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BRIEF REPORT

Fungemia following *Saccharomyces cerevisiae* var. *boulardii* probiotic treatment in an elderly patient

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KEYWORDS
Saccharomyces cerevisiae var. *boulardii*;
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Abstract The yeast *Saccharomyces cerevisiae* var. *boulardii* is a biotherapeutic agent used for the prevention and treatment of several gastrointestinal diseases. We report a case of fungemia in a patient suffering from *Clostridium difficile*-associated diarrhea and treated with metronidazole and a probiotic containing *S. cerevisiae* var. *boulardii*. The yeasts isolated from the blood culture and capsules were identified by MALDI-TOF MS and API ID 32 C as *S. cerevisiae*, and showed the same appearance and color on CHROMAgar Candida. Treatment with fluconazole 400 mg/day was initiated and the probiotic was stopped. The patient was discharged from hospital in good condition and was referred to a rehabilitation center. We suggest that the potential benefit of *S. cerevisiae* var. *boulardii* should be accurately evaluated, especially in elderly patients. Moreover, all physicians should be trained in the use of probiotic agents and enquire whether the use probiotics was included in the patients'medical histories.

PALABRAS CLAVE
Saccharomyces cerevisiae var. *boulardii*;
Fungemia;
Probiótico;
Diarrea asociada a *Clostridium difficile*

Fungemia posterior al tratamiento con *Saccharomyces cerevisiae* var. *boulardii* como probiótico en una paciente añosa

Resumen *Saccharomyces cerevisiae* var. *boulardii* es un agente bioterapéutico usado en la prevención y el tratamiento de varias enfermedades gastrointestinales. Informamos de un caso de fungemia en una paciente con diarrea asociada a *Clostridium difficile*, y tratada con metronidazol y un probiótico que contenía *S. cerevisiae* var. *boulardii*. Las levaduras aisladas a partir del hemocultivo y del contenido de las cápsulas tomadas por la paciente se identificaron como *S. cerevisiae* mediante MALDI-TOF MS y API® ID 32C, las colonias mostraron el mismo color y aspecto en el medio CHROMAgar™ *Candida*. Se instauró un tratamiento con fluconazol

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400 mg/día y se suspendió el probiótico. La paciente fue dada de alta del hospital en buenas condiciones, y remitida a un centro de rehabilitación. Sugerimos que el beneficio potencial del uso de *S. cerevisiae* var. *boulardii* debe ser evaluado en cada paciente, especialmente en personas añosas. El uso de probióticos debería incluirse en los interrogatorios orientados al diagnóstico y formar parte de la historia clínica.

Saccharomyces cerevisiae is considered part of the normal human intestinal flora. *Saccharomyces cerevisiae* var. *boulardii* has been used as a probiotic since the 1950s. The yeast *S. cerevisiae* var. *boulardii* is a biotherapeutic agent used for the prevention and treatment of several gastrointestinal diseases. This probiotic is used worldwide and has been tested for clinical efficacy against several diseases such as antibiotic-associated diarrhea, acute diarrhea in adults, HIV-related diarrhea, *Helicobacter pylori*-related diseases, *Clostridium difficile* and *Salmonella typhi* infections, and Crohn's disease, among others[2,8]. Cases of the use of probiotics immediately preceding or concomitant to the occurrence of fungemia by *S. cerevisiae* are reported in the literature[1,6,14], and can lead to septic shock and an increase in mortality rates, especially in immunocompromised patients[1].

We report a case of *S. cerevisiae* fungemia in an elderly patient suffering from *C. difficile*-associated diarrhea (CDAD), who was treated orally with *S. cerevisiae* var. *boulardii* and metronidazole. The identification of *S. cerevisiae* was confirmed by a proteomic method. The present study questions the safety of this preventive biotherapy.

In March 2017, an 82-year-old woman presented with *C. difficile*-associated diarrhea and was treated with metronidazole 500 mg every 8 h for 14 days and with 200 mg/day probiotic capsules (Floratil®-Temis Lostalo) for six months. She suffered from Alzheimer's disease, arterial hypertension and diabetes as comorbidities.

