Pulmonary Disease Due to *Mycobacterium malmoense*

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A case of pulmonary disease due to *Mycobacterium malmoense* was recently diagnosed in a 43-year-old man from Virginia. This organism was isolated from sputum and bronchial washings. This is the first case of documented human infection due to this organism in the United States.

*Mycobacterium malmoense* was reported as a new pathogenic, non-photochromogenic mycobacterial species by Schröder and Juhlin in 1977 (8). Seven strains derived from gastric lavage, bronchial secretions, sputum, and tissue of four patients in Sweden presented unique characteristics in their lipid pattern and serotype that distinguished the species from other non-photochromogenic mycobacteria. Jenkins and Tsukamura (6) identified 11 patients in England and Wales from whom this species had been isolated. They noted that these strains showed a distinctive lipid pattern that was identical to “provisional species 2” reported by Birn et al. in 1967 (3).

Cases have been reported from England, Wales, and Sweden. No cases of disease due to *M. malmoense* have been documented in the United States. In this paper, we document the isolation and discuss details of treatment and biochemical identification of *M. malmoense* from a person with pulmonary disease in the United States.

**CASE REPORT**

A 43-year-old male from Damascus, Va., consulted his personal physician in June 1981 because of chronic cough and wheezing. A review of his past history revealed no travel outside southwest Virginia and North Carolina (Great Smoky Mountain National Park) for 20 years. Previously, he had worked for 6 months near Cleveland, Ohio, as a woodcutter and 3 months near Oxford, Pa., growing mushrooms. He was otherwise a lifelong resident of Virginia. Employment had included woodcutting, carpentry (constructing houses), drill press operation (briefly), trapping in winter (fox, opossum, etc.), and taxidermy. He had no military service. He had smoked one-and-a-half packs of cigarettes daily for 25 years. During the year before hospital admission, he was treated for chronic bronchitis and asthma. Chest roentgenograms at the time of hospital admission showed a thick-walled, 5-cm cavity in the right upper lobe with small nodular densities in the right midlung. A previous chest film dated 26 September 1975 showed normal results. The patient weighed 159 pounds (ca. 72.3 kg) upon admission.

Cavitory carcinoma was suspected. Scalene lymph node biopsy was negative. Bronchoscopy revealed no abnormalities, but bronchial secretions and subsequent sputum smears contained numerous acid-fast bacilli. A tentative diagnosis of tuberculosis was made, a Mantoux test was applied, and treatment was initiated with isoniazid, 300 mg daily; rifampin, 600 mg daily; and ethambutol, 1,200 mg daily. The patient, his wife, and his children all had nonreactive tuberculin skin tests, so it was suspected that his disease might prove to be a nontuberculous mycobacterial disease.

The patient took his medication faithfully, showed symptomatic improvement, and returned to his work as a carpenter and painter. Between 18 June 1981 and 30 August 1981, six sputum cultures were positive, and the isolates were identified as *M. malmoense* susceptible to rifampin, ethambutol, streptomycin, capreomycin, kanamycin, and ethionamide (for further bacteriological details, see below). Subsequent sputum tests on 9 December 1981 showed a few acid-fast bacilli on smear, and the culture was negative. On 11 February 1982, a smear was negative, and the culture was contaminated. Serial roentgenograms through March 1982 showed a decrease in the size of the cavity with thinning of its wall. The patient continued to smoke while on therapy.

In June 1982, he developed chest pain in the right side, fever, increased cough, and weight loss of 5 lb (ca. 2.3 kg). A chest roentgenogram revealed a new pneumonic infiltrate extending downward from the cavitory area in the right midlung. Bacterial pneumonia was suspected, but a sputum smear showed only leukocytes with no predominant bacteria. The patient was treated with amoxicillin and showed some improvement. Two of five specimens showed acid-fast bacilli (one to nine bacilli per 100 fields), but no growth was obtained by culture. Streptomycin (1 g intramuscularly 5 days a week) was added to the therapeutic regimen, and the isoniazid, rifampin, and ethambutol were continued. The patient also stopped smoking at this time. His cough cleared, his state of well-being improved, and he gained 13 lb (ca. 5.9 kg). The serial roentgenograms showed gradual clearing of the heavy midlung infiltrate between June and October 1982, and it had completely resolved by February 1983. The cavitory lesion continued to show thinning of the wall until the appearance was consistent with a bulla with overlying pleural thickening. Sputum specimens in December 1982 and January 1983 were negative by smear and culture for mycobacteria. The streptomycin was reduced to 1 g intramuscularly three times a week in August 1982, was further reduced to two times a week in December 1982, and was discontinued in February 1983. Oral drugs were discontinued in August 1983. The patient continued to feel well and worked regularly as a carpenter.

**MATERIALS AND METHODS**

Sputum and bronchial washing specimens were processed at the Department of General Services Southwest Regional Laboratory, Abingdon, Va., by the N-acetyl-L-cysteine–sodium hydroxide procedure (10). Smears prepared from the sediment and stained by the Ziehl-Neelsen method...
(10) revealed more than one acid-fast bacillus per oil immersion field.

Sediments of the specimens were inoculated onto Lowenstein-Jensen slants and were incubated at 37°C under normal atmospheric conditions without increased CO₂. After 18 days, 3+ growth of non-photochromogenic acid-fast bacilli was noted. (After the patient was placed on antituberculosis therapy, recovery of the organism from processed sputum took as long as 41 days). Because initial biochemical testing indicated that the mycobacterium was not Mycobacterium tuberculosis (i.e., it was negative for niacin accumulation and negative for nitrate reductase), the cultures were referred to the Department of General Services/Division of Consolidated Laboratory Services and Centers for Disease Control for complete identification.

