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Candida zeylanoides: Another Opportunistic Yeast

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A patient with a long history of scleroderma and gastrointestinal malabsorption requiring total parenteral nutrition was admitted with *Candida zeylanoides* fungemia. The yeast responded to therapy, but on two subsequent admissions for episodes of fever the blood cultures yielded the same yeast. The identity of the *Candida* species was established biochemically by both the API (Analytab) and Vitek system approaches. *C. zeylanoides* ATCC 20356 and ATCC 7351 served as controls for these analyses and for antifungal susceptibility studies and restriction endonuclease analyses of chromosomal DNA. These investigations indicated that representative isolates of the yeasts from the three episodes were identical and differed in several respects from the ATCC strains, which did not share many of the characteristic bands with the DNA restriction fragment analysis. *C. zeylanoides* variants capable of tolerating 35°C can complicate the recovery of patients, especially individuals compromised by their underlying disease.

The growth of medical science has enabled physicians to intercede effectively in a considerable number of disease conditions. Subcutaneous venous access devices must be considered among these advances and are employed for many therapeutic and monitoring purposes. Their presence in patients is complicated on occasion by infection, at times with organisms hitherto unknown in the intimate human biosphere, especially in patients with various degrees of immune compromise. This report describes a recrudescent *Candida zeylanoides* fungemia associated with a Hickman catheter and a collaborative mycological and molecular effort to establish the identity of the etiological agent.

MATERIALS AND METHODS

Pertinent patient information. A 51-year-old woman with an 8-year history of scleroderma and a 3-year history of gastrointestinal malabsorption required placement of a double-lumen Hickman catheter for total parenteral nutrition 3 years prior to the present episode. Her initial admission in September 1989 was preceded by a week of chills and drenching sweats. Her admission temperature was 102.4°F (38.9°C); four of six peripheral blood cultures yielded *C. zeylanoides*. Echocardiography demonstrated a hyperdynamic left ventricle, a small pericardial effusion, and absence of valvular vegetation. Ophthalmological examination was normal. Admission urine culture was negative for bacteria and yeasts. Urine analysis was remarkable for 10 to 20 erythrocytes per high-power field and 1 to 5 leukocytes per high-power field. Therapy with amphotericin B led to repeated negative blood cultures procured from the Hickman catheter and peripheral veins; the fever and other symptoms abated; seven additional blood cultures obtained from the Hickman catheter and peripherally while the patient was on therapy were negative; the Hickman catheter remained in place throughout this period. The patient received a total of 716 mg of amphotericin B. Five months later (February 1990), the patient was readmitted after a 24-h episode of chills and night sweats. Her admission temperature was 101°F (38.3°C), her heart rate was 110, her blood pressure was 100/70, and her heart was hyperdynamic. No murmur was detected. The Hickman catheter entry site did not reveal erythema, edema, or tenderness on palpation. It was without evidence of infection. Two blood cultures from the catheter and peripheral veins again yielded *C. zeylanoides*, and the patient was treated with a total of 691 mg of amphotericin B. A third admission for the same problem was required within a month. The identical organism was isolated from blood.

Laboratory analyses. (i) Blood cultures. Blood cultures were obtained with the adult Isolator Tube (DuPont, Wilmington, Del.). Following centrifugation and supernatant aspiration, the remaining material was distributed in equal aliquots to two chocolate agar plates (BBL Microbiology Systems, Cockeysville, Md.), one 5% sheep blood agar (BBL) plate, one Sabouraud agar (BBL) plate, and a special anaerobic meat broth previously described (5).

(ii) Yeast identification. Yeastlike colonies growing on all of the agar media were Gram stained and analyzed with the API 20C system (Analytab Systems, Inc., Hicksville, N.Y.) (API) and the yeast-ID card (Vitek Systems, Inc., Hazelwood, Mo.). Cornmeal agar was inoculated for detection of pseudomycelium and blastospore formation.

(iii) *Candida* isolates. One *C. zeylanoides* isolate from each of the three episodes of candidemia (strains 89BD10107, NY3971, and 90BD3438), which occurred between September 1989 and March 1990, were studied in greater detail. In addition, two stock isolates of *C. zeylanoides* (ATCC 20356 and ATCC 7351) were included as epidemiologically unrelated control strains. The yeasts were reidentified with the API 20C system and the Vitek yeast identification card. The selected isolates were tested again on cornmeal agar for detection of pseudomycelium and blastospore formation and stored on agar slants at ambient temperature. Each isolate was coded and submitted blindly for antifungal susceptibility testing and typing.

