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# Variations in Fluconazole Susceptibility and DNA Subtyping of Multiple *Candida albicans* Colonies from Patients with AIDS and Oral Candidiasis Suffering One or More Episodes of Infection

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**Five *Candida albicans* colonies from each infection in AIDS patients receiving fluconazole therapy for oropharyngeal candidiasis over a 2-year period were evaluated by antifungal susceptibility testing and DNA subtyping, and the results were correlated with clinical response to determine the occurrence of clinically significant selection of more-resistant *C. albicans* over multiple infections. A total of 534 *C. albicans* isolates were obtained from 38 patients who exhibited 84 episodes of infection. Antifungal susceptibility testing revealed that the MICs for 93% of the isolates were ≤8.0 μg/ml and the MICs for 7% of the isolates were ≥64 μg/ml. DNA subtyping revealed 70 different subtypes, with 78% of patients with one infection exhibiting one DNA subtype and 80% of patients with more than one infection exhibiting multiple DNA subtypes. Also, patients who had multiple infections had lower CD4 counts than those with single infections. Differences between the single-infection group and the multiple-infection group regarding the number of DNA subtypes and CD4 counts were both statistically significant. Of the 74 evaluable infections all were successfully treated with regular-dose (100-mg/day) fluconazole, except for three patients who ultimately responded to higher-dose fluconazole. Only one patient may have shown clinically significant selection of a more-resistant *C. albicans* strain over multiple courses of treatment. Interestingly, MICs reached only 8.0 μg/ml, even though doses of 400 mg of fluconazole were necessary for clinical cure.**

Studies of DNA subtype variation and antifungal resistance among isolates of *Candida albicans* from AIDS patients with multiple episodes of oropharyngeal candidiasis have shown mixed results (1–3, 5, 8, 12, 13, 19). Several authors have shown that AIDS patients are frequently infected with the same subtype over multiple infections (1, 5, 6, 13, 14, 17, 19). In contrast, Korting et al. found with repeat cultures multiple biotypes of *C. albicans* in over 27% of AIDS patients (3). In an earlier study we reported evidence of considerable strain diversity among isolates of *C. albicans* from individuals with AIDS and oropharyngeal candidiasis. Sixty-four percent of these patients treated with fluconazole experienced episodes of infection with two or more different DNA subtypes of *C. albicans*. We also found significant changes in the in vitro susceptibility to fluconazole among isolates from patients with two or more infecting or colonizing subtypes (8, 11). Therefore, in certain individuals this diversity could favor the selection of more-resistant strains. If so, are these more-resistant strains acquired during subsequent infections, are they selected from a subpopulation of an original infection with mixed subtypes, or are they a manifestation of minor genetic variation that may take place within a single strain of *C. albicans* over time (4)? Bart-Delabesse et al. and Sangeorzan et al. have shown that acquisition of new infecting strains does occur (1, 13). Our previous data argues against strain replacement, as we found little cross-infection and a low frequency of shared DNA subtypes among a geographically delimited population (8). Lockhart et al. have conducted elegant studies of patients with recurrent vulvovaginal candidiasis to demonstrate that the main scenario for the relatedness of isolates from sequential episodes of mucosal infection is strain maintenance with or without minor changes in genotype (4). Although strain replacement occurs, it represents a minor scenario. If an infection contains mixed subtypes with different susceptibilities to fluconazole, does this have clinical significance? Even though we have found significant subtype and susceptibility test result diversity, the vast majority of patients both yielded isolates for which the fluconazole MICs were ≤8.0 μg/ml and responded satisfactorily to the standard dosage of medication (8). The purpose of the present study was to examine the subtype diversity of *C. albicans* in AIDS patients with oropharyngeal candidiasis being treated with fluconazole by sampling multiple colonies from culture isolates at the initial and at all relapse infections and by performing DNA subtyping and antifungal susceptibility testing and correlating the data from these tests with the clinical outcomes.

