

## Isolation of *Mycobacterium celatum* from Patients Infected with Human Immunodeficiency Virus

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*Mycobacterium celatum* is a recently described, slowly growing mycobacterium of still undefined clinical relevance. A retrospective study of seven patients was conducted to further elucidate the clinical presentation and prognosis of infection due to *M. celatum* in patients with AIDS. Three patients had an exclusively pulmonary infection and 3 had disseminated infection (including 2 patients with pulmonary and extrapulmonary involvement), and 1 patient had an exclusively extrapulmonary disease. Fever, weight loss, and productive cough lasting for >2 weeks were the most common symptoms. Chest radiographs showed diffuse or focal interstitial infiltrates without cavitation. The recovery of *M. celatum* from one patient was definitively determined to be clinically irrelevant. Our findings indicate that *M. celatum* may cause serious disease in patients with advanced human immunodeficiency virus-related immunosuppression. *M. celatum* infection appears to be responsive to antimycobacterial chemotherapy; however, further studies are needed to establish the optimal drug combination for this indication.

The incidence of disease due to nontuberculous mycobacteria is increasing, especially in patients with HIV-related immunosuppression. During the past 5 years, infection in patients with AIDS by nontuberculous mycobacteria other than *Mycobacterium avium* complex (MAC) has been widely reported [1–3], and the development of 16S rRNA gene fragment sequencing [4] has enabled researchers to explore in depth the genus *Mycobacterium*, discovering a considerable number of yet-undescribed species. However, experience with most of these “new” mycobacteria is limited, and the epidemiological and clinical relevance of these infections in HIV-seropositive patients is still undefined.

*Mycobacterium celatum* was described in 1993 [5]; it appears to be biochemically indistinguishable from MAC but shows a mycolic acid pattern closely related to that of *Mycobacterium xenopi*. The organism could be separated into two types (1 and 2) by restriction fragment length polymorphism; recently, a new type (type 3) has been recovered from patients with AIDS in England [6]. It is interesting that *M. celatum* type 1 cross-reacts with the AccuProbe (Gen-Probe, San Diego) for the *Mycobacterium tuberculosis* complex [7]. *M. celatum* was shown to be clinically relevant in immunocompromised patients [8] as well as in an immunocompetent infant [9]. In this article, seven cases in which *M. celatum* was recovered from different specimens are reviewed, with emphasis on clinical significance, microbiological features, and outcomes.

### Patients and Methods

Sixteen isolates were recovered over a 2-year period from seven patients with AIDS who were hospitalized in the infectious diseases divisions of large city hospitals in Florence, Milan, Bergamo, and Vicenza. Blood, sputum, bronchoalveolar lavage (BAL), and stool specimens were examined by smear and culture for bacteria, fungi, protozoa, and acid-fast organisms. In particular, three sputum samples and one BAL specimen were collected from each patient and submitted to the laboratory for the detection of pulmonary pathogens.

The microbiology workup of the BAL and sputum material was accomplished in the following manner: both were prepared with routine gram Grocott-Gomori methenamine–silver nitrate, modified Kinyoun, and auramine stains and were plated on chocolate agar, charcoal extract, and Sabouraud dextrose agar. Isolation of mycobacteria was achieved on different media (solid, liquid, and biphasic) with use of standard procedures [10]. Culture specimens of peripheral blood were processed by the lysis-centrifugation system (Isolator-10 System, Wampole Laboratories, Cranbury, NJ) prior to inoculation into suitable media.

Biochemical and cultural tests used for conventional identification were performed according to standard methods [10]. The strains were tested with AccuProbe identification kits specific for MAC and *M. tuberculosis* complex. Assays were run on isolate colonies grown on a solid medium, and the unbound probe hydrolysis incubation time was set at 5 minutes instead of the 10 minutes suggested by the manufacturer. The amount of chemiluminescence emitted was estimated with a Leader 50 luminometer (Gen-Probe) and quantified as relative light units (RLU).

This procedure was adopted on the principle that cross-reaction between *M. celatum* type 1 and the AccuProbe for the

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*M. tuberculosis* complex may be used as a tool for the presumptive identification of *M. celatum* type 1. Setting the selection time at 10 minutes is particularly advisable when the AccuProbe identification process is performed on a mycobacterial culture grown in a liquid medium. In this situation, the characteristics of mycobacterial colonies cannot be observed, and false-positive reactions may lead to misidentification. However, if the AccuProbe assay is carried out on a mycobacterial culture grown on a solid medium, the selection time should be kept at 5 minutes.

