MAJOR ARTICLE

Candida dubliniensis at a Cancer Center

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***Candida dubliniensis,* a germ tube–positive yeast first described and identified as a cause of oral candidiasis in patients with acquired immunodeficiency syndrome in Europe in 1995, has an expanding clinical and geographic distribution that appears to be similar to that of the other germ tube–positive yeast, *Candida albicans.* This study determined the frequency, clinical spectrum, drug susceptibility profile, and suitable methods for identification of this emerging pathogen at a cancer center in 1998 and 1999. Twenty-two isolates were recovered from 16 patients with solid-organ or hematologic malignancies or acquired immunodeficiency syndrome. Two patients with cancer had invasive infection, and 14 were colonized with fungus or had superficial fungal infection. All isolates produced germ tubes and chlamydospores at 37°C, did not grow at 45°C, and gave negative reactions with D-xylose and α-methyl-D-glucoside in the API 20 C AUX and ID 32 C yeast identification systems. Phenotypic identification was confirmed by molecular beacon probe technology. All isolates were susceptible to the antifungal drugs amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, and ketoconazole.**

Before 1995, all germ tube–positive *Candida* species were identified as *Candida albicans.* In 1995, a second germ tube–positive *Candida* species, *Candida dubliniensis,* was reported to have colonized the oral cavity and caused oral candidiasis in HIV-positive patients in Ireland [1]. In 2 subsequent retrospective studies from Europe, ~5% of yeast isolates that had initially been identified as *C. albicans* were identified as *C. dubliniensis* [2, 3].

Since the initial report, *C. dubliniensis* has been recovered from patients worldwide from a variety of sources, including the oral cavity, urine, vagina, lung, feces, and sputum, in both HIV-positive and HIV-negative patients [2, 4–7]. Recently, 2 articles reported cases of *C. dubliniensis* fungemia [8, 9]. Most isolates of *C. dubliniensis* have been susceptible in vitro to commonly used antifungal agents. However, resistance to fluconazole has been reported [5, 10, 11], and stable fluconazole resistance can be induced in vitro after exposure to the drug [10].

Since 1995, advances in phenotypic and genotypic methods for yeast identification have helped to define differences between *C. dubliniensis* and *C. albicans* and to establish suitable methods for differentiating the yeasts in the microbiology laboratory. *C. dubliniensis* appears not to grow at 45°C, whereas most isolates of *C. albicans* do [6]. Also, an analysis of yeast identification systems showed that *C. dubliniensis* usually does not assimilate α-methyl-D-glucoside, trehalose, and D-xylose, whereas *C. albicans* usually does [12–14]. DNA-based molecular methods have also helped differentiate the 2 species of *Candida* [15, 16].

To determine the frequency, clinical spectrum, drug susceptibility profiles, and suitable methods for identification of this emerging pathogen at a cancer center, all cases in which *C. dubliniensis* was identified at Memorial Sloan-Kettering Cancer Center (MSKCC), New

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York City, a tertiary-care cancer center, during 1998 and 1999 were reviewed.

PATIENTS AND METHODS

Patients. The names and medical record numbers of all patients with at least 1 culture positive for *C. dubliniensis* in 1998 and 1999 were obtained from MSKCC. The medical records of these patients were abstracted by use of a standardized data extraction form. Invasive fungal infection was defined by clinical evidence of blood or tissue infection and a culture or biopsy specimen from the involved site demonstrating *C. dubliniensis* [17]. Patients were not considered to have an invasive infection when *C. dubliniensis* was isolated in a culture in the absence of any clinical symptoms or signs of infection (colonization) or from a mucosal surface in association with signs of inflammation, ulcerations, plaques, or exudates without evidence of tissue invasion (superficial fungal infection). Exposure to antifungal drugs included the administration of fluconazole, itraconazole, amphotericin B (including lipid-associated forms), clotrimazole troches, or 5-fluorocytosine in the 6 months preceding the isolation of *C. dubliniensis.*

