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Report of Two Fatal Cases of *Mycobacterium mucogenicum* Central Nervous System Infection in Immunocompetent Patients

Toïdi Adékambi, Cedric Foucault, Bernard La Scola, and Michel Drancourt*

Unité des Rickettsies, CNRS UMR 6020 IFR 48, Faculté de Médecine, Université de la Méditerranée, and Assistance Publique-Hôpitaux de Marseille Timone Fédération de Microbiologie Clinique, Marseille, France

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Neurological infections due to rapidly growing mycobacteria (RGM) have rarely been reported. We recently investigated two unrelated immunocompetent patients, one with community-acquired lymphocytic meningitis and the other with cerebral thrombophlebitis. *Mycobacterium mucogenicum* was isolated in pure culture and detected by PCR sequencing of cerebrospinal fluid samples. Both patients eventually died. The two isolates exhibited an overlapping antimicrobial susceptibility pattern. They were susceptible in vitro to tetracyclines, macrolides, quinolones, amikacin, imipenem, cefoxitin, and trimethoprim-sulfamethoxazole and resistant to ceftriaxone. They shared 100% 16S rRNA gene sequence similarity with *M. mucogenicum* ATCC 49650^T over 1,482 bp. Their partial *rpoB* sequences shared 97.8% and 98.1% similarity with *M. mucogenicum* ATCC 49650^T, suggesting that the two isolates were representative of two sequevars of *M. mucogenicum* species. This case report should make clinicians aware that *M. mucogenicum*, an RGM frequently isolated from tap water or from respiratory specimens and mostly without clinical significance, can even be encountered in the central nervous system of immunocompetent patients.

Mycobacterium mucogenicum, formerly known as a "*Mycobacterium chelonae*-like organism," forms a distinct group closely related to *Mycobacterium chelonae-Mycobacterium abscessus* and *Mycobacterium fortuitum* groups among rapidly growing mycobacteria (RGM) (2). As for community-acquired infections, *M. mucogenicum* was isolated from the liver at the autopsy of a patient with granulomatous hepatitis (12) and was found to be responsible for bacteremia in a patient with cirrhosis (18). Recently, *M. mucogenicum* was reported to be the cause of disseminated infection in a patient with idiopathic CD4$^+$ T lymphocytopenia with prolonged fever of unknown origin (21). However, *M. mucogenicum* has mainly been associated with several outbreaks of nosocomial infections, including bacteremia, probably due to water contamination of a central venous catheter during bathing (14); peritonitis due to contamination of a peritoneal dialysis machine (6); and infections associated with hemodialysis (15) traced to tap water used in the dialysis process. Analysis of a collection of 87 clinical isolates showed that 62% of isolates had been recovered from respiratory tracts without clinical significance, while the other isolates were responsible for catheter sepsis, pneumonia, subcutaneous abscess, cellulitis, osteomyelitis, lymphadenitis, surgical wound infection, and peritonitis (22). The central nervous system (CNS) is rarely involved in *M. mucogenicum* neurological manifestation. In a review of reported cases, only two cases have been mentioned without clinical data; both occurred in AIDS patients. For one of the two patients, multiple cerebrospinal fluid (CSF) samples were acid-fast bacillus smear positive for an unrelated reason 3 weeks prior to lumbar puncture (22). We herein report two additional cases of *M. mucogenicum* CNS infection in two unrelated immunocompetent patients.

Case reports. (i) Patient 1. A 23-year-old man who suffered from meningeal syndrome for 2 weeks (headache, vertigo, and nape pain) and a fever of 39°C was admitted to the emergency unit of the University Hospital of Marseilles on 9 July 2004. Laboratory tests revealed a white blood cell count of 7.7×10^9 cells/liter, a hemoglobin count of 15.2 g/dl, and a platelet count of 223×10^9 platelets/liter. Three blood cultures remained sterile. CSF analysis revealed 24 white cells/mm^3 (26% polymorphonuclear leukocytes) and 1 red cell/mm^3. Glucose and protein levels were within the normal range. Serology for human immunodeficiency virus was negative. Gram and Ziehl-Neelsen acid-fast stainings were negative. Bacterial culture was sterile. The patient died 3 days after admission and treatment with perfalgan (1 g/liter) without antibiotherapy. Analysis of the CSF yielded the following results: specific PCRs for cytomegalovirus, herpesvirus types 1 and 2, varicella-zoster virus, human herpesvirus 6, Epstein-Barr virus, and enterovirus were negative. 16S rRNA and *rpoB* gene PCRs were positive. PCR product sequences were found to be 100% and 98.1% similar, respectively, to that of *M. mucogenicum* ATCC 49650^T. This result was confirmed by culture of CSF in Middlebrook 7H9 liquid medium and subculture in Middlebrook and Cohn 7H10 agar. The susceptibility of this isolate to antibiotic agents was assessed using the Etest method (25) and disk test. The results are summarized in Table 1.

