



## Fluconazole Resistance Patterns in *Candida* Species that Colonize Women with HIV Infection



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### ABSTRACT

**Background:** The Women's Interagency HIV Study was established in 1993 to study the natural history of HIV disease among women in the United States. It currently has enrolled 2,895 women testing positive for HIV infection and 972 women without HIV infection recruited from 6 national metropolitan locations. The clinical database information collected for each HIV-positive individual included CD4 cell counts, viral load, and antiviral treatment to evaluate HIV prognosis and related conditions in women.

**Objective:** To provide a baseline for fluconazole treatment prospects in women who test positive for HIV infection. As part of the ongoing Women's Interagency HIV Study project, we investigated the fluconazole susceptibility of *Candida* spp. isolated from women with HIV in comparison to volunteer women without HIV. The implication of antifungal treatment on fluconazole susceptibility was evaluated by reviewing antifungal medication use for the past 2 years in each participant. In addition, genotyping of *Candida* spp. at oral and vaginal sites was monitored for 4 months in 9 patients.

**Methods:** In a cohort of 59 women with HIV and 24 women without HIV, colonization by *Candida albicans* and non-*albicans* species of the oral and vaginal sites was first determined. Fluconazole susceptibility was surveyed *in vitro* according to Clinical and Laboratory Standards Institute protocol. Antifungal drug treatment history was investigated for each patient to correspond with fluconazole susceptibility. Finally, series of isolates from several patients were followed for resistance and susceptibility. Their lineage was verified by genotyping multilocus sequence typing (MLST).

**Results:** A total of 280 *Candida* strains were recovered from oral and vaginal swabs of women with and without HIV infection. We found that patients with HIV were colonized with *Candida* spp. more frequently than women without HIV. The percent of isolates that were susceptibility dose dependent or resistant to fluconazole was higher in *Candida glabrata* compared with *C. albicans* isolates, but higher for *C. albicans* than other published data. Resistance was noted to be more common in vaginal sites. Fluconazole resistance in either species was not associated with relative CD4 cell counts or viral load. However an association with systemic application of fluconazole and resistance was noted.

**Conclusions:** Systemic antifungal therapy, including a vaginal topical regimen in women with HIV infection correlated with reduced fluconazole susceptibility of oral and vaginal isolates. Genotype profiling has disclosed that a majority of isolates from the same individual are clustered together, suggesting the likelihood of an original strain with some microevolution. We observed a change from a susceptibility dose dependent to a resistant phenotype of isolates in 2 women with HIV infection, even though no treatments were received during the 4-month study and the prior 2 years.

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## Introduction

*Candida* species are a major source of fungal infections in patients with HIV/AIDS. The colonization by this organism in the oral mucosa can result in the development of oropharyngeal candidiasis (OPV) in 90% and esophageal candidiasis (EC) at early and late stages of an HIV infection.<sup>1</sup> Vulvovaginal candidiasis (VVC) is more common in women infected with HIV, but is clinically similar to that experienced in HIV-negative women.<sup>2</sup> For both OPV/EC and VVC, ongoing concerns are non-*albicans* *Candida* species and their drug resistance. The current treatment for candidiasis heavily relies on triazoles such as fluconazole, itraconazole, and now posaconazole even though patient responses to these antifungal drugs tend to be slow with a high risk of reinfection.<sup>3</sup> The clinical relapse of candidiasis is obviously dependent on the degree of immunosuppression in individuals infected with HIV and the development of drug resistance.<sup>1</sup> About 5% of patients with HIV/AIDS develop a fluconazole-refractory mucosal candidiasis as described in an 800-case cohort study.<sup>3</sup>

