Peritonitis due to *Blastobotrys proliferans* in a patient undergoing continuous ambulatory peritoneal dialysis

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*Blastobotrys proliferans* is an ascomycetous yeast never reported as a human pathogen. Here we report the case of peritonitis due to *Blastobotrys proliferans* in a 46-year-old man undergoing peritoneal dialysis.

**CASE REPORT**

A 46-year-old Melanesian man, undergoing continuous ambulatory peritoneal dialysis (CAPD) had a past medical history included chronic bronchitis due to cigarette smoking and a certain state of malnutrition. He was on CAPD for the past 3 years and already had three episodes of peritonitis, two due to *Staphylococcus aureus* and one to *Klebsiella pneumonia*, all of which recovered with adapted antibiotherapies.

On January 3rd 2007, he was admitted at the community clinics in Lifou Island (Northeast of the New Caledonia main Island, Grande Terre) for abdominal pain and cloudy peritoneal dialysis bags, which clinically presumed a peritonitis. The peritoneal dialysate fluid white cells count at admission confirmed peritonitis with more than 100/mm$^3$ (90 % polymorphonuclear neutrophils) and a Gram stain revealed numerous Gram positive cocci. The patient was started on a peritonitis protocol consisting of vancomycin (2 g/5 days) together with
ceftazidime (2 g/day), both intraperitoneally. Ceftazidime was stopped when *S. aureus* was identified in cultures from the initial dialysate.

In spite of antibiotherapy, the patient continued to deteriorate with persistent abdominal pain. He was referred to the New Caledonia Territorial Hospital on January 11, 2007. Repeated sampling of the dialysate fluid showed persistent elevation of white cell count at 220 /mm$^3$ (35 % neutrophils, 34 % lymphocytes, 17 % eosinophils) and the presence of numerous yeasts and hyphae growing on Sabouraud agar in less than 24 h. The isolate was sent to the French National Reference Center for Mycoses due to an unusual microscopical aspect (rare segmented hyphae, unusual size and shape of conidia) and lack of identification using carbon assimilation patterns (ID32C, bioMérieux, Marcy-l’Etoile, France).

It was then decided to remove the Tenckoff catheter and to start antifungal therapy by oral fluconazole (100 mg/day), which resulted in a quick clinical improvement and the resolution of the patient’s symptoms. However, treatment was switched to amphotericin B (3 mg/kg/d) for 3 weeks after antifungal drug susceptibility testing results (ATB-Fungus3®, bioMerieux) showed decreased susceptibility to all the antifungals tested, except to amphotericin B. Minimal inhibitory concentrations of amphotericin B (0.5 µg/ml), flucytosine (8 µg/ml), fluconazole (64 µg/ml), voriconazole (2 µg/ml), posaconazole (0.5 µg/ml) and caspofungin (>8 µg/ml) were determined according to the EUCAST microdilution broth reference method (28). The inflammatory markers then returned to normal levels. The clinical symptoms resolved completely with a follow-up of 4 months.

Identification of the species *Blastobotrys proliferans* Marvanová was done based on carbon assimilation pattern (ID32C and 50CH, bioMérieux) and microscopic morphology after slide culture in 2% malt agar medium after 6 days at 25°C using the keys established by de Hoog and colleagues(8, 9). Sequences were determined for the ITS1-5.8S-ITS2 regions (GenBank accession number EF584542) and the D1/D2 variable region of the rDNA gene (GenBank accession number EF58451) using universal primers V9D/LS266 (7, 21) and
Identification of the ascomycetous yeast *B. proliferans* was confirmed by comparison of the D1-D2 region nucleotidic sequence with those published in GenBank (accession number U40098 and DQ442684) (15-17) with 99% of similarity over 590 bp.

