Racial Distribution of Candida dubliniensis Colonization among South Africans

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Candida dubliniensis is a yeast species that has only recently been differentiated from Candida albicans. C. dubliniensis colonization was initially associated with human immunodeficiency virus (HIV)-positive individuals. Because of the large proportion of AIDS patients in South Africa, we tested the generality of this association by assessing the prevalence of C. dubliniensis colonization among 253 black HIV-positive individuals, 66 healthy black individuals, 22 white HIV-positive individuals, and 55 healthy white individuals in South Africa carrying germ tube-positive yeasts in their oral cavities. Molecular fingerprinting with Ca3, a complex DNA fingerprinting probe specific for C. albicans, and Cd25, a complex DNA fingerprinting probe specific for C. dubliniensis, provides the first conclusive evidence of the existence of C. dubliniensis among South African clinical yeast isolates and reveals a higher relative prevalence of this species among white healthy individuals (16%) than among HIV-positive white individuals (9%), black healthy individuals (0%), and black HIV-positive individuals (1.5%). A cluster analysis separated South African C. dubliniensis isolates into two previously described groups, groups I and II, with the majority of isolates clustering in group I. Isolates from white healthy individuals exhibited a higher level of relatedness. A comparison of the C. dubliniensis isolates from South Africa with a general collection of C. dubliniensis isolates collected worldwide revealed no South Africa-specific clade, as has been demonstrated for C. albicans. These results suggest that in South Africa, C. dubliniensis carriage is influenced more by race than by HIV infection status.

Extensive phenotypic and genotypic analyses of atypical oral yeast isolates from human immunodeficiency virus (HIV)-positive patients in Dublin, Ireland, provided evidence that they could be members of a new species, which was subsequently named Candida dubliniensis (26, 28). Phenotypic characteristics that originally led investigators to type isolates of this new species as Candida albicans were the ability to form germ tubes and chlamydospore formation. Atypical phenotypic characteristics that differentiated C. dubliniensis from C. albicans included the failure to grow at 42°C, lack of expression of the enzyme β-glucosidase, different colony color on CHROMagar medium, failure to fluoresce under Wood’s light on methyl blue-Sabouraud agar, and chlamydospore formation on Staib agar (1, 7, 23, 27, 28). Genetic differences between C. albicans and C. dubliniensis were first demonstrated by DNA fingerprinting with C. albicans midrepeat sequence probe 27A, randomly amplified polymorphic DNA analysis with five primers, pulsed-field gel electrophoresis, and rRNA gene nucleotide sequencing (28). Recently, a species-specific complex DNA fingerprinting probe that can be used to distinguish C. dubliniensis from C. albicans and to assess the genetic relatedness and population structure of C. dubliniensis was developed (11).

Although the initial reports of atypical C. albicans isolates that proved to be C. dubliniensis (15, 19, 26) and many of the subsequent reports on this new species involved work done with isolates from HIV-positive patients (12, 13, 23, 27), it is recognized that this species is not restricted to HIV-positive individuals (9, 10, 14, 16, 17). However, it has been generally accepted that C. dubliniensis preferentially colonizes HIV-positive individuals. South Africa has one of the largest HIV-infected populations in the world, with an estimated 4.2 million people currently infected and with this number projected to increase to 7 million by 2005 (29). This increase in the number of HIV-infected individuals has been accompanied by an increase in the number of individuals with oropharyngeal candidiasis (3, 5). An analysis of germ tube-positive isolates from the oral cavities of both HIV-positive patients and healthy individuals revealed a significant number of isolates that did not hybridize to the C. albicans-specific DNA fingerprinting probe Ca3 at high stringency (6). In the study described here, we have analyzed the relative prevalence of C. dubliniensis isolates among both HIV-positive and HIV-negative black and white South Africans. We have also fingerprinted these isolates with the C. dubliniensis-specific fingerprinting probe Cd25. The results indicate a relationship between host race and colonization by C. dubliniensis rather than disease state and colonization in South Africa. In the populations analyzed for the present study, C. dubliniensis was most prevalent in white healthy individuals and less prevalent in black and white HIV-positive individuals, and its prevalence was negligible in black healthy individuals, which is counter to expectations based on the original studies of prevalence in Irish populations.