Treatment failures can occur due to antifungal drug toxicity, but more importantly, drug resistance development.<sup>4</sup> Primary resistance to fluconazole, for example, can result in cross-resistance to other unused triazoles. Resistance to imidazoles such as clotrimazole and ketoconazole has been reported.<sup>1</sup> Thus, a concern in management of candidiasis in this immunocompromised group is the selection of resistant non-*albicans* *Candida* spp. following prophylactic treatment with azoles, which has been confirmed in a high risk population.<sup>4</sup> In fact, the high mortality rate of invasive, bloodborne candidiasis (15%–49%) has decreased little over the past 2 decades despite triazole therapeutics.<sup>4,5</sup> As expected, patients colonized with fluconazole-resistant strains of *Candida* spp also have a higher prevalence of reinfection than those colonized with more susceptible strains.<sup>6</sup> The existing paradigm is that patients treated previously with fluconazole should not be treated with the same drug during a reinfection. However the short- and long-term effects on *Candida* species colonization following the treatment of OPV/EC and VVC candidiasis in patients with HIV/AIDS remain unknown. How the use of specific triazoles at specific doses and duration affect *Candida* colonization at different anatomic locations are not clearly determined, especially after treatment is discontinued. In our study we surveyed fluconazole susceptibility in 280 isolates from our previous study on the probiotic effects on *Candida* spp. colonization in patients with HIV/AIDS.<sup>7</sup> The isolates were obtained from oral and vaginal swabs of 59 women with HIV infection and 24 HIV-negative female volunteers with comparable socioeconomic status. The drug-use history for each individual was followed for 2 years before the current observations. The objective of our study was to provide a baseline for fluconazole susceptibility/resistance in HIV-positive women.

In addition to the susceptibility testing of isolates, we also chose 30 *C. albicans* strains from oral and vaginal cultures of 9 patients to analyze their genotype by multilocus sequence typing (MLST). Combined with fluconazole susceptibilities of both oral and vaginal isolates, MLST provides assurances that persistence of isolates with susceptibilities was not due to replacement by new isolates.

## Materials and Methods

### Study population, clinical data, and infection treatment history

As described in previous studies,<sup>7,10</sup> the patient population included 59 patients with HIV infection and 24 HIV-negative volunteers recruited from the Women's Interagency HIV Study (WIHS) cohort.<sup>8,9</sup> The WIHS was established in 1993 to study the

natural history of HIV disease among women in the United States, and currently has enrolled 2,895 HIV-infected women and 972 HIV-uninfected women recruited from 6 national metropolitan locations. The local institutional review board at each site approved the study protocol and all participants gave written informed consent.

The viral loads and CD4 lymphocyte counts for each patient were determined by standard methods and documented in a previous study.<sup>7</sup> No antibiotics were used by the patients during the study and participants had a short period of probiotic treatment as described previously.<sup>10</sup> Because this study focused on the implications of antifungal drug treatment on the development of resistance, a 2-year retrospective antifungal drug history was reviewed in each patient.

### Strains collection and growth medium

Samples were collected as previously described at 0, 30, 60, 74, and 129 days following a diagnosis of HIV infection.<sup>7,10</sup> All isolates were identified at the species level, and semiquantitation of isolate levels (scale = 0–4) was done as previously described.<sup>7</sup> *C. albicans* SC5314 was also included in MIC and genotyping studies as a reference strain. Yeast extract peptone dextrose (YPD) broth (2% yeast extract, 1% peptone, and 2% glucose) or YPD agar was used to propagate strains from –80°C stock cultures.

### Antifungal activity assays

Roswell Park Memorial Institute 1640 medium (Sigma, St Louis, Missouri) buffered to pH 7.0 with 0.165M 3-(N-morpholino) propanesulfonic acid buffer was used for determinations of isolates fluconazole susceptibility. MIC assays were performed using the standardized broth microdilution protocol (M27-A) of the Clinical and Laboratory Standards Institute (version 2008).<sup>11</sup> Briefly, fluconazole was diluted in the Roswell Park Memorial Institute 1640 medium described above in 96-well microtitre plates to final concentrations of 128 µg/mL to 0.125 µg/mL fluconazole. Isolates were each added to achieve a final concentration of 10<sup>3</sup> cfu/100 µL. The microtitre plates were incubated at 35°C for 48 hours in a humid chamber. MIC<sub>50</sub> values were determined using a plate reader at optical density = 600 nm (Spectra Max 190, Molecular Devices, LLC., Sunnyvale, Californian).

