Prevalence of Vaginal Colonization by Drug-Resistant *Candida* Species in College-Age Women with Previous Exposure to Over-the-Counter Azole Antifungals

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We enrolled 382 college-age women in a cross-sectional survey to investigate the relationship between use of over-the-counter (OTC) azole-based antifungal drugs and vaginal colonization by drug-resistant *Candida*. This study showed no correlation ($P = .506$) between previous OTC exposure and colonization of drug-resistant *Candida* in vaginal flora. However, a small number of resistant *Candida* species isolates were obtained from women with a history of multiple exposures to OTC antifungals; given the widespread use of these products, this may be an emerging concern.

Candida vaginitis is a common problem attributable to overgrowth of *Candida* species; it is estimated that 75% of all women will experience an episode in their lifetime [1, 2]. By the age of 25 years, nearly one-half of all college-age women will have had at least 1 episode of *Candida* vaginitis [3]. *Candida albicans* accounts for 80%–95% of all episodes of *Candida* vaginitis worldwide [1, 2]. Like other topical *Candida* infections, *Candida* vaginitis is treated effectively with azole-based antifungal drugs. However, such therapy can be complicated by the emergence of drug-resistant yeasts [4–6]. Prolonged exposure to fluconazole can shift the predominant vaginal yeast flora from *C. albicans* to more intrinsically azole-resistant species, as has been described for immunosuppressed women [4, 7].

In the 1990s, there was a significant increase in the prevalence of drug-resistant fungal infections due to *Candida* species in patients hospitalized for mucosal or systemic diseases [8–10]. The widespread application of fluconazole or related azole antifungals is postulated to promote selection of resistant subpopulations by shifting colonization to more naturally resistant species, such as *Candida krusei* or *Candida glabrata* [11–13]. Alternatively, azole-resistant subspecies have arisen in vivo and in vitro that show changes in the target enzyme lanosterol 14-α-demethylase, in expression of multidrug efflux pumps, or in both [14–16].

Numerous effective topical vaginal antimycotic agents are available that provide high cure rates with favorable therapeutic indexes. Given the growing correlation between azole antifungal exposure and emergence of drug-resistant *Candida* species, there is a concern that this problem may be exacerbated in healthy women by use of over-the-counter (OTC) products, which became available in the early to mid-1990s for self-treatment of vaginitis [5, 6]. Most notably, because many of the OTC drugs are azole based, it is likely that frequent or prolonged use of these products has the potential to select for widespread drug resistance. In fact, cross-resistance between OTC drugs (miconazole, clotrimazole, and tioconazole) and fluconazole has been observed in clinical isolates of *C. glabrata* and *C. albicans* [17, 18].

To better address this concern, a multicenter, cross-sectional study was initiated at 2 major university health services centers to explore the causal relationship between OTC antifungal use by healthy college-age women and vaginal colonization with fluconazole-resistant *Candida* species

Materials and methods. The multicenter, cross-sectional study was conducted at Columbia University Health and Related Services and Cornell University Health Services from March through July 1999 in New York. All women undergraduate and graduate students who were at least 18 years old and had a pelvic examination scheduled were asked to participate in the study. Of the subjects approached in clinic, 382 women agreed to have administered to them the terms of informed consent and allowed vaginal swabs to be obtained. Enrolled patients were given a brief health history questionnaire, and samples were obtained for culture on the same day.

The samples were collected by clinicians on site from the patient's vaginal pool by use of a sterile cotton swab. Swabs were directly inoculated onto a sterile slant containing Sa-

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Informed consent was obtained from all patients included in this study. Study protocols were approved by the institutional review boards of Columbia University and Cornell University.