(ii) Patient 2. An 82-year-old man was admitted to the emergency unit of the University Hospital of Marseilles, France, on 9 January 2002 with fever, cough, and confusion. His past medical history was marked by diabetes, arterial hypertension, a permanent pacemaker, chronic renal failure, and a femoral vascular graft. On admission, he had a fever of 38°C. Neurological examination showed confusion without any focal neurological sign. Pulmonary auscultation rerevealed crepitated

* Corresponding author. Mailing address: Unité des Rickettsies, Faculté de Médecine, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France. Phone: (33) 04 91 32 43 75. Fax: (33) 04 91 38 77 72. E-mail: Michel.Drancourt@medecine.univ-mrs.fr.

TABLE 1. Antimicrobial susceptibility test results for CSF *M. mucogenicum* isolates

Antibiotic[a]	MIC (μg/ml) for:		
	M. mucogenicum ATCC 49650^T	Strain D3 (patient 1)	Strain Coh (patient 2)
Penicillin	>32	>32	>32
Amoxicillin	1.5	1	0.75
AMC (disc, 20 μg + 10 μg)	<4	<4	<4
Cefoxitin (disc, 30 μg)	<8	<8	<8
Ceftriaxone	>256	>256	>256
Imipenem	0.75	0.50	0.75
Doxycycline	1.5	1	0.125
Minocycline	1.5	2	0.50
Clarithromycin	0.032	0.016	0.125
Erythromycin	1	1	1.5
Azithromycin	0.125	0.50	0.75
Amikacin	1	0.50	1
Tobramycin (disc, 10 μg)	>8	<4	>8
Ciprofloxacin	0.125	0.50	0.25
Ofloxacin	0.75	2	0.75
Sparfloxacin	0.25	0.25	0.50
Rifampin	>32	>32	>32
Metronidazole	>256	>256	>256
Pipemidic acid (disc, 20 μg)	>16	>16	>16
Colistin (disc, 50 μg)	>2	>2	>2
Vancomycin	>256	>256	>256
TMP-SMZ (disc, 1.25 μg + 23.75 μg)	<2	<2	<2

[a] TMP-SMZ, trimethoprim-sulfamethoxazole; AMC, amoxicillin-clavulanate.

rales of the right lung. Laboratory tests showed a white blood cell count of 11.2×10^9 cells /liter, hemoglobin count of 13.1 g/dl, platelet count of 185×10^9 platelets/liter, serum creatinine level of 231 μmol/liter, aspartate aminotransferase level of 219 IU/liter, alanine aminotransferase level of 106 IU/liter, lactate dehydrogenase level of 1,565 IU/liter, creatine kinase level of 176 IU/liter, troponin I (isoform c) level of 25.4 μg/liter, and myoglobin level of 309 μg/liter. A myocardial infarct was suspected, but the electrocardiogram was not contributive because of the presence of the pacemaker. A chest radiograph disclosed alveolar pneumonia of the right lung. Three blood cultures remained sterile. CSF analysis revealed no white cells, 350 red cells/mm^3, a normal glucose level, and a protein level of 0.67 g/liter. A cerebral computed tomography scan showed extensive cerebral thrombophlebitis. Multiple organ failure developed, and the patient died 6 days after admission despite treatment with 2 g/day ceftriaxone in 1 intravenous daily dose combined with 1 g erythromycin three times a day intravenously prescribed for pneumonia and anticoagulation with nadroparin. Serology for human immunodeficiency virus was negative. Gram and Ziehl-Neelsen acid-fast stainings were negative. Bacterial culture was sterile. Further CSF analysis yielded negative results for specific PCR detection of cytomegalovirus, herpesvirus types 1 and 2, varicella-zoster virus, human herpesvirus 6, and Epstein-Barr virus. 16S rRNA and *rpoB* gene-based PCR analyses were positive. PCR product sequences were found to be 100% and 97.8% similar, respectively, to that of *M. mucogenicum* ATCC 49650^T. Culture of CSF in Middlebrook 7H9 liquid medium and subculture in Middlebrook and Cohn 7H10 agar yielded acid-fast bacilli in 5 days. The susceptibility of this isolate to antibiotic agents was assessed using the Etest method (25) and disk test. The results are summarized in Table 1.

