Case report

First report of Cryptococcus laurentii meningitis and a fatal case of Cryptococcus albidus cryptococcaemia in AIDS patients

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We report the first case of Cryptococcus laurentii meningitis and a rare case of Cryptococcus albidus cryptococcaemia in AIDS patients. Both infections were treated with amphotericin B and flucytosine. The C. laurentii meningitis was controlled after 2 weeks of treatment with no evidence of infection 20 months later. The patient with C. albidus cryptococcaemia, despite the amphotericin B/flucytosine combination therapy, died on the 14th day of treatment. The minimum inhibitory concentrations (MICs) for C. laurentii, as determined by Etest on RPMI 1640 agar, were 0.25 µg ml⁻¹ of amphotericin B, 1.25 µg ml⁻¹ flucytosine, 4 µg ml⁻¹ fluconazole, 0.50 µg ml⁻¹ itraconazole and 1.0 µg ml⁻¹ of ketoconazole. The MIC of amphotericin B for C. albidus was 0.5 µg ml⁻¹, flucytosine 1.25 µg ml⁻¹, fluconazole 4 µg ml⁻¹, itraconazole 0.5 µg ml⁻¹ and ketoconazole 0.25 µg ml⁻¹. The agreement of the amphotericin B MIC values obtained in antibiotic medium 3 by the broth microdilution method, with those obtained on casitone medium by Etest, was within a two-dilution range for both isolates. C. laurentii may cause meningitis and may also involve the lungs in AIDS patients.

Keywords AIDS, Cryptococcus albidus, Cryptococcus laurentii, cryptococcaemia, meningitis

Introduction

Cryptococcosis is a serious infection often occurring in immunocompromised hosts and is frequently used synonymously with infection caused by Cryptococcus neoformans [1]. Recent reports have indicated an increasing incidence of the infection among patients with the acquired immunodeficiency syndrome (AIDS) [2, 3]. Despite the recognition of several species of the genus, the non-C. neoformans species are generally regarded as non-pathogenic saprophytes. There are only a few previously reported putative cases [4–8] of cryptococcoses due to C. albidus in immunocompromised patients, and one case of septicaemia in an AIDS patient reported recently [9]. Although six cases of C. albidus meningitis, some more adequately documented than others, have been published we were able to find only three previous reports on C. laurentii extrameningeal infection: one in an immunocompromised patient [10], the second in a patient with chronic uveitis where C. laurentii was the aetiological agent of cryptococcal endophthalmitis [11] and the third in a bone marrow transplant recipient [12]. The former two cases have been the subject of dispute due mainly to the questionable mycological data concerning the identification of the organism [13,14].
We describe two rare manifestations of cryptococcosis due to non-*C. neoformans* species in AIDS patients. To our knowledge, there are no published reports on the isolation of *C. laurentii* from AIDS patients, and therefore it is believed that *C. laurentii* meningitis is the first reported case.

**Case history 1**

A 34-year-old Caucasian heterosexual male, from the island of Lesbos (East of the Aegean sea), a drinker usually of home-made wine, a non-smoker, whose HIV infection had been diagnosed in 1990, had a CD4 count currently on triple antiretroviral therapy (Indinavir, lamivudine, ddC).

**Mycology**

Cerebrospinal fluid (CSF) cultures on Sabouraud glucose agar (SGA) agar at 37 °C revealed the presence of slow-growing hyaline colonies consisting of encapsulated ovoid yeast cells. Five different single colonies were selected from the original plate, streaked onto five different SGA-containing plates and incubated at 30 °C for 48 h. Colonies from each of the five different subcultures were selected for further physiological and biochemical studies. After 3 days at 25 °C in malt extract (OXOID, Basingstoke, UK), cells from each of the five subcultures were identical. They appeared ovoid in short chains of three or four cells, 2±5×3±5–7·8 μm, with capsules ranging between 0·3 and 0·5 μm in thickness. The streak culture after 1 month on malt extract agar (OXOID) was pink to orange, pasty with lobate border.