MATERIALS AND METHODS

Isolation and maintenance of oral yeast isolates. All isolates were collected over a 5-year period, from 1995 to 2000. Oral yeast isolates were obtained from black HIV-positive patients attending AIDS clinics at three hospitals, the Pretoria Academic Hospital, the Kalafong Hospital in Pretoria, and the GaRankuwa Hospital in GaRankuwa; from white HIV-positive patients attending govern-
ment and private clinics (Pretoria, Johannesburg, Cape Town); and from black healthy individuals who were staff at a semiurban oral health center, the Me-
duna Oral and Dental Hospital in GaRankuwa, or who were living and working in either the remote rural area of Mahonisi or Kruger Park in the Northern Province of South Africa. Oral yeast isolates were obtained from white healthy individuals who were staff of an urban dental hospital or who used the oral hygiene service at that hospital in Pretoria. Black and white healthy individuals from whom isolates were obtained had not recently taken medication that could affect oral yeast carriage, had no history of current or recent illness, and had no signs of oral mucosal abnormalities. All patients were dentate (i.e., they did not wear dentures). Samples from all individuals were collected from the dorsal surface of the tongue (4) with a cotton swab. Samples were plated on Sabouraud-
dextrose agar and incubated at 30°C for 4 days. When yeast colonies were ob-
served, a single colony was selected from each sample for further analysis. To test for the capacity to form germ tubes, cells were incubated in medium con-
taining 10% fetal calf serum (HyClone Inc., Logan, Utah). Germ tube formation was assessed microscopically after 12 h of incubation. Only germ tube-positive samples were selected for further analysis. The isolates that were DNA finger-
printed included 253 isolates from black HIV-positive individuals, 22 isolates from white HIV-positive individuals, 66 isolates from healthy black individuals, and 55 isolates from healthy white individuals (Table 1).

DNA fingerprinting. Isolates (Table 1) were first fingerprinted by probing Southern blots with C. albicans-specific DNA probe Ca3 at high stringency (2, 20, 22, 24). Those that showed no or negligible hybridization were then fingerprinted with C. dubliniensis-specific probe Cd25 (11). DNA was purified (21) and di-
gested with EcoRI by previously described methods (22). Electrophoresis was performed in a 0.8% agarose gel at 67 V. DNA from C. dubliniensis strain M6 (11) was run in the outer lanes of each gel as a reference to assist in normalization routines during computer-assisted gel analysis (24). Each gel was run until the blue indicator dye reached a distance of 18 cm from the wells and was then transferred to a Hybond N+ membrane (Amersham, Piscataway, N.J.) through capillary blotting. Salmon sperm DNA was used to prehybridize each Southern blot. Hybridization was performed overnight at 65°C with a randomly primed 32P-labeled Cd25 probe. Hybridization blots were washed at 45°C and autoradiographed on XAR-S film (Eastman Kodak Co., Rochester, N.Y.) with Cronex Lightning-Plus intensifying screens (DuPont Co., Wilmington, Del.).

Cluster analysis of autoradiograms. With the aid of an Astra 1220U flatbed scanner (UMAX Technologies Inc., Fremont, Calif.), each autoradiogram was scanned into the DENDRON software program (24). With the unwarping option of the DENDRON program, distortions were removed and bands were automatically detected, linked, and analyzed. Manual editing of the DENDRON data was performed. A final band data file was then generated for construction of the den-
drograms. Comparison of all pattern pairs was performed by computing the similarity coefficient ($S_{AB}$) by the formula $E/(E + a + b)$, where $E$ is the number of bands shared by strains A and B, a is the number of bands unique to strain A, and b is the number of bands unique to strain B. An $S_{AB}$ value of 0.00 indicates total unrelatedness, and a value of 1.00 represents an identical match of all bands between strains. $S_{AB}$ values increasing from 0.01 to 0.99 represent increasing degrees of genetic relatedness (24).

