CASE REPORT

A 4-year-old girl developed fever to 38.2°C 3 months following autologous stem cell transplantation required after myeloablative chemotherapy to treat stage III neuroblastoma. Her physical examination did not reveal a source for her fever, including a normal-appearing Hickman catheter exit site. Counts were as follows (per liter unless other units are given): hemoglobin, 9.7 g/dl; white blood cells, 3.4 × 10⁹; neutrophils, 2.3 × 10⁹; lymphocytes, 0.5 × 10⁹; monocytes, 0.4 × 10⁹; eosinophils, 0.1 × 10⁹; platelets, 40 × 10⁹. Serum electrolytes, creatinine, hepatic enzymes, and albumin were within normal limits. A bone marrow examination demonstrated hypocellularity consistent with a myelodysplastic syndrome or delayed engraftment. Blood from the Hickman catheter and a peripheral site was collected in BACTEC aerobic pediatric resin bottles (Becton Dickinson, Sparks, Md.). Blood cultures were incubated in the BACTEC 9240 blood culture instrument with a 5-day incubation protocol. Within 48 h, the culture from the Hickman catheter was positive with a branching gram-positive beaded rod that was subsequently identified as an acid-fast bacillus by carbol fuchsin staining. The peripheral blood culture was positive at 96 h (4 days) with the same organism. A bone marrow examination demonstrated hypocellularity consistent with a myelodysplastic syndrome or delayed engraftment. Blood from the Hickman catheter and a peripheral site was collected in BACTEC aerobic pediatric resin bottles (Becton Dickinson, Sparks, Md.). Blood cultures were incubated in the BACTEC 9240 blood culture instrument with a 5-day incubation protocol. Within 48 h, the culture from the Hickman catheter was positive with a branching gram-positive beaded rod that was subsequently identified as an acid-fast bacillus by carbol fuchsin staining. The peripheral blood culture was positive at 96 h (4 days) with the same organism. Repeat cultures, obtained 2 days after the initial blood cultures, were also positive within 48 h from the catheter and 120 h (5 days) from the peripheral sample. The time to positivity of the peripheral and catheter blood cultures suggested a catheter infection. Treatment with azithromycin and rifampin was initiated although the patient remained well with no additional fever after day 2. The catheter was removed, and antibiotic treatment was continued for 5 days. The patient remained afebrile and in her usual state of health. Subsequent blood and bone marrow cultures were sterile.

Initial subcultures from the blood bottles to chocolate, Middlebrook 7H11, and Löwenstein-Jensen agars failed to reveal growth after 2 weeks of incubation at 37°C. Subcultures to these media incubated at 30°C demonstrated yellow-orange-pigmented colonies of acid-fast bacilli after 4 days. Once the organism was recovered on solid media, additional serial subcultures to Löwenstein-Jensen and 7H11 agars demonstrated almost equal amounts of growth at 30 and 37°C within 5 days. PCR amplification and restriction fragment length polymorphism (RFLP) analysis of the hsp65 gene were undertaken to identify the organism by the method of Telenti et al. (14). The resulting RFLP pattern did not match any pattern in our database or the published literature (Fig. 1). Sequence analysis of >1,400 bp of the 16S rRNA gene sequence, should prompt further investigation to establish this organism as a valid mycobacterial species.

The isolate demonstrated 100% sequence identity to “Mycobacterium lacticola” ATCC 9626, a nonestablished, rapidly growing mycobacterial (RGM) species, and 99.3% sequence identity to M. neoaurum ATCC 25795 (a difference of 6 bp and two insertions—Hamming distance of 8). Standardized cutoff values do not exist for defining a novel mycobacterial species by 16S rDNA sequencing. In several studies, cutoff values ranging from 0.8 to 1.5% sequence variation have been used to define potential new species (3, 7, 9). These values, however, are based on analysis of the 5’ 500 bp of the 16S rDNA containing the two most variable regions, rather than the entire gene. These cutoff divergence values would generally be lower if >1,400 bp were analyzed, as the rest of the 16S rDNA sequence is relatively more conserved. Furthermore, there are several examples in mycobacteriology in which proven distinct species differ by zero to four bases throughout the entire gene. This phenomenon is found among both RGM and slowly growing mycobacteria. Slow growers M. kansasii and M. gastri have unique 16S rRNA gene sequence, should prompt further investigation to establish this organism as a valid mycobacterial species.
identical 16S rDNA sequences, and \textit{M. ulcerans} and \textit{M. marinum} differ by two bases in the 3' end of the 16S rDNA sequence. In the latter case, as in several RGM that are closely related by 16S rDNA sequencing, an analysis of the entire 16S rDNA sequence is necessary to detect significant differences between species. For example, over the entire 16S rDNA sequence, the recently described RGM species \textit{M. lacticola} and \textit{M. neoaurum} have 99.3% 16S rDNA sequence identity. Therefore, it is not unreasonable to pursue further characterization of \textit{“M. lacticola”} to determine whether this is a new mycobacterial species.

