

Clonal and Spontaneous Origins of Fluconazole Resistance in *Candida albicans*

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The genotypes and susceptibilities to fluconazole of 78 strains of the human pathogenic yeast *Candida albicans* were compared. The strains comprised two sets of samples from Durham, N.C.: one from patients infected with the human immunodeficiency virus (HIV) and the other from healthy volunteers. For each strain, the MIC of fluconazole was determined by the standard National Committee for Clinical Laboratory Standards protocol. Genotypes were determined by PCR fingerprinting with five separate primers. The analysis revealed little evidence for genotypic clustering according to HIV status or body site. However, a small group of fluconazole-resistant strains isolated from patients infected with HIV formed a distinct cluster. In addition, two fluconazole-resistant strains were isolated from individuals who never took fluconazole, one from a patient infected with HIV and the other from a healthy person. The results suggest both clonal and spontaneous origins of fluconazole resistance in *C. albicans*.

Candidiasis caused by *Candida albicans* is the most common mycosis associated with human immunodeficiency virus (HIV) infection, occurring in up to 90% of patients (10, 19). Fluconazole is a potent antifungal antibiotic that perturbs the biosynthesis of ergosterol by blocking an alpha-14-demethylation step in the biosynthetic pathway. Fluconazole is currently the most widely used antifungal drug because it can be given orally, lacks major side effects, and has broad efficacy against most pathogenic yeasts, including *C. albicans* (25). However, fluconazole-resistant fungal pathogens have become increasingly common (25). Elevated MICs of fluconazole for *C. albicans* have been associated with multiple molecular alterations (reviewed in reference 25). These changes include (i) increased expression of genes that encode multiple drug resistance, ATP-binding cassette transporters, and the target enzyme (ERG11); (ii) nucleotide substitutions at various positions of ERG11; and (iii) decreased heterozygosity at ERG11 due to either mitotic recombination or gene conversion (7, 25).

Because of these multiple and highly variable mechanisms, it is often assumed that resistance to fluconazole arises independently in *C. albicans* and that horizontal transfer of resistant strains among hosts is rare. Indeed, a number of studies have confirmed that the administration of fluconazole may lead to the acquisition of resistance in commensal strains (1, 7, 13). The development of resistance often occurred with a stepwise increase of the MIC (1, 7, 13, 14, 20). However, there is evidence that susceptible commensal strains may be replaced by resistant isolates (1, 16). If isolates of *C. albicans* acquire resistance to fluconazole through a diversity of independent mechanisms, their genotypes should be no more similar to each other than samples of susceptible strains. To our knowledge, no study has unambiguously demonstrated genetic clustering of resistant strains isolated from unrelated hosts.

Advances in molecular biology over the last 2 decades have fostered the development of molecular techniques for genotyping clinical isolates of human pathogenic yeasts, facilitating epidemiological studies (4, 5, 6, 16, 17, 21, 23, 26). Among

these current techniques, PCR fingerprinting and random amplified polymorphic DNA are widely used (1, 4, 5, 6, 14, 16, 17). Both methods have high discriminating power and reproducibility, require little starting material, and are rapid and simple to perform (1, 4, 5, 6, 14, 16, 17).

In this study, PCR fingerprinting, random amplified polymorphic DNA, and in vitro susceptibility testing were used to investigate the origins of fluconazole-resistant strains of *C. albicans* from five patients with AIDS. We analyzed two reference samples comprising strains from multiple hosts from the same geographic area. The inclusion of samples from other patients and healthy persons helped assess the relationships among resistant strains.

MATERIALS AND METHODS

***C. albicans* strains and hosts.** The strains used in this study were obtained from three sources: (i) 5 patients with AIDS who were treated with fluconazole, (ii) 25 HIV-infected patients who were not treated with fluconazole, and (iii) 40 healthy volunteers. Between November 1994 and February 1995 at the Adult Infectious Disease Clinic of Duke University Medical Center, five patients with AIDS developed persistent oropharyngeal candidiasis. These patients were treated with 200 mg of fluconazole/day for up to 3 months or until treatment failure. Strains of *C. albicans* were isolated from the oral cavities of these patients after fluconazole treatment failure. Rectal swabs and, when applicable, vaginal swabs were also taken from all five patients. All swabs were cultured on Inhibitory Mold Agar (Difco, Detroit, Mich.). Isolates of *C. albicans* were identified by the germ tube test, growth at 45°C, and API-20C kits (BioMerieux-Vitek). A total of nine isolates were obtained from these patients (Table 1). All nine strains had the same API-20C profiles, regardless of origin and susceptibility to fluconazole.

Because the developments of fluconazole resistance in five unrelated patients were clustered in time and space, we suspected that these strains might be closely related. As a retrospective study, 29 isolates of *C. albicans* obtained from another 25 HIV-infected patients who were seen at the same clinic during that period of time were retrieved from the Duke University Medical Mycology Research Laboratory stock cultures. As another control, 40 isolates, 1 each from the oral cavities of 40 healthy volunteers, were also analyzed. Table 1 lists the sources of all strains.

