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CASE REPORT/CAS CLINIQUE

Saccharomyces cerevisiae fungemia after probiotic treatment in an intensive care unit patient

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Summary *Saccharomyces cerevisiae* is a common colonizer of the human gastrointestinal system as a benign organism. Enteral supplementation of this yeast as a probiotic product is effective in the treatment of antibiotic associated diarrhae. In rare occasions it can cause invasive infections. We present two fungemia cases in an intensive care unit following probiotic treatment containing *S. boulardii*. We are warning the safety of probiotic treatment in critically ill patients.

Introduction

Saccharomyces cerevisiae (also known as ''baker's yeast'' or ''brewer's yeast'') is widespread in the nature and can be a rare agent of fungemia. *S. cerevisiae* is now included in some diet or healthy foods. *Saccharomyces boulardii*, a subtype of *S. cerevisiae*, is used in probiotic preparation for the prevention and treatment of various diarrheal disorders, such as those associated with *Clostridium difficile* infection or parenteral nutrition [13]. The term ''*boulardii*'' is not an accepted taxonomic classification, according to the current nomenclature like the International Code of Botanical Nomenclature (ICBN). It was shown that *S. boulardii* and *S. cerevisiae* are almost identical as regards their genetic characteristics [29]. It is not possible to differentiate *S. boulardii* from *S. cerevisiae*, because its phenotypic characteristics are not pathognomonic [38].

S. cerevisiae (*boulardii*) fungemia was first reported in 1970 in a patient with a prosthetic mitral valve. Since then, many other fungemia cases caused by this yeast have been

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reported. Although *S. cerevisiae* (*boulardii*) is considered a safe and non-pathogenic biotherapeutic agent, as in *S. boulardii* formulation, several reports show that this fungus may cause severe infections [14]. Probiotics are often regulated as dietary supplements rather than as pharmaceuticals or biological products. However, there are significant concerns with respect to safety in particular populations [4,12,17]. The most important area of concern with probiotic use is the risk of sepsis [23,37]. There are several cases of systemic infections related to probiotic treatment with *S. boulardii* [8,20,22,32] including unexplained fever, fungemia, endocarditis, pneumonia, liver abscess, peritonitis and septic shock. Saccharomyces infection is clinically indistinguishable from invasive candidiasis. Critically ill patients are special cases to be evaluate before the decision of probiotic treatment [9,21,36].

Here we describe two fungemia cases seen in Intensive Care Unit (ICU) after probiotic treatment. We also present a short review of fungemia cases in ICU patients treated with probiotics containing *S. cerevisiae* sub type *boulardii*.

Case 1

A 88-year-old man, with history of confusion, was admitted to the emergency unit. He was transferred to the intensive care unit (ICU) with signs of septic shock. The diagnosis was urosepsis. Treatment was begun as piperacillin-tazobactam. The patient was intubated and placed under mechanical ventilation. Urine culture revealed *Klebsiella pneumonia*. Meropenem treatment was added. Laboratory work-up showed leukocytosis, creatinine increased. The patient then developed diarrhea with a negative stool culture. Probiotic treatment was initiated with 250 mg Reflor® (Biocodex Beauvais, France) containing lyophilisated *S. boulardii* due to persistant diarrhea. On the 24 h of probiotic treatment, patient developed a fever and *S. cerevisiae* (*boulardii*) grew out from two simultaneous blood cultures. Catheter cultures were negative. Probiotic treatment stopped and fluconazole had been started. On the 14th day of piperacillin-tazobactam, 12th day of meropenem, 8th day of fluconazole treatment he was afebrile and cured. He was discharged from hospital.