## MATERIALS AND METHODS

**Patients.** Thirty-eight patients fulfilling the criteria for AIDS of the Centers for Disease Control and Prevention who presented with oropharyngeal candidiasis over a 2-year period were enrolled prospectively and, after informed consent was obtained, were treated with fluconazole. All patients were treated and monitored at the University Health System in affiliation with the University of Texas Health Science Center at San Antonio. Each episode of oropharyngeal candidiasis was documented by subjective complaints of oropharyngeal symptoms, objective clinical presentation of white plaques or erythema, a positive potassium hydroxide preparation, and a positive culture for *Candida*. Each episode was treated orally with fluconazole for 14 days at dosages of 100 to 400 mg/day. If the standard dose

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TABLE 1. Variation in fluconazole MIC among isolates of non-*C. albicans Candida* from patients with oropharyngeal candidiasis

| Patient | Episode | Date (mo/day/yr) | Species | MIC (μg/ml) |
|---|---|---|---|---|
| 4 | 1 | 10/29/92 | *C. glabrata* | 8.0 |
| | | | *C. krusei* | 64.0 |
| | | 11/12/92 | *C. glabrata* | 16.0 |
| | | | *C. krusei* | 64.0 |
| 16 | 1 | 6/18/93 | *C. glabrata* | 8.0 |
| 27 | 6 | 3/7/94 | *C. tropicalis* | 1.0 |
| | 7 | 4/25/94 | *C. tropicalis* | 0.25 |
| 32 | 2 | 9/24/93 | *C. tropicalis* | ND[a] |
| | | 10/11/93 | *C. tropicalis* | 0.5 |
| | 3 | 11/1/93 | *C. tropicalis* | 0.25 |
| | 4 | 2/2/94 | *C. tropicalis* | 1.0 |
| | 5 | 3/7/94 | *C. tropicalis* | ND |
| | 6 | 4/18/94 | *C. tropicalis* | 2.0 |
| 34 | 1 | 4/8/93 | *C. guilliermondii* | 8.0 |
| 35 | 2 | 8/25/93 | *C. krusei* | 64.0 |

[a] ND, not done.

and duration of fluconazole did not achieve clinical cure, then the dose was doubled until the patient no longer had signs or symptoms. At relapse, the patient was treated with the last successful dose used. At the end of successful therapy, samples for culture were taken in the absence of clinical signs and symptoms to determine colonization. Patients were eligible for repeat courses of therapy if a recurrent infection was observed on follow-up. Fifteen of the 38 patients suffered two or more episodes of infection (range, two to eight episodes). Clinical resolution of each episode was defined as the absence of oral lesions on examination. CD4 counts were determined at each infection.

**Isolates.** A total of 534 isolates of *C. albicans* were obtained from 84 episodes of infection. Other *Candida* species in addition to *C. albicans* were found in 12 of the same episodes of infection. Oropharyngeal swabs were plated onto Sabouraud dextrose agar plates (Emmons modification; BBL), and the plates were incubated for 48 h at 25°C. Colonies of yeasts obtained from the primary culture plate were streaked onto Sabouraud dextrose agar for isolation and identified by standard methods (18). Five individual colonies were picked from the primary culture plate of each *C. albicans* culture and saved on Sabouraud dextrose agar slants. These isolates were then used for DNA subtyping and antifungal susceptibility testing.

**DNA typing.** Molecular typing of all isolates was accomplished by restriction endonuclease analysis of genomic DNA with the restriction enzymes *Sfi*I and *Bss*HII followed by pulsed-field gel electrophoresis as described previously (2, 15, 16).

Profiles obtained by restriction endonuclease analysis of genomic DNA were analyzed by visual inspection of photographs of ethidium bromide-stained gels. Photographs were analyzed by three observers to detect similarities and differences in banding patterns. Isolates were considered different if more than two readily detectable bands did not match. In order to achieve maximum strain discrimination, we combined the results of *Sfi*I and *Bss*HII analysis to achieve a composite DNA type, indicated numerically as the *Sfi*I subtype followed by the *Bss*HII subtype (e.g., 1:1, *Sfi*I:*Bss*HII).