In this case, the appearance of mycobacterial colonies (nontuberculous mycobacteria have smooth, sharply edged colonies, while *M. tuberculosis* complex has rough, corded colonies) may help to evaluate AccuProbe results. Thus, a weakly positive reaction in testing of nontuberculous mycobacteria with the *M. tuberculosis* complex AccuProbe strongly suggests that the identification is *M. celatum*.

Mycolic-acid analysis with HPLC and 16s rRNA gene fragment sequencing were carried out as previously described [11–13]. Drug susceptibility patterns were determined by the radiometric system with use of a recently proposed macrodilution method developed for MAC [14]. During therapy and the follow-up period, blood and sputum cultures were performed at 4- to 8-week intervals. Mycobacterial infection was considered cleared if at least two consecutive cultures were found to be negative.

Clinical response was evaluated by assessing the symptoms and signs suggestive of *M. celatum* infection before and during therapy. Clinical, microbiological, and radiological data as well as information on response to treatment and follow-up were obtained from patients' records. All the material was carefully reviewed to evaluate the clinical significance of the findings according to the criteria proposed by the American Thoracic Society [15].

## Results

**Demography.** Of the seven patients enrolled, four had *M. celatum* recovered from respiratory specimens. Three of these patients met the criteria for possible *M. celatum* pulmonary disease. Three other patients had disseminated *M. celatum* infection; of these, two had pulmonary and extrapulmonary involvement. One patient was affected with an exclusively extrapulmonary disease.

All patients had advanced HIV-related immunosuppression, as evidenced by CD4 lymphocyte counts of  $<100/\text{mm}^3$  (mean,  $19/\text{mm}^3$ ; range,  $1\text{--}66/\text{mm}^3$ ). At the time of isolation of *M. celatum*, AIDS had already been diagnosed in all patients, but disseminated *M. celatum* infection was not the initial AIDS-defining illness in any patient.

There were six men and one woman, from 25 to 37 years of age (mean, 31 years). Risk factors for HIV infection included intravenous drug use ( $n = 5$ ), homosexuality ( $n = 1$ ), and heterosexual contact with HIV-infected persons ( $n = 1$ ).

**Clinical features.** Fever (temperature of  $>38.5^\circ\text{C}$ ), weight loss, productive cough, and dyspnea were common symptoms.

The most important ones were fever, noted in all seven patients, and cough and/or dyspnea, observed in all patients whose BAL fluid and/or sputum specimens were positive. The chest radiographic features are summarized in table 1. Two patients had diffuse reticulonodular interstitial infiltrates, and four had focal infiltrates. One of these patients had simultaneous infection with *Pneumocystis carinii*, which was detected in a BAL fluid specimen. Although laboratory diagnosis of nontuberculous mycobacterial infection was achieved in about 2 weeks, *M. celatum* strains were correctly identified (at a reference center) only after considerable time ( $\sim 6$  months) if the initial identification, made exclusively on the basis of biochemical test results, was erroneous.

**Microbiological results.** *M. celatum* was isolated from different sites: 1 isolate was from stool, 2 were from BAL fluid, 3 were from blood, and the remainder were from sputum. Acid-fast smears were positive for three of the six patients from whom respiratory *M. celatum* isolates were recovered. Cultures of respiratory specimens yielded multiple colonies in five of six cases. Four patients' specimens yielded multiple isolates, but *M. celatum* was simultaneously recovered from at least one site other than blood in only two cases. The patient whose stool yielded *M. celatum* did not complain of diarrhea or other gut symptoms. Additional microorganisms were recovered from two of the seven patients at the time of the first isolation of *M. celatum* and included *P. carinii* and *M. xenopi*; both were considered to be clinically significant (table 1).

The strains were isolated on various media, including radiometric liquid media (Bactec 12B and 13A; Becton Dickinson, Towson, MD), conventional solid media (Löwenstein-Jensen and Middlebrook 7H10 and 7H11), and biphasic medium (Septi-Check AFB, Becton Dickinson). An extended panel of biochemical and cultural tests was used for conventional identification. On the basis of these test results, the most likely identification, according to a computer program for identification of mycobacteria [16], appeared to be MAC.