### RESULTS

Relative prevalence of C. dubliniensis among groups. All germ tube-positive isolates were fingerprinted with C. albicans species-specific probe Ca3 at high stringency (20, 22, 24). Those isolates that exhibited weak or negligible hybridization were then fingerprinted with C. dubliniensis species-specific complex probe Cd25 (8, 11). All of the latter isolates exhibited a complex Southern blot hybridization pattern with the Cd25 probe (Fig. 1), demonstrating that they were of the species C.

<table>
<thead>
<tr>
<th>Host race</th>
<th>Host HIV status</th>
<th>Geographical location(s)</th>
<th>Total no. of germ tube-positive isolates</th>
<th>No. (%) of isolates&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>Positive</td>
<td>Pretoria, GaRankuwa</td>
<td>253</td>
<td>249 (98.5)</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>GaRankuwa, Kruger Park, Mahonisi</td>
<td>66</td>
<td>66 (100)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>319</td>
<td>315 (98.7)</td>
</tr>
<tr>
<td>White</td>
<td>Positive</td>
<td>Pretoria, Johannesburg, Cape Town</td>
<td>22</td>
<td>20 (90.9)</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>Pretoria</td>
<td>55</td>
<td>46 (83.6)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>77</td>
<td>66 (85.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> HIV-positive individuals represent HIV-positive and AIDS patients attending clinics for treatment of their condition. Healthy individuals were assumed to be HIV negative on the basis of their state of health. Since this group could have included some individuals who were HIV positive but asymptomatic, we refer to them as “healthy.”

<sup>b</sup> Those germ tube-positive isolates that generated a complex Southern blot hybridization pattern with C. albicans-specific DNA probe Ca3 were typed as C. albicans. Those germ tube-positive isolates that did not hybridize with the Ca3 probe but that did generate a complex Southern blot hybridization pattern with C. dubliniensis-specific DNA probe Cd25 were typed as C. dubliniensis.

**Table 1. Proportion of C. dubliniensis isolates among the different collections of germ tube-positive isolates**

![Image of fingerprinting patterns](http://jcm.asm.org/)

**FIG. 1.** Cd25 fingerprinting patterns of 13 C. dubliniensis isolates collected from South Africans. Strain M6, run in the two end lanes, provides a reference pattern for computer-assisted comparisons of patterns. Note that while the patterns of strains G82, P62, P86, PC35, UP6a, UP12, UP16, UP24a, UP26, UP27a, UP29, and UP36 are similar and representative of the patterns of group I isolates, the pattern of strain G27 is dissimilar and representative of the pattern of group II isolates (11). Patterns represent hybridization of EcoRI-digested DNA with the radioactive Cd25 probe.
The C. dubliniensis patterns of strains included monomorphic bands at 15.6, 4.4, and 1.1 kb (11) (Fig. 1). The majority of germ tube-positive isolates collected from HIV-positive and healthy black individuals were also C. albicans (Table 1). Four of 253 isolates (1.5%) collected from HIV-positive black individuals and 0 of 66 isolates (0%) collected from healthy black individuals typed as C. dubliniensis (Table 1). The proportion of germ tube-positive isolates from all black individuals that typed as C. dubliniensis was 1.3%.