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bouraud dextrose agar (4% [w/v] dextrose, 1% [w/v] peptone, 1.5% [w/v] agar; pH 5.6). These samples were then cultured on petri dishes containing yeast peptone dextrose agar media (1% [w/v] yeast extract, 2% [w/v] Bacto peptone [Difco], 2% [w/v] dextrose, 1.5% [w/v] agar) at 30 ± 2°C for 48 h. Culture-positive samples were plated on CHROMagar (DRG International) and grown at 30 ± 2°C for 24 h. Positive cultures were tested at the New York State Department of Health Mycology Lab, Wadsworth Center, Albany, New York, for *Candida* species identification and antifungal susceptibility testing. All *Candida* samples were maintained on Sabouraud dextrose agar media plates (Becton Dickinson Microbiology Systems) and incubated at 35°C. A germ tube test was performed for presumptive identification of *C. albicans.* Organisms that failed to form a germ tube after a 3-h incubation at 37°C in sterile horse serum were further tested by the auxanographic plate method by use of carbohydrate disks (BBL Microbiology Systems; Difco Laboratories). A broth microdilution test was performed according to the proposed standard guidelines of the National Committee for Clinical Laboratory Standards; microtiter assays to determine level of susceptibility against fluconazole and amphotericin B were performed following the National Committee for Clinical Laboratory Standards guidelines [19].

Fisher's exact test and χ^2 tests were used to compare proportional differences for given categorical and continuous variables between subjects with *Candida*-positive cultures and those with *Candida*-negative cultures. Binomial and Poisson 95% confidence intervals were calculated for prevalence of *Candida* and drug-resistant forms, as appropriate. Analysis was carried out by SAS, version 7 (SAS).

Results. A total of 382 subjects participated in the study, 272 from Columbia and 110 from Cornell; they ranged in age from 18 to 54 years. The mean age ± SD for the participants was 27 ± 5.2 years for students from Columbia and 24 ± 5.1 years for students from Cornell. Students enrolled at Columbia were significantly older and had been in school longer ($P <$ .0001) than Cornell students. The distribution of other characteristics, such as ethnic makeup, previous OTC azole antifungal use, number of vaginal infections in the past 5 years, and presence in the clinic for routine examination, was not distinguishable between centers. The proportion of patients who were symptomatic for vaginitis, the number who had previously used fluconazole, and the percentage of patients who harbored yeast also were not significantly different between the 2 sites.

Approximately 26.4% of all subjects (101 of 382) were identified as harboring yeast in the vaginal flora (table 1). Among the samples from this group, 22.2% (63 of 284 samples) were obtained from asymptomatic women, and 42.4% (28 of 66 samples) were obtained from symptomatic women. Among all yeast isolates, 92.1% (93 samples) were identified as *C. albicans.* Carriage rates in the centers were not significantly different (P = .6113): 25.7% (95% CI, 20.5%–30.9%) and 28.2% (95% CI, 19.8%–36.6%) for Columbia students (70 samples) and for Cornell students (31 samples), respectively. Samples were more likely to be culture positive for yeast that came from symptomatic women (P = .0016) who were in the clinic for nonroutine pelvic examination (P = .0238). There was a marginal association (P = .0579) between isolation of yeast in samples from women with a history of vaginitis episodes. However, among patients with recurring infections (defined as 3 or more per year), there was a significant correlation (P = .0388) with yeast colonization.

Yeast isolates *(C. albicans)* exhibiting resistance (MIC, >64 μg/mL) to fluconazole were recovered from 2 of the 382 women from whom samples were taken. Both students were 30 years old and had histories of vaginitis episodes, with 1 reporting recurring infections within the past year. Neither of the 2 carriers reported previous exposure to fluconazole; however, both patients previously had used azole-based OTC antifungal medication to treat an episode of vaginitis. One patient was symptomatic for vaginitis, and the other was at the clinic for a routine pelvic examination. The presence of 2 fluconazole-resistant isolates indicates a prevalence of 1.98% (95% Poisson CI, 0.24%–7.15%) among the total yeast isolated (101 isolates). From the total sample, 1 woman was found to be colonized with a *C. albicans* isolate that revealed dose-dependent susceptibility to fluconazole (MIC, 16–32 μg/mL). This patient, who was asymptomatic for vaginitis at the time of collection, had frequent yeast infections and had previous exposure to azole-based OTC antifungals. But the patient did not report a history of fluconazole use.