Unlike that originally described [5], our strains produced a weak yellow pigment in the dark, and one of them was able to grow at  $45^\circ\text{C}$ . We also observed that 3-day arylsulfatase activity, determined in the broth of Kubica and Rigdon [17], was positive for only 14.3% of our strains (one of seven). The Bactec NAP test (Becton Dickinson) showed no inhibition by NAP ( $p$ -nitro- $\alpha$ -acetylamino- $\beta$ -hydroxy-propiofenone). The hybridization test performed with the AccuProbe specific for MAC gave negative results.

On the other hand, weak false-positive reaction levels of 30,508–86,219 relative light units (RLU) (mean RLU value, 50,947) were observed with an AccuProbe specific for *M. tuberculosis* complex (cutoff value, 30,000 RLU). A reference strain (*M. tuberculosis* ATCC [American Type Culture Collection] 25177), run in the genetic probe assay as a positive control, gave a value of 580,321 RLU,  $\sim 20$  times the cutoff value.

All the strains showed the same HPLC profile, resembling that of *M. xenopi*. However, a closer comparison of the eluate

**Table 1.** Clinical features of patients with suspected *M. celatum* infection.

Patient no.	Symptoms	Source of isolates of <i>M. celatum</i> (n)	Radiographic findings	Co-isolates (type of specimen)	Chemotherapy	Course of <i>M. celatum</i> infection	Outcome (survival in weeks)
1	Fever, weight loss, anemia, cough	Sputum (2), BAL fluid (1), blood (1)	Interstitial infiltrates, left upper lobe	None	Stm, Ofx, Clof	Clinical and radiographic improvement	Died of wasting syndrome (44)
2	Fever, weight loss, cough, dyspnea	Sputum (2), blood (1)	Diffuse interstitial infiltrates	None	Azm, Eth, Rif	Clinical and radiographic improvement; relapse	Alive (52 as of Jan 96)
3	Fever, weight loss, cough, dyspnea	Sputum (2), BAL fluid (1)	Diffuse interstitial infiltrates	None	Clm, Clof, Rib	Clinical and radiographic improvement	Alive (28 as of Jan 96)
4	Fever, weight loss, hemiplegia	Blood (1)	Normal	None	None	Not applicable (postmortem diagnosis)	Died of cerebral toxoplasmosis (3)
5	Fever, dyspnea	Sputum (1)	Bilateral upper-lobe interstitial infiltrates	<i>P. carinii</i> (BAL fluid)	TMP-SMZ	Clinical and radiographic improvement	Alive (85 as of Jan 96)
6	Fever, weight loss, cough	Sputum (1)	Bilateral lower-lobe interstitial infiltrates	<i>M. xenopi</i> (blood)	Amik, Cpfx, Eth, Rib	Clinical and radiographic improvement	Died of DCMV (40)
7	Fever, cough	Sputum (2), stool (1)	Hilar interstitial infiltrates	None	Amik, Clm, Eth, Rib	Clinical and radiographic improvement	Alive (28 as of Jan 96)

NOTE. Amik = amikacin; Azm = azithromycin; BAL = bronchoalveolar lavage; Clm = clarithromycin; Clof = clofazimine; Cpfx = ciprofloxacin; DCMV = disseminated cytomegalovirus infection; Eth = ethambutol; Ofx = ofloxacin; Rib = rifabutin; Rif = rifampin; Stm = streptomycin; TMP-SMZ = trimethoprim-sulfamethoxazole.

profiles did show minor differences in the second cluster. As previously described [8], such differences represent an important feature for the HPLC-mediated identification of *M. celatum*. Four strains were sent to Prof. E. Böttger at the Institut für Medizinische Mikrobiologie in Hannover, Germany, and were studied by 16s rRNA gene fragment sequencing. This procedure definitively identified all our strains as *M. celatum* type 1.

The MICs ( $\mu\text{g/mL}$ ) able to inhibit the growth of 50% and 90% of *M. celatum* strains are summarized in table 2. The close similarity of growth kinetics between *M. celatum* and MAC easily allowed us to perform the susceptibility testing procedure validated for MAC, which has been adopted for routine use in our laboratories. The time required for the final

reading was 7–8 days for all the strains. Our susceptibility data differ substantially from those reported by Butler et al. [5], who found *M. celatum* to be resistant to many antimycobacterial agents. Since such discrepancies are too striking to be explained exclusively on the basis of the relationship between methods of testing and results, we hypothesize that different groups of *M. celatum* strains may represent separate clones.

**Antimicrobial therapy and outcome.** The clinical outcome of patients with *M. celatum* infection is summarized in table 1. Only 5 patients received antimycobacterial chemotherapy: 3 patients with pulmonary infection and 2 with disseminated infection. Patients were treated with different regimens, all of which included three or four drugs. Clinical improvement, as defined by resolution of fever, respiratory symptoms, and radiographic infiltrates, occurred within 4 weeks following initiation of therapy in all treated patients.

