JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1988, p. 621–624
0095-1137/88/040621-04$02.00/0

Vol. 26, No. 4

Catheter-Associated *Candida utilis* Fungemia in a Patient with Acquired Immunodeficiency Syndrome: Species Verification with a Molecular Probe

AMARYLLIS ALSINA,[1] MARK MASON,[1] RONALD A. UPHOFF,[1] W. STUART RIGGSBY,[1,2] JEFFREY M. BECKER,[1,2]* AND DIANNE MURPHY[3]

Department of Microbiology[1] and Program in Cellular, Molecular, and Developmental Biology,[2] University of Tennessee, and Division of Infectious Diseases, Department of Pediatrics, University of Tennessee Medical Center at Knoxville,[3] Knoxville, Tennessee 37996

Received 5 October 1987/Accepted 22 December 1987

***Candida utilis* was cultured from the blood of a patient with acquired immunodeficiency syndrome. The candidemia was apparently associated with catheter implantation. The isolate was identified initially by standard methods and verified by molecular probing. The pattern of actin-specific restriction fragments obtained from the DNA of the isolate, probed with *C. albicans* actin sequences, corresponded to that of *C. utilis*. This organism adds to the growing list of *Candida* species associated with human disease. Molecular probing offers a definitive identification when an unexpected etiological agent is found.**

Candidiasis is the most common opportunistic fungal infection in humans (11, 16). Immunocompromised individuals, such as patients with acquired immunodeficiency syndrome, are susceptible to infections of this type (10). Seven *Candida* species, *C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, and *C. tropicalis* as well as *Torulopsis glabrata* are regarded as the principal medically important species of *Candida* and *Torulopsis* (11). The issue of the taxonomic status of the genera *Candida* and *Torulopsis* has been described by McGinnis et al. (7). *C. albicans* is the most frequent cause of fungemia, and *C. tropicalis* and *C. parapsilosis* have been noted as increasingly frequent clinical isolates (8). Less common agents of fungemia include *C. lusitaniae* (2, 17), *C. rugosa* (14, 19), and *T. candida* (18), although *C. lusitaniae* may also be regarded as being in the emerging agent category (4).

Candidemia may be the result of a persistent or transient colonization. Intravenous catheters and other foreign devices implanted in a susceptible host are known to act as a nidus for the infectious agent (2, 14, 21). We present in this report a case, in a patient with acquired immunodeficiency syndrome, of catheter-associated candidemia caused by *C. utilis*. This isolate was initially identified by standard methods (3) and subsequently confirmed by molecular probing with the *C. albicans* actin gene (6). To our knowledge, this is the first reported case of a catheter-associated fungemia caused by *C. utilis*. This yeast species is known for its industrial applications (12, 13) and has not been associated with human disease.

CASE REPORT

A 5-year-old male hemophiliac was admitted to the University of Tennessee Medical Center at Knoxville on 27 December 1986 with the diagnosis of acquired immunodeficiency, *C. albicans* esophagitis, *Cryptosporidium* gastroenteritis, and increasing abdominal pain. The patient had been on parenteral hyperalimentation via a central line catheter since July 1986. The patient had had several episodes of pancreatitis and had chronic hepatitis B surface antigenicity. Liver functions were grossly abnormal. He had been on oral nystatin and ketoconazole for *Candida* esophagitis. The patient was neutropenic and mildly thrombocytopenic. Because of recurrent fevers, multiple blood cultures had been drawn between June 1986 and January 1987 for bacterial and fungal cultures and were negative. On 3 January 1987, a fever of 103°F (39.4°C) prompted repeat collection of blood cultures and initiation of nafcillin and gentamicin, which were stopped on 9 January. When the fever returned on 12 January and a physical examination revealed no source for the fever, cultures were obtained and a trial of trimethoprim-sulfamethoxazole was begun. Ongoing evaluation revealed increased radioactive uptake only in the small bowel as would be expected with enteritis.

