Prevalence of *Candida dubliniensis* Isolates in a Yeast Stock Collection

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To establish the historical prevalence of the novel yeast species *Candida dubliniensis*, a survey of 2,589 yeasts originally identified as *Candida albicans* and maintained in a stock collection dating back to the early 1970s was undertaken. A total of 590 yeasts, including 93 (18.5%) β-glucosidase-negative isolates among 502 isolates that showed abnormal colony colors on a differential chromogenic agar and 497 other isolates, were subjected to DNA fingerprinting with the moderately repetitive sequence Ca3. On this basis, 53 yeasts were reidentified as *C. dubliniensis* (including the *C. dubliniensis* type strain, included as a blind control in the panel of yeasts). The 52 newly found isolates came from 36 different persons, and a further 3 *C. dubliniensis* isolates were detected by DNA fingerprinting of previously untested isolates from one of these individuals. The prevalence of *C. dubliniensis* among yeast in oral and fecal samples was significantly higher than that among yeasts from other anatomical sites and was significantly higher among human immunodeficiency virus (HIV)-infected individuals than among known or presumed HIV-negative individuals. However, a single vaginal isolate and two oral isolates from healthy volunteers confirmed that the species is restricted neither to gastrointestinal sites nor to patients with overt disease. The oldest examples of *C. dubliniensis* were from oral samples of three patients in the United Kingdom in 1973 and 1975. In comparison with age-matched control isolates of *C. albicans*, the *C. dubliniensis* isolates showed slightly higher levels of susceptibility in vitro to amphotericin B and flucytosine and slightly lower levels of susceptibility to three azole antifungal agents.

*Candida dubliniensis* is a newly described yeast species that is closely related to *Candida albicans* (25). The new species forms germ tubes and chlamydospores that are almost indistinguishable from those of *C. albicans*, and definitive identification of *C. dubliniensis* requires evidence of nonreactivity of its DNA with the *C. albicans*-specific oligonucleotide probe Ca3 (25). *C. dubliniensis* appears to have a worldwide distribution (23, 24). It has been found primarily in oral samples from persons infected with the human immunodeficiency virus (HIV) (3, 22–25), but it has also been isolated from vaginal samples from HIV-negative and HIV-positive women (23, 25). Isolates of *C. dubliniensis* rapidly develop a stable fluorescein-resistant phenotype on exposure to this antifungal agent in vitro (6).

Recognition of phenotypic characteristics that allow simple detection and differentiation of *C. dubliniensis* remains a problem in routine yeast identification. Colonies of *C. dubliniensis* often have an unusually dark green color within 48 h at 37°C when freshly isolated from clinical material on the differential medium CHROMagar Candida, but this property is not retained in subculture (21). We have observed informally that *C. dubliniensis* colonies usually show a dark green colony phenotype on this medium, even in subcultures, when incubation is prolonged for 4 days or more. However, the normally pale green color of some *C. albicans* colonies can also darken under these circumstances, so that the color is no longer specific to *C. dubliniensis*. Indeed, with prolonged incubation other color aberrations such as yellowing and purpling have occasionally been noted with *C. albicans* isolates (unpublished observations). Another phenotypic characteristic specific for *C. dubliniensis* is a negative result in tests for intracellular β-glucosidase activity (1, 21, 25). However, in its present form this test is too expensive and complex to be used routinely for the screening of yeast isolates on a large scale. Recently, the absence of growth of *C. dubliniensis* at 45°C has been shown to be a simple and reliable characteristic for differentiation of this species from *C. albicans* (17).

The earliest reports of “unusual” and “atypical” *C. albicans* isolates that can now be reliably recognized from their DNA fingerprints as representing isolations of *C. dubliniensis* date back to 1990 (19), although older examples of “atypical” *C. albicans* isolates that may be examples of *C. dubliniensis* are known (22). The oldest confirmed isolate of the species came from a yeast culture collection where it was deposited in 1957 as *Candida stellatoidea* (23, 25). The prevalence of the species in clinical material before the mid-1990s is so far unknown. The present survey was therefore undertaken to examine the prevalence of isolates of *C. dubliniensis* existing in a large stock collection of yeasts predominantly obtained from human material. The findings show that isolates of *C. dubliniensis* from clinical material date back at least as far as 1973 and that although the species seems to be particularly associated with HIV-infected individuals, it has also been isolated in the past from HIV-negative subjects as well.