The majority of germ tube-positive isolates collected from HIV-positive and healthy white individuals were also C. albicans (Table 1). However, a greater proportion of isolates from white individuals than isolates from black individuals were C. dubliniensis. Two of 22 germ tube-positive isolates (9.1%) collected from HIV-positive white individuals and 9 of 55 germ tube-positive isolates (16.4%) collected from healthy white individuals typed as C. dubliniensis (Table 1). The proportion of germ tube-positive isolates from all white individuals that typed as C. dubliniensis was 14.3%, which was significantly higher (P = 5.6 × 10^{-6} by Fisher’s exact test) than the proportion of 1.3% for all black individuals (Table 1). In contrast, the difference in the proportion of C. dubliniensis isolates between healthy and HIV-positive white individuals was insignificant (P > 0.05), and the difference in the proportion of C. dubliniensis isolates between healthy and HIV-positive black individuals was insignificant (P > 0.05).

**Relatedness of C. dubliniensis isolates.** To test first for relatedness among isolates from the different host groups (i.e., HIV-positive individuals versus healthy individuals and black individuals versus white individuals), a dendrogram was generated from the Cd25 Southern blot hybridization patterns (Fig. 1) of the 15 C. dubliniensis isolates collected from South Africans (Fig. 2). The collection consisted of nine isolates from healthy white individuals, four isolates from HIV-positive black individuals, and two isolates from HIV-positive white individuals. The distribution superficially appeared to be random. Isolates from both HIV-positive black and HIV-positive white individuals were dispersed throughout the majority collection of isolates from healthy white individuals (Fig. 2). Since the average S_{AB} for group I isolates computed in the original characterization of the Cd25 probe was 0.80 (11), we used an arbitrary threshold of 0.90, halfway between 0.80 and 1.00, to identify clusters of related isolates (24). Three groups of two or more isolates (groups a, b, and c) were distinguished. Group a and group b each contained two isolates from white healthy individuals (Fig. 2). Group c contained four isolates from healthy white individuals and one isolate from a white HIV-positive individual. All four of the isolates from black HIV-positive individuals, one isolate from a white healthy individual, and one isolate from a white HIV-positive individual did not cluster. These results suggest that C. dubliniensis isolates colonizing healthy white individuals exhibit a degree of relatedness.

In the original characterization of C. dubliniensis-specific DNA fingerprinting probe Cd25, it was demonstrated that a collection of random isolates separated into two deeply rooted clades, group I and group II. Approximately 86% were members of group I, while 14% were members of group II (11). To assess which of the two major C. dubliniensis clades the South African isolates separated into, a mixed dendrogram was generated. The dendrogram included the 57 isolates collected worldwide that were used in the original characterization of the Cd25 probe (11) and the South African collection. The two major clades, group I and group II, separated at an S_{AB} node value of 0.25 (Fig. 3). One South African isolate, isolate G27, from a black HIV-positive individual, coclustered with group II isolates from among the South African isolates (Fig. 3). The remaining 14 isolates coclustered with group I isolates. In the mixed dendrogram, groups b and c discriminated in the dendrogram generated exclusively from South African isolates (Fig. 2) remained intact, but the 2 isolates in group a, isolates UP36 and UP6a, separated (Fig. 3). Except for the isolates clustered in subgroups b and c, the South African isolates were dispersed throughout the mixed dendrogram (Fig. 3). The five South African isolates that did not fall into groups in the exclusive South African isolate dendrogram were all members of group I (Fig. 3). There was no indication in the mixed isolate dendrogram (Fig. 3) of a South African-specific clade, as was found for the species C. albicans (6), suggesting that, in general, the C. dubliniensis isolates from South Africa do not show geographical specificity.