(iv) Antifungal agents. Four antifungal agents were used in this study: amphotericin B (E. R. Squibb & Sons, Princeton,

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N.J.), 5-fluorocytosine (Hoffmann-LaRoche Laboratories, Nutley, N.J.), ketoconazole (Janssen Pharmaceutica Inc., Piscataway, N.J.), and fluconazole (Pfizer, Inc., Groton, Conn.). The antifungal agents were obtained from the manufacturers as standard powders. Concentrated stock solutions (amphotericin B, 5,000 μg/ml; 5-fluorocytosine, 10,000 μg/ml; ketoconazole, 5,000 μg/ml; fluconazole, 5,000 μg/ml) of the antifungal agents were prepared in dimethyl sulfoxide. Serial twofold dilutions of the stock solutions were prepared in sterile RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.), buffered to pH 7 with 0.165 N morpholinepropanesulfonic acid (MOPS) buffer (Sigma). Aliquots (0.1 ml) of each antifungal solution were dispensed into the wells of plastic microdilution plates by using a Quick Spense II dispenser (Sandy Spring Instrument Co., Inc., Germantown, Md.). The final drug concentrations were 0.03 to 32 μg/ml for amphotericin B, 0.12 to 128 μg/ml for 5-fluorocytosine, 0.03 to 32 μg/ml for ketoconazole, and 0.12 to 128 μg/ml for fluconazole. The final concentration of dimethyl sulfoxide in the wells was less than 1%, a concentration that did not affect the growth of the test organisms. The plates were sealed, wrapped in foil, and frozen at −70°C until used in the study. The stability of the antifungal agents at −70°C was monitored by including a quality control strain of *C. tropicalis* (ATCC 13803) with each test run. No deterioration in activity of the test agents was observed during the course of the study.

(v) Antifungal susceptibility studies. MICs were determined in duplicate for the four antifungal agents on all isolates by a broth microdilution procedure. Prior to MIC testing, each isolate was passaged at least twice on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) to ensure viability. The inoculum suspension was prepared by growing each isolate overnight in RPMI 1640, harvesting the cells by centrifugation, and suspending them in sterile distilled water to a concentration of 5×10^5 organisms per ml as determined by hemacytometer counting. The microdilution plates were inoculated by using a Quick Spense inoculator to deliver 5 μl of inoculum suspension into each well. The final inoculum size was 10^4 organisms per ml as verified by hemacytometer counts. After inoculation, the plates were incubated at 30°C in air for a total of 48 h. The MIC for each antifungal agent was read after both 24 and 48 h of incubation as the lowest concentration in which there was no detectable growth. The geometric mean for each duplicate set of MIC determinations was calculated.

(vi) Restriction endonuclease analysis of chromosomal DNA. A suspension of each yeast was placed on Sabouraud dextrose agar (Difco) and incubated at 30°C for 16 h. Two to three medium-size colonies (>1 mm in diameter) were suspended in 2 ml of distilled water. A 30-μl aliquot was added to 5 ml of yeast extract-peptone-dextrose broth (YEPD components available from Difco) and incubated overnight at 30°C with agitation. Spheroplasts were prepared by β-glucuronidase digestion (10,000 U; Sigma G-0876) as described by Forte and Fangman (4). DNA was extracted by the procedure of Scherer and Stevens (13) and concentrated by precipitation in 95% alcohol.

Restriction endonuclease analysis was performed as described by Maniatis et al. (8), with modifications described previously (9, 10). The DNA samples were digested with RNase A (Sigma) for 60 min at 30°C. Each sample was then digested independently with *Hin*dIII at 37°C and *Bst*NI at 65°C (New England BioLabs, Beverly, Mass.) for 4 h as recommended by the manufacturer. The reaction was quenched with 0.1% bromophenol blue–20 mM EDTA–50% glycerol–0.1% xylene cyanol. Electrophoresis was performed by using a horizontal 0.7% agarose gel containing ethidium bromide with TBE buffer (100 mM Tris, 1.0 mM EDTA, 100 mM boric acid) at 45 V for 16 h. Bacteriophage lambda DNA digested with *Hin*dIII was used for molecular weight standards. The gels were photographed by using a UV transilluminator and polarized 107C film.

