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Variations in Fluconazole Susceptibility and Electrophoretic Karyotype among Oral Isolates of *Candida albicans* from Patients with AIDS and Oral Candidiasis

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DNA subtyping by pulsed-field gel electrophoresis and in vitro susceptibility testing were used to study strain variation and fluconazole resistance in *Candida albicans* isolates from patients with AIDS undergoing azole (fluconazole and clotrimazole) therapy for oropharyngeal candidiasis. A total of 29 patients suffered 71 episodes of oropharyngeal candidiasis. Overall, 121 isolates of *C. albicans* recovered throughout the course of treatment of each infection were available for further characterization. DNA subtyping revealed a total of 61 different DNA subtypes. In vitro susceptibility testing of the 121 isolates by using proposed standard methods of the National Committee for Clinical Laboratory Standards revealed MICs of fluconazole ranging from ≤0.125 to >64 μg/ml. The MIC for 50% of isolates tested was 0.25 μg/ml, and the MIC for 90% of isolates tested was 8.0 μg/ml. MICs were ≥64 μg/ml for only 7.4% of the isolates tested. The majority (62%) of the patients with oropharyngeal candidiasis and undergoing azole therapy were infected or colonized with more than one DNA subtype, and the introduction or selection of strains with a more resistant DNA subtype during the course of fluconazole therapy was not uncommon. With one exception, this did not appear to have an adverse effect on clinical outcome. In contrast, for patients with AIDS and oropharyngeal candidiasis infected with a single DNA subtype of *C. albicans*, an increase in fluconazole MICs for the infecting strain was rarely demonstrated over the course of therapy.

Oropharyngeal candidiasis is a common manifestation in immunocompromised patients, including individuals undergoing immunosuppressive therapy for cancer or organ transplantation and those exposed to broad-spectrum antibacterial therapy (1, 6, 11, 14, 19). Most notably, oropharyngeal candidiasis is a major problem in individuals infected with the human immunodeficiency virus (3, 7, 14, 16, 18, 23). Individuals with human immunodeficiency virus infection and AIDS suffer from painful, recurrent oral candidiasis which may be complicated by esophageal candidiasis leading to gastrointestinal bleeding, perforation, or rarely, disseminated candidiasis (7, 18, 19). *Candida albicans* is the species most commonly isolated from patients with these infections.

Treatment of oropharyngeal candidiasis is generally effective and usually involves the use of topical or systemic antifungal therapy with drugs such as the polyenes (amphotericin B and nystatin) and the azoles (clotrimazole, ketoconazole, fluconazole, and itraconazole). Unfortunately, because of the profound and sustained immunosuppression in patients with AIDS, relapse or reinfection is common, and these patients must be followed closely for a clinical recurrence that requires further treatment. For these reasons, patients with AIDS commonly receive antifungal therapy for multiple oral infections with *C. albicans* over a prolonged period of time. The widespread use of antifungal therapy, particularly the azoles, for extended periods has raised concerns regarding the development of resistance among isolates of *Candida* species (6, 8, 11, 14, 23). Recently, concern has been raised regarding the development of resistance to fluconazole; reports indicate that some patients with oropharyngeal and esophageal candidiases are failing fluconazole therapy, despite an initial favorable response to the agent (3, 15, 16, 23). Fluconazole MICs for isolates of *C. albicans* obtained from patients with these infections occasionally are four- to eightfold greater than those for control isolates (3, 15, 16, 23). Unfortunately, because serial isolates from these patients have not usually been saved and characterized, little is known about strain variation and changes in fluconazole susceptibility among isolates of *C. albicans* obtained from patients on long-term antifungal therapy. An unanswered question about fluconazole resistance is whether resistance to fluconazole develops with a single strain during the course of therapy or whether an initially susceptible strain is simply replaced by a genotypically distinct strain which manifests resistance to fluconazole. The objective of the present study was to study strain variation and fluconazole resistance in *C. albicans* isolates from AIDS patients undergoing azole (fluconazole and clotrimazole) therapy for oropharyngeal candidiasis.