Between 13 and 15 January, three separate blood cultures revealed *Streptococcus faecalis*, *Acinetobacter* species, and a yeastlike organism. Trimethoprim-sulfamethoxazole treatment was discontinued, and clindamycin, ampicillin, gentamicin, and amphotericin B treatments were instituted. Because of the underlying bleeding disorder, thrombocytopenia, and platelet dysfunction of the patient, it was elected to leave the central catheter in place and attempt therapy via central catheter administration. The patient became afebrile on 17 January 1987. Amphotericin B was increased by 0.1 mg/kg per day to a maximum dose of 16 mg (0.5 mg/kg per day). After 5 months of therapy, ketoconazole was discontinued on 20 January when the yeast strain was tentatively identified as *C. utilis*. A blood culture from 20 January was still positive for *C. utilis* (amphotericin B dose was 8 mg at that point). On 22 January, the patient experienced another fever of 103°F but was found to have an acute episode of pancreatitis with an amylase level of 375 U/liter and a lipase level of 7,000 U/liter (Ektachem 7000, Kodak Laboratories). The patient continued to have severe bleeding problems requiring multiple infusions of platelets, factor VIII, and intravenous immunoglobulin and continued to be febrile. Cultures of blood samples drawn from the patient on

* Corresponding author.

27 and 30 January and 3, 5, 6, 8, and 10 February were positive for *C. utilis*. Of a total of 17 blood cultures obtained between 14 January and 10 February, there were a total of 11 cultures positive for *C. utilis*. Amphotericin B levels in serum were documented to be between 1 and 2.4 μg/ml. The susceptibility of the organism, as determined both by W. G. Merz at the John Hopkins University and by us, was to 0.5 to 1 μg/ml. Flucytosine was added to the treatment protocol on 9 February. Five days later, the central line was removed, amphotericin B was administered for 2 h, and a new central line was placed. Amphotericin B and flucytosine were continued for nine more days for a total of 32 days of amphotericin B. All antigen and antibody tests for *C. albicans* were negative. The patient experienced an episode of *Escherichia coli* sepsis during the last few days of amphotericin B therapy. Despite continuing fevers between 12 February and 1 March 1987, 20 negative blood cultures were obtained. Between 1 March and the death of the patient on 11 April 1987, 19 more blood cultures yielded only one yeast-positive culture. This yeast strain was identified as *C. parapsilosis* by conventional procedures. Cultures were obtained from both central catheters and peripheral venipunctures.

MATERIALS AND METHODS

Microscopic morphology on Sabouraud glucose agar and cornmeal agar was evaluated by standard methods (3). Carbon assimilation patterns were determined with the API 20C (Analytab Products, Inc., Plainview, N.Y.) yeast identification system (1). Utilization of nitrate as a sole nitrogen source was evaluated by direct inoculation into yeast carbon base broth (Difco Laboratories, Detroit, Mich.) containing 0.5% potassium nitrate.

Molecular probing of the yeast DNA with the *C. albicans* actin gene was performed as described previously (6). The yeast DNA was digested with several hexaschisomers and electrophoresed in agarose. The resulting DNA fragments were transferred to nitrocellulose membranes by the Southern procedure (6) and probed with ^{32}P-labeled actin DNA from *C. albicans*. The resulting patterns were compared with those of various known *Candida* species. All strains used were from the culture collection of W. S. Riggsby or J. M. Becker. The identities of the strains were verified by standard procedures.

Susceptibility testing was performed by the broth dilution method (2). Amphotericin B was tested in yeast nitrogen base (Difco) buffered with 3-[*N*-morpholino]propanesulfonic acid (pH 7) containing 0.5% ammonium sulfate and 2% glucose. *C. albicans* H317 was used as the control strain (15).

