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Optimal Detection and Identification of *Mycobacterium haemophilum* in Specimens from Pediatric Patients with Cervical Lymphadenopathy

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Acid-fast bacilli from pediatric patients with lymphadenopathy were detected in the BACTEC radiometric system and in MB Redox broth, but not on Löwenstein Jensen medium. PCR amplification identified the isolates as *Mycobacterium haemophilum*, which has special nutrition requirements (iron supplements) for growth. Suitable culture medium ensures optimal recovery of this microorganism, avoiding underdiagnosis.

Mycobacterium haemophilum was first described in 1978 as the cause of cutaneous ulcerating lesions in a 51-year-old Israeli woman with Hodgkin's disease (20). Since then, fewer than 70 confirmed cases of infection with this organism have been reported. However, recent studies indicate that it is rapidly emerging as a serious pathogen, particularly in immunocompromised patients (9, 18, 21). *M. haemophilum* has been associated with lesions occurring secondarily to immunosuppressive therapy after transplantation and with AIDS (4, 10–12, 16, 18, 24, 25). It has also been isolated from localized lesions in pediatric patients with cervical lymphadenopathy who otherwise had no underlying immunocompromising factors (1, 5, 18, 22).

The clinical presentation of *M. haemophilum* infection includes painful cutaneous lesions, multiple skin nodules, respiratory symptoms, pneumonitis, and tuberculosis-like granulomas in the lungs. Bacteremia, septic arthritis, and osteomyelitis have also been reported (9, 14, 18, 21). The disease is rare in otherwise healthy patients, in whom it can usually be successfully treated with appropriate antibiotics. However, in patients with impaired cellular immunity, the disease is chronic, disseminated, and sometimes fatal (9, 16, 21).

M. haemophilum is a strongly acid- and alcohol-fast bacillus (20) which grows optimally at 30 to 32°C; it requires an iron supplement (or ferric iron-containing compounds) such as hemin or ferric ammonium citrate for growth (3, 6, 17). The true incidence of infection with *M. haemophilum* may be seriously underestimated because of its distinctive growth requirements (8, 21, 23).

We describe the isolation of *M. haemophilum* from specimens obtained from nine children with cervical lymphadenopathy.

Patients and specimens. Biopsy specimens of nine submandibular lymph nodes and one preauricular lymph node from nine pediatric patients (six males and three females) were examined for bacterial cultures, including culture for mycobacteria.

Media and bacterial isolates. Direct Gram and Ziehl-Neelsen stains were applied. For the mycobacterial cultures, the specimens were inoculated onto BACTEC 460 12B radiometric broth (Becton Dickinson) and Löwenstein-Jensen (L-J) (Heipha Diagnostika Biotest, Heidelberg, Germany) media and incubated at 37°C in all cases. In five of nine cases, specimens were also directly inoculated into MR Redox broth (Heipha Diagnostika, Biotest), a new colorimetric medium for mycobacteria. In addition, subcultures of the initial growth from the clinical samples in BACTEC radiometric broth were performed concomitantly to the same medium, L-J medium, MB Redox broth, and blood agar and then incubated at 30 and 37°C.

Microorganism identification. The following biochemical tests were carried out: niacin, nitrate reductase, semiquantitative catalase, thermostable catalase, Tween-80 hydrolysis, arylsulfatase, acetamide, benzamide, urea, nicotinamide, succinamide, allantoin, and pyrazinamide.

Gen-Probe AccuProbe tests. AccuProbe tests (Gen-Probe, San Diego, Calif.) for *M. tuberculosis*, *M. kansasii*, and *M. avium* were performed according to the manufacturer's instructions.

PCR. Nucleic acids were isolated from mycobacteria growing in MB Redox medium according to a previously published protocol (15). Fragments of the 16S rRNA gene (rDNA) were amplified by PCR using sets of broad-range eubacterial primers combined with mycobacterial genus-specific primers (19). Further characterization to species level was performed by direct DNA cycle sequencing of the 16S rDNA amplicons (19). Sequencing reactions were carried out in triplicate to rule out any polymerase-induced errors.

Results. The principal characteristics of the nine patients are summarized in Table 1. Direct Gram and acid-fast smears were negative in all cases. Growth of acid-fast bacilli was detected in BACTEC radiometric broth within an average of 14 days after incubation. In the five specimens that were also inoculated directly into MB Redox broth, growth of acid-fast bacilli was detected after the same incubation period. No growth was observed on L-J medium even after 10 weeks.

Subcultures from the radiometric broth to the same media, MB Redox broth and L-J medium, yielded growth in both cases after 2 days of incubation at 30°C and after 3 days of incubation at 37°C. Further subcultures from each liquid medium to blood agar showed growth of acid-fast bacilli after 3 days of incubation at 30°C and after 5 days at 37°C. Cultures for all other bacteria were negative.

The only positive biochemical test was cleavage of pyrazinamide. AccuProbe tests for *M. tuberculosis*, *M. kansasii*, and *M. avium* were negative.