MATERIALS AND METHODS

Patients. Twenty-nine patients (28 male and 1 female) who both fulfilled the criteria for AIDS of the Centers for Disease Control and Prevention and had oropharyngeal candidiasis were treated with either fluconazole (62 episodes) or clotrimazole (9 episodes). All patients were treated and followed at the University of Texas Health Science Center in San Antonio. Each episode of oropharyngeal candidiasis was documented by subjective complaints of oropharyngeal dis-

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comfort, objective clinical presentation of white plaques or erythema, a positive potassium hydroxide preparation, and a positive culture for *C. albicans*. A clinical examination and mycological assessment (microscopy and culture) were undertaken pretreatment. Each episode was treated orally with fluconazole for 14 days at dosages of 100 to 400 mg/day. The nine patients (nine episodes) treated with clotrimazole were treated for 14 days at a dosage of 50 mg/day. At the end of 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. Eighteen of the 29 patients suffered two or more episodes of infection (range, two to nine episodes). Clinical resolution of each episode was defined as the absence of oral lesions on examination. The total time that each patient was involved in the study (treatment and follow-up) ranged from 7 to 485 days (mean, 106 days), and the interval between episodes ranged from 10 to 266 days (mean, 52 days).

Isolates. A total of 121 isolates of *C. albicans* were obtained from 71 episodes of infection. Oropharyngeal swabs were plated onto Sabouraud dextrose agar plates (Emmons modification, P87-0; BBL), and the plates were incubated for 48 h at 25°C. Colonies of yeasts and fungi obtained from the primary culture plate were streaked for isolation onto Sabouraud dextrose agar and identified by standard methods (21). Single colonies were picked and saved on Sabouraud dextrose agar slants until needed for DNA typing and antifungal susceptibility testing.

DNA subtyping. Isolates were evaluated for molecular relatedness by pulsed-field gel electrophoresis of chromosome-size DNA molecules to generate an electrophoretic karyotype for each isolate (2, 9, 10, 20). Electrophoretic karyotyping was performed by a modification of the method of Doebbeling et al. (2). Briefly, 5 to 10 colonies of *C. albicans* grown on Sabouraud agar were inoculated in YEPD broth (1% yeast extract, 2% glucose, 2% Bacto Peptone) and were grown overnight at 30°C in a shaking water bath. The cells were pelleted, and 150 μl of the packed cells was transferred to an Eppendorf tube and washed two times with 200 μl of 50 mM EDTA (pH 8.0). The suspension of cells was evenly mixed with 100 μl of yeast cell wall-degrading enzymes (Lyticase L5263, partially purified grade; 1,250 U/ml in 50% [vol/vol] glycerol–0.01 M $NaPO_4$ [pH 7.5]; Sigma Chemical, St. Louis, Mo.), and the mixture was incubated at 37°C for 20 min. Aliquots of 460 μl of 1% low-melting-point agarose (Bio-Rad, Richmond, Calif.) in 125 mM EDTA (pH 7.5) was added to the solution, and the mixture was dispensed into molds to form agarose plugs. The plugs were incubated overnight at 50°C in 1.5 ml of a solution of buffer (0.01 M Tris [pH 7.5], 0.45 M EDTA [pH 8.0], 1% lauroylsarcosine) containing proteinase K (1 mg/ml; protease type XXVIII, 20 U/ml; Sigma). Agarose inserts were washed three times with 3 ml of 50 mM EDTA (pH 8.0) and were incubated overnight at room temperature. Washing was repeated two more times on the following day with the same solution. A slice of 1 to 2 mm from the plugs was inserted into a 1% agarose gel, and the chromosome-size pieces of DNA were resolved by using a contour-clamped homogeneous electric field system (CHEF DRII; Bio-Rad). Electrophoresis was carried out at 150 V and 13°C with pulse intervals of 120 s for 24 h and 240 s for 36 h. After electrophoresis, gels were stained with ethidium bromide and photographed under UV light. *Saccharomyces cerevisiae* chromosome or DNA size standards (Bio-Rad) were included in each gel as standards.

Analysis of electrophoretic karyotypes was performed by visual inspection of photographs of ethidium bromide-stained gels. Each major band and each minor band was identified, and the distance from the origin of the gel relative to those of the molecular mass standards was measured. Isolates were judged to have different DNA subtypes if their electrophoretic karyotype profile differed by one or more bands.

All isolates were analyzed in two or more runs (mean, 5 runs; range, 2 to 15 runs) to allow adjacent migration of DNA samples with similar patterns, to better ascertain pattern relatedness, and to ensure reproducibility. As reported previously (2, 15), reproducibility was excellent (≥95%) for the replicate samples.