DNA extraction. Genomic DNA was extracted from each isolate according to the following protocol. Cultures were grown at 37°C for 24 to 48 h on plates of YEPD agar (1% yeast extract, 2% dextrose, 2% Bacto Peptone, 1.5% agar). A large loopful of cells was transferred to a 1.5-ml microcentrifuge tube containing 0.5 ml of sterile water. The cells were vortexed and centrifuged at 13,000 × g for 2 min. The supernatant fluid was discarded, and the cells were resuspended in 0.5 ml of protoplast buffer (1 M sorbitol, 1% β-mercaptoethanol, 0.25 mg of lysing enzyme [Sigma]/ml, and 0.25 mg of lyticase [Sigma]/ml) and incubated at 37°C for 2 h. The spheroplasts were collected by centrifugation at 5,000 × g for 5 min and lysed by incubation at 65°C in 0.5 ml of lysing buffer (50 mM EDTA, 1% sodium dodecyl sulfate, 0.1 μg of RNase A/ml) for 30 min. One-quarter volume of 7.5 M ammonium acetate (125 μl) and 0.5 ml of chloroform-isoamyl alcohol (24:1) were added. The mixture was vortexed and centrifuged at 13,000 × g for

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TABLE 1. Origins, DNA fingerprinting profiles, and MICs of fluconazole for strains of *C. albicans* used in this study

Patient	Body site	Strain	MIC (µg/ml)	Fingerprinting genotype ^a
AIDS; fluconazole^b				
P1	Oral	P1-oral	64	111011111100010101001
	Rectal	P1-rectal	1	0001011111100010101101
P2	Oral	P2-oral	256	111011111100010101001
	Vaginal	P2-vaginal	256	111011111100010101001
P3	Oral	P3-oral	256	111011111100010101001
P4	Oral	P4-oral	256	111011111100010101001
	Vaginal	P4-vaginal	2	1110110011101010100001
P5	Rectal	P4-rectal	256	111011111100010101001
	Oral	P5-oral	256	111011111100010101100
HIV; no fluconazole^c				
P6	Oral	P6-oral	8	100101111100010101100
	Vaginal	P6-vaginal	2	010101111110010101100
P7	Oral	P7-oral	1	0001011111100010101100
P8	Rectal	P8-rectal	2	0001011111100010101100
P9	Rectal	P9-rectal	2	0001011111101010101100
P10	Rectal	P10-rectal	2	0001011111100011001100
P11	Oral	P11-oral	1	000101111100010101001
	Vaginal	P11-vaginal	1	0001011111100010101001
P12	Rectal	P11-rectal	128	111011111100010101001
	Oral	P12-oral	1	000101111100010101001
P13	Rectal	P12-rectal	1	0001011111100010101001
	Oral	P13-oral	2	000101111100010101001
P14	Oral	P14-oral	2	0001000100000010100100
P15	Oral	P15-oral	1	0001011111100010101001
P16	Oral	P16-oral	2	000101111100010101100
P17	Oral	P17-oral	2	000101111110010100001
P18	Oral	P18-oral	4	000100011110010101001
P19	Oral	P19-oral	1	000101111110000000100
P20	Oral	P20-oral	2	000101111100011001100
P21	Oral	P21-oral	1	1001011111100010101100
P22	Oral	P22-oral	1	0001011111100010101100
P23	Oral	P23-oral	0.5	1110110111101010100001
P24	Oral	P24-oral	2	000101111100010101100
P25	Oral	P25-oral	2	000101111100010101100
P26	Oral	P26-oral	2	111011111100010101100
P27	Oral	P27-oral	2	1001000111100010101100
P28	Oral	P28-oral	2	000101111100010101100
P29	Oral	P29-oral	2	000101111100010101100
P30	Oral	P30-oral	2	000101111100010101100
Healthy; no fluconazole^d				
N1	Oral	N1-oral	1	000101111111010101011
N2	Oral	N2-oral	2	0001011111100010101111
N3	Oral	N3-oral	1	111011111100010101111
N4	Oral	N4-oral	1	0001011111100010101111
N5	Oral	N5-oral	1	0001011111100010101111
N6	Oral	N6-oral	1	000101111100010101111
N7	Oral	N7-oral	0.5	0001011111100010101111
N8	Oral	N8-oral	1	000101111100010101111
N9	Oral	N9-oral	2	0001011111100010101011
N10	Oral	N10-oral	0.5	0001000111100010101100
N11	Oral	N11-oral	1	0001011111100010101100
N12	Oral	N12-oral	1	0001011111100010101100
N13	Oral	N13-oral	1	000101111100010101100
N14	Oral	N14-oral	1	0001011111100010101100
N15	Oral	N15-oral	1	00010101111100010101001
N16	Oral	N16-oral	2	0001011111101001010100
N17	Oral	N17-oral	2	111011111100010101001
N18	Oral	N18-oral	1	000101111110010101100
N19	Oral	N19-oral	1	0001011111100010101001
N20	Oral	N20-oral	1	0001011111100010101100
N21	Oral	N21-oral	1	0001011111100010101001
N22	Oral	N22-oral	1	0001011111100010101001
N23	Oral	N23-oral	1	000101111100010101100
N24	Oral	N24-oral	1	0001011111100010101001
N25	Oral	N25-oral	64	0001011111100010101001
N26	Oral	N26-oral	2	111011111100010101001