Fluconazole breakpoints for each isolate were assigned as susceptible (S), MIC ≤ 8 µg/mL; susceptible dose dependent (SDD), MIC = 16 to 32 µg/mL; or resistant (R), MIC ≥ 64 µg/mL using the M27-A protocol for all isolates. MIC assays in triplicate always included *C. albicans* SC5314 as a reference strain.

### Genotyping assays

DNA from 29 *C. albicans* isolates of 9 HIV-positive patients and SC5314 was extracted from overnight cultures grown in 5 mL YPD broth. The cells were broken with glass beads and treated with phenol-chloroform (1:1). Genomic DNA was collected, dissolved in tris and EDTA buffer, and concentrations were determined using the Nana drop Bioanalyzer 2100 (Agilent Technologies Inc., Columbia, Maryland).

Polymerase chain reaction (PCR) assays were done in a 50 µL volume of primers and a DNA polymerase mixture containing 200 ng DNA from each isolate. The PCR reaction mix was incubated for 2 minutes at 94°C, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 30 seconds, and elongation at 72°C for 1 minute, and an additional 10 minutes at 72°C. DNA fragments in the range of 450 to 550 bp were amplified using Vent DNA polymerase (New England Biolabs, Ipswich, Massachusetts) with 6 sets of primers that have been described elsewhere.<sup>12,13</sup>

The 6 genes chosen for amplification were *AAT1a* and *AAT1b*, highly conserved enzymes in L-aspartate biosynthesis; *PMI1*, synthesis of GDP-mannose; *ADP1*; PDR-subfamily ABC transporter; *ALA1*, alanine-tRNA ligase; and *RPN2*, 26S proteasome regulatory subunit. Amplicons were detected in 1% agarose gels and purified using the PCR purification kit (Qiagen, Gaithersburg, Maryland). One-strand DNA sequence data for each sample were obtained by Sanger DNA sequencing provided by Genewiz Inc., Germantown, Maryland ([www.genewiz.com](http://www.genewiz.com)).

#### Statistical analysis

SAS 9.2 (SAS Institute Inc, Cary, North Carolina) was used for statistical analysis in this study. The similarity among strains from MLST study was analyzed by Cluster X software (The European Bioinformatics Institute). The DNA sequence data of each gene were aligned and polymorphic loci were determined. The numbers of variable loci were scored for each pair of strains. Diversity of strain distribution is shown in a dendrogram created by Mega 5.1 software (Arizona State University).<sup>14</sup>

## Results

#### Colonization by *Candida* spp. is influenced by HIV status

The majority of the patient cohort was African American women who had a higher viral load than the general WIHS cohort (log mean value = 6.12 vs 5.42).<sup>7</sup> During a 4-month survey, 18.6% of patients testing positive for HIV infection (11 out of 59) and 33.3% of patients testing negative for HIV infection (8 out of 24) had negative yeast cultures. Whereas colonization was observed in both patients with and without HIV infection, the overall carriage rate of *Candida* spp. in women testing positive for HIV infection was significantly higher than HIV-negative women from both the oral and vaginal mucosa (83.0% [n = 48 out of 59] vs 66.7% [n = 16 out of 24], respectively;  $P \leq 0.05$ ).<sup>7</sup> Also, 28.8% of HIV patients (n = 17 out of 59) were colonized with non-*albicans* *Candida* spp. compared with 16.7% in the HIV-negative group. Most of the non-*albicans* *Candida* spp. strains (19.11%) were isolates from the vagina of women with HIV infection.