**Antifungal susceptibility testing.** Broth microdilution testing was performed according to National Committee for Clinical Laboratory Standards proposed standard guidelines by using the spectrophotometric method of inoculum preparation, an inoculum concentration of $0.5 \times 10^3$ to $2.5 \times 10^3$ cells/μl, and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma, St. Louis, Mo.) (7, 10). Antifungal agents, including amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole, were obtained from their respective manufacturers. Yeast inocula (100 μl) were added to each well of microdilution trays containing 100 μl of drug solution (2× final concentration). The trays were incubated in air at 35°C and were inspected after 48 h of incubation. The MIC endpoint for each antifungal agent was read visually and was defined for 5-fluorocytosine, fluconazole, and itraconazole as the lowest concentration at which a prominent decrease in turbidity was observed and for amphotericin B as the lowest concentration producing complete inhibition of growth (7, 10). Drug-free and yeast-free controls were included, and quality control was ensured by testing a strain of *Candida parapsilosis* (ATCC 22019) and a strain of *Candida krusei* (ATCC 6528) recommended for this purpose (7, 9, 10). Isolates from the same patient for a given infection were tested the same day.

## RESULTS

A total of 38 patients experienced 84 episodes of infection with oropharyngeal candidiasis and were treated with fluconazole. *C. albicans* was isolated in all of these infections. In twelve infections (seven patients) other *Candida* species were isolated in addition to *C. albicans*. These are summarized in Table 1.

Ten infections were not evaluable for cure because these patients did not return for follow-up after treatment. Of the remaining 74 infections all were successfully treated with 100 mg of fluconazole per day, except for patients 18, 31, and 34. Patient 18, who experienced one infection, did not respond initially to 100 mg of fluconazole but was successfully treated with 200 mg of fluconazole. All ten isolates were the same DNA subtype. For the five isolates from the original infection

TABLE 2. Variation in DNA type and fluconazole MIC among isolates of *C. albicans* from patients with a single episode of oropharyngeal candidiasis

| Patient | Date (mo/day/yr) | DNA type[a] | MIC (μg/ml)[b] |
|---|---|---|---|
| 1 | 9/28/92 | 4:4 | 0.12 (5) |
| 2 | 10/26/92 | 8:7 | 0.25 (5) |
| 3 | 11/12/92 | 9:8 | 128 (5) |
| 4 | 10/29/92 | 10:9 | 0.12 (5) |
| | 11/12/92 | 10:9 | 0.12 (5) |
| 5 | 11/12/92 | 11:10 | 128 (5) |
| 6 | 11/16/92 | 11:11 | 2.0 (5) |
| 7 | 11/9/92 | 12:12 | 128 (5) |
| 8 | 1/4/93 | 13:13 | 1.0 (1) |
| | | 13:13 | 0.5 (4) |
| 9 | 1/5/93 | 14:14 | 2.0 (5) |
| 10 | 2/9/93 | 15:22 | 0.12 (1) |
| | | 15:22 | 0.25 (3) |
| | | 15:22 | 32 (1) |
| | 2/24/93 | 20:23 | 0.12 (5) |
| 11 | 3/1/93 | 6:23 | 0.25 (1) |
| | | 6:23 | 0.12 (1) |
| | | 6:24 | 0.12 (2) |
| | | 6:25 | 0.12 (1) |
| 12 | 2/11/93 | 6:25 | 16 (3) |
| | | 6:25 | 64 (2) |
| 13 | 3/4/93 | 21:26 | 4.0 (2) |
| | | 22:27 | 0.12 (2) |
| | | 23:26 | 0.12 (1) |
| | 3/18/93 | 23:26 | 0.12 (1) |
| | | 22:27 | 0.12 (1) |
| | | 22:27 | 0.25 (2) |
| | | 22:26 | 0.12 (1) |
| 14 | 4/1/93 | 24:28 | 0.25 (5) |
| 15 | 2/1/93 | 35:39 | 0.12 (3) |
| | | 35:39 | 0.25 (2) |
| 16 | 6/3/93 | 36:40 | 0.12 (5) |
| 17 | 6/3/93 | 35:39 | 0.12 (5) |
| 18 | 1/7/93 | 37:41 | 2.0 (5) |
| | 1/21/93 | 37:41 | 4.0 (1) |
| | | 37:41 | 8.0 (4) |
| 19 | 4/22/93 | 1:5 | 2.0 (1) |
| | | 1:5 | 0.12 (4) |
| 20 | 4/8/93 | 2:5 | 0.12 (5) |
| 21 | 4/16/93 | 42:5 | 0.12 (1) |
| | | 42:5 | 0.25 (2) |
| | | 42:5 | 1.0 (2) |
| | 4/30/93 | 43:48 | 0.12 (3) |
| 22 | 10/14/93 | 7:29 | 0.25 (1) |
| | | 7:29 | 0.5 (4) |
| 23 | 10/19/93 | 50:56 | 0.12 (1) |
| | | 51:57 | 0.12 (5) |