Continued

TABLE 1—Continued

Patient	Body site	Strain	MIC (µg/ml)	Fingerprinting genotype ^a
N27	Oral	N27-oral	0.5	0001010111100010101100
N28	Oral	N28-oral	2	0001011111100010101001
N29	Oral	N29-oral	1	0001011111100010101100
N30	Oral	N30-oral	1	111011111100010101001
N31	Oral	N31-oral	1	0001010111100010101100
N32	Oral	N32-oral	1	0001011111100010101100
N33	Oral	N33-oral	1	0001011111100010101100
N34	Oral	N34-oral	1	0001011111100010101011
N35	Oral	N35-oral	2	0001011111100010101101
N36	Oral	N36-oral	2	1110110111101010101001
N37	Oral	N37-oral	1	11101111111010101001001
N38	Oral	N38-oral	1	1010110111101010100001
N39	Oral	N39-oral	1	1110110111101010101001
N40	Oral	N40-oral	1	1110110100001010101001

^a Composite fingerprinting profiles were determined by scoring bands from five PCR single primers. There are 22 digits for each strain. From left to right, digits 1 to 7 are bands generated by primer OPA-03, corresponding to PCR fragments of the following sizes: 400, 460, 600, 660, 710, 1,150, and 1,450 bp, respectively. Digits 8 to 11 are bands produced by primer T3B, corresponding to PCR fragments of 740, 850, 1,000, and 1,200 bp, respectively. Digits 12 and 13 are bands from primer Tel01, corresponding to PCR fragments of 480 and 600 bp, respectively. Digits 14 to 19 are products of (GACA)₄, corresponding to PCR fragments of 490, 580, 700, 740, 1,050, and 1,200 bp, respectively. Digits 20 to 22 are from the primer M13 core sequence, corresponding to PCR fragments of 590, 1,500, and 1,800 bp, respectively. 1, presence of the PCR product; 0, absence of the fragment.

^b Patients with AIDS who had fluconazole treatment failure.

^c HIV-infected patients with no fluconazole treatment.

^d Healthy individuals with no symptoms of candidiasis and no fluconazole treatment.

10 min or until the upper layer was clarified. The supernatant was transferred to a new 1.5-ml microcentrifuge tube, 0.5 ml of ice-cold isopropanol was added, the tube contents were mixed gently by inversion and centrifuged at 13,000 × g for 2 min, and the supernatant fluid was discarded. The DNA was washed with 70% ethanol, vacuum dried, resuspended in 60 µl of Tris-EDTA, and stored at -20°C. By this method, a loopful of cells yielded approximately 5 µg of genomic DNA.

PCR fingerprinting. Five oligonucleotides were used as single primers for PCR fingerprinting of all strains: (i) the M13 phage core sequence (5'-GAGG GTGGCGTCT-3') (17); (ii) the intergenic spacer repeat of tRNA, T3B (5'-AGG TCG CGG GTT CGA ATC C-3') (15); (iii) the simple repeat sequences (GACA)₄: (5'-GAC AGA CAG ACA GAC A-3') (17); (iv) the telomeric core sequence, TELO1 (5'-TGG GTG TGT GGG TGT GTG GGT GTG-3') (1); and (v) the oligonucleotide 5'-AGTCAGCCAC-3' (OPA-03 from Operon Technologies). All PCRs were performed in a Perkin-Elmer thermal cycler (model 9600). For primers M13, T3B, (GACA)₄, and TELO1, the PCR was performed with an initial denaturation of 97°C for 3 min followed by 40 cycles of 20 s at 93°C, 60 s at 50°C, and 20 s at 72°C and a final cycle of 5 min at 72°C. For primer OPA-03, the PCR was performed with an initial denaturation of 97°C for 3 min followed by 45 cycles of 60 s at 93°C, 60 s at 36°C, and 120 s at 72°C and a final cycle of 5 min at 72°C.

The amplification products were separated by electrophoresis in 1.5% agarose gels in 1× TAE buffer for 13 h at 2 V/cm. Amplification products were detected by staining with ethidium bromide (0.5 µg/ml) and were visualized under UV light. The electrophoretic bands were sized and scored manually. For all isolates, each DNA fragment was scored as present (1) or absent (0). For the 5 patients with AIDS who were treated with fluconazole, 11 HIV-infected patients, and 2 healthy volunteers, we initially analyzed multiple isolates from the same body site of each host. A total of 115 strains were genotyped and compared. However, in every case where multiple isolates were obtained from the same body site of individual patients, they had identical genotypes and MICs of fluconazole. Therefore, only one isolate from each body site of each person was included in the analysis. A total of 78 isolates were compared.