Antifungal susceptibility testing. The susceptibilities of *C. albicans* isolates to fluconazole was performed by using proposed standard methods of the National Committee for Clinical Laboratory Standards (4, 5, 12, 13). Briefly, isolates were tested by a broth macrodilution method with RPMI 1640 medium (BioWhitaker, Walkersville, Md.) buffered to pH 7.0 with MOPS (3-[*N*-morpholino]-propanesulfonic acid) buffer, an inoculum of 10^3 cells per ml, and incubation at 35°C for 48 h. The range of fluconazole concentrations tested was 0.125 to 64 μg/ml. Following incubation, the growth in each tube was scored as follows: 0, optically clear; 1+, slightly hazy; 2+, prominent reduction in turbidity compared with that of the drug-free control (80% inhibition endpoint); 3+, slight reduction in turbidity compared with that of the drug-free control; 4+, no reduction in turbidity compared with that of the drug-free control. As recommended by Espinel-Ingroff et al. (4), the MIC was defined as the lowest fluconazole concentration in which the growth score was 2+ (80% inhibition) or less following 48 h of incubation. The intralaboratory reproducibility of this method has been shown to be >95% within a fourfold concentration range (4, 5). Thus, in the present study a significant increase in the fluconazole MIC was judged to occur when the MIC increased by fourfold or more for isolates tested in parallel.

RESULTS

A total of 29 patients suffered 71 episodes of oropharyngeal candidiasis. Sixty-two of the episodes (22 patients) were treated with fluconazole (Tables 1 and 2), and nine of the episodes (9 patients) were treated with clotrimazole (Table 3). Two patients (patients 16 and 25) were treated with both clotrimazole (initial episode) and fluconazole (subsequent episodes).

With one exception, all episodes of infection in all patients responded clinically to treatment with 100 mg of fluconazole per day. The exception was patient 20 (Table 1), whose dosage had to be increased to 400 mg/day at infection episode 9. This corresponded to an increase in the fluconazole MIC from 8.0 to >64 μg/ml. Although patients 7 and 23 (Table 2) were also colonized or infected with strains of *C. albicans* for which fluconazole MICs were ≥64 μg/ml, they responded clinically to 100 mg of fluconazole. All patients except patients 3 and 18 receiving clotrimazole (Table 3) achieved clinical cure.

Overall, 121 isolates of *C. albicans* recovered from the beginning and throughout the course of treatment of each infection were available for further characterization. DNA subtyping of these isolates revealed a total of 61 different DNA subtypes (Fig. 1). Eleven patients (38%; 27 episodes of infection) were infected or colonized with a single DNA

TABLE 1. Variation in fluconazole MICs for strains from patients infected or colonized with a single DNA subtype of *C. albicans* and undergoing therapy with fluconazole

Patient no.	Infection episode no.	Isolate	DNA subtype	Fluconazole MIC (μg/ml)
2	1	R-111	T	≤0.125
	2	R-158	T	≤0.125
	3	R-170	T	≤0.125
5	1	R-1013	A	0.25
	1	R-1034	A	0.25
	2	R-1848	A	0.25
	2	R-1854	A	0.25
19	1	R-1701	P	0.25
	2	R-1717	P	≤0.125
20	1	R-1587	V	0.25
	3	R-1731	V	1.0
	3	R-1758	V	8.0
	4	R-1761	V	8.0
	5	R-1794	V	8.0
	5	R-1797	V	8.0
	6	R-1832	V	8.0
	6	R-1845	V	8.0
	7	R-1885	V	8.0
	7	R-1928	V	8.0
	8	R-1946	V	8.0
	9	91-1427	V	8.0
	9	91-1514	V	>64
22	1	R-1786	II	≤0.125
	2	R-1857	II	0.25
24	1	R-1810	I	≤0.125
	2	R-1856	I	≤0.125
	3	R-1920	I	≤0.125
28	1	R-1957	HHH	1.0
	1	R-1964	HHH	4.0
	2	R-2046	HHH	2.0
29	1	R-1958	III	≤0.125
	2	R-2063	III	≤0.125
	2	R-2065	III	≤0.125

subtype throughout each episode of infection (Tables 1 and 3). Eighteen patients (62%; 42 episodes of infection) were infected or colonized with two or more different DNA subtypes (Tables 2 and 3).

In vitro susceptibility testing of the 121 isolates to fluconazole revealed a range of MICs (≤0.125 to >64 μg/ml) (Tables 1 to 3). The MIC for 50% of isolates tested was 0.25 μg/ml, and the MIC for 90% of isolates tested was 8.0 μg/ml. MICs of ≥64 μg/ml were found for only 7.4% (9 of 121) of the isolates tested.