[a] Composite DNA type indicated numerically as the *Sfi*I subtype followed by the *Bss*HII subtype.

[b] The number in parentheses is the number of isolates with the given DNA type for which the MIC was as indicated.

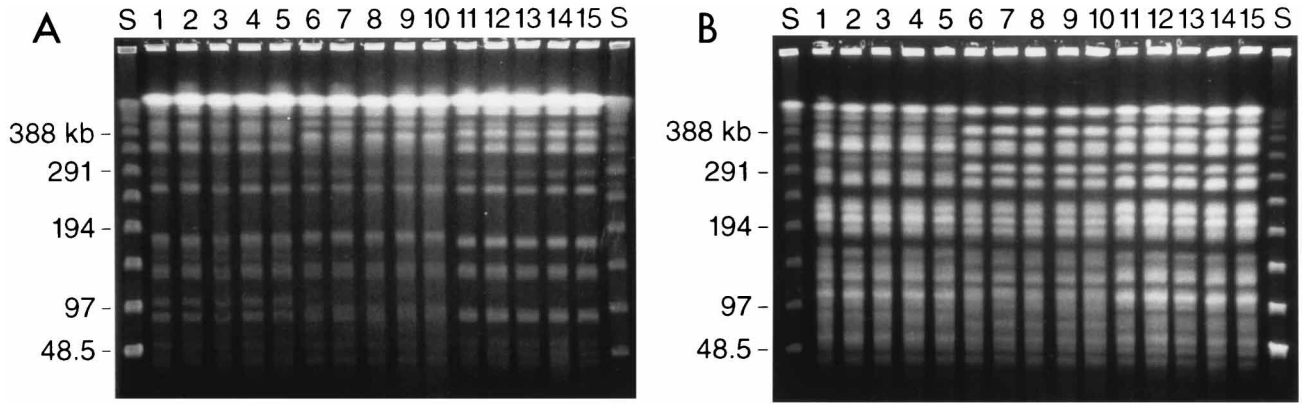


FIG. 1. Representative DNA profiles of *C. albicans* isolates from each of three patients (five isolates each). (A) Profiles obtained with restriction enzyme *Sfi*I; (B) profiles obtained with *Bss*HII. Lanes: S, lambda phage molecular size standards (kilobases); 1 to 5, five colonies from episode 5 in patient 26 (DNA type 52:6); 6 to 10, five colonies from episode 4 in patient 28 (DNA type 26:29); 11 to 15, five colonies from episode 8 in patient 27 (DNA type 15:21).