#### Fluconazole susceptibility profiles of isolates

*C. albicans* and *C. glabrata* accounted for 261 of the 280 isolates. MIC values for fluconazole of 227 *C. albicans* and 34 *C. glabrata* isolates were determined (Table I). We chose MIC<sub>50</sub> determinations instead of using MIC<sub>90</sub> because of an obvious trailing effect, defined as a persistent growth of *Candida* spp. with drug concentrations above the MIC<sub>50</sub> after 48 hours of incubation. This observation was not unexpected because most of the isolates were from patients with HIV infection. The overall frequencies at each breakpoint for fluconazole of *C. albicans* were susceptible, 81.9%; R, 10.6%; and SDD, 7.5%. In contrast to *C. albicans*, *C. glabrata* isolates

**Table I**

Fluconazole susceptibility profiles of *Candida* spp. isolates from patients with and without HIV, in oral or vaginal candidiasis.

Number of isolates tested (% for each MIC)			
MIC <sub>50</sub> (µg/mL)	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis/C. tropicalis/C. spp.</i>
0.125-8	186 (81.9)	15 (44.1)	12 (63.2)
16-32	17 (7.5)	11 (32.4)	0
≥ 64	24 (10.6)	8 (23.5)	7 (36.8)
Total	227	34	19

**Table II**

Oral and vaginal isolates with reduced fluconazole susceptibility (*Candida albicans* and *Candida glabrata*), by HIV status.

MIC <sub>50</sub> (µg/mL)	HIV+		HIV-	
	Vaginal	Oral	Vaginal	Oral
0.125-8	49 (68.1)	106 (76.8)	13 (100)	33 (86.8)
16-32	13 (18.1)	15 (10.9)		
≥ 64	10 (13.8)	17 (12.3)		5 (13.2)
Total	72	138	13	38

were less susceptible to fluconazole (frequency of 44.1%) and had increased resistance (frequency of 23.5%), whereas SDD was 32.4%. The MIC profiles of strains from each patient remained similar for the entire course of this study in patients positive for HIV infection (n = 39) compared with those patients for whom MIC values changed (n = 11). In the latter group, the MICs of fluconazole from isolates of 2 patients with HIV (patients 5 and 7) increased from SDD to R.

#### Isolates of *Candida* spp. from the group of women testing positive for HIV are less susceptible to fluconazole

Resistance to fluconazole of our isolates described in Table I was higher than most other published data ( $\leq 3\%$  for *C. albicans*).<sup>15</sup> Vaginal isolates of *C. albicans* and *C. glabrata* from the group of women testing positive for HIV were more resistant to fluconazole compared with women without HIV. We found that in comparison of 100% susceptibility of the group of women without HIV, only 68.1% of vaginal isolates recovered from the group of women with HIV were susceptible to fluconazole for both species (Figure 1). At same time, 18.1% were SDD and 13.8% were resistant to fluconazole among vaginal isolates. On the other hand, the percentage of resistance to fluconazole in oral isolates was similar in both patients with HIV infection and HIV-negative volunteers (12.3% vs 13.2%) in Table II.

#### The MIC profiles of isolates are not related to CD4 cell counts

Untreated HIV infection leads to a progressive reduction in the number of T cells that express CD4. Patients testing positive for HIV infection with CD4 counts > 500 cells/mm<sup>3</sup> are not at risk for opportunistic infections; however, when that number drops to 200 to 500 cells/mm<sup>3</sup>, candidiasis occurs. In our study the isolates from patients with CD4 counts above 500 cells/mm<sup>3</sup> were mostly susceptible to fluconazole. Exceptions were 4 patients, 3 of whom tested positive for HIV infection, and 1 who tested negative for HIV infection. We then compared MIC profiles of each resistant isolate to patient CD4 counts (cells/mm<sup>3</sup>) and viral load (Table III). We found that fluconazole resistance in *C. glabrata* isolates occurred regardless of the relative CD4 cell counts or viral load (Table III). All resistant *C. albicans* strains were from patients with CD4 counts  $\leq 700$  mm<sup>3</sup>/mL except 1 case, and 5 out of 9 patients with CD4 counts  $\leq 500$  mm<sup>3</sup>/mL.

