JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1996, p. 753–755 Vol. 34, No. 3
0095-1137/96/$04.00+0

Emergence of Fluconazole-Resistant Strains of *Blastoschizomyces capitatus* Causing Nosocomial Infections in Cancer Patients

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Received 17 July 1995/Returned for modification 16 August 1995/Accepted 15 December 1995

***Blastoschizomyces capitatus* strains resistant to fluconazole were isolated in three cancer patients. All of the strains had identical genomic DNA restriction profiles. Our findings give evidence for the emergence of fluconazole-resistant *B. capitatus* and suggest a nosocomial acquisition emanating from a common source within the hospital environment.**

Cytotoxic chemotherapy, neutropenia, prolonged antibiotic treatment, corticosteroids, and central catheterization are risk factors of invasive fungal diseases in cancer patients (4). Among fungal organisms, *Blastoschizomyces capitatus*, formerly named *Trichosporon capitatum* or *Geotricum capitatum*, has been recognized as an uncommon yet increasingly seen agent of disseminated infection (2, 5, 6). It can be distinguished from other similar yeastlike organisms for its formation of annelloconidia that undergo schizogonic division to form arthroconidiumlike structures and its inability to utilize urea, as well as its resistance to cycloheximide (12, 14). Data from the literature show that *B. capitatus* is usually susceptible in vitro to azole agents (1, 9). Although recent reports have documented the development of fluconazole-resistant strains of *Candida* spp. (13), nosocomial infections due to fluconazole-resistant *B. capitatus* have not been described. We report here the emergence of fluconazole-resistant strains of *B. capitatus* causing nosocomial infections in cancer patients.

Patient reports. (i) Patient I. Patient I was a 71-year-old male with advanced prostatic cancer and a complicated medical history with episodes of serious bacterial infections of the gastrointestinal and genitourinary tracts. The patient was submitted to a prolonged treatment with quinolones. In July 1993, he presented symptoms of dysuria, with a body temperature of <38°C. Laboratory tests revealed anemia and neutropenia with no renal insufficiency. A suprapubic aspirate of urine was submitted for microbiological culture. Urine culture yielded a yeastlike fungus count of more than 100,000 colonies per ml, which were identified as *B. capitatus*. Despite treatment with oral fluconazole (150 mg twice a day), there was no response of clinical signs and symptoms and/or microbiological laboratory data. However, a modification of the clinical course appeared to be positive when amphotericin B was administered intravenously.

Patient II. Patient II was a 49-year-old male with acute lymphoblastic leukemia. In November 1993, he was treated with vincristine, prednisone, daunoblastin, and methotrexate. At that time, the patient presented with an episode of hyperpyrexia. Prophylactic antimicrobial treatment consisted of ofloxacin and nystatin. Fever improved with the administration of amikacin and ceftazidime. Empirical antibiotic therapy was discontinued after 18 days. A new onset of fever (>38°C) recurred on day 47 of granulocytopenia associated with dyspnea and hypotension. Amikacin plus ceftazidime was promptly administered. The patient died within 24 h because of a clinical deterioration and septic shock. Three separate blood cultures revealed gram-negative bacteremia, with a synchronous fungemia. The bacteria were identified as *Pseudomonas aeruginosa*, whereas the fungi were morphologically and physiologically consistent with *B. capitatus*.

Patient III. Patient III was a 38-year-old male with myeloma and was treated with three VAMP (vincristine, cytosine-arabinoside, methotrexate, and prednisone) courses. In March 1994, autologous rescue was taken into consideration in an attempt to eredicate the persistent disease. Therefore, the patient was submitted to high doses of cyclophosphamide to induce recruitment of hematopoietic stem cells in the peripheral blood. The pretransplant conditioning (myeloablative) regimen consisted of melphalan and fractionated total body irradiation. Autologous peripheral hematopoietic stem cells were rapidly thawed and reinfused without further manipulation. His prophylactic antimicrobial treatment consisted of ciprofloxacin and oral fluconazole (150 mg once a day). On day 5 of granulocytopenia during the aplastic phase, the patient developed fever of >38.5°C which was associated with a progressive oral mucositis nonresponsive to amikacin and ceftazidime. After 7 days of antibiotic therapy, empiric intravenous amphotericin B was administered. Prior to antifungal treatment, two blood cultures were drawn which revealed a yeastlike fungus that was identified as *B. capitatus*. After the administration of amphotericin B, the blood cultures remained persistently negative.