Blood cultures were obtained and processed in the following manner. Both peripheral cutaneous venipunctures and catheter specimens were obtained after the site was prepared with a 10% povidone-iodine solution which was allowed to dry. Sterile technique and equipment were also used. The specimen was then injected into a Bactec blood collection bottle (aerobic blood culture bottle containing tryptic soy broth). The bottles were sampled every day for radioactive ^{14}C production. Once a growth index of 35 was obtained, a portion of the specimen was aseptically removed and evaluated with a Gram stain. If any organism resembling yeasts was identified, further samples were inoculated onto 5% sheep blood agar, chocolate agar, and Sabouraud glucose agar. If no organism was seen, a sample was inoculated onto chocolate agar.

Tests for antibody against *Candida* mannan and cytoplasmic components were performed by Smith and Kline Biologics Laboratories, Philadelphia, Pa. Antibody tests were also done by the Centers for Disease Control, Atlanta, Ga., by immunodiffusion and latex agglutination. Antigen tests were done by the Centers for Disease Control by the enzyme-linked immunosorbent assay.

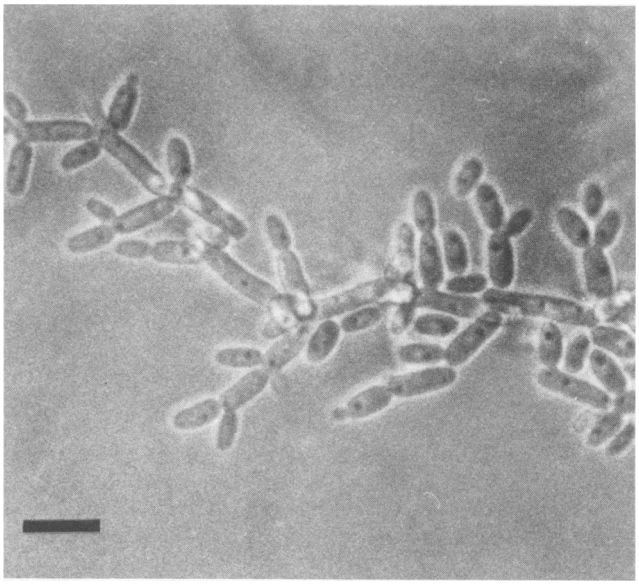

FIG. 1. Micrograph of clinical isolate grown on cornmeal agar. Bar, 10 μm.

RESULTS

The morphological and physiological data gathered on the isolate correlated well with those of *C. utilis* (9). On Sabouraud glucose agar, the yeasts appeared as smooth, cream colonies after 24 h of incubation at 30°C. The yeasts did not form germ tubes. Microscopic examination revealed globose to ovoid cells. Cultures on cornmeal agar inoculated by the Dalmau method showed filamentous growth consisting of chains of elongated blastoconidia. Ovoid cells were also present (Fig. 1). This yeast strain was able to grow at 37°C, and the MIC of amphotericin B was 0.52 μg/ml.

The biocode resulting from the API 20C assimilation test (6400033) was not found in the API 20C profile index. Indeed, *C. utilis* is not part of the API 20C data base. Direct readings from the API 20C strip indicated that this yeast strain was able to assimilate glucose, glycerol, xylose, maltose, sucrose, melezitose, and raffinose and none of the remaining 11 carbon compounds. These characteristics were compatible with those of *C. utilis* except for cellobiose assimilation, which is noted as positive according to the standard description (9).

Molecular probing verified the identity of the isolate as *C. utilis*. The various DNA fragments containing actin gene sequences generated after treatment of cellular DNA with restriction endonucleases are shown in Fig. 2. The restriction pattern obtained with each enzyme corresponded to those obtained with an authentic strain of *C. utilis* (ATCC 9226). The pattern generated from probing the clinical isolate did not correspond to those previously reported (6) for *C. albicans* H317, *C. krusei* ATCC 6258, *C. guilliermondii* SR1207, *C. parapsilosis* ATCC 22019, *C. pseudotropicalis* ATCC 2512, *C. stellatoidea* ATCC 11006, or *C. tropicalis* ATCC 13803.