To better explain the high resistance to fluconazole in this group of patients with HIV infection, we traced the antifungal drug treatment for the previous 2 years for each participant because only 1 patient with HIV received fluconazole treatment during our 4-month surveillance study. As shown in Table III, 6 of 12 patients testing positive for HIV infection with high MICs (SDD or R) had received antifungal drugs during the past 2 years. Yet the frequency of antifungal drug use in patients with HIV infection was 32% during the past 2 years (19 out of 59) (Table IV). These

**Table III**

Fluconazole susceptibility of dose dependent/resistant isolates and the relationship to CD4 cell counts and viral load among HIV-positive patients.

Candida spp.	MIC <sub>50</sub> (µg/mL)		CD4 (cells/mm <sup>3</sup> )	Viral load (copies)	Antifungals used
	V-strain	O-strain			
<i>C. albicans</i>	32	64	111	1,160,000	-
<i>C. albicans</i>	64	32	160	1,330,000	-
<i>C. albicans</i>	64	64	208	2,950	FCZ
<i>C. tropicalis</i>	64	64	290	80	FCZ
<i>C. albicans</i>	64	64	428	48	-
<i>C. albicans</i>	16	-	535	76	FCZ
<i>C. albicans</i>	64	-	602	48	-
<i>C. albicans</i>	64	64	697	48	FCZ
<i>C. albicans</i>	64	-	1080	48	-
<i>C. glabrata</i>	32	16	890	108	FCZ + MCZ
<i>C. glabrata</i>	16	16	1,091	48	-
<i>C. glabrata</i>	64	64	1,225	48	FCZ

FCZ = fluconazole prescribed at least once in past 2 years; MCZ = miconazole typical used at least once in past 2 years; O-strain = oral isolates of *Candida* spp; V-strain = vaginal isolates of *Candida* spp.

patients were given systemic fluconazole alone or also treated with miconazole. Although a similar percentage of antifungal drugs had been used in HIV-negative volunteers (37.5%; n = 9 out of 24), the treatment modes were mostly restricted to miconazole topical use (Table IV). Only a single volunteer without HIV received fluconazole.

#### MLST characterization of oral and vaginal isolates

We typed 30 isolates of *C. albicans* from 9 of HIV infected patients with oral or vaginal candidiasis by MLST.<sup>12–14,16</sup> The objective of these experiments was to look for persistence and changes in susceptibility to fluconazole at times during the full course of this study and also determine if oral and vaginal isolates from the same patient were genotypically similar. PCR amplification of 6 housekeeping genes was used to determine relatedness among isolates. We found 49 polymorphic loci among the 30 strains by MLST (Figure 1). The majority of these isolates are members of clade I of *C. albicans* using a threshold of 0.002 and *C. albicans* SC5314 as a reference strain. Isolates from Patients 2, 4, 5, 8, and 9 are likely the same strain with some microevolution. From Patient 7, the 30-day isolate clearly represents a different strain from a different clade. Patient 1 isolates appeared to be divergent and represent different strains.

Vaginal isolates were more divergent than oral isolates, suggesting that, in general, a separate microevolution between the oral and vaginal niches occurred with frequent recolonization from 1 niche to the other.

Persistent fluconazole resistance and/or SDD phenotypes were noted in Patients 1, 5, and 7. Of these 3 patients, R or SDD was observed in oral (Patient 1) or vaginal (Patient 7) isolates only. Patient 5 oral isolates initially were SDD (MD) but by 120 days became resistant (MR) although fluconazole therapy was not used during this study. In the same patient, cluster-similar isolates from the vagina were SDD (MD) (Figure 1). The vaginal isolate from Patient 7 also changed from SDD to R during the 4-month surveillance. All other isolates from the remaining patients were susceptible to fluconazole throughout the course of this study (Figure 1).