There were 24 episodes of infection in eight patients who were treated with fluconazole and in which a single DNA subtype of *C. albicans* was isolated from each episode of infection (Table 1 and Fig. 2). These 24 episodes represented 33 isolates and eight different DNA subtypes (Table 1). Each patient carried his or her own unique subtype which was isolated at the onset of (and frequently throughout) each episode of infection. Antifungal susceptibility testing revealed that a significant increase (fourfold or greater) in the fluconazole MICs for isolates recovered over the course of treatment occurred in only two (25%) of the eight patients (patients 20 and 28; Table 1).

TABLE 2. Variation in fluconazole MICs for strains from patients infected or colonized with multiple DNA subtypes of *C. albicans* and undergoing therapy with fluconazole

Patient no.	Infection episode no.	Isolate	DNA subtype	Fluconazole MIC (μg/ml)
4	1	R-1031	YY	≤0.125
	1	R-1040	XX	≤0.125
	1	R-1103	XX	≤0.125
6	1	R-1796	W	≤0.125
	2	R-1835	W	2.0
	3	R-1871	W	2.0
	4	R-1896	WW	0.25
7	1	R-1804	D	8.0
	1	R-1811	E	64
	2	R-1846	D	4.0
	2	R-1852	F	64
	3	R-1878	G	0.5
	3	R-1881	F	64
	4	R-1947	H	8.0
9	1	R-1588	J	≤0.125
	1	R-1702	K	≤0.125
	2	R-1809	L	≤0.125
	2	R-1834	M	1.0
	3	R-1853	L	0.25
11	1	R-1200	N	0.25
	2	R-1585	O	8.0
	2	R-1589	O	8.0
12	1	R-1272	R	0.25
	1	R-1275	S	0.25
	1	R-1276	U	2.0
14	1	R-1016	JJJ	≤0.125
	1	R-1024	BB	0.25
	1	R-1037	JJ	0.25
	1	R-1044	JJJ	≤0.125
16	2	R-1704	UU	0.25
	3	R-1729	HH	≤0.125
	3	R-1756	MM	0.25
	4	R-1783	UU	0.25
	4	R-1790	W	0.25
17	1	R-1038	Y	0.25
		R-1043	ZZ	0.25
21	1	R-1278	X	≤0.125
	2	R-1703	NN	≤0.125
	3	R-1762	KK	0.25
	4	R-1833	NN	≤0.125
	5	R-1879	NN	≤0.125
	6	R-1932	NN	≤0.125
23	1	R-1705	PP	>64
	2	R-1730	OO	>64
	3	R-1756	W	0.25
	3	R-1760	QQ	>64
	4	R-1784	RR	>64
	5	R-1891	OO	>64
25	2	R-1700	XX	0.25
	3	R-1759	Z	0.25
26	1	R-1919	BBB	0.25
	1	R-1933	CCC	2.0
	2	R-1945	DDD	4.0
	2	R-1963	DDD	2.0
	3	R-2045	CCC	0.25
	3	R-2064	CCC	0.25
27	1	R-1956	EEE	1.0
	1	R-1961	EEE	1.0
	2	R-2047	FFF	1.0
	2	R-2062	GGG	4.0

TABLE 3. Variation in DNA subtypes and fluconazole susceptibilities of *C. albicans* isolated from patients undergoing therapy with clotrimazole

Patient no.	Isolate[a]	DNA subtype	Fluconazole MIC (μg/ml)
1	R-1109	B	0.25
	R-1119	C	0.25
	R-1179	B	0.25
3	R-1235	Q	≤0.125
	R-1250	Q	0.25
	R-1257	Q	≤0.125
8	R-1011	GG	0.5
	R-1032	GG	0.25
10	R-1114	JJJ	≤0.125
	R-1124	JJJ	≤0.125
	R-1198	JJJ	≤0.125
13	R-1106	LL	0.5
	R-1108	SS	0.25
	R-1112	SS	0.25
15	R-1104	CC	0.25
	R-1105	TT	≤0.125
	R-1107	TT	≤0.125
	R-1113	TT	≤0.125
16	R-1079	UU	0.25
	R-1098	UU	0.25
18	R-994	EE	4.0
	R-1009	EE	4.0
	R-1015	EE	4.0
	R-1023	AAA	8.0
	R-1036	FF	4.0
25	R-1281	XX	≤0.125
	R-1308	DD	≤0.125
	R-1309	DD	≤0.125

[a] All isolates were from one infection episode.