MATERIALS AND METHODS

Cultures and reidentification. The 2,589 yeasts studied had been stored since the early 1970s. The isolates had originally been identified phenotypically as *C. albicans* on the basis of a positive germ tube test, together with other standard morphologic and physiologic criteria when necessary. A total of 1,349 isolates had been maintained in sterile distilled water (7), and 1,240 had been kept at −70°C in 10% glycerol. Most of the yeasts were originally isolated from clinical material; a small number came from other culture collections or from inanimate sources. Some of the yeasts have been the subject of previous publications, including several epidemiologic studies of *C. albicans* from various patient groups, anatomic sources, and geographical locations (2, 8, 9, 11–14, 16, 19). Among the 2,589 yeasts, 81 (3.1%) were originally isolated in the 1970s, 990 (38.2%) were originally isolated in the 1980s, and 1,216 (47.0%) were originally isolated between 1990 and 1996. For 302 of the specimens (11.7%) the date of isolation was unknown.

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isolation was unknown. The number of yeasts from 1995 and 1996 was unusually small, only 66 isolates, since most of the C. albicans and C. dubliniensis isolates obtained in those years were the subject of a prospective study that has already been described (21) and were therefore not included as part of the present retrospective study. The exception was the type strain of C. dubliniensis, which was included in the panel under its collection number as a control for the blind detection of C. dubliniensis.

The majority of the yeasts were isolated in European countries (2,089 isolations; 80.7%), with those from the United Kingdom (30.9% of isolates) and Belgium (29.1%) being particularly heavily represented. Oropharyngeal rinses or swabs were the most common source material for the isolates (41.5%), with female genitalia (12.3%), feces or anal swabs (7.4%), and the skin (6.5%) the next most commonly represented sites of isolation. The isolates came mainly from patients in gynecology or genitourinary clinics (19.3%), hematology units (16.7%), and specialized HIV clinics (15.2%), while intravenous drug abusers (7.6% of the isolates) and dermatology patients (4.6%) were also well represented among the clinical settings for individuals from whom the isolates were obtained. Strains were not preselected. Since all isolates originally identified as C. albicans were screened, repeated isolations from one or more anatomical sites in the same patient were not excluded.

The yeasts were cultured on CHROMagar Candida (CHROMagar Microbiology, Paris, France) and were incubated at 37°C for 4 to 6 days. Isolates whose green colony color on CHROMagar Candida differed from the characteristic leaf-green hue of C. albicans (10) were regarded as possible isolates of C. dubliniensis and were further characterized by tests for intracellular β-glucosidase activity (1, 21) and DNA fingerprinting by Southern blotting with the moderately repetitive, C. albicans-specific oligonucleotide sequence Ca3 (20, 21). A random selection of 896 isolates with the typical C. albicans green colony color was also tested by one or both of these methods. All isolates that gave negative results in the β-glucosidase tests were retested to confirm the negative result; only isolates that were negative on retesting were recorded as testing true negative by the test. All isolates that were tested by DNA fingerprinting and that showed only weak hybridization with the Ca3 probe in the region of high-molecular-weight bands were identified as C. dubliniensis. The tests were done by operators who knew only the stock reference number of each isolate and who were therefore blinded for possible biases concerning sources and other information about the isolates at the times that the retrospective screening tests were done.

Susceptibility testing. Isolates of C. dubliniensis were tested for susceptibility to amphotericin B, fluconazole, itraconazole, and ketoconazole by a spectrophotometric broth microdilution method (15) based on the U.S. National Committee for Clinical Laboratory Standards M27 reference method. For each C. dubliniensis isolate tested, a C. albicans isolate from the stock collection was tested in parallel; in each case the control isolate was the C. albicans isolate with the closest possible stock number to the corresponding C. dubliniensis isolate.