the fluconazole MIC was 2.0 μg/ml. However, the next culture, taken when 100 mg of fluconazole had failed, showed one isolate for which the MIC was 4.0 μg/ml and four isolates for which the MIC was 8.0 μg/ml. Patient 34 yielded a single strain (DNA subtype) of *C. albicans*, for which the MIC was 0.25 μg/ml, at the initial infection. At 2 weeks without clinical cure, *Candida guilliermondii*, for which the MIC was 8.0 μg/ml, was isolated. The dose of fluconazole was increased to 200 mg, with clinical cure. Subsequently, the patient relapsed and was treated with 200 mg of fluconazole but no culture was obtained prior to treatment. At 2 weeks the patient was still without clinical cure, and the culture obtained at this time revealed that all five isolates were the same *C. albicans* subtype as that for the first infection and that the MIC was 0.25 μg/ml for all of them. The dosage of fluconazole was increased to 400 mg per day, with clinical cure. However, the end-of-therapy culture was positive for colonization with a new subtype of *C. albicans*, for which the MIC was 8.0 μg/ml. Patient 31 responded to 100 mg of fluconazole at the initial infection, when all five isolates were the same DNA subtype and the MIC for each was 0.12 μg/ml. However, at the first relapse infection the fluconazole dosage had to be increased to 200 mg per day for clinical cure. DNA subtypes and MICs, however, remained the same as those for the initial infection.

Among the 534 isolates of *C. albicans* recovered throughout the study, 70 different DNA subtypes were identified. Thirty-two patients (84%) showed a single DNA subtype with their first infection and 20 patients (54%) were infected or colonized with one DNA subtype throughout each infection. Eighteen patients (46%) were infected or colonized with two or more different DNA subtypes over all infections.

In vitro susceptibility testing of the 534 isolates with fluconazole showed a broad range of MICs (≤0.125 to ≥64 μg/ml). The MICs for 494 (93%) of the isolates were ≤8.0 μg/ml, and the MICs for 36 (7%) were ≥64 μg/ml. Five patients (13%) exhibited isolates resistant to fluconazole at their first infection in this study. (According to National Committee for Clinical Laboratory Standards recommendations we considered isolates for which the MICs were ≤8.0 μg/ml as susceptible and those for which the MICs were ≥64 μg/ml as resistant.) Two additional fluconazole-resistant subtypes were detected (at the end of therapy in one patient and in a subsequent episode in one patient). The patients with resistant isolates displayed a variable clinical pattern. Patients 3 and 7 did not return for follow-up and so were not evaluated for clinical cure. Patients 5, 12, 26, and 37 all achieved clinical cure with a 100-mg/day dosage. Patients 12, 26, and 37 had a mixture of susceptible and resistant isolates. Patient 5 had only resistant isolates.

Twenty-three patients (61%) experienced a single episode of oropharyngeal candidiasis (Table 2). Eighteen (78%) of these showed only one DNA subtype. Of the five patients with greater than one DNA subtype, one patient had four types, one had three types, and two had two types. Two groups of two patients each shared the same subtype. In the patients with only one episode of infection, for 118 (85%) isolates the MICs of fluconazole were ≤8.0 μg/ml and for 17 (12%) isolates the MICs were ≥64 μg/ml. The mean CD4 count for this group was 182/mm$^3$ with a range of 3.0 to 695/mm$^3$. Representative DNA profiles from three patients in this group are shown in Fig. 1.

Fifteen patients (39%) experienced more than one episode (range, 2 to 8; total, 61) of oropharyngeal candidiasis (Table 3). Twelve (80%) of these patients exhibited more than one DNA subtype (range, 2 to 8). No patients had the same subtype. For 376 (95%) isolates from these patients the MICs of fluconazole were ≤8.0 μg/ml, and for 19 (5%) isolates the MICs were ≥64 μg/ml. The mean CD4 count for this group was 82/mm$^3$ with a range of 4.0 to 529/mm$^3$.

Statistical analysis was performed to evaluate differences in DNA subtype frequency and CD4 count between the group with single infections and the group with multiple infections. By using chi-square analysis, the multiple-infection group was found to be more likely to exhibit multiple DNA subtypes than the single-infection group, with a $P$ value of 0.0014. By using Student's $t$ test analysis the multiple-infection group was found to have significantly lower CD4 counts than the single-infection group, with a $P$ value of 0.016.