Testing susceptibility to fluconazole. The MICs of fluconazole were determined by a standard broth microdilution method, M-27A, recommended by the National Committee for Clinical Laboratory Standards (NCCLS); endpoints were read at 48 h (18). The MICs of fluconazole for all strains were also determined by a statistical method based on the colony size (27). For all strains, the MICs obtained by these two methods differed by no more than a twofold dilution, confirming that the rapid and statistical colony size method is comparable to the NCCLS method. However, to be consistent with most other studies, only MIC results obtained by the NCCLS method were presented. Any strain for which the MIC of fluconazole was ≥64 µg/ml was considered resistant.

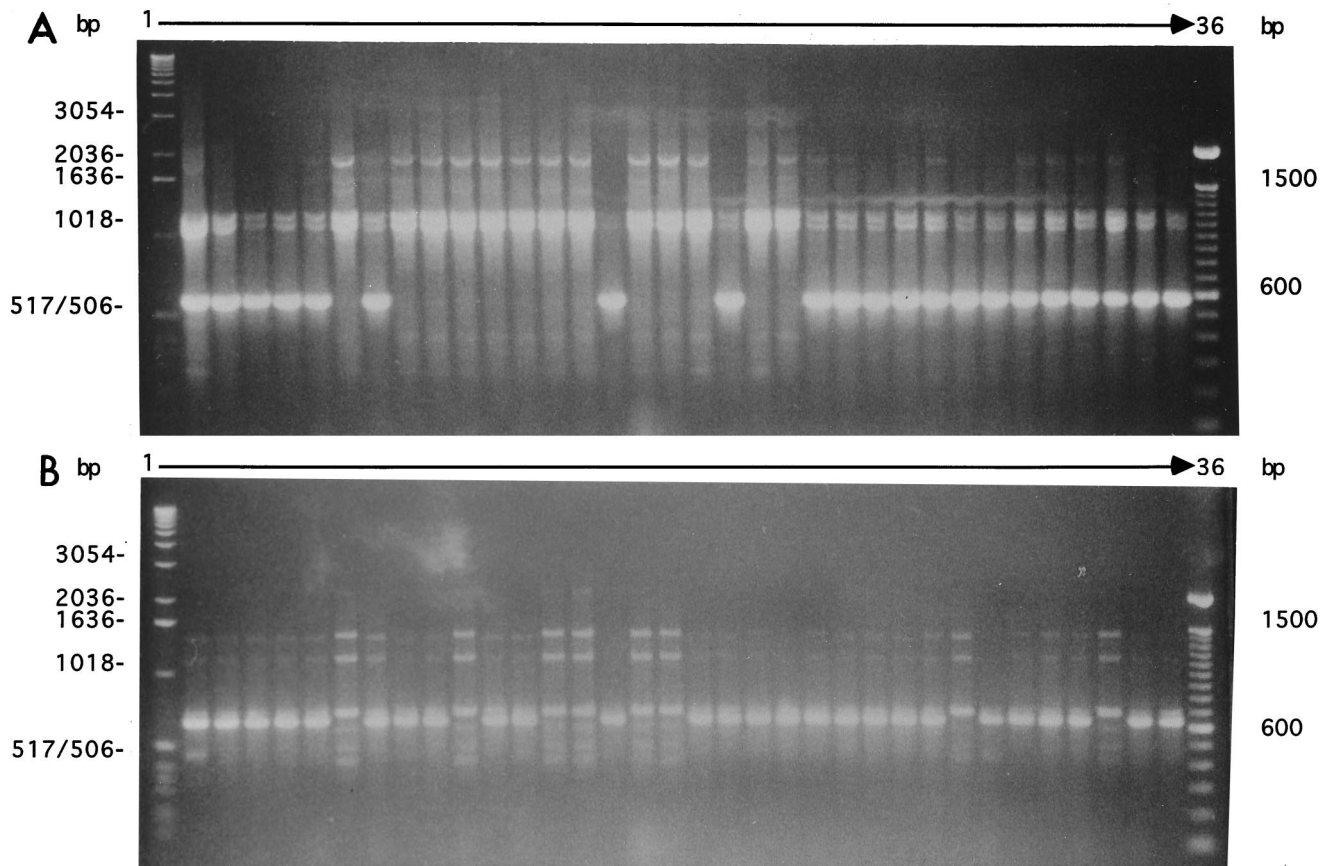


FIG. 1. Examples of electrophoretic separation of PCR fingerprints obtained by amplifying genomic DNA from 34 strains of yeast isolated in this study using the M13 core sequence (5'-GAGGGTGGCCGTTCT-3') (A) and PA03 (5'-AGTCAGCCAC-3') (B) as single primers. Lanes 1 and 36 are 1-kbp and 100-bp DNA ladders from GIBCO-BRL, respectively. Lanes 2 to 35 are strains in the following order: P6-vaginal, P7-oral, P8-rectal, P9-rectal, P10-rectal, P11-oral, P11-rectal, P11-vaginal, P12-oral, P12-rectal, P2-oral, P3-oral, P14-oral, P4-oral, P4-rectal, P15-oral, P16-oral, P17-oral, P18-oral, P19-oral, P20-oral, P22-oral, P24-oral, P25-oral, P26-oral, P27-oral, P28-oral, P29-oral, P30-oral, P5-oral, P6-oral, and P21-oral.