RESULTS

Reidentification of C. dubliniensis isolates. Among the 2,589 yeasts cultured on CHROMagar Candida, 502 (19.4%) demonstrated some abnormality of the green colony color characteristic of C. albicans after prolonged incubation at 37°C. Among these 502 isolates, 93 (18.5%) were negative in tests for β-glucosidase activity. Among the 2,087 yeasts whose colonies retained the color characteristic of C. albicans even after prolonged incubation, 896 were randomly tested for intracellular β-glucosidase activity and 30 (3.3%) were negative. These results indicate a significant association between abnormal colony color and negative β-glucosidase tests ($\chi^2 = 92; P < 0.0001$).

A total of 590 isolates, including all β-glucosidase-negative isolates, was studied by DNA fingerprinting with the Ca3 probe. Of these, 53 (9.0%) gave hybridization patterns characteristic of C. dubliniensis; the remaining 537 all gave strong Ca3 hybridization with patterns confirming their original identification as C. albicans. All of the 53 isolates reidentified by DNA fingerprinting as C. dubliniensis (these included the C. dubliniensis type strain) were negative in the test for β-glucosidase activity, and 51 of them had given abnormal green colony colors on the differential medium. Of the 537 isolates shown to be C. albicans by DNA fingerprinting, 406 had shown an abnormal green color on CHROMagar Candida medium, with 41 of these negative for β-glucosidase activity. Of the 131 C. albicans isolates that gave a normal green colony color, 26 were negative for β-glucosidase activity.

When the details for the 53 isolates reidentified as C. dubliniensis were analyzed (see below) a further three isolates of the species were found in the stock collection. These gave normal green colonies on the differential medium, but they were negative for β-glucosidase activity. The final total of C. dubliniensis isolates found by rescrutiny of the yeast stock collection was therefore 56, comprising 55 newly reidentified isolates and the C. dubliniensis type strain. The overall prevalence of C. dubliniensis among the whole panel of putative C. albicans isolates selected for DNA fingerprinting was therefore 55 of 2,588, or 2.1%.

C. dubliniensis: epidemiologic aspects. Table 1 presents the frequency of occurrence of the 55 newly reidentified C. dubliniensis isolations as a function of date of isolation, country of isolation, anatomical site of isolation, and clinical setting of the patient for the samples for which this information was known. The majority of C. dubliniensis isolates came from oral and fecal samples, with only a single isolate from a vulvovaginal sample and none from deep organs, skin, or nail ($\chi^2 = 19.4; P < 0.01$). There was also a statistically significant difference in the prevalence of C. dubliniensis between patient types, with the highest prevalence among isolates from HIV-infected patients ($\chi^2 = 15.0; P < 0.05$). With the exception of the small number of samples from the period from 1980 to 1984, about 10 to 12% of the yeasts originally deposited in the culture collection as C. albicans in each time period since 1970 and selected for DNA fingerprinting were now reidentified as C. dubliniensis. Some differences in the prevalence of C. dubliniensis isolates between countries of origin were evident, but these were not statistically significant ($\chi^2 = 9.2; P > 0.05$).