No pseudomycelium was produced on slide culture on potato agar. Assimilation tests were performed using the API ID 32 yeast identification system. The species profile was compatible with that of *C. laurentii* in the API APILAB database (bioMérieux, Marcy d’Etoile, France). Nitrate assimilation was negative and the strain was capable of producing starch. All tests were subsequently run simultaneously with the NCCLS 3760 *C. laurentii* reference strain (National Collection for Pathogenic Fungi, Public Health Laboratory Service (PHLS), Bristol, UK) and all tests confirmed the identity of our isolate. The minimal inhibitory concentration (MICs) of antifungal agents was determined using Etest (AB Biodisk, Solna, Sweden), according to the manufacturer’s instructions on RPMI 1640 agar (Sigma, Deisenhofen, Germany), with l-glutamine but without bicarbonate, buffered with 0·165 M morpholinopropanesulphonic acid (MOPS) (Sigma) at pH 7. The MIC of amphotericin B (AmB) was 0·25 μg ml⁻¹, fluconazole (FC) 1·25 μg ml⁻¹, itraconazole (IT) 0·5 μg ml⁻¹ and of ketoconazole (KE) 1·0 μg ml⁻¹.

The amphotericin B MIC using Etest on Casitone agar (Difco, Detroit, USA), buffered with phosphate buffer, was 0·19 μg ml⁻¹. The microdilution method, according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for drug concentrations and inoculum size [15] in antibiotic medium 3 (OXOID) supplemented with 2% glucose, revealed an amphotericin B (Squibb & Sons, UK) MIC of 0·15 μg ml⁻¹. The quality control strains ATCC 90028 and 90018 MICs were 1·0 μg ml⁻¹ and 1·5 μg ml⁻¹, respectively.
Case history 2

A 47-year-old Caucasian heterosexual female with AIDS from the North West of Greece, whose HIV infection had been diagnosed in 1992 and whose CD4 count at that time was $220 \times 10^3 \text{ cells}^{-1}$, was receiving zidovudine treatment, to which ddI was added in January 1995. Her clinical course was complicated by central nervous system toxoplasmosis (August 1995), wasting syndrome, fever of unknown aetiology and myelodysplastic syndrome (January 1996). In March 1996 she presented with a 20-day history of high fever (41°C). Her blood pressure at presentation was 90/70 mmHg, pulse 112 min$^{-1}$, with signs of meningeal involvement. On examination she was acutely ill and marasmic and weighed 32 kg. The haematocrit was 22% with a haemoglobin of 10.3 g dl$^{-1}$, the leucocyte count was $2 \times 10^5 \text{ cells}^{-1}$ and the platelet count was $90 \times 10^3 \text{ cells}^{-1}$. The CD4 cell count at presentation was $5 \times 10^2 \text{ cells}^{-1}$. The CSF cell count was $1 \times 10^6 \text{ cells}^{-1}$ with normal protein and glucose and the microscopic examination of CSF such as starch formation were positive and confirmed identification. On completion of identification, all tests were run simultaneously with those for NCPF 38 C. albidus (PHLS, Bristol, UK) and the results confirmed the identity of our isolate. Susceptibility testing was performed with Etest, as described previously. The MICs recorded were for AmB 0.5 μg ml$^{-1}$, FC 1.5 μg ml$^{-1}$, FL 4 μg ml$^{-1}$, IT 0.5 μg ml$^{-1}$ and KE 0.25 μg ml$^{-1}$. The amphotericin B MIC on casitone agar was 0.25 μg ml$^{-1}$. The broth microdilution method [15] revealed an amphotericin B MIC of 0.31 μg ml$^{-1}$ in antibiotic medium 2 supplemented with 2% glucose.

Discussion

Fungal infections caused by C. neoformans are most commonly seen in patients with defective T-lymphocyte function. In the setting of AIDS many uncommon fungal diseases are frequently reported [3]. However, reports of infection due to other species of Cryptococcus such as C. albidus, C. laurentii and C. luteolus are extremely rare [3–11] and poorly substantiated [13,14]. In the present study evaluation of clinical data suggested that C. laurentii lung involvement could be associated with meningeal infection. This observation has been reported for the first time in an AIDS patient.

It must be noted, however, that C. albidus is usually isolated from the skin of healthy individuals and from indoor and outdoor air. In addition, C. laurentii is a saprobiic organism and a known contaminant during the fermentation process of wine and beer [16]. Both these species have very rarely been reported to cause disease [10,11]. Despite the confirmed exclusive growth of C. laurentii and C. albidus, precautions were taken in each case to exclude the possibility of contamination of clinical material through repeated isolation of the organisms from the clinical specimens. The likelihood of the involvement of C. neoformans and the transient presence of the saprobiic non-C. neoformans species in the clinical specimens were excluded by repeatedly testing individual colonies from subsequent subcultures of the original isolates. All individual colonies demonstrated the same morphological and biochemical characters conforming to those of C. laurentii and C. albidus, respectively.

