

## *Mycobacterium malmoense* Bacteremia in Two AIDS Patients

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**We report two cases of *Mycobacterium malmoense* bacteremia in two patients with AIDS. These are the first reported cases of disseminated *M. malmoense* in human immunodeficiency virus patients occurring in the United States. This slow-growing organism can cause invasive disease mimicking *Mycobacterium avium* complex infection; recognition and identification of this organism by mycobacteriology laboratories are essential for appropriate diagnosis and therapy of disseminated disease.**

Disseminated nontuberculous mycobacterial infections appear in patients with advanced human immunodeficiency virus (HIV) disease and are associated with increased mortality and morbidity. We report *Mycobacterium malmoense* bacteremia in two patients with AIDS; this organism is uncommonly isolated in mycobacteriology laboratories and is rarely associated with invasive disease in immunocompetent hosts (13).

**Case reports.** Patient 1 is a 26-year-old man who was admitted to the hospital in December 1993 with low-grade fever, productive cough, night sweats, and progressive weight loss. The physical examination revealed an emaciated young adult in no acute respiratory distress. Oral thrush was noted; lungs were clear to auscultation, and the extremities did not show any clubbing or cyanosis. Chest X ray showed a right upper lobe infiltrate and cavitation, and the patient was started on intravenous penicillin G. Sputum for acid fast bacillus (AFB) concentrated smear and culture (three samples) were ordered. A repeat chest X ray 10 days after admission showed partial resolution of the infiltrate and a decrease in size of the cavitation. A scheduled bronchoscopy was canceled because of the clinical and radiological improvement of the patient's condition. No AFB organisms were seen on the concentrated smears; however, one of the three samples grew two colonies after 7 weeks of incubation in BACTEC 12B bottles. Samples were also subcultured on Lowenstein-Jensen and 7H10 media. Both colonies were reported as suggestive of *Mycobacterium avium* complex (MAC) on the basis of colony morphology and microscopy. Gen-probe for MAC was negative. However, at that time, the manufacturer stated in the product insert that the probe could not detect all MAC strains, and the isolate was identified as *M. avium* on the basis of the differential result for Tween 80, which was initially found to be negative. Antimycobacterial therapy was not initiated because of absence of evidence of invasive disease. The patient was discharged and given oral penicillin for the treatment of lung abscess, which was to be monitored in chest clinic. The patient underwent HIV testing in early April 1994 and was found to be seropositive. He was then readmitted to the hospital with dry cough, shortness of breath, and fever. Chest X-ray findings were remarkable for resolving upper lobe cavitation. He was treated with intravenous trimethoprim-sulfamethoxazole for a presumed *Pneumocystis carinii* pneumonia and showed improvement clinically and radiographically. Three sputum samples

did not grow any AFB organisms after 8 weeks of incubation. Stool for AFB culture grew five colonies after 7 weeks of incubation; they were reported as suggestive of MAC on the basis of macroscopic and microscopic morphology. No biochemical or Gen-probe tests for MAC were done on this isolate. The patient was seen in immunodeficiency clinic in August 1994 for fever and weight loss. The CD4 count was 20 cells per  $\mu$ l. Chest X ray showed complete resolution of the right lung cavity and infiltrate. Blood was sent for AFB culture, and the patient was started empirically on rifabutin (300 mg orally [p.o.] once a day [q.d.]), azithromycin (500 mg p.o. q.d.), and ciprofloxacin (500 mg p.o. twice a day) for a suspected disseminated MAC infection. AFB organisms were isolated after 8 days of incubation in BACTEC 13A (Becton Dickinson, Cockeysville, Md.) blood culture bottles. A slow-growing nonchromogenic mycobacterium was recovered; Gen-probe results for MAC were negative. The catalase reaction (at 68°C; pH 7.0) was weakly positive; the nitrate reduction and urease tests were negative; the tellurite and aryl-sulfatase tests were positive. Based on a positive Tween 80 hydrolysis reaction, the preliminary identification was *M. malmoense*. At this time, the isolate previously recovered from sputum was reexamined and was found to be weakly Tween hydrolysis positive, and an amended report was subsequently issued. The identification of the organism was confirmed by the New York state health laboratory. The patient's symptoms resolved on antimicrobial therapy; however, a repeat AFB blood culture taken 4 months later grew *M. malmoense* after 22 days of incubation in BACTEC 13A bottles.

