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Pulmonary infection due to *Pseudozyma aphidis* in a patient with burkitt lymphoma: first case report

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ABSTRACT

Fungal infections are being increasingly reported in patients with malignancies. *Pseudozyma aphidis* is an opportunistic yeast usually isolated from plants and rarely from human samples. In this study, we report the first case of pulmonary infection due to *P. aphidis* in a Burkitt lymphoma patient.

1. Introduction

Pulmonary fungal infections often occur in patients immunocompromised by various conditions such as cancer and neutropenia (Biswas et al., 2010). *Pseudozyma aphidis* is an unusual pathogen commonly isolated from plants (Sugita et al., 2003). This is a ustilaginomycetes anamorphic yeast. There are so far only 1 case report of human infection by *P. aphidis* in blood and central venous catheter (Lin et al., 2008). Here, we reported a first case of pulmonary infection due to *P. aphidis*.

2. Case report

A 17-year-old man treated with chemotherapeutics for Burkitt lymphoma was hospitalized in a public pediatric oncology center in Recife, Brazil. The patient used in chemotherapy treatment cyclophosphamide, vincristine, methotrexate, ifosfamide, carboplatin, etoposide, doxorubicin, and prednisone. After the initiation of chemotherapy, the patient presented a persistent severe febrile neutropenia (neutrophil counts <100/mm^3). Broad-spectrum antibiotics and antifungal prophylaxis with fluconazole (Pfizer, São Paulo, Brazil) (400 mg/day) for 15 days and, afterwards, with caspofungin (Merck Research Laboratories, Whitehouse Station, NJ, USA) (0.5 mg/day) for 23 days were stated. After 38 days of hospitalization and chemotherapy treatment, the neutropenic patient developed fever as high as 39 °C, cough, bloody sputum, and a chest X-ray–detected bilateral infiltrates and pleural effusion. The hemogram counts were 80/mm^3 for neutrophils and 29,000/mm^3 for platelets. Caspofungin therapy was suspended. Blood cultures were collected for the bacteriologic diagnosis; however, all were negatives. After the symptoms worsened, the patient was admitted to the intensive care unit of the same hospital with respiratory insufficiency due to a probable pulmonary infection.

Venous blood samples were collected aseptically from the central and peripheral veins by venipuncture, and 3 samples of pleural fluid were aseptically collected via thoracentesis. The samples were processed immediately after collection by standard methods of mycologic diagnosis (direct examination and culture).

Microbiological identification was achieved using traditional taxonomy according to the criteria described by Barnett et al. (2000) and Boekhout and Fell (1998), through the VITEK 2 system (BioMerieux, Brazil) and by sequencing fragments of the internal transcribed spacer region of the rDNA using the primers ITS-1 and ITS-4 (Gonçalves et al., 2012).

Antifungal susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) M27-A3 document (CLSI, 2008). The antifungal drugs liposomal amphotericin B (AMB; United Medical, São Paulo, Brazil), anidulafungin (ANI; Pfizer), caspofungin (CAS; Caspofungin MSD, Merck), fluconazole (FLZ; (Pfizer), itraconazole (ITZ; Janssen, Titusville, NJ, USA), and voriconazole (VRZ;

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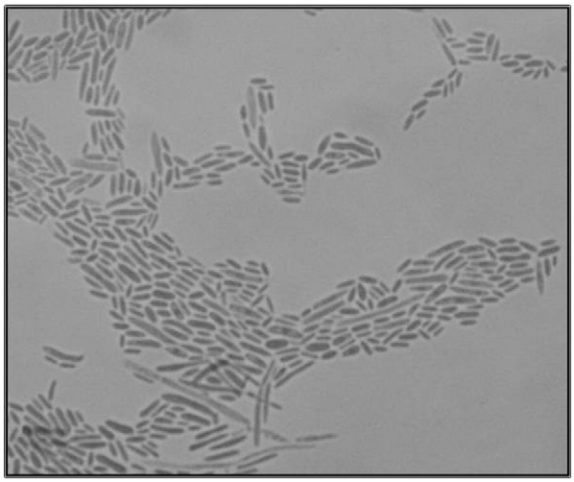

Fig. 1. Microscopic examination of culture on Sabouraud dextrose agar media after 7 days of incubation at 37 °C showing elongated spindle-shaped blastoconidia consistent with *Pseudozyma* species.

Pfizer) were tested. The quality control was performed by testing CLSI-recommended strain *C. parapsilosis* ATCC 22019.

Blood cultures were negative. The direct examinations of the pleural fluid showed various hyaline blastoconidia, and the cultures after 7 days of growth at 30 °C and 37 °C on Sabouraud dextrose agar (Difco; BD Diagnostics, Franklin Lakes, NJ, USA) media, showed moist yeast colonies, brownish yellow and wrinkled in all samples. Microscopic examination of the culture showed elongated spindle-shaped blastoconidia (see Fig. 1). The organism was positive for urea hydrolysis and for assimilation of galactose, D-xylose, D-arabinose, sucrose, lactose, Meα-D-glucoside, maltose, raffinose, soluble starch, trehalose, and nitrate, and was negative for assimilation of cellobiose and inulin. According to these morphologic and biochemical characteristics, this isolate was identified with *P. aphidis*. The VITEK 2 system showed the same assimilation profile described by Barnett et al. (2000) for *P. aphidis*.