The known details for the 55 isolates of C. dubliniensis are

<table>
<thead>
<tr>
<th>Date of isolation</th>
<th>Isolation detail</th>
<th>No. tested for DNA patterns</th>
<th>C. dubliniensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1980</td>
<td>41</td>
<td>4</td>
<td>9.8</td>
</tr>
<tr>
<td>1980–1984</td>
<td>25</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>1985–1989</td>
<td>262</td>
<td>29</td>
<td>11.1</td>
</tr>
<tr>
<td>1990–1994</td>
<td>151</td>
<td>19</td>
<td>12.6</td>
</tr>
<tr>
<td>Region or country of isolation</td>
<td>No. tested for DNA patterns</td>
<td>C. dubliniensis</td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>93</td>
<td>4</td>
<td>4.3</td>
</tr>
<tr>
<td>Belgium</td>
<td>81</td>
<td>4</td>
<td>4.9</td>
</tr>
<tr>
<td>France</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Germany</td>
<td>28</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Spain</td>
<td>70</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>243</td>
<td>35</td>
<td>14.4</td>
</tr>
<tr>
<td>Other</td>
<td>38</td>
<td>2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* All isolates negative for β-glucosidase activity plus a random selection of β-glucosidase-positive isolates were tested for reactivity with oligosaccharide probe Ca3 in Southern blots.
Isolate reference no. | Date | Country | Source | Clinical setting | Details |
--- | --- | --- | --- | --- | --- |
73/089 | 1973 | United Kingdom | Oropharynx | Cardiovascular | Open-heart surgery patient SH27, Leeds General Infirmary (11) |
75/004 | 1975 | United Kingdom | Oropharynx | Diabetes | Diabetic patient reference no. OD003, Leeds General Infirmary (12) |
70/008 | 1978 | United Kingdom | Oropharynx | Healthy | Healthy undergraduate medical student TC, University of Leicester |
81/080 | 1981 | United Kingdom | Oropharynx | Healthy | Healthy undergraduate medical student TUNJ, University of Leicester |
81/217 | 1981 | United States | Oropharynx | Unknown | Received from Baylor Medical Center, Houston, Texas (9) |
82/006 | 1982 | United Kingdom | Oropharynx | Dental surgery | Received from Sheffield University Dental School |
11 | 1985 | United Kingdom | Oropharynx | Intensive care | Patient EH; surveillance culture, Bangor, Wales |
514 | 1985 | United Kingdom | Vagina | Intensive care | Patient EK; surveillance culture, Bangor, Wales |
251 | 1985 | United Kingdom | Feces | Hematology | Patient BO; surveillance culture, 26 November 1985, Royal Free Hospital, London (13) |
457 | 1985 | United Kingdom | Feces | Hematology | Patient BO; surveillance culture, 26 November 1985, Royal Free Hospital, London (13) |
1003 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 10, Valencia (5, 14) |
1046 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 19, Valencia (5, 14) |
950 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 30, Madrid (5, 14) |
959 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 30, Madrid (5, 14) |
961 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 39, Valencia (5, 14) |
952 | 1986 | Spain | Oropharynx | Drug addict | Intravenous drug abuser patient 39, Valencia (5, 14) |
456 | 1986 | United Kingdom | ? | Hematology | Sample 93577, other details unknown; Royal Free Hospital, London (13) |
895 | 1986 | United Kingdom | Sputum | Hematology | Patient BB; surveillance culture 12 April 1986 Royal Free Hospital, London (13) |
872 | 1986 | United Kingdom | Sputum | Hematology | Patient BB; surveillance culture 18 June 1986 Royal Free Hospital, London (13) |
879 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 19 June 1986 Royal Free Hospital, London (13) |
696 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 24 April 1986 Royal Free Hospital, London (13) |
710 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 12 June 1986 Royal Free Hospital, London (13) |
684 & 685 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 17 April 1986 Royal Free Hospital, London (13) |
675 | 1986 | United Kingdom | Feces | Hematology | Patient BB; surveillance culture 22 April 1986 Royal Free Hospital, London (13) |
632 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 12 June 1986 Royal Free Hospital, London (13) |
892 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 12 June 1986 Royal Free Hospital, London (13) |
893 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 12 June 1986 Royal Free Hospital, London (13) |
875 | 1986 | United Kingdom | Feces | Hematology | Patient BB; surveillance culture 14 June 1986 Royal Free Hospital, London (13) |
883 | 1986 | United Kingdom | Feces | Hematology | Patient BB; surveillance culture 16 June 1986 Royal Free Hospital, London (13) |
904 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 14 July 1986 Royal Free Hospital, London (13) |
910 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 14 July 1986 Royal Free Hospital, London (13) |
902 | 1986 | United Kingdom | Oropharynx | Hematology | Patient BB; surveillance culture 21 July 1986 Royal Free Hospital, London (13) |
88/014 | 1988 | United Kingdom | Oropharynx | HIV infection | Patient TT, Leicester University Medical School, Leicester (19) |
89/014 | 1988 | United Kingdom | Oropharynx | HIV infection | Patient TT, Leicester University Medical School, Leicester (19) |
90/013 | 1989 | United Kingdom | Oropharynx | HIV infection | Patient TJB Leicester University Medical School, Leicester (19) |
90/015 | 1990 | United Kingdom | Oropharynx | HIV infection | Patient TJB Leicester University Medical School, Leicester (19) |
90/033 | 1990 | United Kingdom | Oropharynx | HIV infection | Patient TJB Leicester University Medical School, Leicester (19) |
88/029 | 1990 | United Kingdom | Oropharynx | HIV infection | Patient NE Leicester University Medical School, Leicester (19) |
90/006 | 1990 | United Kingdom | Oropharynx | HIV infection | Patient NE Leicester University Medical School, Leicester (19) |
J920710 | 1992 | Belgium | Oropharynx | HIV infection | Received from Université Catholique de Louvain, Brussels |
J930644 | 1992 | Canada | Oropharynx | HIV infection | Received from Laboratoire du Santé Publique, Québec |
J930666 | 1992 | Canada | Oropharynx | HIV infection | Received from Laboratoire du Santé Publique, Québec |
B69819/1 | 1992 | France | Oropharynx | HIV infection | Patient KN, Hôpitaux Saint-Louis, Paris |
B71507 | 1992 | Ireland | Oropharynx | HIV infection | Received from Dental Hospital, Trinity College, Dublin |
J930713 | 1993 | Belgium | Oropharynx | HIV infection | Received from Université Catholique de Louvain, Brussels |
J930936 | 1993 | Germany | Oropharynx | HIV infection | Patient PT, Johann Wolfgang Goethe Universitat, Frankfurt |
J930953 | 1993 | Germany | Oropharynx | HIV infection | Patient IW, Johann Wolfgang Goethe Universitat, Frankfurt |
J931021 | 1993 | Belgium | Oropharynx | HIV infection | Patient IC, Johann Wolfgang Goethe Universitat, Frankfurt |
J931111 | 1993 | United Kingdom | Oropharynx | HIV infection | Patient 093c, Chelsea & Westminster Health Care Trust, London |
J931113 | 1993 | United Kingdom | Oropharynx | HIV infection | Patient 296c Chelsea & Westminster Health Care Trust, London |
J930822 | 1993 | United States | Oropharynx | HIV infection | Received from Audie L. Murphy VA Hospital, San Antonio, Texas |
J932634 | 1994 | Belgium | Oropharynx | Genitourinary infection | Patient 770322, gynecology clinic, Turnhout |
J940613 | 1994 | Belgium | Oropharynx | Genitourinary infection | Patient IC50, gynecology clinic, Antwerp |
J941841 | 1994 | The Netherlands | Oropharynx | Hematology | Surveillance culture, University Hospital, Nijmegen |