#### Discussion

In general, reduced susceptibility to fluconazole was prevalent in this patient cohort. We found that resistance to fluconazole among isolates was 10.6% and 23.5% for *C. albicans* and *C. glabrata*,

Meanwhile, SDD to fluconazole was 7.5% and 32.4% for *C. albicans* and *C. glabrata*, respectively. The latter is interpreted as those strains with MICs of 16 to 32 µg/mL to fluconazole. The clinical implication of SDD with high MICs *in vitro* is to achieve maximal fluconazole levels in blood and tissue to inhibit fungal growth.<sup>17–19</sup> With higher resistance and SDD occurred among *C. glabrata* isolates, *C. albicans* isolates were more susceptible (81.9%) to fluconazole than *C. glabrata* (44.1%). In patients who tested positive for HIV, the less susceptible strains were especially isolated from vaginal cultures.

No clear correlation between MIC profiles and viral load or CD4 counts has been found in patients testing positive for HIV infection. Most isolates were susceptible to fluconazole at CD4 counts > 500 cells/mm<sup>3</sup>. However, except for 1 isolate, all resistant *C. albicans* strains were isolated from patients with CD4 counts ≤ 700 cells/mm<sup>3</sup>.<sup>7</sup> The higher amount of resistance in the HIV-positive group may imply an association with inappropriate therapy, defined as delay in treatment, wrong dosage, or treatment of a patient with a resistant isolate.<sup>17</sup> In fact, a retrospective review of patient history 2 years before this study showed that about 32% of patients positive for HIV infection had oral fluconazole treatment alone or with antifungal topical agents. In contrast to a higher frequency of fluconazole use in the group of patients testing positive for HIV, the percentage of fluconazole treatment was only 4% in individuals without HIV, although some of these individuals received topical miconazole treatment. Intriguingly 7 patients with resistant isolates were colonized from the first day of surveillance and, possibly, the isolates were transmitted from vaginal to oral sites or oppositely from oral to a vaginal site. The implication is that optimal use of fluconazole may need adherence to established methods of treatment.

From 30 isolates of 9 patients, fluconazole susceptibility profiles from 6 of the 9 patients appeared to be stable during the 4-month surveillance. All isolates from those 6 patients remained susceptible to fluconazole. Resistance and SDD were noted in 3 of the 9 patient isolates, with progression from SDD to R in the oral isolates. For patients in whom both oral and vaginal isolates were