Clustering analysis. Similarity coefficients among all isolates were calculated as the ratio of matches over the total number of bands scored from the composite DNA fingerprinting patterns derived from all five primers (22). The unweighted pair group method with arithmetic mean (UPGMA) phenogram showing the similarities of all 78 isolates was generated based on the pairwise similarity coefficient matrix (9). The calculations of similarity coefficients and the generation of UPGMA phenograms were done with the statistical package PAUP4d64 (24). The within- and between-group similarities were calculated as the arithmetic mean of all pairwise distances. Student's *t* test was used to compare genetic similarities between different groups of isolates (22). Two comparisons were performed: the sample of *C. albicans* isolates from HIV-infected patients was compared with the sample from healthy volunteers, and the samples of fluconazole-susceptible and -resistant isolates from the HIV-infected patients were compared.

RESULTS

Patterns of fluconazole susceptibilities. Seven of the nine strains from patients with AIDS who were treated with fluconazole were resistant to fluconazole in vitro. These included all five oral isolates, one rectal (P4-rectal) isolates, and one vaginal (P2-vaginal) isolate. Two other strains, P1-rectal and P4-vaginal, were susceptible to fluconazole. The MICs of fluconazole for these two strains were 1 and 2 $\mu\text{g/ml}$, respectively (Table 1).

For individuals never administered fluconazole, the distributions of MIC values were similar for strains isolated from patients infected with HIV and from healthy people without HIV infection (Table 1). As expected, most strains from persons never treated with fluconazole were susceptible. However,

two isolates were resistant to fluconazole (MIC ≥ 64 $\mu\text{g/ml}$); one was a rectal isolate from a patient with HIV (P11), and the other was from the oral cavity of a healthy person (N25).

DNA fingerprinting. All five primers generated polymorphic bands among the 78 strains. Among the five primers, 22 polymorphic bands were scored, and a total of 30 unique fingerprinting profiles were found. Primer OPA-03 produced eight polymorphic bands; (GACA)₄ produced six bands; and T3B, M13, and TELO1 generated four, three, and two polymorphic bands, respectively (Table 1). A representative picture of the PCR products for primers OPA-03 and M13 is presented in Fig. 1. No PCR primer identified all 30 fingerprints or genotypes. Primer OPA-03 identified nine genotypes, primers (GACA)₄ and M13 identified five genotypes each, and primers TELO1 and T3B identified four and three genotypes, respectively (Table 1). Four genotypes were shared between strains from healthy and HIV-infected hosts (Fig. 2). These four genotypes included a total of 42 isolates. Twelve genotypes were unique to strains from HIV-infected patients, and 14 to those from healthy people.

When the genotypes of the strains from healthy and from HIV-infected subjects were compared, there was no significant genetic difference (Table 2). Among the strains from healthy people, of the 22 bands scored, the average number of band differences between pairs of isolates in this sample was about 4.5. As shown in Table 2, this average difference was comparable to that of the strains from HIV-infected persons (mean