Case 2

A 38-year-old female was admitted to the hospital with weight loss and respiratory distress. She was diagnosed pneumonia and piperacillin-tazobactam and ciprofloxacin initiated. On the second day of her hospitalization, she was suddenly lost her consciousness. She was transferred to ICU and intubated. On the 48 h in the ICU, patient developed diarrhea and the stool culture was negative for pathogenic bacteria. Probiotic treatment with 250 mg Reflor® was started. During the probiotic treatment, leukocytosis and tachycardia developed. Repeated two blood cultures revealed *S. cerevisiae* (*boulardii*). Catheter cultures were negative. Seven days after treatment with fluconazole had been started, the patient developed septic shock and died.

Mycological studies

Yeast colonies were isolated from blood cultures of the patients in BacT/ALERT® 3D instrument of automated microbial detection system (BioMerieux, France). Yeast colonies were subcultured onto Sabouraud dextrose agar (SDA) plates. *S. cerevisiae* was identified using multiple methods. Corn meal-tween 80 agar morphology and yeast identification system API®ID32 C (BioMerieux, Marcy l'Etoile, France) were used. Ascospores were visualized in acetate ascospor agar plates. Probiotic-associated fungemia was pre-diagnosed. For the confirmation of case identification, probiotic strain was isolated in Sabouraud dextrose broth (SDB) and subcultured onto SDA plate. At that step of the workflow, reference strains of *S. cerevisiae* ATCC 9763/DSM1333 and *S. cerevisiae* DSM 70487 were also included. Patients' isolates (2), reference strains (2) and probiotic strain were re-identified by using Matrix-Assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (Bruker Daltonics Microflex MALDI-TOF/MS). Five colonies from Sabouraud agar plates were suspended in 300 μL of water and inactivated by addition of 900 μL of 96% ethanol. The cells were spun down, and the pellet was air-dried at room temperature, resuspended in 50 μL of 70% formic acid, and extracted by addition of an equal volume of acetonitrile and thorough mixing. Cellular debris was removed by centrifugation (17 000 × g for 2 min), and 1 μL of the clear supernatant was spotted onto a polished steel carrier, allowed to dry, overlaid with 1 μL of HCCA matrix (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid; Bruker Daltonics) and allowed to dry again. Measurement was performed with the MALDI BioTyper 2.0 (library version 3.0) and spectra were detected in positive linear mode, with a mass range of 2—20 kDa. Identification score of *S. cerevisiae* was > 2.000 for all isolates [2].

Genomic identification was performed for the confirmation of phenotypic identification of the five isolates. Genomic DNAs were isolated from the yeast colonies. Fungal barcod gene ITS located in 5.8S region of rRNA cistron was sequenced using primers ITS1F 5′-CTT GGT CAT TTA GAG GAA GTA-3′ and ITS4R 5′-TCC TCC GCT TAT TGA TAT GC-3′ [33]. Polymerase chain reaction (PCR) under the following conditions was used for the amplification of ITS region: 950°C for 5 min followed by 35 cycles of 950°C for 15 s, 550°C for 1 min, and 720°C for 30 s, a final elongation step at 720°C for 10 min was included. The PCR products were sequenced using an ABI Prism TM 310 Genetic Analyzer (Applied Biosystems, USA) and a BigDye® Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) according to the manufacturer's instructions. Sequenced DNA products were purified using Sephadex G-50. The sequence data analyzed using the National Center for Biotechnology Information (NCBI, Bethesda, Md., USA) BLAST system (available at http://www.ncbi.nlm.nih.gov/BLAST/). Fungal DNA isolated from yeast colonies was identified as *S. cerevisiae* (*boulardii*).

Clinical isolates, probiotic strain and reference strains were compared by Arbitrary Primed Polymerase Chain Reaction (AP-PCR) analysis, in order to clear strain homology. AP-PCR under the following conditions was performed using 5′-GACA-3′ primer (Sentegen Biotech, Ankara, Turkey): 950 C for 5 min followed by 35 cycles of 950°C for 30 s, 400°C for 1 min, and 720°C for 30 s, a final elongation step at 720°C for 10 min was included. The AP-PCR products were visualized under UV-transilluminator on high-resolution grade agarose (NuSieve™ Agarose) [24]. Genotyping revealed that

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