Follow-up sputum and blood cultures were performed between 2 and 9 months after initiation of therapy; cultures for four patients remained negative. Clinical, radiological, and microbiological evidence of relapse was documented in one patient after 9 months of therapy. For this patient, treatment was changed to administration of clarithromycin, ciprofloxacin, and pyrazinamide, and the patient is still alive. Of the remaining 4 patients who received antimycobacterial therapy, 2 died of unrelated complications within 10–11 months of diagnosis and 2 are still alive. Necropsies were not done.

One patient with *M. celatum* infection did not receive any antimycobacterial therapy; the patient died of cerebral toxoplasmosis, and *M. celatum* grew in a blood culture after death. One patient, whose sputum yielded few *M. celatum* colonies but who had concurrent *P. carinii* pneumonia, was treated with a full course of trimethoprim-sulfamethoxazole. In this case, clinical and radiological resolution of pulmonary involvement

**Table 2.** Activity of 12 antimicrobial agents against seven *M. celatum* strains.

Drug	MIC value ( $\mu\text{g/mL}$ )		
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
Amikacin	1	2	0.25–2
Azithromycin	2	8	1–8
Ciprofloxacin	0.5	2	0.25–2
Clarithromycin	0.25	1	0.12–1
Clofazimine	0.12	0.25	0.06–0.25
Ethambutol	1	2	0.5–2
Isoniazid	0.5	0.5	0.25–0.5
Ofloxacin	0.5	2	0.12–2
Rifabutin	0.5	1	0.2–1
Rifampin	128	>128	...
Sparfloxacin	0.25	0.5	0.12–0.5
Streptomycin	0.25	1	0.25–1

was observed following therapy, and cultures for mycobacteria remained negative during the whole follow-up period (85 weeks), showing the initial isolation of *M. celatum* to be clinically irrelevant.

## Discussion

Our findings show that *M. celatum* is a cause of potentially treatable disease in patients with advanced HIV infection. *M. celatum* was isolated from blood and/or respiratory specimens from seven patients with HIV infection and low CD4 lymphocyte counts. *M. celatum* was considered to be clinically relevant in all patients except one. Clinical, radiographic, and microbiological evidence of mycobacterial disease did not persist following antimycobacterial therapy.

In patients with AIDS, *M. celatum* infection seems to have clinical features sometimes resembling MAC or *M. tuberculosis* infections. In fact, three patients had *M. celatum* isolated from respiratory specimens only, and three had disseminated disease mimicking MAC infection. Our findings suggest that *M. celatum* caused pulmonary disease and not merely colonization in our patients. Indeed, most of them met the American Thoracic Society criteria for the diagnosis of nontuberculous mycobacterial disease.

The patients' radiographs showed evidence of pulmonary disease that could not be attributed to other causes and that was associated with the repeated isolation of multiple colonies of the same mycobacterial strain. Cultures for all patients treated for pulmonary disease yielded multiple colonies of *M. celatum*, and two of three patients had at least two positive cultures.

Finally, treatment with antimycobacterial therapy resulted in clinical and radiographic improvement in all treated patients for a considerably long period. Although the treatment regimens were different, they were all effective. Ethambutol, rifabutin, clarithromycin, clofazimine, and amikacin were mainly used. Our in vitro susceptibility data seem to correlate with patients' clinical improvement, as the MICs of many drugs were below the concentrations achievable in serum during therapy.

It is noteworthy that evidence of relapse was documented for one patient, as well as in another case previously described [8, 18], whose treatment regimens included rifampin; actually, one of the most striking features of *M. celatum* is that the organism appears to be highly resistant to rifampin. From a microbiological standpoint, the newly described species *M. celatum* should be suspected when a mycobacterium, behaving in conventional tests as MAC or *M. xenopi*, fails hybridization with probes specific for MAC.

Additional test findings for presumptive identification include strong resistance to rifampin, a "bird's nest" microscopic appearance on smears from broth cultures, optimal

growth temperature of 37°C instead of 45°C, and a weakly positive reaction with probes specific for *M. tuberculosis* complex. At present, only genetic sequencing and mycolic-acid analysis with HPLC can confirm the identification. Finally, our results suggest that treatment with a combination of three or four drugs should result in a considerable reduction in the morbidity associated with this disease.

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