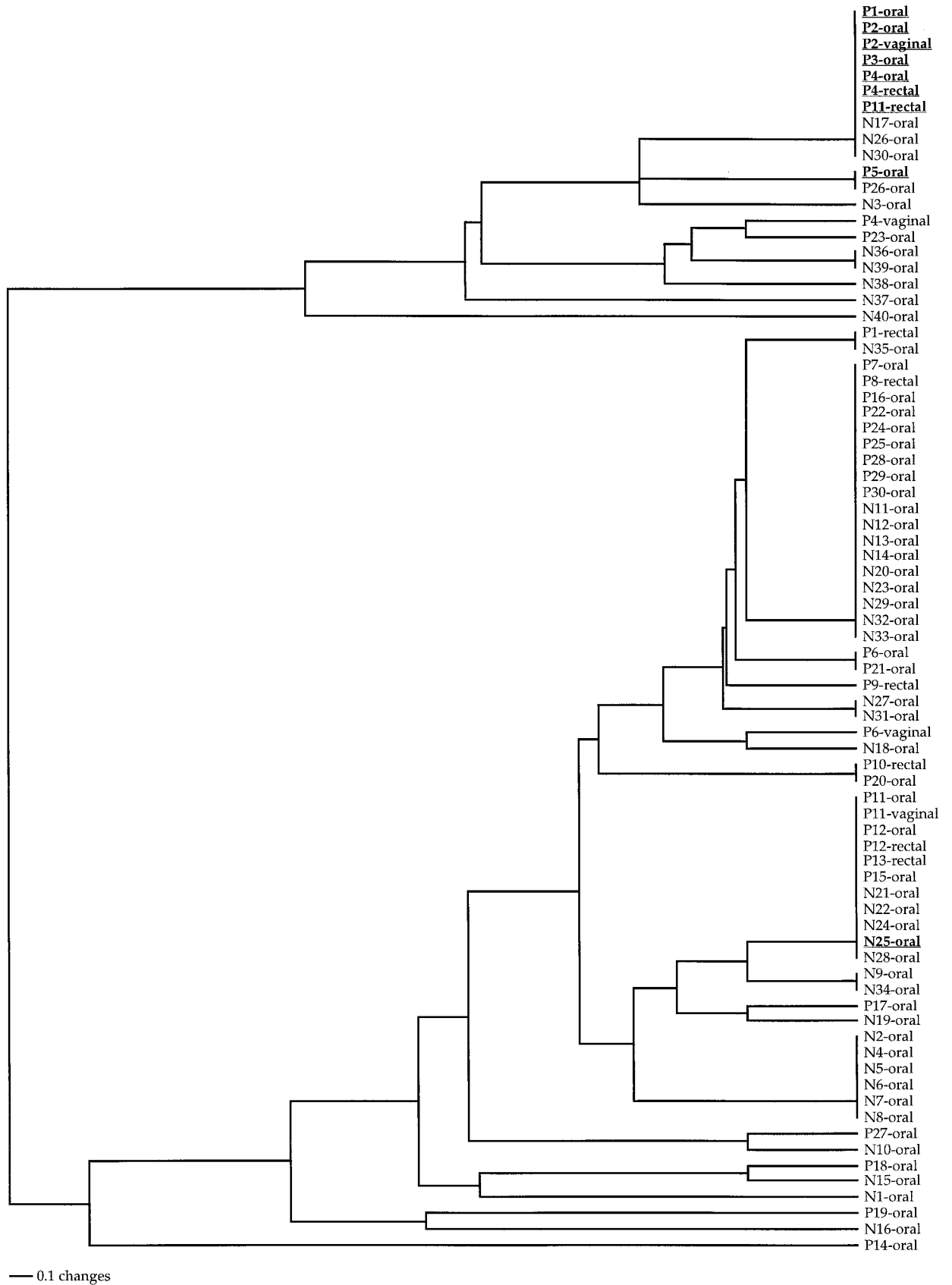


FIG. 2. UPGMA phenogram of all 78 strains of *C. albicans* analyzed in this study. The strain names correspond to those in Table 1. Fluconazole-resistant strains are in boldface and underlined.

TABLE 2. Mean dissimilarity between pairs of *C. albicans* strains from Durham, N.C., that differ either in host condition or fluconazole susceptibility

Strain group	No. of pairwise comparisons	Mean dissimilarity ^a ± SD
Healthy-HIV^b		
Within healthy volunteer group	780	4.47497 ± 3.38002
Within HIV-infected patient group	703	4.64154 ± 3.15047
Between healthy and HIV-infected groups	1,520	4.61053 ± 3.20276
Resistant-sensitive^c		
Within fluconazole-resistant group	28	0.5 ± 0.88192
Within fluconazole-sensitive group	435	3.85057 ± 3.14155
Between resistant and sensitive groups	240	6.55833 ± 2.07514

^a The mean number of band differences out of the total 22 polymorphic bands scored in the whole sample (sample size, $n = 78$).

^b Strains from within and between healthy volunteers ($n = 40$) and HIV-infected patients ($n = 38$). There was no significant genetic difference between strains of *C. albicans* from the two groups of hosts ($P > 0.9$).

^c Strains from patients with HIV infection that are either resistant ($n = 8$) or sensitive ($n = 30$) to fluconazole. There was a significant genetic difference between fluconazole-resistant and -sensitive strains ($P < 0.001$).

band difference, 4.6), as well as for comparisons between strains from healthy and HIV-infected people (mean band difference, 4.6).

Strains from different body sites of the same host. Strains from different body sites of the same patient can be highly variable in both genotype and fluconazole susceptibility (Table 1 and Fig. 3). For example, two HIV-infected subjects (Table 1, P4 and P11) had isolates from three body sites (oral, vaginal, and rectal). Neither patient had isolates with identical PCR fingerprints at all three sites (Fig. 3). Patient 4 had oral and rectal isolates with identical genotypes, while the vaginal isolate was genetically different; the oral and rectal strains were both resistant to fluconazole, while the vaginal isolate was susceptible. In contrast, patient 11, who was not treated with fluconazole, had identical oral and vaginal strains, and both were susceptible to fluconazole. Interestingly, the rectal isolate from patient 11 had a different genotype and was resistant to fluconazole (Table 1 and Fig. 3).

Four patients (P1, P2, P6, and P12) had isolates from two body sites. In patient 1, the oral isolate was genetically different from the rectal isolate, and each strain had a different MIC of fluconazole. Patient 2 had oral and rectal isolates, both of which were genetically identical and resistant to fluconazole. With patient 6, the oral isolate was genetically different and had a higher MIC of fluconazole than the vaginal isolate. With patient 12, the oral and rectal isolates had identical genotypes, and both were susceptible to fluconazole (Table 1).