MATERIALS AND METHODS

Phenotypic characterization of the isolate. CSF specimens were microscopically observed following Gram and Ziehl-Neelsen stainings and inoculated into BACTEC 9000MB broth according to the manufacturer's instructions (BD Biosciences, Sparks, MD). Mycobacterial isolates were subcultured onto Middlebrook 7H10 agar, egg-based Lowenstein-Jensen (LJ) slants (bioMérieux, La Balme-les-Grottes, France), and 5% sheep blood agar (BioTechnologie Appliquée, Dinan, France), and cultures were inspected twice weekly. Colony morphology and pigmentation as well as growth at various temperatures (30, 37, and 42°C) on 5% sheep blood agar, Middlebrook 7H10 agar, and LJ slants were noted. As for antimicrobial susceptibility testing, suspensions of the isolates were prepared by emulsifying colonies grown on 5% sheep blood agar slants in 5 ml sterile water to achieve a density equal to a 1.0 McFarland turbidity standard by visual examination. Suspensions were mixed vigorously on a vortex mixer for 20 s and then inoculated onto the entire surface of a 5% sheep blood agar plate. The MICs of penicillin, amoxicillin, imipenem, cefotaxime, ceftriaxone, rifampin, ciprofloxacin, ofloxacin, sparfloxacin, doxycycline, minocycline, erythromycin, clarithromycin, azythromycin, amikacin, metronidazole, teicoplanin, and vancomycin were determined by incubation with the respective Etest (AB Biodisk, Solna, Sweden) at 30°C for 3 days. For all drugs, the MIC was recorded as the point of intersection between the zone edge and the Etest strip (25). An additional disk diffusion method on 5% sheep blood agar for 3 days at 30°C was used to determine the MIC of trimethoprim-sulfamethoxazole (1.25 μg/23.75 μg), tobramycin (10 μg), amoxicillin-clavulanate (20 μg/10 μg), pipemidic acid (30 μg), and cefoxitin (30 μg). The MICs of antimicrobial agents tested were determined according to the breakpoints recommended by the CLSI (formerly NCCLS) (16, 17) and those proposed by Brown-Elliott and Wallace (7). Every test was done three times, with each repeat performed on a separate day in order to ensure the reproducibility of the results.

Sequence analysis. DNA was extracted from colonies grown on 5% sheep blood agar using the Fast-prep device and the FastDNA kit according to the recommendations of the manufacturer (BIO 101 Inc., Carlsbad, CA). We performed PCR amplification and sequencing of 1,482 bp of the 16S rRNA gene (24) and 723 bp of the *rpoB* gene (1, 4). Products of sequencing reactions were recorded using an ABI Prism 3100 DNA sequencer according to the supplier's standard protocol (Perkin-Elmer Applied Biosystems, Foster City, CA). The percentage of similarity between sequences was determined using the Clustal W program supported by the PBIL website (http://npsa-pbil.ibcp.fr/cgi-bin/npsa).

Nucleotide sequence accession numbers. The GenBank accession numbers of the sequences herein determined for *M. mucogenicum* strain D3 are DQ068744 (16S rRNA) and DQ068743 (*rpo*B), and those determined for *M. mucogenicum* strain Coh are DQ068745 (16S rRNA) and DQ068742 (*rpoB*).

RESULTS AND DISCUSSION

We detected the presence of *M. mucogenicum* in the CSF of two unrelated immunocompetent patients within a 2.5-year period. It is unlikely that detection was the result of contamination. In our area, *M. mucogenicum* was not reported as a contaminant in hospitals and laboratory settings. It was detected from CSF, a normally sterile fluid aseptically collected from patients. Also, both isolates were investigated separately, while no other such isolate was investigated in the laboratory during this long period of time. Finally, the presence of *M. mucogenicum* in the CSF was ensured in both cases by two unrelated methods, PCR sequence in one laboratory and culture in a second, 10-km-distant laboratory. In both cases, accurate identification of the isolate was ensured by using 16S rRNA and *rpoB* gene PCR sequencing. The original *rpoB* sequence confirmed that the two isolates did not merely result from laboratory contamination due to the fact that *M. mucogenicum* ATCC 45650^T was also available in the laboratory.