Once the identity of our isolate was known, additional \textit{hsp65} RFLP and sequence analyses were performed and the biochemical profile of the organism was also determined. \textit{“M. lacticola”} ATCC 9626 and \textit{M. neoaurum} demonstrated almost identical \textit{hsp65} RFLP patterns. There was a slight difference in the size of the lower-molecular-weight band of the HaeIII digest (Fig. 1). The BstEII RFLP pattern of the patient isolate was identical to that of \textit{M. neoaurum} and \textit{“M. lacticola”}; however, the HaeIII pattern was distinctly different (Fig. 1). Our isolate was missing a 172-bp band and exhibited two bands of 94 and 87 bp, indicating the presence of an additional restriction site(s) within the larger HaeIII fragment. Analysis of the \textit{hsp65} DNA sequence was undertaken to confirm the locations of the HaeIII sites in the patient isolate and to compare this sequence to those of \textit{M. neoaurum} and \textit{“M. lacticola”} ATCC 9626. A 441-bp fragment of \textit{hsp65} was amplified with primers TB11 and TB12 (14). Forward and reverse sequencing of the PCR product was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, Calif.) and primers TB11 and TB12. The samples were run on an ABI Prism 310 Genetic Analyzer, and the resulting sequences were compared for identity (Fig. 2).

Substitution of a C for a G at position 306 in the patient isolate resulted in the creation of an additional HaeIII restriction site (CGCC → GGCC), confirming the band sizes seen on RFLP analysis. The expected RFLP band sizes are shown in the Table 1. The slight difference in the lower-molecular-weight HaeIII band, seen visually in the HaeIII digest of \textit{“M. lacticola”} ATCC 9626 and \textit{M. neoaurum}, was confirmed by sequence analysis (Table 1). This small difference is apparent when the two isolates are run side by side on the gel.

The patient isolate had 99.2% \textit{hsp65} sequence identity (3-bp difference) with \textit{“M. lacticola”} ATCC 9626 and 98.7% sequence identity with \textit{M. neoaurum} (5-bp difference). The sequence identity between \textit{“M. lacticola”} ATCC 9626 and \textit{M. neoaurum} was 99.5% (2-bp difference). The lack of complete identity between \textit{“M. lacticola”} ATCC 9626 and our isolate is not entirely unexpected since \textit{hsp65} is more variable than the 16S rDNA (10). Intraspecific variation in the \textit{hsp65} sequence has been reported for other RGM species such as \textit{M. abscessus}, \textit{M. fortuitum}, and \textit{M. chelonae} (11). In some instances, the

![Image](http://jcm.asm.org/ Downloaded from)

**FIG. 1.** \textit{hsp65} RFLP patterns of mycobacterial isolates. \textit{hsp65} PCR products were digested with the restriction enzymes BstEII (lanes 1 to 3) and HaeIII (lanes 5 to 7). Digests were run on a 9% polyacrylamide gel and stained with ethidium bromide. Lanes: 1 and 5, \textit{M. neoaurum}; 2 and 6, \textit{“M. lacticola”} ATCC 9626; 3 and 7, patient isolate; 4, OX174 HinfI molecular weight marker.