Microscopic examination of the referred culture revealed short, occasionally beaded, acid-fast bacilli which were not arranged in serpentine cords. On Lowenstein-Jensen medium, colonies were grayish white, dysgonic, smooth, and thin. When subcultured to Middlebrook and Cohn 7H-10 agar, the colonies were thin and transparent with an irregular edge but became more dome shaped and opaque with age.

Subcultures were slow growing (an average of 17 days) and remained colorless after exposure to light. Laboratory tests by previously described methods (10) indicated that the mycobacterium gave negative results for niacin accumulation, nitrate reductase, urease, and 3-day and 2-week arylsulfatase tests. Variable results were obtained for the tellurite reduction and pyrazinamidase tests. No growth was observed on Lowenstein-Jensen medium containing 5% sodium chloride. Although the mycobacterium showed catalase activity (<45 mm of foam) at 37°C, no catalase activity was observed after it was heated to 68°C. The organism was able to hydrolyze Tween 80 with a markedly positive color reaction within 2 days and was able to grow in the presence of 10 μg of thiopehne 2-carboxylic acid hydrazide per ml and 10 μg of thiacetazone per ml. Lipid analysis performed on these two isolates by P. A. Jenkins, University Hospital of Wales, confirmed that the cultures were M. malmoense.

Antimicrobial susceptibility tests were performed by the Centers for Disease Control. Both isolates were susceptible to 2 and 10 μg of streptomycin per ml, 5 μg of ethambutol per ml, 1 μg of rifampin per ml, 5 μg of ethionamide per ml, and 10 μg of capreomycin per ml and were resistant to 1 and 5 μg of isoniazid per ml, 2 μg of para-aminosalicylic acid per ml, and 50 μg of pyrazinamide per ml. One isolate was susceptible to 30 μg of cycloserine per ml and resistant to 5 μg of kanamycin per ml; the other isolate tested had the reverse pattern.

RESULTS AND DISCUSSION

The details of the case described here fulfill the criteria of the American Thoracic Society (1, 13) for nontuberculous mycobacterial disease. Specifically, the chest roentgenogram of the patient showed evidence of disease, and sputum specimens submitted for culture repeatedly grew multiple colonies of the same Mycobacterium strain in the absence of another pathogen. To our knowledge, this report is the first documented case of disease due to M. malmoense in the United States. Although the organism has been reported previously, no details were given, and therefore the criteria needed to establish nontuberculous mycobacterial disease were not met (5). M. malmoense was first reported in four patients from Malmö and Lund, Sweden, in the taxonomic study of Schröder and Juhlin (8). Subsequently, Jenkins and Tsukamura reported nine patients from England and Wales (6).

In the reports mentioned above, lipid analysis and seroagglutination were used in addition to cultural and biochemical criteria to identify these mycobacteria. Because these former methods are not routinely available in most hospital or reference laboratories, the identification of M. malmoense can represent a particular problem to clinical laboratories. With the procedures for identification of mycobacteria proposed in a number of different references (1, 7, 10), a culture of M. malmoense would be noted initially as slow growing and nonpigmented (grayish white). The first biochemical test to be done would include determinations for niacin accumulation, nitrate reductase, and catalase after heating at 68°C (heat-stable catalase). All these tests should be negative for M. malmoense. At this point in testing, all laboratories performing biochemical tests would be able to classify the organism as a nontuberculous mycobacterium. Laboratories that perform complete identifications of mycobacteria would place the organism in the non-photochromogen group (1, 7, 10).

Laboratory identification of M. malmoense may be difficult, and the organism has been confused with several other slowly growing, nonpigmented Mycobacterium species (2, 4). It can be differentiated from MAIS-intermediate group organisms by its lack of pigment, low semiquantitative catalase activity, and marked ability to hydrolyze Tween 80. M. malmoense may be differentiated from the Mycobacterium avium complex by its hydrolysis of Tween 80. Separation of M. malmoense and the Mycobacterium terrae complex may be accomplished by using the semiquantitative catalase and nitrate reductase tests. M. malmoense does not reduce nitrate and it produces <45 mm of foam in the semiquantitative catalase test, whereas the M. terrae complex produces >45 mm of foam and may be positive for nitrate reductase (1, 9). Growth in the presence of thiacetazone and a negative 2-week arylsulfatase reaction distinguish M. malmoense from Mycobacterium gastri (11, 12).

The appropriate chemotherapy for mycobacterial diseases due to M. malmoense is unknown. However, based on experience with tuberculosis and other mycobacterial diseases, it seems reasonable to advise multiple drug therapy based on in vitro susceptibility data. Because so few cases have been documented and because patients customarily receive more than one antituberculosis drug for various periods of time, it has not been possible to determine the efficacy of any particular drug regimen. Previous reports of drug susceptibility patterns indicate that M. malmoense should be susceptible to ethionamide, ethambutol, kanamycin, and cycloserine and resistant to other antitymbacterial drugs (8). This susceptibility to several antituberculous drugs may aid in separating M. malmoense from other non-photochromatogens because the latter are highly drug resistant. The isolates from our patient also were susceptible to ethionamide and ethambutol as well as rifampin, streptomycin, and capreomycin. In our study, variable results were obtained for susceptibility to kanamycin and cycloserine. The discrepancies between drug susceptibility test results of this report and those of Schröder and Juhlin (8) point out the need to continue documenting drug patterns of nontuberculous mycobacteria to determine whether it will be possible to predict appropriate therapy. At the present time, this patient appears to have responded to therapy and is still being followed.
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LITERATURE CITED