**DISCUSSION**

Although C. dubliniensis has been shown to colonize healthy individuals in a variety of geographical locales throughout the world, it has repeatedly been shown to colonize AIDS patients preferentially. Indeed, the first studies that helped resolve this species were performed with isolates from HIV-positive patients in Dublin, Ireland (26, 28). Subsequent studies supported the conclusion that C. dubliniensis preferentially, but not exclusively, colonized HIV-positive individuals (10, 14, 17, 25). Prevalence rates of between 15 and 30% have been reported in HIV-positive populations worldwide, while prevalence rates in healthy individuals have been below 5% (14). We have tested the generality of this difference by measuring the frequency of C. dubliniensis isolates in the oral cavities of individuals from South Africa, in which a large and increasing
The proportion of the population is HIV positive (29). In a previous analysis of the species and strain specificities among yeast isolates from South Africans, we demonstrated that while approximately 80% of yeast isolates from black HIV-positive patients were of the species \textit{C. albicans}, only 58 and 67% of yeast isolates from healthy black and healthy white individuals, respectively, were \textit{C. albicans} (6). In addition, we demonstrated that more than 50% of \textit{C. albicans} isolates from both HIV-positive and healthy black South Africans belonged to a South African-specific clade and that 35% of \textit{C. albicans} isolates from healthy white South Africans belonged to this clade (6). This clade was found to be represented at negligible levels in the United States and low levels in Europe (6, 18). These findings indicated that dramatic differences in species distributions between HIV-positive and healthy populations, as well as between black and white populations, exist in South Africa. In the previous study (6), we identified a minority of germ tube-positive isolates that did not hybridize with the Ca3 probe at high stringency. Here, we have tested whether any of the latter isolates plus additional germ tube-positive, Ca3-negative isolates collected from white HIV-positive patients in South Africa were of the species \textit{C. dubliniensis} and whether \textit{C. dubliniensis} preferentially colonized HIV-positive patients in this geographical locale.

Our results are actually contrary to those that would have been expected on the basis of the original and most extensive studies with Irish cohorts, summarized by Ponton et al. (17). We have found that in South Africa, \textit{C. dubliniensis} preferentially colonizes healthy white individuals. The frequency of colonization of healthy white individuals was 16%, while the frequency of colonization of healthy black individuals was 0%. The proportion of HIV-positive white individuals colonized with \textit{C. dubliniensis} was 9%, while the proportion of HIV-positive black individuals was 1.5%. These pronounced differences could be racially based or could be the result of cultural differences that include habitat and diet.

The differences observed could not be explained by changes in distribution during sampling because all of our samples were collected during the same time window, over a 5-year period, by the same procedure. These results also could not be explained by differences in geographical location, since the majority of black HIV-positive individuals (218 of 253) were from Pretoria, the same geographical locale as the 55 healthy white individuals. It should be noted that our numbers do not represent the real prevalence of \textit{C. dubliniensis} in the South African population because it is well known that \textit{C. dubliniensis} is often recovered in mixed cultures with other \textit{Candida} yeasts and a single isolate was analyzed from each individual (14, 26, 27). Our results therefore reflect the relative prevalence and are still indicative of epidemiological differences between these populations.

Our results demonstrate colonization of the oral cavities of South Africans with \textit{C. dubliniensis}. They also indicate that, contrary to expectations, \textit{C. dubliniensis} preferentially colonizes healthy white individuals and does not seem to colonize healthy black individuals from the same geographical locale. Finally, these data demonstrate a second characteristic of fungal colonization that differs between South African white and black individuals. In addition to a significant difference in the proportion of \textit{C. albicans} isolates representing the South African-specific clade that colonize the oral cavity, the proportion of \textit{C. dubliniensis} isolates that colonize the oral cavities of white and black individuals differs significantly, most notably among healthy individuals.

![FIG. 3. Mixed dendrogram of the 15 \textit{C. dubliniensis} isolates from South Africa and 57 isolates collected worldwide and used in the original characterization of Cd25 (11). The two major clades, group I and group II, are delineated. In addition, isolates in subgroups a, b, and c, identified in the dendrogram generated exclusively for South African \textit{C. dubliniensis} isolates (Fig. 2), are noted. W-Hthy, strains from white healthy individuals; W-HIV, strains from white HIV-positive individuals; B-HIV, strains from black HIV-positive individuals.](http://jcm.asm.org/)
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