## DISCUSSION

This study attempted to sample a broad cross section of DNA subtypes of *C. albicans* at baseline and over multiple infections. The technique of selecting five individual colonies from each culture was chosen at random. It is possible that significant subtypes were present in these cultures but were not detected with the method we used. In evaluating DNA subtype diversity of oropharyngeal candidiasis infections in AIDS patients, the results of this study paralleled our earlier study and showed that the majority of patients had a single DNA subtype that was susceptible (MIC of ≤8.0 μg/ml) to fluconazole in the

TABLE 3. Variation in DNA type and fluconazole MIC among isolates of *C. albicans* from patients with multiple episodes of oropharyngeal candidiasis

| Patient | Episode | Date (mo/day/yr) | DNA type[a] | MIC (μg/ml)[b] |
|---|---|---|---|---|
| 24 | 1 | 9/21/92 | 1:1 | 1.0 (5) |
| | 2 | 4/19/93 | 2:2 | 0.12 (5) |
| 25 | 1 | 9/28/92 | 2:3 | 0.25 (5) |
| | 2 | 12/28/92 | 3:3 | 0.25 (5) |
| 26 | 1 | 10/26/92 | 5:5 | 0.25 (3) |
| | | | 5:5 | 128 (2) |
| | 2 | 1/11/93 | 5:5 | 128 (5) |
| | | 1/25/93 | 6:6 | 0.5 (5) |
| | 3 | 3/4/93 | 7:5 | 0.25 (5) |
| | 4 | 6/14/93 | 7:5 | 0.25 (2) |
| | | | 7:5 | 0.5 (1) |
| | | | 7:5 | 128 (2) |
| | 5 | 10/1/93 | 52:6 | 0.12 (5) |
| 27 | 1 | 9/21/92 | 15:15 | 0.25 (2) |
| | | | 16:16 | 0.25 (1) |
| | | | 16:16 | 0.5 (2) |
| | | 10/5/92 | 16:16 | 0.25 (2) |
| | | | 17:17 | 0.25 (3) |
| | 2 | 11/2/92 | 15:18 | 2.0 (2) |
| | | | 17:17 | 0.12 (1) |
| | | | 18:18 | 2.0 (1) |
| | | | 19:19 | 0.12 (1) |
| | | 11/17/92 | 17:17 | 0.12 (5) |
| | 3 | 12/14/92 | 15:20 | 0.12 (5) |
| | | 1/5/93 | 17:17 | 1.0 (4) |
| | | | 17:17 | 0.12 (1) |
| | 4 | 4/29/93 | 15:21 | 0.12 (5) |
| | | 5/10/93 | 17:17 | 2.0 (1) |
| | | | 17:17 | 1.0 (2) |
| | | | 17:17 | 0.5 (2) |
| | 5 | 7/12/93 | 15:21 | 0.12 (5) |
| | | 7/26/93 | 17:17 | 0.12 (3) |
| | | | 17:17 | 0.25 (2) |
| | 6 | 8/23/93 | 15:21 | 0.12 (5) |
| | | 9/7/93 | 17:17 | 0.25 (2) |
| | | | 17:17 | 0.12 (2) |
| | | | 17:17 | 8.0 (1) |
| | 7 | 3/15/93 | 15:21 | 0.12 (5) |
| | | 3/28/93 | 17:17 | 0.12 (5) |
| | 8 | 2/21/93 | 15:21 | 0.12 (5) |
| 28 | 1 | 9/28/92 | 26:29 | 0.12 (5) |
| | 2 | 1/27/93 | 26:29 | 0.12 (5) |
| | 3 | 6/21/93 | 26:29 | 0.25 (5) |