In September, she was admitted to the Dr. Julio Mendez Hospital, in the city of Buenos Aires, for a gastrostomy feeding tube placement procedure. The patient developed a fever 48 h upon admission. Laboratory tests revealed the following: white blood cell count (WBC) 13 800 cells/ml and 86.2% neutrophils, platelet count 309 000 cells/ml, hemoglobin 11 g/dl, glycemia 134 mg/dl, creatinine 0.44 mg/dl, alkaline phosphatase 119 U/1, aspartate aminotransferase (AST) 44 U/1 and alanine aminotransferase (ALT) 22 U/1.

After obtaining blood (from the peripheral vein) and urine for culture, empirical treatment with vancomycin (1 g every 12 h) and piperacillin tazobactam (4.5 g every 6 h) was started. Persistent fever was observed despite the use of broad-spectrum antibiotics. Yeasts were isolated by the BacT/Alert (BioMériux, France) system from two sets of blood culture. The urine culture was negative. The yeast was cultured in a differential chromogenic medium (CHROMagar Candida, France). Antibiotics were discontinued and treatment with fluconazole 400 mg/day was initiated.

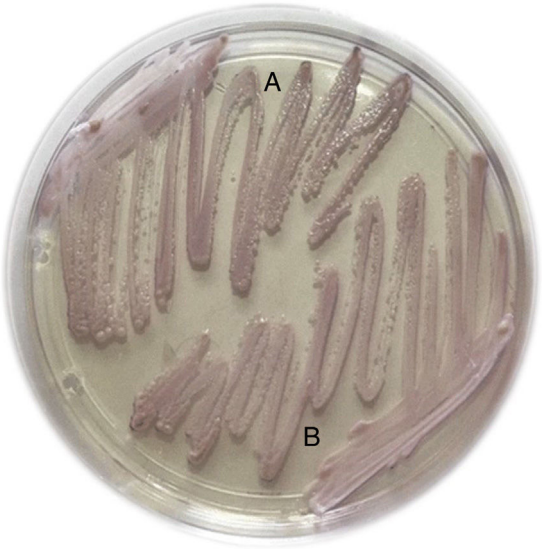

Figure 1 Appearance of yeast colonies on CHROMAgar Candida (CHROMagar Company, France) isolated from the blood culture (A) and capsule contents (B).

Carbohydrate assimilation tests using a commercially available API ID 32C kit (BioMérieux, France) matched the *S. cerevisiae* profile.

Capsules of the probiotic and the yeast isolated from the blood culture were sent to the medical mycology laboratory. Strains of *S. cerevisiae* isolated from the blood culture and capsule contents were cultivated in CHROMAgar Candida (CHROMagar Company, Paris, France). They showed the same appearance and color (Fig. 1).

Strains were identified with Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker® Daltonics, Bremen, Germany) and the API ID 32C kit (BioMérieux, France). MALDI-TOF analysis was performed with an initial protein extraction step using sterile water, ethanol, formic acid and acetonitrile.

The corresponding mass spectra of both isolates were analyzed, and *S. cerevisiae* was identified (The score achieved was >2). Scores of 2.0 are accepted for species assignment, and scores 1.7–2.0 are accepted for identification at the genus level. Scores below 1.7 are regarded as unreliable. The use of carbohydrate assimilation tests with API ID 32C matched the *S. cerevisiae* profile with 99.7 ID (Identification Percentage) and 0.81 T (T index). Both

isolates showed the same profile and were able to assimilate glucose, galactose, maltose, sucrose and raffinose. The sporulation study on ascospore agar[10] was negative in both strains isolated[7].

Fluconazole was administered for 18 days and the probiotic treatment was stopped. Follow-up blood cultures were negative. The ultrasound study and computed tomography scan of the abdomen did not show any pathologic alterations. The patient was afebrile with normal WBC counts; therefore, she was discharged from hospital and referred to a rehabilitation center.

This study was reviewed and approved by the Bioethics Committee at Julio Mendez Hospital, in line with the Declaration of Helsinki and the International Conference for Harmonization. The local ethics committee agreed that the findings in this report were based on normal clinical practice and were therefore suitable for dissemination. A written informed consent was obtained from the patient enrolled in the present study.

S. cerevisiae var. *boulardii* is usually administered to prevent and treat antibiotic-associated diarrhea and CDAD infection and to improve inflammatory bowel diseases through immunomodulation[2,8].

