

## Fatal Septicemia Due to Amphotericin B-Resistant *Candida lusitanae*

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Five yeast strains, causally associated with septicemia and death in a patient after peritonitis, were identified as *Candida lusitanae* van Uden et do Carmo-Sousa by standard methods. The organism was initially susceptible to 5-fluorocytosine but strongly resistant to amphotericin B, requiring 50 µg/ml for complete inhibition at 48 h.

Known for more than 140 years (2), yeast infections, particularly systemic iatrogenic mycoses, have increased considerably in prevalence and significance in contemporary medicine. The number of etiological agents has increased, and approximately 25 yeasts species, among the 600 known at the present time, are frequently associated with humans or are responsible for human infections (1, 2). Pappagianis et al. (6) and Holzschu et al. (5) have recently reported the first case of *Candida lusitanae* van Uden et do Carmo-Sousa opportunistic infection in a 47-year-old man with a multiple myeloma, as well as the development of resistance to amphotericin B during the treatment. This report relates an additional case of *C. lusitanae* human infection in which the yeast exhibited resistance to amphotericin B before therapy.

Five strains identified as *C. lusitanae* at the Laboratory of Mycology, Pasteur Institute in Lyon, were isolated from an inpatient experiencing a peritoneal syndrome and requiring surgery for peritonitis resulting from a perforated retroileal appendicitis. After surgery, the patient, receiving metronidazole and cefotaxime, recovered normally and was afebrile 23 days later, beginning to eat and to stand up. At day 30, the fever grew to 40°C without any other clinical manifestation but with a rapid deterioration of the general state. Three strains of *C. lusitanae* were isolated from each of three blood cultures collected 31 days after the operation and before the establishment of the antifungal therapy. The two other strains were isolated from indwelling catheters. Identifications were based on the phenotypic criteria described by van der Walt (9), i.e., morphological characters, carbohydrate and nitrogen assimilation (using the method of agar medium in petri dishes with

impregnated disks), carbohydrate fermentation (using the method of sugar-peptone water with inverted Durham tube), and presence of ascospores when the organisms were grown on sodium acetate medium and stained by the method of Küfferath-Wirtz (9). Additionally, resistance to cycloheximide and ability to reduce tetrazolium triphenyl chloride were tested (2).

Determination of susceptibility was accomplished with the agar dilution test method (8), using Sabouraud agar medium for amphotericin B and a synthetic medium, carbon auxanogram (Institut Pasteur Production) with 5% agar and 0.15% asparagine (4), for 5-fluorocytosine. For each antifungal, a series of twofold dilutions was made from stock solutions containing 10,000 µg/ml to give final concentrations ranging from 0.1 to 100 µg/ml. Stock solutions were prepared in distilled water with standard 5-fluorocytosine powder (Produits Roche S.A., Neuilly-sur-Seine) and in dimethyl sulfoxide with standard amphotericin B (Squibb Laboratories, Neuilly-sur-Seine). The inoculum was harvested from 24-h cultures grown at 28°C on Sabouraud agar medium, suspended in sterile saline, adjusted to 10<sup>5</sup> yeast cells per ml, and then seeded in streaks at the agar media surfaces. *Saccharomyces cerevisiae* Hansen ATCC 36375 and *Candida albicans* ATCC 24433 were used as control organisms. Readings were taken after 48 h of incubation at 28°C, and the minimum inhibitory concentration was determined as the lowest drug concentration completely inhibiting yeast growth.

Serological diagnosis was done by indirect immunofluorescence and electrosyneresis. The indirect immunofluorescence was performed in a quantitative reaction (P. Ambroise-Thomas, doctoral thesis, University of Lyon, 1969), using a standard strain of *C. albicans* (Robin) Berk-

hout and with a strain of *C. lusitaniae* isolated from the patient's blood. A search for precipitins in electrosyneresis (counterimmunoelectrophoresis) was done with somatic and metabolic antigens (Institut Pasteur Production) of *C. albicans* (3; N. Absi, doctoral thesis, University of Lyon, 1979). Three milliliters of an equal mixture of 1% agarose and 1% Noble agar, dissolved in Veronal buffer (0.01 M, pH 8.2), was applied to a microscope slide (2.5 by 7.5 cm), and 3-mm wells were cut into the agar. Each serum was tested with three concentrations of antigen. Each cathodic antigen well was 6 mm from the corresponding anodic serum well. A control serum was used in each test. Electrophoresis was performed at room temperature with 0.01 M Veronal buffer, pH 7.2, at a constant current of 6 mA per slide for 90 min. Readings were made after electrophoresis and after staining with Coomassie brilliant blue.

The five yeast strains exhibited identical characteristics and were identified as *C. lusitaniae*. The morphological characters corresponded to those previously designated for this species. All strains assimilated glucose, galactose, maltose, sucrose, xylose, trehalose, cellobiose, and ammonium sulfate; they did not assimilate lactose, melibiose, raffinose, or potassium nitrate. Glucose, galactose, and trehalose were fermented, whereas maltose, lactose, sucrose, and raffinose were not (10). These organisms did not grow on media containing cycloheximide, exhibited pink colonies on triphenyl tetrazolium chloride agar, and did not produce sexual spores on sodium acetate medium.

These strains of *C. lusitaniae* were susceptible to 5-fluorocytosine with a minimum inhibitory concentration  $\leq 0.10$   $\mu\text{g/ml}$ , but they were strongly resistant to amphotericin B, requiring 50  $\mu\text{g/ml}$  for complete inhibition at 48 h. The search for precipitins was positive by using electrosyneresis with *C. albicans* metabolic antigen. In indirect immunofluorescence, a titer of 80 was obtained with a standard strain of *C. albicans*, and a titer of 160 was obtained with a strain of *C. lusitaniae* isolated from the patient. In spite of small differences in serological titers, this may further underline the pathogenic role of *C. lusitaniae*.

Because of the results of physiological studies, *C. lusitaniae* was at first erroneously identified as *Candida parapsilosis* (Ashford) Langeron et Talice by Pappagianis et al. (6) and as *Candida tropicalis* (Castellani) Berkhout in this study. The main differential characters of *C. lusitaniae* seem to be the assimilation of cellobiose and trehalose fermentation (both negative

for *C. parapsilosis*) and the complete lack of growth on cycloheximide-containing media, even after 48 h of incubation (7), the pink staining on triphenyl tetrazolium chloride agar, and the absence of maltose and sucrose fermentation (all opposite for *C. tropicalis*). Contrary to Holzschu et al. (5), we think there is little difficulty distinguishing *C. lusitaniae* from *Candida freyschusii* Buckley et van Uden and *Candida obtusa* (Dietrichson) van Huden et do Carmo-Sousa ex van Uden et Buckley, because these last two yeasts do not ferment or assimilate galactose.

*C. lusitaniae* is associated with humans and other warm-blooded animals (10). In this second observation of a *C. lusitaniae* human infection, the significance of this species in contributing to the demise of the patient as well as the particular resistance of these strains to amphotericin B, a rather rare phenomenon (6), should be underscored. Consequently, this species enlarges the already substantial spectrum of opportunistic yeasts and makes it desirable to reevaluate the criteria used for the identification of yeasts in clinical microbiology laboratories.

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