**FIG. 2.** Sequence alignment of a 441-bp fragment of \textit{hsp65} from \textit{M. neoaurum}, \textit{“M. lacticola”} ATCC 9626, and the patient isolate of \textit{“M. lacticola.”} Sequence variations are noted with the symbol ■, and the HaeIII sites are shaded. Nucleotide 1 represents position 396 of the \textit{M. tuberculosis} \textit{hsp65} sequence (13).

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<thead>
<tr>
<th>Organism</th>
<th>BstEII</th>
<th>HaeIII</th>
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<tbody>
<tr>
<td>Patient isolate</td>
<td>310, 116</td>
<td>145, 94, 87</td>
</tr>
<tr>
<td>\textit{“M. lacticola”}</td>
<td>310, 116</td>
<td>172, 145</td>
</tr>
<tr>
<td>\textit{M. neoaurum}</td>
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new species, as is the case with the M. fortuitum third bivariant complex (10, 11). It is difficult to determine the percentage of variation that would be expected for a given species unless a number of strains of each species are analyzed. As with 16S rDNA sequencing, there are no standardized cutoff values for determining species identity with hsp65 sequencing although this is being actively pursued (A. McNabb, D. Eisler, K. Adie, M. Amos, M. Rodrigues, and J. Isaac-Renton, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C220, 2003). What can be gleaned from the present analysis is that the hsp65 sequence of the clinical isolate is more closely related to that of “M. lacticola” ATCC 9626 than to that of M. neoaurum. The significance of the sequence differences between the clinical isolate and “M. lacticola” ATCC 9626 remains to be determined.

No biochemical data are available, to our knowledge, on isolates identified as “M. lacticola.” We determined a limited biochemical profile of the clinical isolate and compared it with that of M. neoaurum as reported in the literature (8, 14–16, 18).

The colonial morphology of the clinical isolate revealed a smooth, pigmented, acid-fast organism that grew within 2 days at temperatures of 25, 30, and 37°C, but not at 42°C. Biochemical test results were positive for tolerance to 5% NaCl, iron uptake, heat-stable (68°C) catalase, and urease. Results were negative for sodium citrate as the sole carbon source, growth on MacConkey agar without crystal violet, 14-day arylsulfatase, semiquantitative catalase (<45 mm), nitrate, and niacin. Acid was produced from mannitol, inositol, and fructose but not from sorbitol. The biochemicals selected for testing were those that appeared to give fairly consistent results with M. neoaurum. However, as is typical with biochemical testing, variability has been reported with some tests, such as growth at 42°C, Tween 80 hydrolysis, and 14-day arylsulfatase (1, 4, 8, 18). Our results were in general concordance with the M. neoaurum profile based on the limited number of tests compared. However, our isolate differed from isolates of M. neoaurum (as identified by 16S rDNA sequencing) by a negative result in the nitrate test (1, 18).

The clinical presentation of “M. lacticola” infection, as reported here, is typical for the reported cases of M. neoaurum infection (1, 4–6, 8, 18). All have involved infection of indwelling catheters, primarily in immunosuppressed patients. It is reassuring that unlike infections caused by other RGM such as M. fortuitum and M. chelonae (5), these infections have not required debridement of infected tissues surrounding the catheter site. The optimal antibiotic regimen and specific recommendations regarding catheter removal have not yet been determined. In most cases, the indwelling device was removed.

In only two of the reported cases of M. neoaurum infection was the organism identified definitively by 16S rDNA sequencing (1, 18). Therefore, it is possible that some of the reported isolates were actually “M. lacticola.” Interestingly, an outside laboratory sent us two strains of M. neoaurum (identified biochemically) that were isolated from blood and/or catheter tips. One of the isolates demonstrated the RFLP pattern of “M. lacticola” ATCC 9626.

Although pigmented RGM are uncommon blood isolates, they should be considered as possible pathogens when isolated in the setting of indwelling medical devices. It is our hope that the report of this case will prompt further characterization of “M. lacticola” as a potential new mycobacterial species capable of causing clinically significant infections.

**Nucleotide sequence accession numbers.** The hsp65 sequences of “M. lacticola” ATCC 9626 and the patient isolate of “M. lacticola” have been deposited in the GenBank sequence database under accession numbers AY341032 and AY341033, respectively.

**REFERENCES**