Patient 2 is a 31-year-old male with AIDS (CD4 count, 4 cells per  $\mu$ l) who was admitted in April 1995 with fever, weight loss, fatigue, headache, and increased forgetfulness. His medical history was pertinent for *P. carinii* pneumonia in 1993, cryptococcal meningitis in 1994, and a recent admission for thrombocytopenia and leukopenia. His medications included rifabutin (300 mg p.o. q.d.), fluconazole (200 mg p.o. q.d.), zidovudine (200 mg p.o. three times daily), and aerosolized pentamidine (300 mg monthly). On physical examination, the patient was obtunded; his lungs were clear to auscultation; the abdomen was soft with no hepatosplenomegaly. Neurologic examination was significant for marked weakness of the right lower extremity. A computerized tomography scan of the head showed a ring enhancing lesion in the left basal ganglia with slight mass effect and focal enhancement in right thalamic and left parietal areas. He was started on sulfadiazine (1.5 g p.o. every 6 h) and pyrimethamine (50 mg p.o. q.d.) for empirical

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TABLE 1. In vitro susceptibilities of *M. malmoense* to anti-MAC drug panel

Patient no.	Susceptibility <sup>a</sup> to compound (μg/ml)					
	Amikacin (8.0)	Ciprofloxacin (8.0)	Ethambutol (8.0)	Clarithromycin (4)	Clofazimine (2.0)	Rifabutin (0.5)
1	S	S	S	S	S	R
2	S	S	S	S	S	R

<sup>a</sup> S, susceptible; R, resistant.

treatment of central nervous system toxoplasmosis. However, his fever persisted and he did not show any neurological improvement. The patient had a generalized tonic clonic seizure 4 days after admission. A repeat computerized tomography scan of the head did not show any progression of the central nervous system lesions. Because of the patient's persistently elevated temperature, blood samples for AFB cultures were collected. The patient developed coffee-ground emesis and spiked a fever of 41°C on day 12 of admission. He rapidly became severely hypoxic and hypotensive. He expired the same day, and postmortem examination was refused by the family. The blood cultures grew AFB organisms after 24 days of incubation in BACTEC 13A bottles. The catalase reaction was weakly positive; the nitrate reduction and urease tests were negative; the tellurite and Tween 80 hydrolysis tests were positive. Gen-probe for MAC was negative. The organism was identified as *M. malmoense*.

The in vitro susceptibilities of the two blood isolates to antituberculous and anti-MAC drugs are shown in Tables 1 and 2. Susceptibility studies were done by the BACTEC method (8).

**Discussion.** *M. malmoense* has been isolated from the respiratory tracts of patients with pulmonary disease, especially those with cavitory lesions. It is rarely associated with disseminated disease; those patients reported to have systemic manifestations had an underlying immunodeficiency (13). Few cases of *M. malmoense* colonization of the respiratory tract have been reported for HIV patients (2, 11). In addition, invasive pulmonary infection has been described (3, 12, 13). To our knowledge, these are the first two reported cases of documented *M. malmoense* bacteremia or disseminated disease in HIV patients from the United States. The organism has been isolated from patients with advanced HIV disease; most of them, including our patients, had a CD4 count of ≤100 cells per μl. Our patients were severely immunocompromised secondary to advanced HIV disease, which made them susceptible to infection by this organism. Our patients' symptoms mimicked those of disseminated MAC infection. Fever and weight loss were present for both patients. Moreover, dissemination was verified by the recovery of the organism from blood. The route of entry was probably the respiratory tract for patient 1, because of underlying pulmonary cavitory disease. Unfortunately, no sputum or stool for AFB cultures was collected from patient 2. The possible source of entry for this patient might have been either the respiratory or the gastrointestinal tract.