The duration of cancer or HIV infection was marked from the time of diagnosis of the underlying primary disease to the time of *C. dubliniensis* isolation. The duration of follow-up was marked from the time *C. dubliniensis* was isolated to the time of the last follow-up, through 31 January 2000, or to the time of death or loss to follow-up. For the assessment of outcome, death was defined as death during the hospitalization during which *C. dubliniensis* was first identified or death within 90 days of initial isolation of *C. dubliniensis.*

Yeast isolates. To determine the prevalence and incidence of *C. dubliniensis* at MSKCC in 1998, 100 randomly selected isolates of germ tube–positive yeasts were prospectively identified by the phenotypic microbiological methods described below. In addition, 131 germ tube–positive yeasts isolated from sterile sites previously identified as *C. albicans* were retrospectively examined. Finally, beginning in April 1999, all germ tube–positive yeasts were routinely identified as either *C. albicans* or *C. dubliniensis.*

Phenotypic identification tests. Tests to presumptively identify yeast included germ-tube formation at 37°C in horse serum (Life Technologies), chlamydospore production on cornmeal agar with polysorbate 80 (Becton Dickinson), growth at 45°C on Sabouraud dextrose agar (Becton Dickinson), and the substrate reactivity profiles obtained with the API 20 C AUX and ID 32 C systems (bioMérieux Vitek).

Genotypic identification tests. A panel of clinical isolates of *C. albicans* and *C. dubliniensis* was prepared at MSKCC and sent for blinded molecular evaluation to the Public Health Research Institute, New York. Molecular evaluation of the ribosomal ITS2 region of the yeast was used to independently verify the species identification obtained by phenotypic characterization [18, 19]. After PCR amplification of the ITS2 region of the yeast, species-specific DNA sequence information was assessed with the use of both molecular beacons and restriction enzymes [20].

Antifungal susceptibility testing. Tests were performed at the New York State Mycology Laboratory according to the National Committee for Clinical Laboratory Standards M27-A broth microdilution protocol [21]. *Candida krusei* ATCC 62258 and *Candida parapsilosis* ATCC 22019 were used as quality-control strains. Amphotericin B and 5-fluorocytosine were purchased commercially (Sigma Chemical). Fluconazole, ketoconazole, and itraconazole were received as gifts from Pfizer Inc. and Janssen Pharmaceutica, respectively. Stock drug solutions were made either in dimethyl sulfoxide or sterile water. RPMI 1640 containing MOPS (morpholinepropanesulfonic acid) buffer and without sodium bicarbonate was used as the drug diluent for preparation of stock microtiter plates, which were stored at −70°C. *C. dubliniensis* isolates were stored on potato dextrose agar slants at −70°C. Isolates for susceptibility testing were cultured serially twice overnight on modified Sabouraud dextrose agar plates at room temperature, and the inoculum was standardized spectrophotometrically. The microtiter plates, containing 100 μL each of drug dilution and inoculum per well, were incubated at 35°C, and the presence or absence of growth was examined visually at 48 h. The amphotericin B MIC was set at the drug concentration that completely inhibited yeast growth, whereas the MIC for the other drugs was set at the lowest concentration (80% MIC), which caused an apparent inhibition of yeast growth when compared to the growth control well.

RESULTS

Yeast isolates. In the 3 study periods and approaches, 22 (2%) of the 974 germ tube–positive yeasts were identified as *C. dubliniensis.*

Patients. Twenty-two isolates from 16 patients were identified (table 1). The median age of patients was 46 years, and 9 patients were men. Seven patients had solid-organ malignancies, 6 patients had hematologic malignancies, and 3 patients had AIDS. Six patients had received antifungal drugs before the isolation of *C. dubliniensis.* The median time from the diagnosis of cancer to the isolation of *C. dubliniensis* was ~3 years. The median time from the diagnosis of HIV infection to the isolation of *C. dubliniensis* was ~10 years. In 1 patient, thrush caused by *C. dubliniensis* was present at the time of diagnosis of HIV infection.

Table 1. Characteristics of 16 patients with *Candida dubliniensis* who were treated at Memorial Sloan-Kettering Cancer Center.