**Table IV**

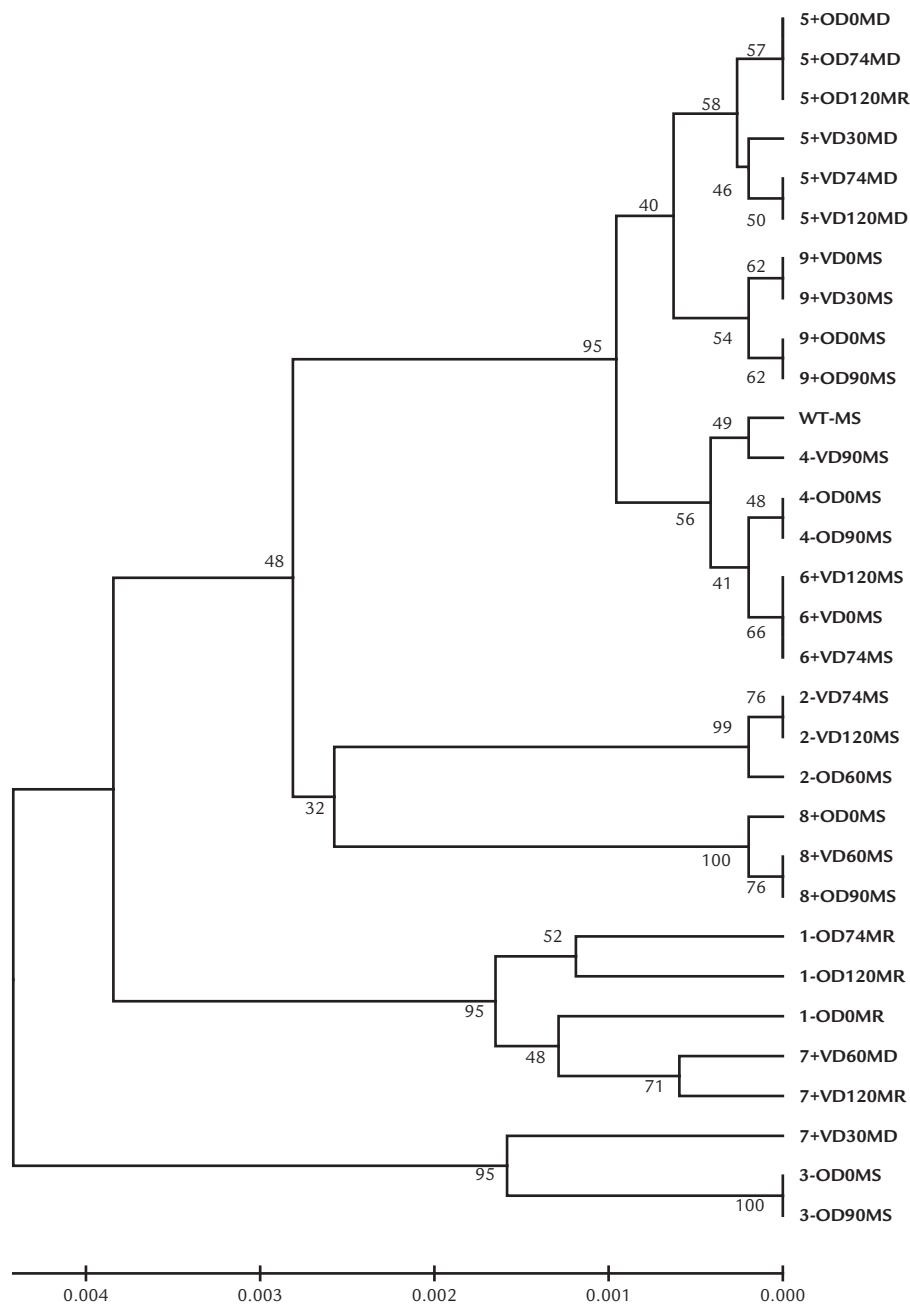
MICs of oral and vaginal isolates with a patient history of antifungal use during the past 2 years.

HIV+ (n = 19/59)				HIV- (n = 9/24)			
Vaginal	Oral	Candida spp. (V/O)	Drug used <sup>*</sup>	Vaginal	Oral	Candida spp. (V/O)	Drug used <sup>†</sup>
16.0	-	alb /-	FCZ	64.0	0.125	gla /alb	MCZ
-	-	- / -	FCZ	0.25	-	par /-	FCZ
1.0	0.25	alb/alb	FCZ	-	0.125	- / alb	MCZ
-	4.0	-/alb	FCZ	8.0	-	kru /-	MCZ
64.0	64.0	trp/trp	FCZ	-	0.125	- / alb	MCZ
64.0	64.0	alb/alb	FCZ	-	1.0	- / alb	MCZ
-	2.0	- / gla	FCZ	0.5	0.5	alb/alb	MCZ
-	0.5	- / gla	FCZ	-	-	- / -	MCZ
2.0	2.0	alb/alb	FCZ+MCZ	-	64.0	- /alb	MCZ
4.0	-	gla / -	FCZ				
0.5	4.0	fam/alb	FCZ+MCZ				
64.0	64.0	par/alb	FCZ				
0.5	-	alb / -	MCZ				
-	4.0	- / alb	FCZ				
1.0	2.0	alb/alb	FCZ				
0.25	0.125	alb/alb	FCZ+MCZ				
64.0	64.0	gla/gla	FCZ				
32.0	16.0	gla/alb	FCZ+MCZ				
0.25	0.25	alb/alb	FCZ				