| | | 7/1/93 | 27:30 | 0.12 (5) |
| | 4 | 2/7/94 | 26:29 | 0.5 (5) |
| 29 | 1 | 1/14/93 | 28:31 | 4.0 (1) |
| | | | 28:31 | 8.0 (4) |
| | 2 | 5/12/93 | 29:32 | 0.12 (5) |
| | 3 | 6/21/93 | 30:33 | 0.25 (5) |
| | | 7/9/93 | 31:34 | 4.0 (5) |
| | 4 | 7/20/93 | 31:34 | 4.0 (5) |
| | 5 | 8/16/93 | 31:34 | 4.0 (5) |
| 30 | 1 | 1/14/93 | 32:35 | 0.12 (5) |
| | | 1/27/93 | 33:36 | 4.0 (5) |
| | 2 | 3/18/93 | 32:37 | 0.12 (5) |
| | 3 | 6/7/93 | 32:37 | 0.12 (5) |
| | 4 | 10/28/93 | 32:37 | 0.12 (5) |
| | 5 | 2/11/94 | 32:37 | 0.12 (5) |
| 31 | 1 | 4/29/93 | 15:23 | 0.12 (5) |
| | 2 | 7/19/93 | 15:23 | 0.12 (5) |
| | | 8/2/93 | 15:23 | 0.12 (5) |
| 32 | 1 | 2/22/93 | 39:43 | 0.12 (5) |
| | 2 | 10/11/93 | 39:43 | 0.12 (5) |
| | 3 | 11/2/93 | 39:43 | 0.12 (5) |
| | 4 | 12/9/93 | 39:43 | 0.12 (5) |
| | | 12/27/93 | 39:44 | 2.0 (3) |
| | | | 39:44 | 4.0 (2) |
| | 5 | 1/8/94 | 39:43 | 0.25 (3) |
| | | | 39:45 | 0.25 (2) |
| | 6 | 2/21/94 | 39:46 | 1.0 (5) |
| 33 | 1 | 9/2/93 | 40:47 | 0.12 (5) |
| | | 9/16/93 | 41:47 | 0.12 (5) |
| | 2 | 12/30/93 | 41:47 | 0.12 (5) |
| 34 | 1 | 3/26/93 | 44:49 | 0.25 (5) |
| | 2 | 6/18/93 | 44:49 | 0.25 (5) |
| | | 7/1/93 | 45:50 | 8.0 (5) |
| 35 | 1 | 6/14/93 | 46:51 | 0.12 (5) |
| | | 6/28/93 | 46:51 | 0.12 (5) |
| | 2 | 8/16/93 | 46:51 | 0.25 (5) |
| | | 8/30/93 | 46:51 | 0.12 (5) |
| | 3 | 11/1/93 | 46:51 | 0.12 (4) |
| | | | 46:53 | 0.12 (1) |
| | 4 | 2/17/94 | 47:53 | 0.12 (3) |
| | | | 47:53 | 0.25 (2) |
| | 5 | 3/29/94 | 47:53 | 0.12 (5) |
| 36 | 1 | 2/16/93 | 48:54 | 0.12 (5) |
| | 2 | 11/8/93 | 48:54 | 0.12 (5) |
| | 3 | 3/7/94 | 48:54 | 0.12 (5) |
| 37 | 1 | 9/27/93 | 51:58 | 0.12 (5) |
| | | 10/11/93 | 51:58 | 256 (5) |
| | 2 | 11/22/93 | 51:58 | 0.25 (5) |
| | 3 | 1/24/94 | 51:58 | 256 (5) |
| 38 | 1 | 10/29/92 | 15:23 | 0.5 (4) |
| | | | 15:23 | 0.25 (1) |
| | 2 | 1/7/93 | 15:23 | 0.25 (1) |
| | | | 15:23 | 1.0 (4) |
| | 3 | 3/18/93 | 15:23 | 1.0 (3) |
| | | | 15:23 | 2.0 (2) |
| | 4 | 6/1/93 | 15:23 | 0.25 (1) |
| | | | 15:23 | 0.5 (4) |
| | 5 | 7/29/93 | 49:55 | 4.0 (5) |
| | 6 | 9/27/93 | 15:23 | 0.12 (4) |
| | | | 15:23 | 1.0 (1) |
| | 7 | 1/27/94 | 15:23 | 1.0 (5) |

[a] Composite DNA type indicated numerically as the *Sfi*I subtype followed by the *Bss*HII subtype.