Patient	Age (years)/ sex	Underlying disease	Source of culture sample	Previous antifungal exposure	Outcome
1	48/M	Lung cancer	Sputum	No	Death
2	81/F	CML	Stool	No	Death
3	48/F	Lung cancer; Hodgkin's disease	Sputum	No	Alive
4	65/F	Breast cancer	Urine	No	Alive
5	46/M	NHL, S/P BMT	Stool	Yes	Alive
6	75/M	Ampullar tubulovillous adenoma	Bile	No	Alive
7	58/F	Breast cancer	Bronchial washing	No	Alive
8	46/M	AIDS; COPD	Sputum	Yes	Alive
9	28/F	HIV	Tongue	No	Alive
10	29/M	NHL	Sputum, stool	Yes	Alive
11	31/M	AIDS; CML, S/P BMT (>15 years previously)	Sputum	Yes	Alive
12	31/M	CML, S/P BMT	Tracheal aspirate, stool	Yes	Death
13	71/M	NHL	Mouth	No	Death
14	29/M	Rectal cancer	Pelvic abscess	No	Alive
15[a]	1/F	Neuroblastoma	Blood	Yes	Alive
16[a]	50/F	Multiple myeloma	Synovial fluid	No	Death

NOTE. Positive cultures from the same source from the same patients are not duplicated in the table. CML, chronic myelogenous leukemia; COPD, chronic obstructive pulmonary disease; F, female; M, male; NHL, non-Hodgkin's lymphoma; S/P BMT, status post bone marrow transplant.

[a] Patients with invasive disease.

Two of the 16 patients had invasive *C. dubliniensis* infection, and 14 patients were thought either to be colonized with *C. dubliniensis* or to have superficial fungal infection originating from the oropharynx. The 2 patients with invasive disease had cancer. The first patient was a 1-year-old girl with adrenal neuroblastoma that metastasized to the liver. She was admitted with fever and chemotherapy-induced neutropenia. Two days after admission, she became septic and was transferred to the intensive care unit. *C. dubliniensis* was recovered from 2 sets of blood cultures. The patient was treated successfully with iv lipid-associated amphotericin B. The second patient was a 50-year-old woman with refractory multiple myeloma who was admitted with persistent right knee effusion. Arthrocentesis failed to show malignant cells and demonstrated the presence of inflammatory arthritis. Cultures of synovial fluid were positive for *C. dubliniensis*. The patient was treated with iv lipid-associated amphotericin B. She died 3 weeks later of sepsis.

Five (31%) of the 16 patients died, including 1 patient with invasive disease. None of the deaths appeared to be caused by infection with *C. dubliniensis,* although autopsy was performed on only 2 patients.

Phenotypic identification. All isolates of *C. dubliniensis* produced germ tubes and chlamydospores at 37°C, did not grow at 45°C, and gave assimilation profiles with the API 20 C AUX and ID 32 C systems, including negative reactions for D-xylose and α-methyl-D-glucoside, which is suggestive of *C. dubliniensis*.

Genotypic identification. Both molecular beacon and restriction enzyme analyses distinguished between reference *Candida* species and confirmed all *C. dubliniensis* strains identified by phenotype in this study.

Antifungal susceptibility tests. All the isolates of *C. dubliniensis* were susceptible to the antifungal drugs amphotericin B (MIC, 0.12–1.0 μg/mL), 5-fluorocytosine (MIC, <0.06–0.25 μg/mL), fluconazole (MIC, 0.06–2.0 μg/mL), itraconazole (MIC, <0.03–0.06 μg/mL), and ketoconazole (MIC, <0.03–0.03 μg/mL).

DISCUSSION

C. dubliniensis appears to be a minor component of the normal oral flora of humans. Fewer than 10% of germ tube–positive yeasts in culture collections or from the oral cavities of healthy individuals have been identified as *C. dubliniensis* [22]. However, as with other yeasts, immunosuppression and the use of antimicrobials permit *C. dubliniensis* to increase

Table 2. Summary of reported cases of invasive infection caused by *Candida dubliniensis*.