Significant clustering of fluconazole-resistant strains from HIV-infected patients. In the analysis of genetic similarity of strains from HIV-infected patients, strains resistant to fluconazole were genetically more similar to each other than they were to fluconazole-susceptible strains (Table 2 and Fig. 3). This difference is statistically significant based on Student's *t* test ($P < 0.001$). Specifically, the average number of band differences between fluconazole-resistant strains was 0.5, and that between fluconazole-susceptible strains was 3.85. However, the average difference between susceptible and resistant strains was about 6.5, much greater than the within-group differences (Table 2). The finding that resistant strains from different hosts were genetically more similar to each other than even strains from different body sites of the same hosts strongly

suggests a clonal origin of fluconazole resistance among this group of strains (viz., P1-oral, P2-oral, P2-vaginal, P3-oral, P4-oral, P4-rectal, and P11-rectal).

DISCUSSION

In this study, we identified a significant genetic cluster of fluconazole-resistant strains of the pathogenic yeast *C. albicans* from unrelated hosts who were infected with HIV. Interestingly, while seven of the eight resistant strains were from HIV-infected patients treated with fluconazole, one resistant strain was isolated from a patient who was not treated with fluconazole or any other azole. A similar finding was reported by Goff et al. (8). They isolated fluconazole-resistant strains of *C. albicans* from immunocompromised patients with various diseases, but none were infected with HIV and none had been treated with azoles. However, genetic analysis was not performed on their strains (8). These eight fluconazole-resistant isolates are very similar if not identical in genotype (Fig. 3).

Of the 40 strains from healthy hosts, one was resistant to fluconazole. This resistant strain (N25-oral) is genetically very different from the other eight resistant strains isolated from HIV-infected patients (Table 1 and Fig. 2). This result suggests that the origin of fluconazole resistance in strain N25-oral is different from and independent of those of the other eight resistant isolates analyzed in this study.

While the independent origin of fluconazole resistance in *C. albicans* has been repeatedly demonstrated in many studies, to our knowledge there is no report of the recovery of a resistant isolate from a healthy person never administered fluconazole. This finding belies the common assumption that isolates of *C. albicans* from persons who were never exposed to fluconazole will be susceptible to fluconazole. Although the frequency of de novo resistance in the absence of fluconazole may not be high, the isolation of this strain and the report from Goff et al. (8) should alert clinical laboratory personnel to consider determining the MIC of fluconazole for a patient's isolate of *C. albicans* before fluconazole is administered to treat candidiasis.

The genetic cluster of resistant strains from unrelated patients is intriguing because of the preponderance of evidence supporting the concept that resistant strains arise independently (1, 7, 13, 14, 16, 20, 25). There are two possible explanations for this clustering: (i) all resistant strains in this cluster were derived independently from previously susceptible but genetically similar strains, and/or (ii) the mutation to resistance arose only once and became widely distributed as a result of horizontal, clonal spread.

Different persons can harbor the same or very similar genotypes of *C. albicans* (3, 16, 26) (Table 1 and Fig. 3). If the yeasts that initially colonized the four patients with AIDS (P1, P2, P3, and P4) were similar, resistant strains may have developed independently after treatment with fluconazole. (Pretreatment isolates are not available from these patients.) However, independent origins of resistance in these strains are unlikely because fluconazole-susceptible strains were recovered from other body sites of two (P1 and P4) of the four patients, and the susceptible strains were genetically different from the resistant strains from the same patient.

In most other studies, strains from different body sites of the same person were usually more similar to each other than strains from different people. In this report, we found a similar pattern for fluconazole-susceptible isolates from patients P11 (P11-oral and P11-vaginal) and P12 (P12-oral and P12-rectal) (Table 1 and Fig. 2). Genetically dissimilar strains were only obtained from patients who carried strains for which the MICs of fluconazole were different (P1, P4, P6, and P11). In addition,