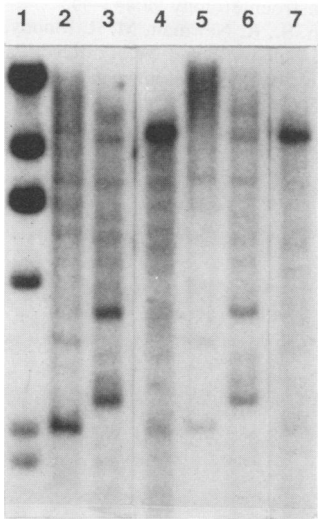

FIG. 2. Restriction fragment analysis. DNA isolated from *C. utilis* ATCC 9226 (lanes 2, 3, and 4) or from the clinical isolate (lanes 4, 5, and 6) was digested with restriction endonuclease *Eco*RI (lanes 2 and 5), *Hin*dIII (lanes 3 and 6), or *Pvu*II (lanes 4 and 7). Southern blotting was done as described previously (6). Lane 1 contains a *Hin*dIII digest of bacteriophage lambda DNA as markers.

DISCUSSION

Persistent episodes of candidemia have been associated with the presence of intravenous catheters (14, 21). The immunosuppressed state of the patient with acquired immunodeficiency syndrome in our report obviously facilitated the colonization observed. How the patient acquired the organism is not clear. All fluids used for injection were inspected and cultured and none grew *C. utilis*. Only rarely has *C. utilis* been isolated as a contaminant from clinical materials (e.g., sputum, vaginal discharge) (12). This organism has been shown to have low pathogenicity even in immunosuppressed mice (5).

While the catheter remained, the patient experienced persistent candidemia even though the in vitro MIC indicated susceptibility to amphotericin B at levels documented to be present in the serum of the patient. Patients with acquired immunodeficiency syndrome have been noted to have difficulty in clearing *Candida* infections even when on appropriate and normally adequate antifungal therapy (20). When the intravenous catheter was removed, the infection cleared with only nine additional days of amphotericin B therapy.

Until now, *C. utilis* had not been associated, to our knowledge, with human infection. Since we based our initial identification on morphological and physiological data which contained one anomalous assimilation character, we decided to verify it by methods based on genotypic properties. The pattern of actin-specific restriction fragments obtained from the nuclear DNA of the isolate corresponded to that of *C. utilis*. This molecular probe provides definitive identification of *Candida* species. It may be particularly useful when standard methods do not allow a precise identification, or, as here, when an unexpected etiological agent is involved. In an era in which medical therapy involves increasingly extensive use of antibiotics and immunosuppressive drugs, novel infectious agents may be expected to emerge. In recent years, for example, documented cases of fungemia caused by yeasts such as *T. candida* and *C. rugosa* have been reported (14, 18, 19). This case of *C. utilis* fungemia adds to the list of novel agents related to human disease. It further stresses the importance to vigilence for emerging new opportunistic fungal pathogens.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-14387 from the National Institute of Allergy and Infectious Diseases.

We thank Diane Walker, senior laboratory technician at the University of Tennessee Medical Center at Knoxville, for her persistent and astute efforts and observations.