- = no *Candida* spp. was isolated during 4 months surveillance; alb = *Candida albicans*; fam = *Candida famata*; gla = *Candida glabrata*; kru = *Candida krusei*; trp = *Candida tropicalis*; FCZ = fluconazole; MCZ = miconazole.

\* Nineteen HIV-positive patients were prescribed with FCZ at least once during past 2 years.

† Nine HIV-negative individuals were prescribed once or multiple times MCZ for vaginal topical use during past 2 years.



**Figure 1.** The relatedness of 30 clinical isolates and 1 standard strain (WT) of *Candida albicans* based on the multilocus sequence typing genotyping. This unweighted pair-group method with arithmetic averages dendrogram was generated with Clustalx 1.81 and Mega 5.1. 1–9 = Patients 1 to 9; +/- = HIV positive/negative; D# = isolation day; MD = fluconazole dose-dependent susceptible; MR = fluconazole resistant; MS = fluconazole susceptible; V/O = vaginal isolates or oral isolates.

sampled, isolates were apparently of the same genotype and had similar susceptibility profiles except for the progression from SDD to R in Patient 5. Cross-transmission among both sites could be an explanation of isolate similarity.

The change in fluconazole susceptibility of an isolate from SDD to R in Patient 5 is intriguing in light of the lack of fluconazole treatment during the 4-month surveillance. *C. albicans* is known to undergo a loss of heterozygosity that can be associated with conversion to lower fluconazole susceptibility.<sup>15</sup> Loss of heterozygosity can be reversed by a number of processes, including gene conversion. We suggest that a gain-of-function mutation has occurred in this isolate.<sup>13</sup> Mrr1p and Tac1p, regulators of azole efflux pumps, are 2 candidates, both of which could contribute to the decreased susceptibility that only was recognized during our routine susceptibility assays.

Vaginal isolates of *Candida* spp. in patients with HIV infection displayed an increase in drug resistance. Antifungal over-the-counter treatments may have contributed to the increased resistance. The risk of transfer of resistant isolates *Candida* spp. from a vaginal to an oral site (or its reverse) in patients with HIV infection will require additional study. Taken together, the higher frequency of resistance of *C. albicans* and non-*albicans* *Candida* spp. in the group of patients with HIV is challenging therapeutically and should encourage the development of new antifungal drugs, alterations in drug dosing, or encourage routine susceptibility testing in patients with candidiasis.<sup>16–18</sup> Colonization and drug susceptibility studies of these patients provided a good baseline for ongoing projects, especially in regard to treatment intervention or to monitor reinfection in patients.

## Conclusions

Vaginal isolates of *Candida* spp. in patients testing positive for HIV infection displayed an increase in drug resistance. Thirty-two percent of women with HIV infection had received oral fluconazole and antifungal over-the-counter treatments that may have contributed to the increased resistance seen in this group of patients. The results also suggested that the transfer of resistant isolates of *Candida* spp. from a vaginal to an oral site may have occurred.

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Dr. Zhang, L and Dr. She, X were responsible for drug activity screens and genotyping experiments. Dr. She, X and Dr. Li, D were responsible for the integrity of the data and the accuracy of the data analysis. Study concept and design are initiated by Dr. Calderone, R and Merenstein, D, MD. Clinical isolates were collected by Hamilton, P. Clinical treatment history was provided by Wang C. Patient recruitment for this study was assigned to Blackmon, M and Hu, H. The manuscript was written by Dr. Li, D and Dr. Calderone, R. The first two authors contributed equally to this work.

## Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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