Unlike previously reported cases of fungaemia where non-specific symptoms were described and laboratory diagnosis was based on a single positive blood culture [6,14] C. albidus fungaemia in this case was characterized by typical symptoms and the organism was isolated from two consecutive blood cultures. In previously reported cases no Cryptococcus-like cells were

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found in CSF [5,10]. In the present case of *C. laurentii* meningoencephalitis typical encapsulated yeast cells were detected upon microscopic examination of CSF with India ink.

Moreover, both our isolates grew at 37 °C upon incubation of the clinical samples. This is compatible with that recorded for most strains of *C. albicans* and *C. laurentii* that can grow at 37 °C [16]. It is noteworthy, however, that after several subcultures both isolates grew poorly at 37 °C and profusely at 28–30 °C.

Despite the repeated isolation of both non-*C. neoformans* species from clinical material and their identification with physiological and biochemical tests, it was necessary to obtain further confirmation of the identity of the isolates by comparison of our isolates with reference strains. Both isolates were deposited to the National Collection of Pathogenic Fungi, at the PHLS, Bristol, UK and were assigned the following numbers: for *C. laurentii* NCPF 8225 and for *C. albicans* NCPF 8249. Although *C. albicans* has been reported to share many biochemical characteristics and some capsular antigens with *C. neoformans*, cryptococcal antigen latex agglutination testing has been reported to yield negative results in cases of *C. albicans* infection [8]. In both cases presented here the *in vitro* latex agglutination tests for cryptococcal antigen were negative.

The treatment of choice for the more common meningeal and extrameningeal cryptococcal infections due to *C. neoformans* is amphotericin B and flucytosine. This combination therapy has been reported to control 90% of patients, including those with AIDS, whose CSF was sterile after 2 weeks of treatment [15–21].

Treatment regimens comprising amphotericin B combined with flucytosine have been found to be effective and well tolerated in first-line therapy in patients with AIDS-associated meningeal cryptococcosis [22].

Both our patients were treated with the conventional combination therapy of amphotericin B and flucytosine. Thus far the combination of fluconazole and flucytosine has been employed in treating *C. neoformans* infections. In all these cases clinical data were supported by *in vitro* studies on a large number of *C. neoformans* strains [23–25].

Different treatment schemes have not been employed on infections attributable to any other *Cryptococcus* species, except in a previously reported case of ocular mycosis attributed to *C. laurentii*, where fluconazole monotherapy was sufficient to treat the infection [11].

The *C. laurentii* meningoencephalitis we report here responded well to the combination therapy of amphotericin B and flucytosine within 14 days, and no relapse was detected until March 1998. The patient is still on low-dose fluconazole maintenance treatment of 200 mg day⁻¹. In contrast our patient, who was infected with *C. albicans*, was at the latest stage of AIDS and the *C. albicans* septicaemia was the terminal event.

This is the first time that non-*C. neoformans* clinical isolates have been evaluated as to their susceptibility to antifungals. Interestingly, the Etest MICs of amphotericin B on RPMI 1640 agar were higher, 0.25 μg ml⁻¹ and 0.5 μg ml⁻¹ for *C. laurentii* and *C. albicans*, respectively, that those recorded on Casitone medium (0.19 μg ml⁻¹ and 0.25 μg ml⁻¹) for each *Cryptococcus* species, respectively. The microdilution method performed in antibiotic medium 3 revealed MIC values closer to those obtained by the Etest on Casitone medium, 0.15 μg ml⁻¹ and 0.37 μg ml⁻¹ for *C. laurentii* and *C. albicans*, respectively. This indicates that the agreement between the MIC values of amphotericin B obtained on casitone medium by Etest and in antibiotic medium 3 by the microdilution method are in better agreement than those obtained by the Etest on RPMI 1640.

Cryptococcosis attributable to non-*C. neoformans* species requires extensive investigation in order to be substantiated. Provided the possibility of contamination of clinical specimens with such species is excluded, the potential of saprobic cryptococcal species to infect AIDS patients cannot be eliminated.

The epidemic of AIDS has set new challenges to the clinician and the mycologist by broadening and intensifying studies on the emerging opportunistic infections. It has been estimated that for every million AIDS patients 50 000–100 000 will contract cryptococcosis [2]. The unusual emerging cryptococcal, as well as other, fungal infections [26] will require novel strategies in the surveillance of patients at risk, in preventive measures and in basic and applied research.

**Acknowledgements**

We thank Dr C. K. Campbell, Mycology Reference Laboratory, PHLS, Bristol, UK, for his help in confirming the identity of our isolates. Professor H. M. Moutsopoulos MD for his comments and Ms M. Logotheti for technical help.

**References**


Cryptococcus laurentii and C. albidus in AIDS


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