* Isolates are arranged in chronological order except when isolates from the same patient are grouped together.

+ Isolates were found by rescrutiny of all remaining isolates from patient LB.

Table 2. Details about C. dubliniensis strains reidentified from 2,587 isolates deposited as C. albicans in yeast culture collection

Presented in Table 2. The earliest isolate now reidentified as C. dubliniensis came from an oral surveillance culture from a patient undergoing open-heart surgery in Leeds, England, in 1975 (11). Two other, diabetic outpatients in Leeds were the sources of isolates of C. dubliniensis in 1975. Two isolates of C. dubliniensis were obtained from healthy undergraduate medical students in 1978 and 1981. Many student and staff volunteers were occasionally sampled for oral yeast carriage during that period to provide fresh isolates of Candida spp. for experimental use, but only 28 of such isolates were deposited in the stock collection. Two isolates of C. dubliniensis came from Wales, and one each came from Texas and Sheffield, United Kingdom, between 1981 and 1985. These included the first isolate from a nonoral site in the present survey: a vaginal isolate from a patient in an intensive therapy unit. For patients EH and EK, no other Candida-positive cultures were represented in the stock collection.

In 1986, six isolates now reidentified as C. dubliniensis were...
isolated from intravenous drug abusers as part of surveys conducted in Spanish clinics (5, 14). Two of these were separate isolations made from the same patient, patient 39. The other four C. dubliniensis-positive isolates were single examples from four separate patients.