There were 38 episodes of infection in 14 patients treated with fluconazole and from whom multiple (two or more) different DNA subtypes were isolated (Table 2 and Fig. 3). These 38 episodes represented 60 isolates and 41 different DNA subtypes (Table 2). Each patient was infected or colonized by more than one subtype (range, two to five subtypes) throughout the period of study. Three subtypes were isolated from more than one patient, as follows: subtype W from patients 16 and 23, subtype WW from patients 4 and 25, and subtype JJJ from patients 10 and 14. Antifungal susceptibility testing revealed that a significant increase (fourfold or greater) in the fluconazole MICs for isolates recovered over the course of treatment occurred in 8 (57%) of the 14 patients (patients 6, 7, 9, 11, 12, 23, 26, and 27). Frequently, the more resistant isolate had a DNA subtype different from that observed previously. In only one instance (patient 6) was a significant increase in the fluconazole MIC observed within the same DNA subtype.

Nine patients were treated with clotrimazole for single episodes of infection (Table 3). Two of these patients (patients 16 and 25) were treated subsequently with fluconazole for new episodes of infection (Table 2). These nine episodes of infection comprised 28 isolates and 15 DNA subtypes. Four patients (patients 3, 8, 10, and 16) were infected or colonized with a single DNA subtype throughout the episode of infection, while the remainder (patients 1, 13, 15, 18, and 25) were infected or colonized with isolates exhibiting more than one DNA subtype (range, two to three subtypes) throughout the episode of infection. Antifungal susceptibility testing indicated that a significant increase in fluconazole (or clotrimazole; data not shown) MICs failed to occur for isolates recovered over the course of treatment.

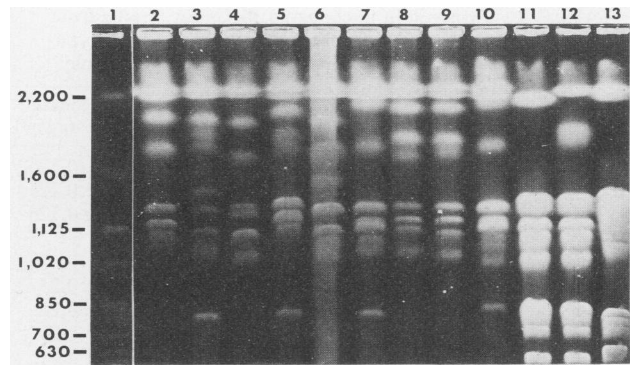

FIG. 1. Contour-clamped homogeneous electric field electrophoresis of *C. albicans* isolates from eight different patients. Lane 1, *S. cerevisiae* chromosome or DNA size standards (in kilobases); lane 2, DNA subtype A (patient 5); lane 3, DNA subtype YY (patient 4); lane 4, DNA subtype II (patient 22); lane 5, DNA subtype DD (patient 25); lane 6, DNA subtype D (patient 7); lane 7, DNA subtype K (patient 9); lane 8, DNA subtype W (patient 16); lane 9, DNA subtype MM (patient 16); lane 10, DNA subtype L (patient 9); lane 11, DNA subtype F (patient 7); lane 12, DNA subtype E (patient 7); lane 13, DNA subtype FF (patient 18).

DISCUSSION

The results of the present study extend those of previous investigators regarding strain variation and antifungal resistance among isolates of *C. albicans* from patients with AIDS and oropharyngeal candidiasis. In the present study, we

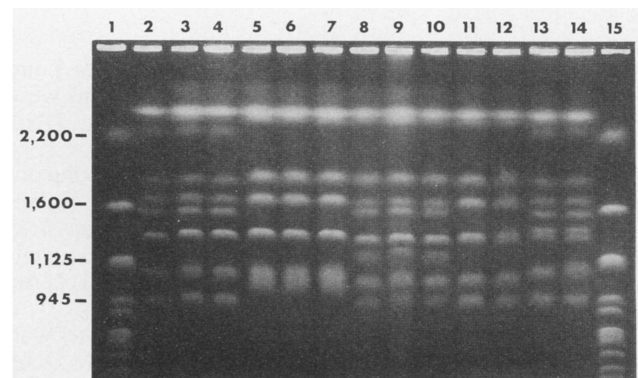

FIG. 2. Contour-clamped homogeneous electric field electrophoresis of *C. albicans* isolates from multiple episodes of infection in each of five different patients. Lanes 1 and 15, *S. cerevisiae* chromosome or DNA size standards (in kilobases), lanes 2 to 4, DNA subtype Q from three different infection episodes in patient 3; lanes 5 to 7, DNA subtype III from three different infection episodes in patient 29; lanes 8 to 10, DNA subtype HHH from three different infection episodes in patient 28; lanes 11 and 12, DNA subtype P from two different infection episodes in patient 19; lanes 13 and 14, DNA subtype GG from two different infection episodes in patient 8.

