differentiate between the two species.

Sensitivity patterns observed for the Helsinki strains included resistance to isoniazid, rifampin, pyrazinamide, and cycloserine and susceptibility to streptomycin, ethionamide, ethambutol, and capreomycin (2) based on the methodology described by Canetti et al. in 1969 (7). It was also stated that susceptibility to ethambutol in combination with resistance to cycloserine is not commonly observed in other species of mycobacteria (12). Other than for members of the *M. tuberculosis* complex, no standardized methods are available for the susceptibility testing of mycobacterial species. Furthermore, the clinical efficiency and outcome of antimicrobial treatment of NTM infections in correlation with susceptibility results have yet to be studied extensively (9, 10). Interpretations of MICs determined for *M. avium* isolates have been suggested (8, 10, 15) and are the only basis available for a tentative interpretation of the susceptibility patterns of the *M. branderi* isolates in this study. The finger infection resolved following treatment with ciprofloxacin. However, the lung infection did not improve, suggesting the possibility of another disease or inadequate treatment.

Although *M. branderi* has previously been isolated from respiratory tract specimens, this is the first reported case of isolation from a wound infection. The isolation of *M. branderi* as a sole pathogen from a hand infection indicates that this organism may be more pathogenic than previously recognized.

![FIG. 1. Clarification of discrepancies and ambiguity detected with the only available nucleotide entry of the 16S rRNA gene sequence of *M. branderi* in the GenBank database. The top row shows the sequence of ATCC 51789 (accession no. X82234); the bottom row shows the sequence obtained for *M. branderi* ATCC 51789, ATCC 51788, isolate 1, and isolate 2 from our institution. *E. coli* positions within 16S rRNA gene are nucleotides 1016 to 1024 (A), nucleotides 1141 to 1149 (B), and nucleotides 1163 to 1171 (C). Dashes indicate identical nucleotides.](download.png)

![TABLE 2. Antimicrobial sensitivity results for the two clinical isolates of *M. branderi*](download.png)
Additional studies are required to further characterize *M. branderi* and understand the role of this species as a human pathogen.

REFERENCES