[b] The number in parentheses is the number of isolates with the given DNA type for which the MIC was as indicated.

pretreatment culture of their initial infection while under study. Also, over the course of treatment of the initial and subsequent infections, resistant subtypes (MIC of ≥64 μg/ml) were very uncommon. In some cases different DNA subtypes and fluconazole MICs were observed among isolates obtained from a single culture, and in other cases they emerged in subsequent cultures. However, for the vast majority of isolates, the MICs were ≤8.0 μg/ml and the subtypes for which the MICs were higher were not commonly selected following exposure to fluconazole. Also, as in our previous study, subtype diversity was more common in patients with multiple episodes of oropharyngeal candidiasis than in those with a single episode. Also, multiple episodes appeared related to increasing immunosuppression as measured by CD4 count.

Clinical response was excellent and usually correlated with the MICs of fluconazole, but there were exceptions. One patient with only resistant isolates responded to regular (100 mg per day) fluconazole dosing. Three patients with mixed susceptible and resistant isolates also were clinically cured on 100 mg of fluconazole. It is possible that the susceptible isolates were the primary cause of the clinical infection and the resistant isolates only represented colonization.

The three patients that required increased doses of fluconazole presented an interesting pattern. One patient (patient 31) needed 200-mg dosing for clinical cure, although this patient had only one DNA subtype throughout and the MICs of fluconazole for this subtype were very low. One patient (pa-

tient 18) showed isolates for which the MICs increased from the initial culture through one treatment failure culture, even though all DNA subtypes were the same. The highest MIC reached only 8.0 μg/ml, but 200 mg of fluconazole was required for clinical cure. This patient was severely immunosuppressed (CD4 count = 11/mm$^3$), which may account for the need for a higher dose. Patient 34 appears to be the only patient in this study where a more resistant *Candida* strain may have been selected in subsequent infections. Although the initial infection appeared to be due to a strain of *C. albicans* that was susceptible to fluconazole (MIC = 0.25 μg/ml), subsequent episodes of infection required higher doses of fluconazole and were associated with isolation of strains of *C. guilliermondii* and *C. albicans* for which the fluconazole MICs were elevated (8.0 μg/ml). Relative immunosuppression may have played a role here as well, with a CD4 count of 14/mm$^3$.

As we and others have shown, there is significant variation among *C. albicans* isolates with respect to DNA subtype and antifungal susceptibility in AIDS patients with oropharyngeal candidiasis. This variation is most common in those with multiple recurrences of infection and probably is a reflection of multiple exposures to different subtypes in an increasingly immunocompromised host. Alternatively, the appearance of different DNA subtypes may represent either microevolution or "substrain shuffling" as described by Lockhart et al. (4). These investigators employed a highly sensitive DNA fingerprinting method and sophisticated computer analysis to demonstrate the presence of relatively stable subgenotypes in the infecting population for women with recurrent vaginitis. They observed a shuffling process that occurs between subgenotypes in sequential infections and that accounts for genotypic variation in sequential isolates obtained from these patients.

Exposure to fluconazole or other antifungals may play a role in the selection of different subtypes, but in this study it does not appear to have commonly selected for more-resistant subtypes. Also, the appearance of stable resistance was not observed in this study. This suggests that although a resistant clone may appear in an episode of oropharyngeal candidiasis, it may not persist or recur once clinical response has been achieved by either higher dosing or alternative agents. Thus, subsequent episodes may not involve the previous resistant subtypes and may respond to fluconazole (patients 26 and 37, Table 3). In such instances antifungal susceptibility testing may be very useful in directing therapy of recurrent oropharyngeal candidiasis. Treatment strategies may involve cycling of different antifungal agents in order to preserve efficacy and to avoid the selection of a stably resistant clone.

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