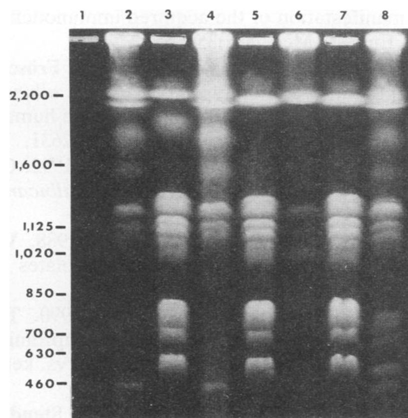

FIG. 3. Contour-clamped homogeneous electric field electrophoresis of *C. albicans* isolates from recurrent infections in a single patient (patient 7; Table 2). Lane 1, *S. cerevisiae* chromosome or DNA size standards (in kilobases); lanes 2 and 4, DNA subtype D; lanes 5 and 7, DNA subtype F; lanes 3, 6, and 8, DNA subtypes E, G, and H, respectively.

found that the majority (62%) of AIDS patients with oropharyngeal candidiasis and undergoing azole therapy were infected or colonized with more than one strain of *C. albicans*, that the same strain was rarely shared among two or more individuals, and that the introduction or selection of a more resistant strain during the course of fluconazole therapy was not uncommon. By comparison, an earlier study by Whelan et al. (22) found that *C. albicans* isolates from patients with AIDS shared a number of genotypic and phenotypic characteristics and did not vary appreciably from isolates obtained from individuals without AIDS. Using restriction fragment length polymorphism analysis, they reported that patients with AIDS are frequently infected with the same strain and that repeat isolates from individual patients were generally the same strain, suggesting relapse rather than reinfection. More recently, Schmid et al. (17) used Southern hybridization analysis with a *C. albicans*-specific probe and a more sophisticated analysis of restriction fragment length polymorphism patterns and found that strains of *C. albicans* from patients with AIDS were more closely related than isolates from individuals without AIDS. They, too, found that repeat isolates from the same patient were usually the same strain; however, they did not find convincing evidence for cross infection among these patients. Those studies did not examine the antifungal susceptibilities of the isolates.

Korting et al. (8) examined the antifungal susceptibilities as well as the biotype profiles of *C. albicans* isolates from 62 patients with AIDS. They found a predominant biotype among these isolates; however, repeat cultures revealed the introduction of a new biotype in 27.3% of patients. Antifungal susceptibility testing revealed higher MICs of amphotericin B, flucytosine, ketoconazole, and itraconazole for isolates of *C. albicans* from patients with more advanced stages of human immunodeficiency virus infection and more prolonged exposure to antifungal agents. These results are consistent with the findings of the present study and suggest that long-term antifungal therapy for oral candidiasis in patients with AIDS may result in decreased susceptibility to commonly used agents.

In contrast to the studies of Whelan et al. (22) and Schmid et al. (17), we found evidence of considerable strain diversity among isolates of *C. albicans* obtained from individuals with AIDS and oropharyngeal candidiasis. Although 38% of patients in the present study were infected or colonized with the same DNA subtype on sequential cultures, the majority (62%) of patients experienced episodes of infection with two or more different DNA subtypes of *C. albicans*. Differences in patient populations, frequency of cultures, duration of follow-up, intensity of exposure to specific antifungal agents, and methods of DNA subtyping may all contribute to the different conclusions of the studies described above.

In addition to the variation in DNA subtypes, we also noted significant changes in the in vitro susceptibility to fluconazole among isolates from patients with two or more infecting or colonizing strains of *C. albicans*. These differences in antifungal susceptibilities support the apparent genetic diversity determined by DNA subtyping methods and suggest that in certain individuals, this diversity could predispose the selection of more resistant strains. The clinical significance of these findings is unclear because most of the significant increases in MICs occurred among isolates for which MICs were ≤8.0 μg/ml, and the patients infected with those isolates had a satisfactory clinical response to fluconazole. A notable exception to this was patient 20 (Table 1). Even though the patient was infected with a single DNA subtype, the isolate became progressively more resistant to fluconazole; this required an increase in the daily dose of fluconazole from 100 to 400 mg in order to control the infection. The clinical and microbiologic details of this case are reported elsewhere (15); however, it is notable that in concert with the clinical course of infection, the infecting strain became increasingly resistant to fluconazole; MICs for the strain increased from 0.25 to >64 μg/ml over several episodes of infection and therapy.

