Fatal Case of Polymicrobial Meningitis Caused by Cryptococcus liquefaciens and Mycobacterium tuberculosis Complex in a Human Immunodeficiency Virus-Infected Patient

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We describe a fatal case of polymicrobial meningitis in a human immunodeficiency virus-infected patient from Guatemala caused by Cryptococcus liquefaciens and Mycobacterium tuberculosis complex. Central nervous system infections caused concurrently by these species are extremely rare. This is also the first report of disseminated disease caused by C. liquefaciens.

CASE REPORT

A 31-year-old woman was admitted to the San Juan de Dios Hospital in Guatemala City in October 2012 with a history of weight loss and an occipital headache of 1 month of evolution accompanied by postprandial vomiting, fever, asthenia, and adynamia. Upon physical examination, she appeared to be chronically ill. Cardiopulmonary and abdominal evaluations were normal. Neurologically, the patient had bilateral papilledema; she was disoriented in time, space, and person, and her speech was incoherent. Muscular strength was 4/5 in four extremities, she had osteotendinous reflexes in 2/4 left hemibody and 3/4 right hemibody, was Kernig’s sign positive and Brudzinski’s sign positive, and had intact cranial nerves.

Because of the weight loss and suspected meningitis, a test for HIV was performed using the Determine HIV-1/2 test (Abbot Diagnostic Division, Hoofdorp, The Netherlands), and the result was positive for HIV-1. Therefore, meningitis was the AIDS-defining illness in this patient. On day 4, hematologic analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture revealed a decreased glucose level (39 mg/dl) and increased protein level (67.3 mg/dl). Cytological examination revealed 15 leukocytes/mm³ with a predominance of mononuclear cells (80%) over polymorphonuclear cells (20%). Direct microscopic examination of CSF with Gram stain, Ziehl-Neelsen stain, and India ink was performed, but no microorganisms were observed. A test using commercially available agglutination latex to detect cryptococcal antigen (International Immuno Diagnostics, Foster City, CA) was negative, but PCR for detection of mycobacterial DNA had a positive result. CSF cultures were negative for bacteria. However, a basidiomycetous yeast and a mycobacterium isolate were recovered separately from CSF using specific media. On Sabouraud dextrose agar (Merck Millipore), yeast-like colonies that were cream colored and mucoid with a glossy, smooth surface were grown after 3 days of incubation at 25°C. In addition, on Löwenstein-Jensen medium (bioMérieux, Marcy l’Etoile, France), buff to yellow, rough wrinkled colonies characteristic of mycobacteria were grown after 25 days of incubation at 37°C.

Initially, the yeast isolate was identified as Cryptococcus albidus by an API strip assimilation test (API ID32; bioMérieux, Marcy l’Etoile, France), and by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen, Germany), with a score of 1.766, which indicates probable genus identification. After sequencing of the internal transcribed spacer (ITS), the isolate could not be differentiated from Cryptococcus liquefaciens and Cryptococcus albidosimilis (Fig. 1A). However, with the sequence of the D1/D2 domains of the 28S rRNA region, the isolate was finally confirmed as C. liquefaciens (Fig. 1B) (1). In vitro antifungal susceptibility testing performed by microdilution using Sensititre (YeastOne; TREK Diagnostic Systems, Inc., Cleveland, OH) showed that C. liquefaciens presented a low amphotericin B MIC (1 μg/ml) but remarkably higher MICs for 5-flucytosine (256 μg/ml), posaconazole (>8 μg/ml), itraconazole (>16 μg/ml), voriconazole (>8 μg/ml), and fluconazole (>256 μg/ml).

The mycobacterial isolate was identified by the GenoType Mycobacterium CM test (Hain Lifescience, Germany) as Mycobacterium tuberculosis complex, and it was found to be susceptible to isoniazid (0.1 μg/ml), rifampin (1 μg/ml), ethambutol (5 μg/ml), and streptomycin (1 μg/ml) using the automated system Bactec MGIT 960 (BD Diagnostics). Identification to the species level was not pursued given that M. tuberculosis is the most common species...
causing tuberculosis in humans and because the treatment regimen for this disease does not differ among the species of the complex.

Immediately after the yeast isolate was recovered from CSF, the patient was administered amphotericin B deoxycholate (0.7 mg/kg/day). She was also treated with isoniazid (300 mg/day), rifampin (600 mg/day), ethambutol (1,000 mg/day), and pyrazinamide (1,200 mg/day). However, the condition of the patient deteriorated, and the woman died after 35 days. An autopsy was not performed.

To our knowledge, this is the first report of a fatal case of meningitis caused concurrently by *C. liquefaciens* and *M. tuberculosis* complex in a patient with advanced immunodeficiency due to HIV infection. Independently, cryptococcal and mycobacterial species are a common cause of meningitis among patients with HIV/AIDS because of the propensity of these pathogens to invade the central nervous system (CNS) after dissemination from the lungs, which are the initial site of infection by these microorganisms (2, 3). However, meningitis caused simultaneously by both species is very atypical. Few cases of polymicrobial infections involving these species have been reported in immunosuppressed individuals, including HIV-infected patients, a patient with lupus erythematosus, and a patient with Waldenström’s macroglobulinemia, among others (4–6).