Between 1985 and 1986, 674 yeast-positive samples from 153 hematology patients were studied as part of a survey for C. albicans biotypes (13). Among these samples, 15 were now found to contain C. dubliniensis during the blinded screening process. However, as indicated in Table 2, the species was isolated from only three different patients (plus a single isolate from a source for which details are unknown). Two isolates came from patient BO, three came from patient BB, and nine came from patient LB. Rescrutiny of the available data for these patients showed that only the two isolates now reidentified as C. dubliniensis were ever stocked from patient BO, and they in fact both represented an isolation from a single fecal sample, dated 26 November 1985, that was stored twice in the stock collection, under different reference numbers, for unknown reasons. For patient BB, five different positive cultures had been obtained, of which four have survived in storage since 1986. The fourth isolate from this patient was reidentified and rechecked by the β-glucosidase test and with the Ca3 fingerprinting gel: its characteristics were definitely those of C. albicans, not C. dubliniensis. For patient LB, 12 positive yeast cultures had been stocked in distilled water, of which 2 represented a single isolate and were stocked twice with different reference numbers. Retesting of the three isolates from patient LB that had not been detected as C. dubliniensis in the blinded screening showed that these were also isolates of C. dubliniensis according to their DNA fingerprinting patterns. These three isolates were also negative for β-glucosidase activity, but they had not shown colony color abnormalities in the blinded screening. Hematology patient LB was therefore the source of 12 C. dubliniensis isolates from 11 fecal or oral specimens obtained over a 3-month period in 1986.

From a survey of oral Candida isolates from AIDS patients in the United Kingdom (19), seven yeasts were now reidentified as C. dubliniensis (Table 2). They came from three patients: patients TT, JB, and NE. Three other isolates in the collection from patient TT were confirmed as C. albicans, but the four isolates from patient JB and the two isolates from patient NE that are listed in Table 2 were the only isolates from these individuals available in the stock collection. Among the 15 C. dubliniensis isolates found in the collection among cultures stocked since 1990, each came from a separate patient, all but three of whom were HIV positive.

Susceptibility testing. Some differences in antifungal susceptibilities between the C. dubliniensis isolates and their matched C. albicans controls were apparent. In total, 58 isolates of each species were tested in two separate runs of susceptibility determinations. The C. dubliniensis isolates tested included 49 of the 55 strains reidentified in the present study, the C. dubliniensis type strain, and 8 isolates obtained in 1995 in a prospective study of Candida carriage in HIV-positive patients (21). Few of the isolates were resistant to any of the five agents tested except for fluconazole and fluconosine. One isolate of C. dubliniensis was susceptible to fluconosine only at 32 μg/ml, and another was inhibited by fluconazol only at 64 μg/ml. Among the 58 C. albicans isolates, 4 were resistant to fluconosine (MIC ≥64 μg/ml) and 3 were resistant to fluconazole (MIC, ≥64 μg/ml). The geometric mean MICs for the panels of isolates suggested a trend toward higher levels of susceptibility to amphotericin B (0.25 versus 0.43 μg/ml) and fluconosine (0.074 versus 0.21 μg/ml) for the C. dubliniensis isolates than for the C. albicans isolates. For the three azole antifungal agents, by contrast, the geometric mean MICs were consistently higher for the C. dubliniensis isolates than for the C. albicans isolates: fluconazole, 0.83 versus 0.34 μg/ml; itraconazole, 0.027 versus 0.009 μg/ml; ketoconazole, 0.017 versus 0.007 μg/ml.

DISCUSSION

This study has shown that C. dubliniensis isolates dating back to 1973 could be found by retesting of a collection of 2,589 yeasts, mostly of clinical origin. In total, 55 isolates of C. dubliniensis from 36 different persons were found by the process of reidentification. C. dubliniensis was reidentified under blinded test conditions more than once in different samples from the same patient and even from the same sample restocked under different reference numbers in two instances. This finding suggests that the quality of the stock collection was high and that the older isolates now identified as C. dubliniensis are genuine early examples of the species. If some or all of the C. dubliniensis isolates found in this study had been the result of cross contamination of specimens now or at previous times of reisolation and restockage of the yeasts, then they would be expected to have assumed a more random distribution in the collection rather than an association with specimens from particular patients.

Primary recognition of C. dubliniensis remains a technical problem. In the present study the green color of yeast colonies after prolonged incubation on a differential medium was intended to be used as a primary indicator of yeast phenotypes likely to be different from C. albicans, with the more complex test for intracellular β-glucosidase activity used as a secondary test to exclude C. albicans isolates. However, the colony color was not a reliable criterion for the detection of C. dubliniensis among the stock yeast isolates. Two isolates of the species gave normal colony colors in the blind phase of screening; three further isolates with normal colony colors were subsequently found to be C. dubliniensis by specific rescruity of isolates from one patient of particular interest.