Among the 5 *C. glabrata* isolates recovered from the total sample, only 1 expressed intermediate dose-dependent fluconazole resistance. The carrier was an asymptomatic 26-year-old woman who reported that she had never experienced an episode of yeast infection or exposure to OTC azole medication or fluconazole. The isolate from this woman showed evidence of resistance to amphotericin B (MIC, >1 μg/mL).

Fifty-eight percent (220 of 382) of the women in this study reported use of OTC medication to treat episodes of vaginitis. Of those 220 women, 28.6% (63 women) were colonized with yeast. There was no statistically significant association between the use of OTC medication for vaginal yeast infections and occurrence of fluconazole resistance (P = .6403) in the study population. The association between prior OTC azole antifungal use and fluconazole resistance when the analysis was controlled for previous exposure to fluconazole was not significant (P = .6291). Interestingly, all 3 patients harboring *C. albicans* isolates with decreased susceptibility to fluconazole (2 resistant isolates and 1 isolate with dose-dependent susceptibility) reported previous OTC azole antifungal use.

Discussion. In this study, we demonstrate that previous

Table 1. Demographic and clinical characteristics of 382 women enrolled in a study of over-the-counter treatment of *Candida* infection.

Characteristic	All subjects (n = 382)	Subjects colonized with yeast (n = 101)	Uncolonized subjects (n = 281)	P[a]
Age in years[b]				.1887
Mean	25.7	24.8	26.1	
18–21	84 (22.1)	28 (27.7)	56 (20.0)	
22–25	119 (31.2)	29 (28.7)	90 (32.1)	
26–35	159 (41.7)	42 (41.6)	117 (41.8)	
⩾36	19 (5.0)	2 (2.0)	17 (6.1)	
Years of post–high school education, mean ± SD	4.5 ± 1.1	4.2 ± 1.3	4.6 ± 1.0	.0886
Ethnic group[c]				
Non-Hispanic white	264 (69.1)	71 (70.3)	193 (68.7)	.8027
Non-Hispanic black	27 (7.1)	8 (7.9)	19 (6.8)	.6572
Asian/Pacific Island	47 (12.3)	8 (7.9)	39 (13.9)	.1568
Hispanic	7 (1.8)	0 (0.0)	7 (2.5)	.1969
Other	37 (9.7)	14 (13.9)	23 (8.2)	.1163
Routine pelvic examination[d]				.0238
Yes	246 (64.4)	51 (50.5)	195 (69.4)	
No	67 (17.5)	23 (22.8)	44 (15.7)	
Unknown	69 (18.1)	27 (26.7)	42 (14.9)	
Symptomatic for vaginitis[d]				.0016
Yes	66 (17.3)	28 (27.7)	38 (13.5)	
No	284 (74.3)	63 (62.4)	221 (78.7)	
Unknown	32 (8.4)	10 (9.9)	22 (7.8)	
Vaginal infections, no.[e]				.0579
0	135 (35.3)	27 (26.7)	108 (38.4)	
1–2	119 (31.1)	32 (31.7)	87 (31.0)	
3–5	69 (18.1)	18 (17.8)	51 (18.2)	
>5	56 (14.7)	23 (22.8)	33 (11.7)	
Unknown	3 (0.8)	1 (1.0)	2 (0.7)	
Recurring vaginitis[f]				.0388
Yes	74 (19.4)	27 (26.7)	47 (16.7)	
No	300 (78.5)	72 (71.3)	228 (81.1)	
Unknown	8 (2.1)	2 (2.0)	6 (2.1)	
Use of over-the-counter azole antifungal[d]				.2912
Yes	220 (57.6)	63 (62.4)	157 (55.9)	
No	162 (42.4)	38 (37.6)	124 (44.1)	
Use of fluconazole[d]				.2879
Yes	46 (12.0)	15 (14.9)	31 (11.0)	
No	328 (85.9)	83 (82.2)	245 (87.2)	
Unknown	8 (2.1)	3 (3.0)	5 (1.8)	

NOTE. Data are no. (%) of subjects, unless otherwise noted.