LITERATURE CITED

1. **Buesching, W. J., K. Kurek, and G. D. Roberts.** 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. J. Clin. Microbiol. **4**:565–569.
2. **Christenson, J. C., A. Guruswamy, G. Mukwaya, and P. J. Rettig.** 1987. *Candida lusitaniae*: an emerging human pathogen. Pediatr. Infect. Dis. J. **6**:755–757.
3. **Cooper, B. H., and M. Silva-Hutner.** 1985. Yeasts of medical importance, p. 526–541. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. **Hadfield, T. L., M. B. Smith, R. E. Winn, M. G. Rinaldi, and C. Guerra.** 1987. Mycoses caused by *Candida lusitaniae*. Rev. Infect. Dis. **9**:1006–1012.
5. **Holzshu, D. L., F. W. Chandler, L. Ajello, and D. G. Ahearn.** 1979. Evaluation of industrial yeasts for pathogenicity. Sabouraudia **17**:71–78.
6. **Mason, M. M., B. A. Lasker, and W. S. Riggsby.** 1987. Molecular probe for identification of medically important *Candida* species and *Torulopsis glabrata*. J. Clin. Microbiol. **25**:563–566.
7. **McGinnis, M. R., L. Ajello, E. S. Beneke, E. Drouhet, N. L. Goodman, C. J. Halde, L. D. Haley, J. Kane, G. A. Land, A. A. Padhye, D. H. Pincus, M. G. Rinaldi, A. L. Rogers, I. F. Salkin, W. A. Schell, and I. Weitzmann.** 1984. Taxonomic and nomenclatural evaluation of the genera *Candida* and *Torulopsis*. J. Clin. Microbiol. **20**:813–814.
8. **Merz, W. G., J. E. Karp, D. Schron, and R. Saral.** 1986. Increased incidence of fungemia caused by *Candida krusei*. J. Clin. Microbiol. **24**:581–584.
9. **Meyer, S. A., D. G. Ahearn, and D. Yarrow.** 1984. Genus 4. *Candida* Berkhout, p. 822. *In* N. J. W. Kreger-van Rij (ed.), The yeasts: a taxonomic study, 3rd ed. Elsevier Science Publishers B. V., Amsterdam.
10. **Mildvan, D., U. Mathur, R. W. Enlow, P. L. Romain, R. J. Winchester, C. Colp, H. Singman, B. R. Adelsberg, and I. Spigland.** 1982. Opportunistic infections and immune deficiency in homosexual men. Ann. Intern. Med. **96**:700–704.
11. **Odds, F. C.** 1979. Candida and candidosis. Leicester University Press, Leicester, England.
12. **Phaff, H. J.** 1985. Biology of yeasts other than *Saccharomyces*, p. 537–562. *In* A. L. Demain and N. A. Solomon (ed.), Biology of industrial microorganisms. Benjamin Cummings Publishing Co., Inc., Menlo Park, Calif.
13. **Prior, B., S. Kilian, and P. Lategan.** 1980. Growth of *Candida utilis* on ethanol and isopropanol. Arch. Microbiol. **125**:133–136.
14. **Reinhardt, J. F., P. J. Ruane, L. J. Walker, and L. George.** 1985. Intravenous catheter-associated fungemia due to *Candida rugosa*. J. Clin. Microbiol. **22**:1056–1057.
15. **Riggsby, W. S., L. J. Torres-Bauza, J. W. Wills, and T. M. Townes.** 1982. DNA content, kinetic complexity and the ploidy question in *Candida albicans*. Mol. Cell. Biol. **2**:853–862.
16. **Rippon, J. W.** 1982. Medical mycology, p. 484–531. The W. B. Saunders Co., Philadelphia.
17. **Sanchez, P. J., and B. H. Cooper.** 1987. *Candida lusitaniae*: sepsis and meningitis in a neonate. Pediatr. Infect. Dis. J. **6**:758–759.
18. **St.-Germain, G., and M. Laverdiere.** 1986. *Torulopsis candida*,

a new opportunistic pathogen. J. Clin. Microbiol. **24**:884–885.
19. **Sugar, A. M., and D. A. Stevens.** 1985. *Candida rugosa* in immunocompromised infection. Cancer **56**:318–320.
20. **Tavitian, A., J.-P. Raufman, L. E. Rosenthal, J. Weber, C. A. Webber, and H. P. Dincsoy.** 1986. Ketoconazole-resistant *Candida* esophagitis in patients with acquired immunodeficiency syndrome. Gastroenterology **90**:443–445.
21. **Tchekmedyian, S., K. Newman, M. R. Moody, W. Costerton, J. Aisner, S. C. Schimpff, and W. P. Reed.** 1986. Case report: special studies of the Hickman catheter of a patient with recurrent bacteremia and candidemia. Am. J. Med. Sci. **29**: 419–424.