Of particular interest in the present study was the observation that although relapses of oropharyngeal candidiasis with the same DNA subtype suggest failure to eradicate the infecting organism, only rarely (as in patients 6, 20, and 28) does this failure appear to be due to the development of relative resistance to fluconazole over time. In contrast, individuals infected with more than one DNA subtype of *C. albicans* frequently became infected or colonized with a new and more resistant subtype either during the course of therapy or following therapy. Whether this is a reflection of new acquisition of a more resistant subtype from an exogenous source or the selection of a resistant subpopulation from an originally mixed infection cannot be answered from the results of the present study. The lack of apparent cross infection among this geographically delimited population, suggested by the low frequency of shared DNA subtypes, speaks against the first possibility. The alternative explanation, that these individuals were initially infected with a mixed population of organisms, is intriguing. The fact that different DNA subtypes from the same patient may have significantly different susceptibilities to fluconazole is potentially clinically significant. Future studies should include sampling of multiple colonies from pre- and posttreatment cultures in order to address this important issue.

The persistence of the same genotype (DNA subtype) in a given patient over time certainly may suggest that individuals with AIDS develop candidiasis because of local or systemic immune dysfunction rather than the presence of a particular *C. albicans* strain (22). However, the observations that infection or colonization in many of the patients with AIDS treated with fluconazole may be due to more than one DNA subtype and that the emergence of strains that are relatively resistant to fluconazole may be more frequent in these patients introduce the possibility that antifungal resis-

tance may play a role in the overall infection process. The substitution of a more resistant strain for a more susceptible strain in the face of intense antimicrobial pressure has precedence with other infections and microorganisms and is a function of the organism and not the host. This does not mean that these strains are more or less virulent than any other strain.

These findings underscore the great complexity of candidal infections in immunocompromised patients. The diversity observed among isolates of *C. albicans* obtained from patients with AIDS undergoing intensive antifungal therapy is impressive. The potential for development of fluconazole resistance (or resistance to any other antifungal agent) by a single strain or acquisition of a resistant strain from cross infection or the environment or for the possibility of selection of a resistant strain from a mixed population of *C. albicans* during therapy is both worrisome and fascinating. For each of these possibilities, different strategies for prevention and optimal therapy will be required. Clarification of these various possibilities will require additional studies designed to detect the potential resistance of a de novo mixed infection and the emergence of resistance in an originally susceptible strain. These studies must follow patients longitudinally and include both molecular typing and antifungal susceptibility testing.