A BLAST search produced a 100% match between the *P. aphidis* ITS rDNA sequences in the GenBank database. The DNA sequence was submitted to GenBank with the accession number of JQ743064. The isolate was submitted to a stock collection of the Department of Mycology, Federal University of Pernambuco, Brazil, with record number 6351.

The antifungal susceptibility test showed MIC values of 0.25 μg/mL for AMB, 4.0 μg/mL for ANI, 4.0 μg/mL for CAS, 4.0 μg/mL for FLZ, 0.25 μg/mL for ITZ, and 0.03 μg/mL for VRZ. Thus based on MIC values presented in the document M27-A3 for other yeasts, the isolate was susceptible to AMB, FLZ, ITZ, and VRZ, and resistant to ANI and CAS.

Accordingly, the patient was treated with a 10-day course of intravenous amphotericin B lipid complex (Bagó, Brazil) (5 mg/kg per day) and, afterwards, with a 15-day course of oral voriconazole (Pfizer) (400 mg/day). This antifungal therapy resulted in a successful clinical improvement as verified by chest X-ray and the absence of clinical manifestations.

3. Discussion

The percentage of patients who develop invasive fungal infections has increased dramatically in recent decades. Most of these infections occur in patients with hematologic malignancies (Montagna et al., 2012). This increase is attributed to host defense impairment due to intensive cytotoxic chemotherapies, hematopoietic stem cell transplantation, ablative radiation therapy, and use of corticosteroids, cyclosporine, and new immunosuppressive agents (Muhlemann et al., 2005).

During the last 20 years, other opportunistic fungal pathogens, such as *Fusarium* spp. and Zygomycetes, have also emerged (Kontoyiannis et al., 2005), whereas infections caused by other fungi are still rare (Girmenia et al., 2005).

P. aphidis is an anamorphic yeast related to the smut fungi in the genus *Ustilago*. This yeast is mainly isolated with a phytopathogen (Sugita et al., 2003). However, invasive human infections due to this species are rarely reported and little is known about its pathogenicity (Lin et al., 2008).

Sugita et al. (2003) reported the isolation of *P. aphidis* from a blood culture taken from a Thai patient, and Lin et al. (2008) described a case of central venous catheter (CVC) infection in a child with short gut syndrome. Still, a brain abscess associated with *Pseudozyma* species in a patient with astrocytoma was reported by Hwang et al. (2010). Infections due to *Ustilago* species have been reported in patients with chronic skin rash (Teo and Tay, 2006), hypersensitivity pneumonitis (Yoshida et al., 1996), and CVC infection (Patel et al., 1995). In the case reported by Yoshida et al. (1996), the patient was infected by *U. esculenta* and showed the same clinical aspects as in our patient. The analysis of the cases of human infection due to *Pseudozyma* species described in the literature is shown in Table 1.

Due to the rarity of *Pseudozyma* species infection in humans, no MIC breakpoints exist for this organism; limited susceptibility data suggested that some *Pseudozyma* species are susceptible to AMB (Sugita et al., 2003). In the case described for Lin et al. (2008), the *P. aphidis* isolate was susceptible to FLZ. In this article, the isolate was susceptible to AMB, FLZ, ITZ, and VRZ, but resistant to ANI and CAS. The results suggest a clinical and laboratorial resistance for echinocandin due to the occurrence of *P. aphidis* infection after treatment with CAS and antifungal susceptibility in vitro test results. Others studies reported *C. guilliermondii* and *C. parapsilosis* with decreased susceptibility to echinocandins (Munro, 2010).

To the best of our knowledge, our patient is the first report of pulmonary infection due to *P. aphidis*. We believe that the patient was infected through inhalation of conidia in the hospital environment. Due to immunosuppression caused by chemotherapy, reducing the

Table 1
Cases of human infection due to *Pseudozyma* species described in the literature.

Author	Isolate	Underlying disease	Age	Symptoms	Clinical sample	Treatment	Outcome
Sugita et al. (2003)	*Pseudozyma parantarctica* *P. thailandica*	Leptospirosis and aseptic meningitis Acute asthmatic attack and respiratory failure	21 years old 52 years old	Not reported	Blood obtained from 3 patients	Not reported	Not reported
Lin et al. (2008)	*P. aphidis*	Short gut syndrome	7 years old	Intermittent fever, chills, malaise, and fatigue	Blood Central venous catheter	Fluconazole (dose not mentioned), itraconazole (5 mg/kg per day)	Cure
Hwang et al. (2010)	*Pseudozyma* sp.	Astrocytoma	78 years old	Weakness in right leg, mass in cerebral lobe, and fever	Biopsy examination from cerebral abscess	Antifungal therapy not initiated	Death due to multi-organ failure

number of neutrophils allowed the germination of conidia in the respiratory tract and subsequent infection.

In summary, pulmonary infection due to *P. aphidis* can occur in patients with Burkitt lymphoma and the treatment of this mycosis with amphotericin B associated with voriconazole proved to be effective. Our case indicates that physicians caring for cancer patients need to be aware of these unusual yeasts as a potential source of respiratory infection.

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