Diagnostic procedures. Seven isolates of yeastlike strains were obtained from the three patients. Two strains were isolated from the urine of patient I, respectively, before and after oral fluconazole administration. The remaining five strains were blood isolates from patient II (three strains) and patient III (two strains).

Cream-colored yeastlike colonies were grown on Sabouraud glucose agar (Difco Laboratories, Detroit, Mich.). The microscopic observation of a small amount of growth on Sabouraud glucose agar revealed arthroconidium-like structures. The col-

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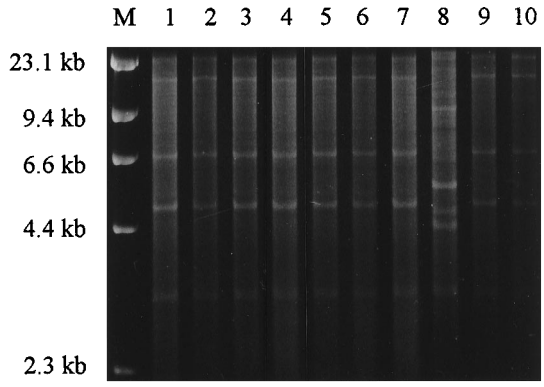

FIG. 1. Restriction endonuclease patterns (*Eco*RI digests of whole chromosomal DNA) of clinical isolates and ATCC reference strains of *B. capitatus*. Lanes: M, size markers (lambda DNA digested with *Hin*dIII); 1 and 2, urinary isolates from patient I; 3 to 5, blood isolates from patient II; 6 and 7, blood isolates from patient III; 8, ATCC 62963 (clinical isolate); 9, ATCC 62964 (clinical isolate); 10, ATCC 28576 (human isolate).

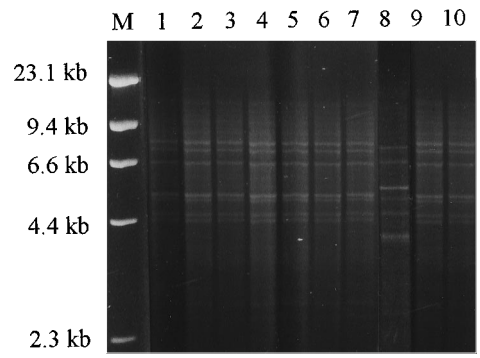

FIG. 2. Restriction endonuclease patterns (*Hin*dIII digests of whole chromosomal DNA) of clinical isolates and ATCC reference strains of *B. capitatus*. Lanes: M, size markers (lambda DNA digested with *Hin*dIII); 1 and 2, urinary isolates from patient I; 3 to 5, blood isolates from patient II; 6 and 7, blood isolates from patient III; 8, ATCC 62963 (clinical isolate); 9, ATCC 62964 (clinical isolate); 10, ATCC 28576 (human isolate).

ony features and microscopic morphology at initial examination were compatible with those of *B. capitatus*. Observations of these fungi developing on potato dextrose agar plates (Unipath S.p.A., Garbagnate Milanese, Milan, Italy) and on cornmeal agar (Unipath) slide cultures confirmed this identification. Isolated yeastlike organisms were unable to utilize potassium nitrate as the sole nitrogen source with a nitrate test medium as reported by others (11). With Christensen urea agar (Unipath), the isolates were unable to hydrolyze urea and, moreover, showed resistance to cycloheximide when plated onto Mycosel (BBL Microbiology Systems, Cockeysville, Md.). Finally, when the ATB 32 C (Api System; BioMerieux Italia, Rome, Italy) and Auto Microbic system yeast biochemical card (Vitek System; BioMerieux Italia) were used, the isolates were identified as *T. capitatum* (*G. capitatum*), which is now correctly known as *B. capitatus*. Antifungal susceptibility testing performed according to the National Committee for Clinical Laboratory Standard guidelines (10) showed that all seven isolates were fluconazole resistant in vitro (MICs, $\geq$32 μg/ml). In one patient (patient I), there was evidence of a clinical failure of fluconazole treatment.