**DISCUSSION**

Peritonitis remains a common complication of peritoneal dialysis and occurs at an overall average rate of one episode every 29 months (32). The most common aetiology is bacterial peritonitis, with *S. aureus* being the most frequently implicated species. However, in New Caledonia, the frequency of peritonitis is higher, due to poor housing conditions, reaching the rate of one episode every 16 to 20 months. Fungal peritonitis is a less frequent (4 to 6% of all the peritonitis in this context) (1), but more severe complication, requiring Tenckoff catheter removal and switch to definitive haemodialysis. History of antibiotherapy for bacterial peritonitis within the 4 weeks preceding fungal peritonitis is often but not systematically reported (29). Risk factors also identified for development of fungal peritonitis include recent bacterial peritonitis (3), and lupus (13, 30). Our patient’s history thus conforms to these reports. Outcome of fungal peritonitis appears to be more favourable in children (33) and in patients with residual renal function (18).

*Candida* species are the most common fungi isolated (6, 26, 27). Peritonitis due to various filamentous fungi is also reported. *Aspergillus* spp. are responsible for a severe form of peritonitis, frequently lethal, and require a prompt removal of the Tenckoff catheter while starting intravenous amphotericin B (2, 22, 23). Zygomycetes remain an uncommon cause of peritonitis associated with a high mortality rate of 57% (23). Other filamentous fungi and yeasts are even less frequently reported (*Fusarium, Trichoderma, Penicillium, Paecilomyces, Curvularia, Acremonium, Rhodotorula, Trichosporon*) (4, 5, 10-12, 14, 19, 20, 25, 31). To our knowledge, *B. proliferans* has never been reported as a cause of infection in humans or animals even though the strain CBS 293.84 stored at the
Centraal Bureau voor Schimmelcultures (Utrecht, the Netherlands) is indicated as recovered from a “cystic lesion of ankle in a man”. The reservoir of *B. proliferans* is unknown.

Until recently, the dimorphic genus *Blastobotrys* was treated as a hyphomycete, close to the ascomycetous genus *Sporothrix*. What distinguishes both genera is conidiogenesis. In fact, *Blastobotrys* species have distinct mother cells (primary conidia) and secondary conidia whereas in *Sporothrix* species there is no visible differentiation between conidia of first and second order. The species *B. proliferans* has conidiophores bearing pear-shaped mother cells, containing a conspicuous body (Figure 1a). The mother cells are single, each crowned with secondary conidia. Globose, lateral conidia (Figure 1b), and hyaline, thick-walled, terminal and intercalary chlamydospores (Figure 1c) are present. *Blastobotrys proliferans* is different from all other *Blastobotrys* species by its proliferating mother cells and the refractive bodies in those mother cells (8). It grows with most carbon sources, does not assimilate nitrate and ferments glucose. Growth in the presence of melibiose, raffinose and at 37°C are characteristic of *B. proliferans* in the genus.

In the purpose of phylogenetic analysis, Kurtzman and Robnett (17) have re-examined relationship between *Blastobotryx, Arxula, Sympodiomyces* and several *Candida* species with a multigene analysis. They have demonstrated that *Blastobotrys, Arxula, Sympodiomyces* and some *Candida* species were members of the same clade. The multigene sequence analysis showed also *Trichomonascus* to represent the ascosporic state of this clade. Finally, *Blastobotrys* is considered as anamorphic members of the Saccharomycetales treated under the yeasts, while *Sporothrix* belongs to the Ophiostomatales.

Of note, despite the decreased in vitro susceptibility of the isolate to fluconazole assessed by two techniques, clinical improvement was observed rapidly after the introduction of oral fluconazole. This corroborates the usual lack of correlation between in vitro susceptibility testing results and clinical efficacy. However, it
does not mean that fluconazole should be the first choice for the treatment of fungal peritonitis due to uncommon species.

CONCLUSION
Clinical features of fungal peritonitis are not different from those of bacterial peritonitis but are less frequent. Persistence of clinical or biological abnormalities despite adequate antibiotherapy for bacterial peritonitis should prompt new sampling and suspicion of fungal peritonitis. Identification of the pathogen is always required to adapt the treatment. Infections due to uncommon fungi are most frequently seen in immunocompromised patients but are also an emerging threat in those with end stage renal failure. Whether *B. proliferans* represents a new source of human infection is unknown.
Figure 1: *Blastobotrys proliferans*, culture on 2% malt agar, examined with Nomarski interphase contrast (x100)

a. Conidiophores bearing mother cell with refractive body and secondary conidia
b. Lateral conidia (arrows)
c. Chlamydospores (arrow)
REFERENCES