Reference, patient age (years)/sex	Source of culture sample	Underlying disease	Clinical symptoms	Treatment	Outcome[a]
Meis et al. [8]					
39/F	Blood	CML, S/P Allo-BMT	Fever, ascites	iv Fluconazole	Death (3 weeks)
5/M	Blood	Nasopharyngeal rhabdomyosarcoma	Fever, diarrhea	iv Fluconazole	Death (1 year)
8/F	Blood	Sickle cell anemia, S/P Allo-BMT	Sepsis with ARF	AmB + 5-FC; fluconazole	Resolved
Brandt et al. [9]					
74/M	Blood	CLL	Multiple organ failure	NA	Death (1 day)
32/F	Blood	End-stage liver disease	Gastrointestinal bleeding, ascites	iv Fluconazole	Death (24 days)
39/M	Blood	End-stage liver disease	ARF, ascites	iv Fluconazole	Death (5 days)
37/F	Blood	AIDS	Fever, chills	Oral fluconazole	NA
Current report					
1/F	Blood	Neuroblastoma	Fever	Lipid AmB	Resolved
50/F	Synovial fluid	Multiple myeloma	Arthritis of the knee	Lipid AmB	Death (3 weeks)

NOTE. 5-FC, 5-fluorocytosine; AmB, amphotericin B; ARF, acute renal failure; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; F, female; lipid AmB, lipid-associated AmB; M, male; NA, not available; S/P Allo-BMT, status post allogeneic bone marrow transplantation.

[a] Numbers within parentheses reflect the time from the isolation of *C. dubliniensis* to death.

in numbers and eventually to cause oral candidiasis. Approximately 25% of HIV-infected patients may be colonized with the yeast, and *C. dubliniensis* has been isolated from the oral cavity of ~30% of patients with AIDS and oral candidiasis [23]. *C. dubliniensis* was implicated as a pathogen in linear gingival erythema in an HIV-infected child [24]. Although several independent reports have described the recovery of *C. dubliniensis* from patients with HIV infection and from those without since 1995, it was not until recently that invasive disease was reported. In 1999, *C. dubliniensis* was reported as a cause of fungemia in 2 recipients of bone marrow transplantations and in 1 patient with chemotherapy-induced neutropenia [8]. In the year 2000, 4 cases of *C. dubliniensis* were reported; 2 patients had end-stage liver disease, 1 patient had chronic lymphocytic leukemia, and 1 patient was HIV positive [9] (table 2).

In the present study, ~2% (22 of 974) of cases of infection by germ tube–positive yeast were identified as caused by *C. dubliniensis*, although this study was not exclusively prospective. Sixteen patients from whom the yeast was isolated had hematologic malignancies, solid-organ tumors, or AIDS. The organism was isolated not only from oral secretions but also from blood, bile, synovial fluid, sputum, bronchial washings, urine, stool, and abscess fluid. Fourteen patients either were colonized with the yeast or had superficial fungal infection. Two patients had invasive fungal infection, thus bringing the number of reported cases of invasive disease to 9 (table 2).

The majority of *C. dubliniensis* isolates have been susceptible in vitro to commonly used antifungal agents. However, isolates with reduced susceptibility to fluconazole have been recovered from patients with AIDS who had previously been exposed to fluconazole. In one study, 20% of oral isolates recovered from patients with AIDS who had been exposed to fluconazole were resistant to fluconazole [10]. In the same study it was also shown that *C. dubliniensis* can develop resistance to fluconazole after direct exposure to the drug in vitro. Sequential exposure of fluconazole-susceptible clinical isolates to increasing concentrations of fluconazole in an agar medium resulted in the recovery of yeast that expressed a stable fluconazole-resistant phenotype. It was suggested that *C. dubliniensis* may encode multidrug transporters similar to those encoded by *C. albicans* genes *MDR1*, *CDR1*, and *CDR2* [25]. In the present study, all isolates were sensitive to all antifungals tested, including fluconazole, even isolates from patients who had been exposed to fluconazole.

Thus, *C. dubliniensis* is an emerging opportunistic fungal pathogen that can cause invasive disease in patients with a variety of clinical conditions, including cancer and HIV infection. It appears that the clinical spectrum of *C. dubliniensis* resembles that of *C. albicans*. However, the in vitro suggestion that *C. dubliniensis* may be able to develop azole resistance should lead to heightened efforts to look for this germ tube–positive yeast. Future studies will determine whether this pathogen will become a significant cause of illness among immunocompromised patients

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