(vii) Analysis of DNA restriction fragment patterns. Photographs of ethidium bromide-stained gels were analyzed to detect differences in banding patterns. Molecular weights were interpolated with respect to the lambda phage standards. Any difference of a readily detected band between the sample lanes was considered significant.

TABLE 1. Antimicrobial susceptibility of *C. zeylanoides*

Test strain	MIC (μg/ml) of:							
	5-Fluorocytosine		Amphotericin B		Fluconazole		Ketoconazole	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
89BD10107	<0.13	<0.13	0.5	1.0	2.0	4.0	0.12	0.25
NY3971	<0.13	<0.13	0.5	2.0	2.0	4.0	0.12	0.5
90BD3438	<0.13	<0.13	0.5	1.0	2.0	4.0	0.12	0.173
ATCC 20356	32.0	>128.0	1.0	2.0	8.0	16.0	0.5	1.0
ATCC 7351	<0.13	<0.13	0.12	1.0	4.0	8.0	0.12	0.12

RESULTS

All of the candidal isolates from the patient gave identical reaction with the various test systems. All of the yeasts assimilated glucose, glycerol, 2-keto-D-gluconate, sorbitol, *N*-acetylglucosamine, and trehalose (API code, 6102140; AutoMicrobic System code, 001103040). The yeast did not assimilate L-arabinose, xylose, adonitol, xylitol, galactose, inositol, methyl-D-glucoside, cellobiose, lactose, maltose, sucrose, melezitose, or raffinose. In addition, the Vitek yeast biochemical card demonstrated failure of the yeast to elaborate urease and assimilate dulcitol, palatinose, erythritol, or melibiose; the organism did not grow in the presence of cycloheximide. The clinical isolates differed from the description of Kreeger-van Rij (7) by luxuriant growth at 35 and 37°C; the control strains did not grow at the elevated temperatures but grew only at 30°C. Strain ATCC 7351 yielded an identical code with the API profile, while ATCC 20356 achieved only a good likelihood and low selectivity with this test (600100). On cornmeal agar, the clinical isolates produced a pseudomycelium consisting of chains of cells frequently curved, bearing oval, somewhat elongate blastospores, singly, in small clusters, and in short chains. The pseudomycelium gave a feathered appearance at low power, exactly as described by Kreeger-van Rij (7). The identity of the isolates was confirmed through the courtesy of Michael R. McGinnis (University of Texas Medical Branch at Galveston).

The antifungal susceptibility profile of the patient bloodstream isolates and the control strains is summarized in Table 1. Despite repeated and prolonged exposure to amphotericin B (total dose, 1,407 mg), the clinical isolates remained susceptible to that agent. The antifungal susceptibility profiles of the bloodstream isolates were identical and demonstrated that these organisms were highly susceptible to the four agents tested. Likewise, control strain ATCC 7351 had a high-susceptibility profile which was virtually identical to those of the three clinical isolates. In contrast,

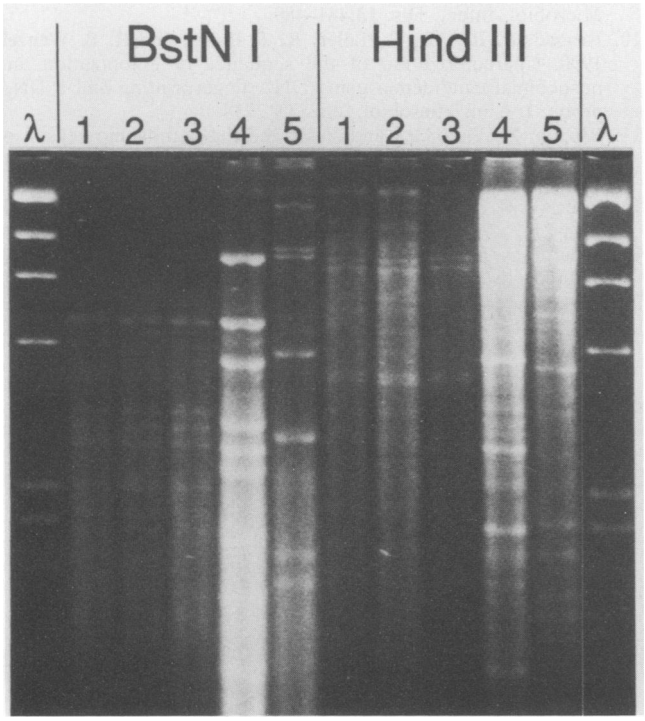

FIG. 1. DNA restriction fragment analysis of *C. zeylanoides* isolates. The photograph demonstrates *Bst*NI and *Hin*dIII digest patterns of three clinical bloodstream isolates (lanes 1 to 3) and two epidemiologically unrelated control strains (lane 4, ATCC 20356; lane 5, ATCC 7351). Lambda (λ) molecular weight markers are on the right and left. Note the complex banding patterns of all isolates and the similarity of the bloodstream isolates to each other.