The diagnosis of nontuberculous mycobacterial meningitis was previously classified as definitive when nontuberculous

mycobacteria (NTM) were isolated in CSF samples from patients with clinical and biochemical features of meningitis, with or without supporting histological data. For those cases in which clinical data are lacking, the CSF samples and specimens obtained at autopsy were also considered definitive as probable meningitis when cultures were positive for NTM, clinical manifestations of meningitis were present, CSF biochemical profiles were normal or unreported, and other pathogens were identified concomitantly in CSF cultures or histological studies and were considered as doubtful when NTM were isolated from CSF in the absence of other supporting data (11). Patient 1, described herein, had meningitis suggestive of an infectious origin, and the sole CSF sample received by the laboratory was positive for *M. mucogenicum*. The bacterium was detected by PCR directly from CSF and was isolated in pure culture. The absence of any alternative pathogenic agent supports the potential clinical relevance of this isolate, which was recovered from a sterile site and in the presence of a biochemical profile consistent with lymphocytic meningitis. This case could thus be regarded as definitive. Since patient 1 presented with subacute febrile meningeal syndrome 2 weeks prior the hospitalization, it is unlikely that *M. mucogenicum* meningitis was nosocomial. It is more probable that acquisition occurred through the ingestion of, or contact with, a contaminated environmental water source outside of the hospital setting. It is unknown what sources of drinking water were used by the patient prior to his illness. *M. mucogenicum* has been identified as a water contaminant in the hospital setting (6, 14, 19) as well as in drinking water (10). The frequent presence of *M. mucogenicum* in tap water and processed water probably reflects its presence in natural water supplies and its relative resistance to standard disinfectants such as chlorine, formaldehyde, glutaraldehyde, and iodine (8, 9, 13, 19). Fortunately, nosocomial disease as a consequence of the presence of this species in hospital water systems is rare given the basis of previous reports of disease (22, 23). Its presence, however, may result in the contamination or transient colonization seen in sputum samples and is a potential source of contamination whenever tap water is used for laboratory studies or patient care.

M. mucogenicum has been described as the cause of various human infections but, to our knowledge, not as the etiologic agent of cerebral thrombophlebitis in immunocompetent patients, as was the case with patient 2. Negative blood culture demonstrated no evidence of disseminated disease, but the patient had a disseminated form of the disease with pulmonary infiltrates and neurological manifestations. The patient showed no clinical improvement, presented lung diseases (infiltrate lesions), and died after ceftriaxone and erythromycin treatment. Susceptibility data obtained from this isolate showed resistance to ceftriaxone and susceptibility to erythromycin (Table 1). However, the clinical relevance of in vitro ceftriaxone and erythromycin susceptibility testing of *M. mucogenicum* is unknown and is not the recommended treatment for RGM (7).

M. mucogenicum was designated as a new species in 1995 owing to its unique 16S rRNA gene sequence (20). We isolated two mycobacteria from CSF which shared 100% 16S rRNA gene sequence similarity with *M. mucogenicum* ATCC 45650^T, and these isolates were therefore suspected to belong to this species. Isolation of NTM from a sterile fluid such as CSF is a significant finding. Therefore, further investigations were done by performing *rpoB* gene sequencing, which, as we have shown previously, allows the description of highly discriminatory nucleotide positions within *M. mucogenicum* isolates (1). *rpoB* gene sequences of isolates D3 and Coh shared 97.8% and 98.1% similarity with that of the *M. mucogenicum* ATCC 45650^T, corresponding to 16-bp and 14-bp differences, respectively, over the partial 723-bp *rpoB* fragment. We previously proposed partial *rpoB* sequencing as a powerful tool for the accurate identification of RGM, and we showed that an RGM isolate belonged to a known species if it exhibited $<2\%$ sequence divergence with the reference strain for that species (1, 3, 5). We therefore confirmed that isolates D3 and Coh were two sequevars for *M. mucogenicum* ATCC 45650^T. Likewise, antibiotic susceptibility was similar to that of reference strain *M. mucogenicum* ATCC 45650^T (Table 1) and closely related species (4). They were susceptible in vitro to tetracyclines, macrolides, quinolones, amikacin, imipenem, and cefoxitin as well as to trimethoprim-sulfamethoxazole. Their colonies on Middlebrook 7H10 agar were smooth and mucoid, and no pigment was produced. Their growth occurred at 30 and 37°C but not at 42°C on 5% sheep blood agar, Middlebrook 7H10 agar, and LJ slants.

Also, *rpoB* gene sequence data pointed out subtle genetic heterogeneities within the two isolates. The use of the *rpoB* gene for genetic analysis would be helpful to better assess the clinical relevance of the isolates of *M. mucogenicum* and suggests a foreseeable heterogeneity of this species. Our data suggest that when *M. mucogenicum* is isolated in immunosuppressed or immunocompetent patients with neurological manifestations, it should not be routinely considered to be a contaminant. Finally, this case report should make clinicians aware that *M. mucogenicum*, an RGM frequently isolated from tap water or from respiratory specimens and mostly without clinical significance, can even be encountered in the CNS of immunocompetent patients.

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