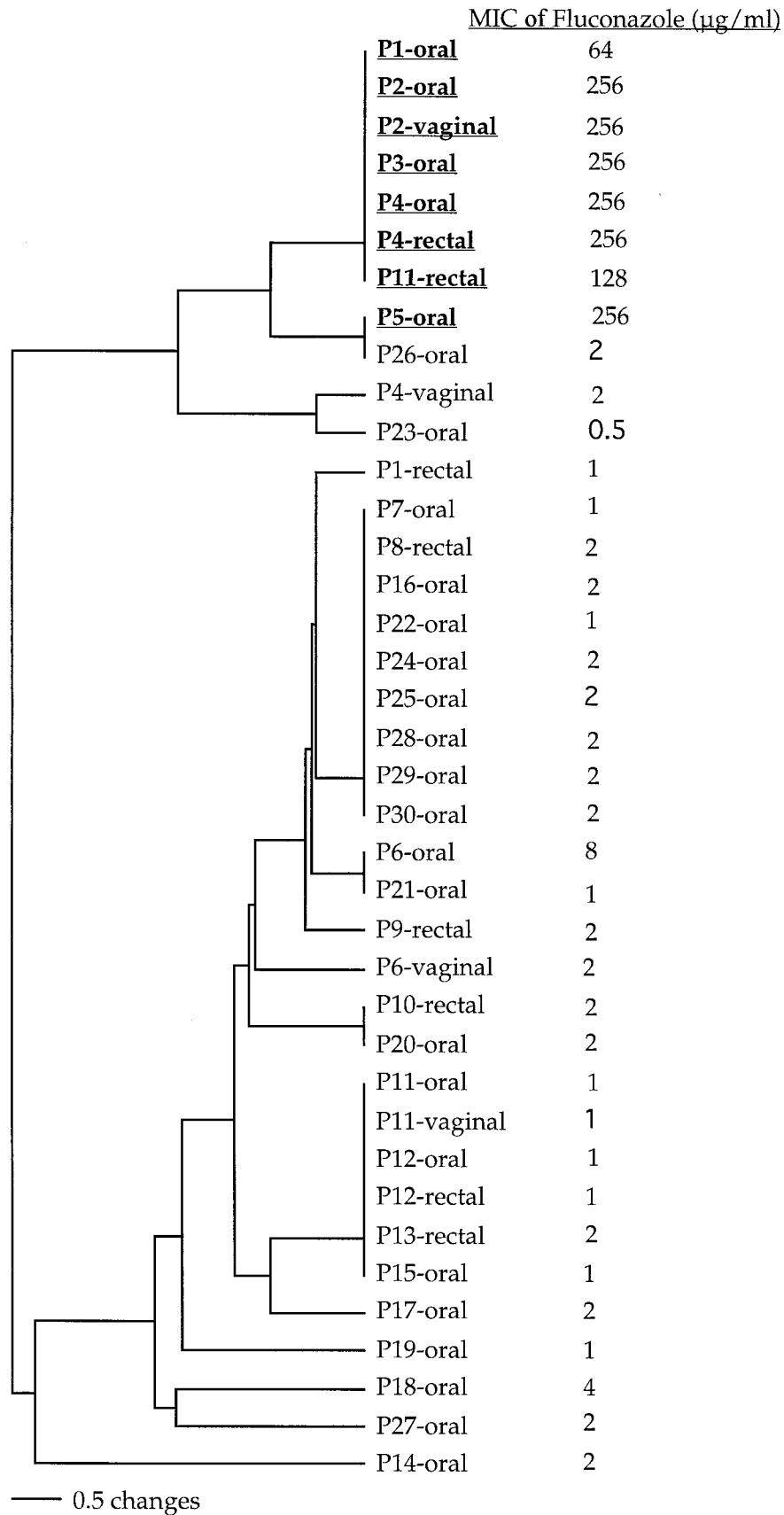


FIG. 3. UPGMA phenogram of the 38 strains of *C. albicans* from HIV-infected patients analyzed in this study. The strain names correspond to those in Table 1. Fluconazole-resistant strains are boldface and underlined. The right-hand column indicates the MICs of fluconazole as determined by the NCCLS protocol.

the hypothesis of independent origin is hard to reconcile with the origin of the fluconazole-resistant strain P11-rectal. This strain had the same genotype as six other resistant strains, but it came from a patient never treated with fluconazole.

An alternative hypothesis to explain the distribution of a resistant genotype among five patients is the clonal spread of a single resistant mutant. This explanation is consistent with the data that seven of the eight resistant strains possess the same genotype, which was not recovered from other HIV-infected patients (Fig. 2). Furthermore, when multiple strains were obtained from different body sites of the same person, resistant strains were always more similar to resistant strains from other persons than to susceptible strains from a different body site of the same person.

If the second hypothesis is true, the route of spread for this resistant genotype is intriguing. So far, the only documented direct transmission of a fluconazole-resistant strains of *C. albicans* occurred between two sexual partners (2). Transmission of fluconazole-resistant strains of *C. albicans* among unrelated, nonintimate individuals has not been conclusively demonstrated. On the other hand, it has been shown that healthcare workers in the clinical setting could facilitate the spread of *C. albicans* (11), as well as that of antibiotic-resistant bacteria (12). Therefore, the clinical environment common to all the patients with AIDS and the HIV-infected persons in this study might have contributed to the spread of the resistant genotype. Since surveillance cultures of the yeast microfloras at the clinical environments and among healthcare workers were not conducted, this possibility cannot be directly tested.

It should be noted, however, that the data did not suggest specific DNA fingerprints unique to resistant strains in the whole sample of 78 isolates. Indeed, the three genotypes found among the resistant strains were shared by susceptible strains from healthy persons, HIV-infected patients, or both (Fig. 2). Consistent with other studies, this result indicated that DNA fingerprinting alone could not predict whether a strain was resistant or susceptible to fluconazole (1, 3, 20).

When isolates of *C. albicans* from HIV-infected and healthy subjects were compared, the two samples had similar genetic diversities (Table 2) and a distribution of MICs of fluconazole similar to that for isolates from persons who had not been treated with fluconazole (Table 1). Each sample had one isolate that was resistant to fluconazole, which suggests that fluconazole-resistant strains may exist as members of the commensal yeast microfloras of both immunocompromised and immunocompetent people. Therefore, like antibiotic resistance in bacteria (12), resistance to fluconazole in *C. albicans* could persist and spread clonally among humans.

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