*M. malmoense* is commonly isolated in Northern Europe, especially in England and Sweden. On the other hand, very few cases of colonization or infection have been reported from the United States (10). Our patients lived in Buffalo, N.Y., and did not have any recent travel history. Our laboratory did not initially identify the sputum isolate as *M. malmoense*; this is probably related to the lower sensitivity of earlier MAC probes and the unfamiliarity with the organism due to its infrequent

isolation in U.S. laboratories. Biochemical tests are essential in the identification of this organism, and reliance on colony morphology and pigmentation is not sufficient. Limiting the diagnostic tests to decrease the cost of identification of AFB organisms may result in misidentification of *M. malmoense*. Full identification of AFB organisms is necessary even for cultures recovered from nonsterile sites.

The conventional methods developed to recover *Mycobacterium tuberculosis* are adequate for growth and identification of most of the nontuberculous mycobacteria; however, fastidious organisms, including *M. malmoense*, may need special media to help recover the organism. The standard Lowenstein-Jensen medium may not provide optimal conditions for *M. malmoense* growth. Ispahani and Baker reported the importance of prolonged incubation of 8 to 12 weeks for *M. malmoense* recovery (5). In addition, Katila et al. demonstrated the use of pyruvate containing Lowenstein-Jensen-egg medium with reduced pH for improved detection of *M. malmoense* (7). Multiple biochemical tests along with chromogenicity and colony morphology are used to interpret culture results. Nontuberculous nonchromogenic mycobacteria that may be confused with *M. malmoense* include MAC *Mycobacterium shimoidei*, *Mycobacterium gastri*, and *Mycobacterium terrae* complex. A key biochemical test to distinguish *M. malmoense* from MAC is the Tween 80 hydrolysis reaction; MAC does not hydrolyze Tween. In addition, *M. malmoense* exhibits slower growth than MAC on Lowenstein-Jensen medium. *M. shimoidei*, *M. gastri*, and *M. terrae* complex hydrolyze Tween; however, they test acid phosphatase positive in contrast to *M. malmoense* (9, 10). The semiquantitative test for catalase may help in differentiating *M. malmoense* from *M. terrae* complex; *M. malmoense* produces a column of bubbles <45 mm high. Although biochemical analysis is reliable for identification of *M. malmoense*, glycolipid analysis by thin-layer chromatography and cellular fatty-acid analysis by gas chromatography are more specific (6, 9). However, the last two methods are not accessible for most mycobacteriology laboratories.

There is no established antimicrobial regimen for treatment of disseminated *M. malmoense*. In addition, in vitro susceptibility testing does not necessarily correlate with the in vivo activity of the antimicrobial agent or the clinical outcome (1). Like most of the other nontuberculous mycobacteria, *M. malmoense* strains are resistant in vitro to isoniazid, rifampin, and ethambutol (4). It is interesting that both strains were resistant to rifabutin in vitro, a drug widely used for MAC prophylaxis. We treated patient 1 with azithromycin, rifabutin, and ciprofloxacin, a regimen usually used in treating disseminated MAC infection. Patient 1 exhibited symptoms of chronic infection; his fever recurred while he was on antimicrobial therapy, and the organism was recovered again from blood.

This is the first report of *M. malmoense* bacteremia in AIDS patients in the United States. The standard mycobacterial culture techniques currently followed in the majority of laborato-

TABLE 2. In vitro susceptibilities of *M. malmoense* to antituberculous drug panel

Patient no.	Susceptibility <sup>a</sup> to compound (μg/ml)					
	Isoniazid (0.2)	Rifampin (1.0)	Ethambutol (5.0)	Pyrazinamide (100)	Para-aminosalicylate (2.0)	Streptomycin (2.0)
1	R	R	R	R	R	S
2	R	R	R	R	S	S

<sup>a</sup> S, susceptible; R, resistant.

ries are capable of recovering the organism; however, laboratories need to be cognizant of the difficulty in the culture and identification of slow-growing mycobacteria, especially *M. malmoense*. Media and growth parameters have to be adjusted to recover some of the difficult-to-grow nontuberculous mycobacteria. Finally, microbiologists should consider *M. malmoense* when Gen-probe-MAC-negative organisms are isolated from AIDS patients. A positive Tween hydrolysis reaction helps differentiate Gen-probe-MAC-negative MAC organisms from *M. malmoense*. Misidentification of *M. malmoense* as MAC may account for underreporting of the former organism in the AIDS population in the United States.

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