There is, however, a risk of fungemia due to the administration of *S. cerevisiae* var. *boulardii*, particularly in critically ill patients[2,11,15] mainly immunocompromised individuals and those requiring a central venous catheter[14]. Since the 1990s, invasive fungal infections have been reported in patients treated with *S. cerevisiae* var. *boulardii* as a probiotic[6] as well as in patients in close physical proximity to those being treated[1]. In the patient herein studied, the identified risk factors for fungemia were treatment with a probiotic containing *S. cerevisiae* var. *boulardii*, the use of broad-spectrum antibiotics, diabetes and age-associated immune dysfunction. The fungemia was probably due to damage induced by the gastrostomy feeding tube placement and digestive translocation by the oral administration of *S. cerevisiae* var. *boulardii*. Portals of entry for this yeast include translocation of ingested microorganisms from the enteral or oral mucosa and contamination of intravenous catheter insertion sites[1,8].

In this patient, the fungemia was detected 180 days after the administration of the probiotic. In a review of the literature including 60 cases of *S. cerevisiae* fungemia, a median of 10 ± 62.3 days (range, 4–300 days) was observed after the administration of the biotherapeutic agent[14]. The patient recovered when she stopped ingesting health food containing yeasts and was treated with fluconazole. The antifungal agent of choice for treatment of *Saccharomyces* species has not been finally established; however, amphotericin B and fluconazole seems to be preferable[14].

Likewise, several cases described in the literature, even those of immunocompromised patients, were reported to attain a rapid response once the treatment with the probiotic preparation and/or the antifungal therapy was terminated and the central venous catheter was removed[1]. Altogether, these observations underscore the prognostic importance of the rapid diagnosis of fungemia.

The isolates obtained from the patient and the probiotic (Floratil®) were identified as *S. cerevisiae* by the MALDI-TOF analysis. Characterization of *Saccharomyces boulardii* as a separate species was supported by the lack of galactose and sporulation as compared to *S. cerevisiae*[12]. However, a narrowed biochemical profile of *S. boulardii* with galactose, maltose and a raffinose-positive phenotype was reported by McCullough et al.[13] Despite some differences found in phenotyping, genotyping and proteomic studies[7], *S. cerevisiae* var. *boulardii* was definitively regarded as a member of the species *S. cerevisiae*[9]. Molecular phylogenetic and typing techniques suggested that *S. cerevisiae* var. *boulardii* forms a separate cluster but belongs to the species *S. cerevisiae*[14]. These findings have also been supported by other clinical studies, in which *S. cerevisiae* recovered from patients and *S. cerevisiae* var. *boulardii* strains isolated from probiotic preparations were proved to be genomically identical[1,11,14].

It is important to highlight that this report presents limitations due to the unavailability of molecular DNA-based analyses to confirm that the yeast isolated from the blood culture was the same as that found in the probiotic. However, other clinical studies conducted in similar situations showed that the *S. cerevisiae* found in the culture obtained from the patient and the *S. cerevisiae* var. *boulardii* in the probiotic administered were genetically identical[11,14].

Physicians are often undereducated about probiotic agents and do not ask their patients whether they have received probiotics as part of their treatment, thus they may be unaware of their patients' utilization of these agents. Both guidelines on the therapy of CDAD provided by the Infectious Diseases Society of America's (IDSA)[3] and by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)[4] do not yet recommend the use of probiotics as an adjunctive measure to prevent or treat CDAD.

In the cases of fungemia caused by *S. cerevisiae*, it is necessary to determine whether the patient is being treated with one of these biotherapeutic agents. It is important to emphasize the role of this probiotic, because it was responsible for 40.2% of invasive *Saccharomyces* infections reported in the literature[6]. Physicians and hospital systems need to be vigilant for potential rare cases of adverse events[6].

Furthermore, the largest randomized, double-blind placebo-controlled study to date that has been recently published found no benefits in the administration of the *S. cerevisiae* var. *boulardii* probiotic to prevent antibiotic-associated diarrhea in a population of hospitalized patients who received systemic antibiotic treatment[5].

S. cerevisiae var. *boulardii* fungemia may be underestimated as an iatrogenic infection. Even if the clinical impact of the infection is moderate, the potential benefit of *S. cerevisiae* var *boulardii* should be well evaluated accordingly, especially in elderly patients with underlying diseases that have predisposing factors for fungemia.

Conflict of interest

The authors declare that they have no conflicts of interest.

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