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TABLE 1. Principal characteristics of the nine study patients[a]

Patient no.	Age (yr)/sex	Clinical data	Response to PPD[b] (5 IU)	Pathology	Outcome
1	9/F	6 weeks[c]; right cervical lymphadenitis; lesion size, 2 by 2 cm	13	Granulomatous lymphadenitis	Cured after 6 mo
2	1/M	4 weeks; right preauricular lymphadenitis; lesion size, 1 by 1 cm	6	Fine-needle aspiration showed granulomatous lymphadenitis	Cured after 5 mo
3	3/M	3 weeks; left submandibular lymphadenitis; lesion size, 3 by 3 cm	20	Fine-needle aspiration showed granulomatous lymphadenitis	Cured after 5 mo
4	2/M	4 weeks; left submandibular lymphadenitis; lesion size, 3 by 3 cm	Negative	Fine-needle aspiration showed granulomatous lymphadenitis	Cured after 6 mo
5	2/M	1 week; left submandibular lymphadenitis; lesion size, 3 by 4 cm	30	Suppurative lymphadenitis	Cured after 6 mo
6	4/F	1 week; right submandibular lymphadenitis; lesion size, 5 by 3 cm	14	Suppurative lymphadenitis	Cured after 5 mo
7	6/F	8 weeks; left submandibular lymphadenitis; lesion size, 3 by 3 cm	25	Granulomatous lymphadenitis	Cured after 3 mo
8	4/M	6 weeks; left submandibular lymphadenitis; lesion size, 4.5 by 1.5 cm	15	Granulomatous lymphadenitis	Cured after 6 mo
9	10/M	6 weeks; right submandibular lymphadenitis; lesion size, 5 by 5 cm	Negative	Granulomatous lymphadenitis	Cured after 4 mo

[a] All patients were treated with cephalosporins or β-lactam antibiotics before hospitalization, without response.
[b] PPD, purified protein derivative. The diameter of the induration (in millimeters) is listed.
[c] Length of hospitalization.

On PCR amplification, the sequence found was identical for all four tested samples. Comparison of the nucleic acids with entries in the GenBank database yielded a 99.8% agreement with the sequence of *M. haemophilum* (accession number U06638) (Fig. 1) and 98.5% agreement with the sequence of *M. haemophilum* (accession number L24800).

Discussion. *M. haemophilum* has been described as a human pathogen in less than 70 confirmed cases within the last 20 years. According to the published studies, affected patients can be divided into two broad categories. The main risk group consists of patients who are severely immunocompromised and in whom *M. haemophilum* occurs as an opportunistic infection. Indeed, the earliest reports documented infection in persons with either lymphoma or renal transplants (2, 13, 20). Today, patients with AIDS are the largest reported group with this infection (7, 9, 21), and bone marrow transplant recipients have been added to the list of individuals at risk (24). Indeed, any condition resulting in marked suppression of cell-mediated immunity is likely to predispose patients to *M. haemophilum* infection. As reported in previous papers, material from superficial lesions, including lymph nodes and joint fluid, as well as deep-tissue and bone infections, should also be considered.

The second risk group category consists of immunocompetent children in whom *M. haemophilum* infection induces cervical and perihilar lymphadenitis, clinically similar to that induced by infection with *M. avium* complex, *M. tuberculosis*, and *M. scrofulaceum* (21). *M. haemophilum* adenitis has been reported in children in Australia, Canada, and the United States (1, 5, 18, 22). More recently, these nine sporadic cases detected at our Center, within a period of 1 year, support the assumption that *M. haemophilum* infection may occur more frequently than reported in the medical literature. The true incidence of *M. haemophilum* may be underestimated either because it is not reported or because some laboratories have not changed their routine procedures for some specimens to include a broth with an iron supplement to be incubated at 30°C.

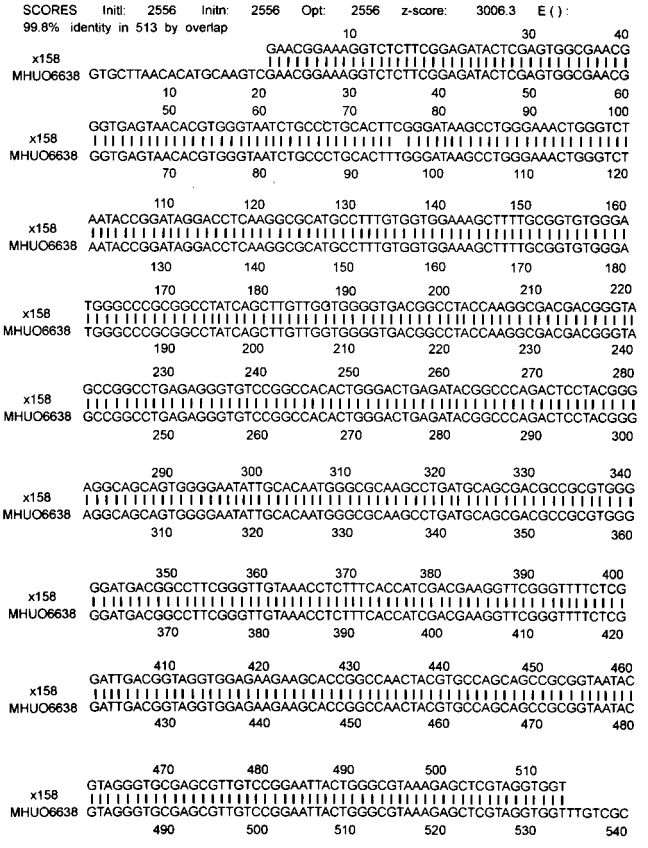

FIG. 1. Comparison of the sequence of the 16S rDNAs of the isolates investigated (×158) with the sequence of *M. haemophilum* (strain MHUO6638). Agreement between the nucleotides is shown by the vertical lines.

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