the profile of control strain ATCC 20356 was significantly different from those of the other isolates, with resistance to 5-fluorocytosine (MIC, >128 μg/ml) and fluconazole (MIC, 16 μg/ml).

DNA restriction fragment analysis with *Bst*NI or *Hin*dIII (Fig. 1) demonstrated essentially identical patterns among all three bloodstream isolates. Each of the epidemiologically unrelated control isolates had a unique *Bst*NI and *Hin*dIII pattern and was distinctly different from the blood stream isolates. Thus, the three blood stream isolates were closely related and represented the same strain (subtype) of *C. zeylanoides* and each of the epidemiologically unrelated isolates represented a distinctly different subtype of this species.

DISCUSSION

Infectious complications caused by *Candida* species have been documented especially in patients receiving parenteral nutrition, corticosteroid or immunosuppressive therapies, in substance-abusing patients, in patients following extensive surgery, and in individuals on long-term antimicrobial therapy, especially those afflicted with various neoplasms (2, 6, 11). However, *C. zeylanoides* has been reported only once involving the bloodstream (12) and once as the cause of arthritis and transient fungemia (1). Kreeger-van Rij (7) has indicated that this yeast does not grow at 37°C, an observation substantiated by the behavior of the American Type Culture Collection strains. However, the two earlier reports and our observations indicate that the ability to proliferate at body temperature is within the capability of this *Candida* species. Similarly, the assimilation and morphological studies indicate that the clinical isolates belong to this species. *C. tropicalis* strains may, at times, give poor assimilation results when isolated (7) and, under those circumstances, resemble *C. zeylanoides*. On continued cultivation, however, *C. tropicalis* returns to its normal biochemical pattern. The clinical strains reported here remained constant in their behavior with respect to their biochemical activities. Similarities in certain of the reactions elicited required differentiation of *C. zeylanoides* from *C. guillermondi*, *C. humicola*, and *C. lipolytica*, yeasts that assimilate glycerol readily. *C. zeylanoides* is less active than *C. guillermondi* and *C. humicola*; it does not assimilate arabinose, galactose, and methyl-D-glucoside, substrates readily consumed by the two other species. *C. lipolytica* is less active than *C. zeylanoides*; fewer strains of that yeast ferment sorbitol or assimilate *N*-acetylglucosamine, and none utilize trehalose.

The API profiles of the American Type Culture Collection cultures indicate an element of uncertainty in the recognition of the species, since only the API code for ATCC 7351 gave a profile identical to that of the clinical strains while ATCC 20356 differed appreciably by failing to assimilate 2-keto-D-gluconate, sorbitol, and trehalose. The same stock culture was more resistant to all of the antifungal agents, in contrast to the greater agreement between the clinical isolates and ATCC 7351 in this respect.

The results of the DNA typing studies confirm the clinical impression that this patient suffered at least two relapses of her initial infection (a total of three septic episodes). The demonstration of identical DNA restriction fragment profiles in isolates from each of the three septic episodes and the fact that they were significantly different from DNA profiles of epidemiologically unrelated strains of *C. zeylanoides* strongly suggest continued infection with the same strain of *C. zeylanoides* in this patient. Like other *Candida* spp., *C. zeylanoides* probably persisted attached to the Hickman catheter despite its susceptibility to amphotericin B, the agent used for therapy. The inability of antimicrobial agents to penetrate microbial biofilms has been demonstrated for bacteria (3). The survival of *C. zeylanoides* for 6 months suggests that biofilm formation on the Hickman catheter played a role in these repeated episodes. The recovery of this new intruder into the intimate biosphere suggests that other species of the genus unknown in clinical specimens may complicate the recovery of patients in the future, especially when the yeasts are provided the opportunity to colonize and form biofilms on devices supporting the therapy and nutrition of patients.

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