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Repeated isolation of *Cryptococcus laurentii* from the oropharynx of an immunocompromized patient

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Abstract

Cryptococcus laurentii is one of the non-*neoformans* cryptococci that have rarely been isolated from humans. We report a case of repeated colonization of the oropharynx by *Cr. laurentii* in a patient with erythroleukaemia. The isolate was identified by phenotypic and genotypic tests and showed resistance to fluconazole.

Key words: *Cryptococcus laurentii*, colonization, case report

Introduction

The increasing use of cytotoxic chemotherapy, broad-spectrum antibiotics and immunosuppressants in leukaemia patients has led to a higher incidence of fungal infections [1, 2].

Among the yeasts, *Candida* species are most frequently isolated. However, a changing spectrum towards non-*Candida* species, i.e. *Cryptococcus* spp., has been observed [1, 3]. Although *Cr. neoformans* is the major cause of cryptococcosis, more uncommon opportunistic species such as *Cr. laurentii* may also cause infection in immunocompromized patients [4]. The spectrum of clinical manifestations associated with *Cr. laurentii* ranges from skin lesions to fungaemia [4]. The source of infection is mostly indoor or outdoor air [5].

We report a case of repeated oropharyngeal isolation of *Cr. laurentii* from a patient with erythroleukaemia during treatment with fluconazole.

Materials and methods

In July 1999, a 45-year old man suffering from erythroleukaemia (FAB M6) was admitted to the hospital with high fever. All microbiological investigations on blood, urine and repeatedly taken sputum samples remained negative. After a long history of extensive use of fluconazole (from October 1998 until September 1999 he received a total dose of >10 g), the patient was again treated with intraveneous fluconazole (200 mg daily), as well as with antivirals, antibacterials and cytostatics. His blood counts became low, i.e. hemoglobin 8.0 g/dl, leucocytes 150 per μl and platelets 9000 per μl but the white cell count gradually recovered, revealing a majority of leukaemic blasts and only a limited neutrophil count. The therapy-induced oropharyngeal mucositis clinically graded severe.

Oropharyngeal exudates and nose swabs were taken twice a week. On July 26 and 29, *Cr. laurentii* and *C. albicans* were isolated from the exudates. Meanwhile, the patient suffered from severe respiratory failure and the sampling of bronchoalveolar lavage fluid for diagnosis of a deep-seated fungal infection could no longer be justified.

A new oropharyngeal exudate on August 2 again contained a high number of *Cr. laurentii*. When the radiological examination showed a confluent infiltrate in the right and subsequently the left lung, fluconazole was replaced by amphotericin B (60 mg daily). The infiltrates improved slowly and had almost completely resolved on August 20. Radiological stagnation of the residual lesions was subsequently observed. Although

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the patient's condition stabilized, the oropharyngeal exudates continued to reveal low numbers of *Cr. laurentii*.

The patient became preterminal the following weeks and antimicrobial therapy was stopped a few days before he died from acute respiratory distress syndrome on September 2. Unfortunately, a mycological examination of post-mortem tissue samples could not be performed.

Results

Yeasts were isolated in a previously described enzymatic two-step procedure for the detection of *Candida* species [6]. The first isolate was identified as *C. albicans*. The second isolate was subcultured on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich., USA) at 37 °C to yield cream-colored colonies with a smooth mucoid texture. Culture on Cornmeal agar (Difco) supplemented with Tween 80 showed budding yeasts only, with pseudohyphae absent. An India ink preparation revealed a narrow but distinct capsule around the cells. Phenoloxidase activity after growth for 48 h at 37 °C on bird seed agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) was absent. The above observations were consistent with the genus *Cryptococcus*. The physiological pattern of the strain based on standard yeast identification methodology [7] was identical with that of CBS 139, the type strain of *Cr. laurentii*, and CBS 2174, CBS 7235, and CBS 7140, all representative isolates of *Cr. laurentii*. Noteworthy were the assimilation of creatinine and D-proline, a positive reaction on CGB medium [8], and the ability to grow at 37 °C. In these reactions, the isolate resembles *Cr. neoformans* var. *gattii*. However, melanin production on L-DOPA and norepinephrine media was negative.

The D1/D2 sequence of the isolate proved identical with that of CBS 139 (Genbank AF075469), the type strain of *Cr. laurentii* [9, 10], thus confirming the phenotypic identification.

Antifungal susceptibility testing on the isolate was performed twice with Fungitest® (Sanofi Pasteur, Marnes-la-Coquette, France) to obtain minimal inhibitory concentrations (MIC, μg/ml). The organism was susceptible to 5-flucytosine (2 μg ml^{-1}), amphotericin B (2 μg ml^{-1}) and miconazole (0.5 μg ml^{-1}), unlike to fluconazole (64 μg ml^{-1}), ketoconazole (4 μg ml^{-1}) and itraconazole (4 μg ml^{-1}).

Discussion

We have repeatedly isolated *Cr. laurentii* from a neutropenic patient with erythroleukaemia. As far as we know, this is the first report on the occurrence of this organism in Belgium. Because of the lack of bronchoalveolar lavage results, it is speculative to attribute the signs of pulmonary invasion in the patient to this fungus. The fact that *Cr. laurentii* was repeatedly found in oropharyngeal exudates, even during treatment with fluconazole, indicates that it was continuously present in the patient, at least as a colonizer. Its presence could not be confirmed in blood or urine. We suggest that the persistence of the isolate is mainly due to the severe immunocompromized status of the patient. In addition, the previous history of prolonged administration of fluconazole may have contributed to a selection of a resistant population of *Cr. laurentii*.

The presence of other fungi, e.g. *Aspergillus* spp., could not be ruled out, despite the lack of specific signs of aspergillosis and the failure to isolate it from any of the clinical samples, including oropharyngeal exudates. Our data do not warrant the establishment of a definitive relationship between the presence of *Cr. laurentii* and the patient's clinical picture. Nevertheless, we feel that the present report on a persistent oropharyngeal colonization by *Cr. laurentii* highlights the need to pay attention to uncommon colonizing yeasts. Susceptible patients, especially those at risk following immunosuppressive therapies, might inadvertently come in contact with such species and develop nosocomial infections.

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