The test for β-glucosidase activity, while not specific for C. dubliniensis (we found 67 β-glucosidase-negative isolates of C. albicans among 537 isolates tested), nevertheless gave negative results for all 56 C. dubliniensis isolates tested. Indeed, β-glucosidase-positive strains of C. dubliniensis have not yet been described anywhere, to our knowledge (1, 21–23). We tested a total of 1,398 yeasts from the stock collection for β-glucosidase activity over a period of 10 months; to have tested all 2,589 yeasts would have been prohibitively expensive in terms of both cost and the labor involved. We can be confident that all C. dubliniensis isolates among those 1,398 isolates were detected, since all β-glucosidase-negative isolates were subjected to DNA fingerprinting. However, there remain 1,191 yeast isolates among which there may still be examples of C. dubliniensis that were not detected because they gave normal colony colors on the differential medium. Since C. dubliniensis often gives atypical results in carbohydrate assimilation tests (21–25), it is also possible that isolates of C. dubliniensis identified as species other than C. albicans have been deposited in our collection, although the morphologic phenotype of C. dubliniensis should have minimized such occurrences.

Since this study was completed and first submitted for publication, Pinjon and colleagues (17) have shown that growth (C. albicans) versus nongrowth (C. dubliniensis) of germ tube-forming yeasts at 45°C is a reliable and simple test for the differentiation of the two species. Differential growth at 45°C could have been used as a cheap and simple means of detecting possible C. dubliniensis isolates in our collection.

Sullivan et al. (24) established that the distribution of C. dub-
*C. dubliniensis* is widespread—probably worldwide—and that the species has been found in patients outside the groups of HIV-positive individuals in whom its high degree of prevalence led to its initial discovery (22–24). Almost all studies of *C. dubliniensis* so far have been of oral isolates. Among oral isolates from patients with and without HIV infection, Coleman et al. (3) reported an overall prevalence of 26.4% among 382 HIV-positive patients and 61% among HIV-negative patients. In the present study, isolates from HIV-positive patients were certainly the richest single source of *C. dubliniensis* (Table 2), and the five Spanish drug abusers from whom *C. dubliniensis* was isolated may well also have included some HIV-positive individuals. However, we also frequently found the species among patients with hematologic malignancies undergoing or about to undergo chemotherapy. *C. dubliniensis* has recently been found to be the cause of three cases of candidemia among patients in this clinical setting (4).

The occurrence of *C. dubliniensis* in fecal samples is also a novel finding of the present study. The single vaginal isolate and the two oral isolates from healthy student volunteers suggest that the distribution of *C. dubliniensis* may in fact be very similar to that of *C. albicans*. The standard practice of many laboratories of reporting as “*C. albicans*” all yeast isolates that form germ tubes in serum is an obstacle to the routine recognition of *C. dubliniensis*. Definition of the true prevalence of this species in material from patients and from healthy subjects will depend on improvements in techniques for the differential recognition of *C. dubliniensis* and *C. albicans*.

Like Moran and colleagues (6), we found most of our isolates of *C. dubliniensis* to be susceptible to systemically used antifungal agents. Those investigators found fluconazole MICs at or above the 16 µg/ml for 4 of 20 *C. dubliniensis* isolates. The prevalence of such isolates among our test panel was 3 among 58 isolates. A single isolate was resistant to both fluconazole and itraconazole according to the breakpoints established by the National Committee for Clinical Laboratory Standards (MICs, 64 and 1.0 µg/ml, respectively) (18). We did not attempt experiments similar to those of Moran et al. (6) to evaluate the ease of induction of fluconazole resistance in our *C. dubliniensis* isolates.

The oldest known isolate of *C. dubliniensis* so far confirmed by DNA fingerprinting had been deposited in a stock collection as “*C. stellatoidea*” in 1957 (22, 24). Our survey confirms that *C. dubliniensis* was present in clinical samples decades before the new species was first described. It has been encountered in primary cultures requiring new strategies for routine identification in clinical laboratories: differential growth at 45°C provides a straightforward test for the presumptive differentiation of germ tube-positive yeast isolates. Determination of the clinical importance of *C. dubliniensis* requires further study.

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