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REFERENCES

1. **Clift, R. A.** 1984. Candidiasis in the transplant patient. Am. J. Med. **77:**34–38.
2. **Doebbeling, B. N., P. F. Lehman, R. J. Hollis, L. C. Wu, A. F. Widmer, A. Voss, and M. A. Pfaller.** 1993. Comparison of pulsed-field gradient gel electrophoresis with isoenzyme profiles as a typing system for *Candida tropicalis*. Clin. Infect. Dis. **16:**377–383.
3. **Dupont, B., L. Improvisi, M. Eliaszewicz, G. Pialoux, and the GEMO.** 1992. Resistance of *Candida albicans* to fluconazole (FcZ) in AIDS patients. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1203.
4. **Espinel-Ingroff, A., C. W. Kish, Jr., T. M. Kerkering, R. A. Fromtling, K. Bartizal, J. N. Galgiani, K. Villareal, M. A. Pfaller, T. Gerarden, M. G. Rinaldi, and A. Fothergill.** 1992. Collaborative comparison of broth macrodilution and microdilution antifungal susceptibility tests. J. Clin. Microbiol. **30:**3138–3145.
5. **Fromtling, R. A., J. N. Galgiani, M. A. Pfaller, A. Espinel-Ingroff, K. F. Bartizal, M. S. Bartlett, B. A. Body, C. Frey, G. Hall, G. D. Roberts, F. B. Nolte, F. C. Odds, M. G. Rinaldi, A. M. Sugar, and K. Villareal.** 1993. Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. Antimicrob. Agents Chemother. **37:**39–45.
6. **Goodman, J. L., D. J. Winston, R. A. Greenfield, P. H. Chandrasekar, B. Fox, H. Kaizer, R. K. Shadduck, T. C. Shea, P. Stiff, D. J. Friedman, W. G. Powderly, J. L. Silber, H. Horowitz, A. Lichtin, S. N. Wolff, K. F. Mangan, S. M. Silver, D. Weisdorf, W. G. Ho, G. Gilbert, and D. Buell.** 1992. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N. Engl. J. Med. **326:**845–851.
7. **Klein, R. S., A. H. Carol, C. B. Small, B. Moll, M. Lesser, and G. H. Friedland.** 1984. Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome. N. Engl. J. Med. **311:**354–358.
8. **Korting, H. C., M. Ollert, A. Georgii, and M. Fröschl.** 1988. In vitro susceptibilities and biotypes of *Candida albicans* isolates from the oral cavities of patients infected with human immunodeficiency virus. J. Clin. Microbiol. **26:**2626–2631.
9. **Magee, P. T., L. Bowdin, and J. Staudinger.** 1992. Comparison of molecular typing methods for *Candida albicans*. J. Clin. Microbiol. **30:**2674–2679.
10. **Merz, W. G., C. Connelly, and P. Itieter.** 1988. Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. J. Clin. Microbiol. **26:**842–845.
11. **Meunier, F., M. Aoun, and M. Gerard.** 1990. Therapy for oropharyngeal candidiasis in the immunocompromised host: a randomized double-blind study of fluconazole vs. ketoconazole. Rev. Infect. Dis. **12**(Suppl 3)**:**S364–S368.
12. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard. Document M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **Pfaller, M. A., M. G. Rinaldi, J. N. Galgiani, M. S. Bartlett, B. A. Body, A. Espinel-Ingroff, R. A. Fromtling, G. S. Hall, C. E. Hughes, F. C. Odds, and A. M. Sugar.** 1990. Collaborative investigation of variables in susceptibility testing of yeasts. Antimicrob. Agents Chemother. **34:**1648–1654.
14. **Quintiliani, R., N. J. Owens, R. A. Quercia, J. J. Klimek, and C. H. Nightingale.** 1984. Treatment and prevention of oropharyngeal candidiasis. Am. J. Med. **77:**44–48.
15. **Redding, S., J. Smith, G. Farinacci, M. Rinaldi, A. Fothergill, J. Rhine-Chalbert, and M. Pfaller.** Development of resistance to fluconazole among isolates of *Candida albicans* obtained during treatment of oropharyngeal candidiasis in AIDS: documentation by in vitro susceptibility testing and DNA subtype analysis. Clin. Infect. Dis., in press.
16. **Rodriguez-Tudela, J. L., F. Laguna, J. V. Martinez-Suarez, R. Chaves, and F. Dronda.** 1992. Fluconazole resistance of *Candida albicans* isolates from AIDS patients receiving prolonged therapy. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1204.
17. **Schmid, J., F. C. Odds, M. J. Wiselka, K. G. Nicholson, and D. R. Soll.** 1992. Genetic similarity and maintenance of *Candida albicans* strains from a group of AIDS patients, demonstrated by DNA fingerprinting. J. Clin. Microbiol. **30:**935–941.
18. **Tavitian, A., J. P. Raufman, and L. E. Rosenthal.** 1986. Oral candidiasis as a marker for esophageal candidiasis in the acquired immunodeficiency syndrome. Ann. Intern. Med. **104:**54–55.
19. **Trier, J. S., and D. J. Bjorkman.** 1984. Esophageal, gastric, and intestinal candidiasis. Am. J. Med. **77:**39–43.
20. **Vazquez, J. A., A. Beckley, J. D. Sobel, and M. J. Zervos.** 1991. Comparison of restriction enzyme analysis and pulsed-field gradient gel electrophoresis as typing systems for *Candida albicans*. J. Clin. Microbiol. **29:**962–967.
21. **Warren, N. G., and H. J. Shadomy.** 1991. Yeasts of medical importance, p. 617–629. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
22. **Whelan, W. L., D. R. Kirsch, K. J. Kwon-Chung, S. M. Wahl, and P. D. Smith.** 1990. *Candida albicans* in patients with the acquired immunodeficiency syndrome: absence of a novel or hypervirulent strain. J. Infect. Dis. **162:**513–518.
23. **Willocks, L., C. L. S. Leen, R. P. Brettle, D. Urquhart, T. B. Russell, and L. J. R. Milne.** 1991. Fluconazole resistance in AIDS patients. J. Antimicrob. Chemother. **28:**937–939.