Whole-cell DNA was isolated according to the method described by Scherer and Stevens (15). Whole-cell DNA was digested with *Eco*RI and *Hin*dIII (Boehringer, Mannheim, Germany) in 40 μl of the reaction mixture (50 mM NaCl, 100 mM Tris hydrochloride, 10 mM $MgCl_2$ [pH 7.5]) at 37°C for 3 h. The fragments were separated by electrophoresis through 0.9% agarose in Tris-borate-EDTA buffer, were stained with ethidium bromide, and were photographed with Polaroid type 557 film.

A total of seven isolates of *B. capitatus* were examined by genomic DNA restriction endonuclease analysis and compared with three American Type Culture Collection (ATCC) reference strains of *B. capitatus*. The ATCC strains were all human isolates (one from sputum [ATCC 28576] and two from clinical samples [ATCC 62963 and 62964]). Both *Eco*RI (Fig. 1) and *Hin*dIII (Fig. 2) restriction patterns were identical among all the strains but one (ATCC 62963). The latter finding suggests a sufficient discriminatory power through our molecular typing system, since it allowed the differentiation of two clinical isolates of *B. capitatus*, namely, ATCC 62963 and 62964, which apparently are epidemiologically related (7).

Our isolates, as well as two human isolates from the ATCC, share the same genomic profile. Their *Eco*RI restriction pattern is defined by five bands with the following molecular sizes, which were computed with Gel Compar software (Applied Maths, Kortrijk, Belgium) through an exponential model: 22.1, 16.7, 7.2, 4.7, and 3.2 kb. The predominant *Hin*dIII restriction pattern is characterized by seven major bands: 7.5, 7.1, 6.6, 5.2, 5.0, 4.5, and 4.4 kb.

With *Candida albicans* and *Candida glabrata*, well-documented cases of resistance emerging during fluconazole treatment have been reported (3, 13, 16, 17), and it has also been suggested that a correlation between fluconazole resistance and the total quantity of fluconazole administered exists (8). Thus, patients might acquire fluconazole-resistant *B. capitatus* either as a result of prolonged fluconazole treatment or by nosocomial spreading of isolates. The former hypothesis may be ruled out, since the first recognized fluconazole-resistant strain was isolated from the genitourinary tract of patient I before treatment with fluconazole was started, and patient II was prophylactically treated with nystatin only and subsequently died. Indeed, a hospital acquisition of fluconazole-resistant *B. capitatus* better explains our results, given that no patient was colonized with *B. capitatus* on admission; all of the isolates had an identical genomic profile, suggesting a common parental strain; the frequency of infection due to *B. capitatus* was far higher than that expected, with all of the incidences clustered within a very short period and with no more *B. capitatus* infection detected presently at our department.

Thus, our findings show the emergence of fluconazole-resistant *B. capitatus* and suggest a nosocomial acquisition of the strains.

These findings raise particular concern about selective pressure in our hospitals because of the current widespread use of fluconazole as a prophylactic and therapeutic agent that might enhance, even dramatically in particular circumstances (such as immunocompromised patients), the appearance of fluconazole-resistant *B. capitatus*.

Therefore, currently accredited strategies for prevention and treatment of fungal infection need to be reviewed, taking into account the emergence of a larger number of resistant strains, whether they are selected during the course of therapy or are acquired from the hospital environment.

We thank C. Girmenia (Dipartimento di Biopatologia Umana, Università La Sapienza, Rome, Italy) and A. Nanetti (Istituto di Microbiologia, Università di Bologna, Bologna, Italy) for confirming the identity of *B. capitatus*.

This work was supported in part by the Associazione Donatori Sangue, Pescara, Italy.

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