Cryptococcosis alone is considered one of the AIDS-defining conditions: although it can also occur in other immunocompromised populations, it is among the most common fungal infections that cause mortality in patients with HIV (2). It is also one of the most important opportunistic pathogens causing meningitis, pneumonia, or both (7). Regarding the etiological agent, the most common cryptococcal species isolated from AIDS patients is *C. neoformans*; infections by other species, such as *C. albidus* or *C. lauritii*, are uncommon (8). Interestingly, this is the first time that *C. liquefaciens* has been isolated from CSF of an HIV-infected patient. In nature, this species has been described as a free-living saprophytic yeast, while clinically it has been found in rare cases together with *Cryptococcus diffluens* colonizing the skin of patients with atopic dermatitis, although its role in this skin condition is still not clear, and its clinical relevance remains unknown (9).

More recently, *C. liquefaciens* was reported in a case of central venous catheter-related fungemia in a non-HIV patient who recovered after prophylaxis with fluconazole and voriconazole (10). Similar to this nonfatal fungemia case, the cryptococcal isolate herein reported, initially recognized as *C. albidus*, was correctly identified as *C. liquefaciens* only with molecular analysis.

FIG 1 Sequence-based identification of *Cryptococcus liquefaciens*. Shown are results from maximum likelihood analysis of ITS (A) and D1/D2 regions of the large subunit rRNA gene (B) sequences of *C. liquefaciens* isolate 273-12 from Guatemala (highlighted) compared with sequences from Centraalbureau voor Schimmelcultures (CBS) strains (1). Bootstrap values are given on the nodes. CBS strain numbers and GenBank accession numbers are indicated after each species name.

![Sequence-based identification of *Cryptococcus liquefaciens*](http://jcm.asm.org/)
cation at the species level was not accurate by either the API strip system or MALDI-TOF MS because the biochemical reactions and protein spectra of *C.* liquefaciens are not included in any of the databases of these two methodologies. Morphologically and even physiologically, species of the genus *Cryptococcus* are very easily mistaken. *C.* liquefaciens differs from *C.* albidus only by the ability to assimilate methyl-α-D-glucoside and galactitol and from *C.* diffluens, its closest relative, by the ability to assimilate galactitol and saccharic acid (9). Currently, the distinction from any species of the *C.* albidus clade is best obtained by D1/D2 sequence comparison (1) (Fig. 1). Considering that related species are therefore hardly distinguished in the absence of sequencing, many cases of disseminated disease reported as being caused by *C.* albidus in fact may be caused by closely related species, which indicates that not only *C.* liquefaciens but also other relatives could be more common than currently thought. The reduced susceptibility to the main antifungal drugs for the treatment of cryptococcosis that *C.* liquefaciens showed in this study and the resistance of this species to 5-fluorocytosine already reported (10) highlight the need for a prompt and accurate identification of possibly nonsusceptible emerging pathogens.

Besides *C.* liquefaciens, *M.* tuberculosis complex was also encountered causing fulminant meningitis in the female patient. CNS tuberculosis is frequently found in regions where the incidence of pulmonary tuberculosis is high and where there are factors inherent in the community, like poverty and malnutrition. However, coinfection with HIV dwarfs these risk factors and predisposes to the development of tuberculous meningitis (3). In 2013, it was estimated that worldwide 9 million people developed tuberculosis and 1.5 million died of the disease, of which 360,000 deaths were associated with HIV infection (11). Importantly, tuberculous meningitis is difficult to diagnose and treat, and it is one of the most serious forms of extrapulmonary infection caused by *M.* tuberculosis, causing death or severe sequelae in over half of the cases, despite specific treatment (12).

In conclusion, this report suggests that the simultaneous presence of *C.* liquefaciens and *M.* tuberculosis complex causing disseminated disease is an unusual but very serious condition that should be deemed possible within HIV-infected patients. Given that the presenting clinical features of polymicrobial meningitis are nonspecific, delays in the correct identification of the causative agents and in starting appropriate treatment exacerbate the prognosis and considerably increase the risk of mortality. The interaction of *C.* liquefaciens and *M.* tuberculosis complex causing disease, together with the cell immunity deficits of the patient, may have led to a complicated pathological condition that exceeded the capacity of the host response and caused the death of the patient. Finally, the not so straightforward identification of unusual species, such as *C.* liquefaciens, in HIV-infected individuals and the reduced susceptibility of this pathogen to the currently available antifungal drugs make its surveillance and the determination of its real impact as an etiologic agent of disseminated disease significantly important.

**Nucleotide sequence accession numbers.** The nucleotide sequences for the ITS and the D1/D2 regions have been deposited in GenBank under accession no. KM501502 and KM501501, respectively.

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The authors have no conflicts of interest.

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