[a] Fisher's exact test and χ^2 tests were used, as appropriate.
[b] Age data were available for 381 patients.
[c] P values are for the comparison of each ethnic group with all other ethic groups.
[d] Only the rows for "Yes" and "No" were used to calculate P values.
[e] In the past 5 years.
[f] Three or more vaginal infections in the past year.

exposure to OTC azole antifungal drugs is not associated with widespread colonization of drug-resistant *Candida* species in the vaginal flora of largely healthy college-age women. In contrast to oropharyngeal candidiasis in patients with AIDS, azole resistance has only been reported in 1 case of vaginitis caused by *C. albicans* [20]. In vitro resistance remains rare among isolates from women with vaginitis due to *C. albicans,* even among isolates from HIV-positive women, in whom there is a tendency for such infections to occur in the oral cavity. The same is true of patients with recurrent vulvovaginal candidiasis in which vaginal isolates remain susceptible to azole-based antifungal drugs and do not show increased resistance to any drug despite long-term exposure to azoles [21]. The results of this study support this finding (table 1).

The widespread use of azoles has led to an increase in the prevalence of fluconazole-resistant non–*C. albicans* species, especially *C. glabrata* [4, 7, 8, 18, 22, 23]. Of the 5 *C. glabrata* isolates recovered, only 1 expressed intermediate dose-dependent (MIC, >32 μg/mL) fluconazole resistance. The source was an asymptomatic 26-year-old woman who had no history of either a yeast infection or exposure to azole antifungals. It is noteworthy that this isolate was cross-resistant to amphotericin B (MIC, >1 μg/mL). Although such findings are unusual, similar findings have been reported for fluconazole-resistant *C. albicans* isolates recovered from both HIV-positive and healthy people who had never taken fluconazole or other azoles [24, 25].

The presence of asymptomatic women colonized with azole-resistant strains has important implications for therapeutic management of future vaginitis episodes in these women. Perhaps of more concern is the possibility that a primary drug-resistant colonizing strain could predominate at later time if the women become immunosuppressed. Furthermore, the spread of these primary drug-resistant colonizers by sexual transmission is a distinct possibility [26]. If candidiasis is caused by the host's original commensal strain or strains, knowledge of the yeast microflora of high-risk patients before the manifestation of candidiasis (e.g., susceptibility to different antifungal drugs) could lead to improved strategies for prophylaxis and treatment [23].

Another concern is the transmission of drug-resistant commensal colonizing strains to family members. Muller et al. [27] reported that transmission of azole-resistant strains from symptomatic HIV-infected patients with oropharyngeal candidiasis to asymptomatic family members is possible and may represent a previously underappreciated risk for families that include members infected with HIV. The acquisition of an azole-resistant strain of *C. albicans* by an asymptomatic patient has important clinical implications, especially in high-risk populations where presentation of oropharyngeal candidiasis may result in therapy refractory to azoles.

Yeast were isolated from 26.4% (101) of the women (table 1) in this study, which is consistent with previous point-prevalence studies, in which asymptomatic vaginal colonization with *Candida* was observed in 10%–55% of healthy adult women [28]. Despite exposure to azole antifungals of >50% among all patients, *C. albicans* was the predominant species found, accounting for 92.1% (93) of all isolates. The selection of non–*C. albicans* species, especially *C. glabrata,* in HIV-positive women receiving fluconazole therapy is well established, although *C. albicans* is still the predominant pathogen, at >85% of all isolates from HIV-seronegative women [2].

Our study has a number of limitations. First, detailed clinical chart reviews were not available to us, which might limit the associations we can suggest between clinical risk factors and drug-resistant yeast colonization. In addition, there may have been an element of recall bias regarding previous exposure to azole-based OTC. However, in either instance, we would expect our estimates to point away from an association between previous OTC usage and drug-resistant colonization. We believe neither to be the case in this study. The importance of the lack of statistical association seen in our study is limited by the small sample size; a true lack of association may not be indicated by our results. However, this study provides a baseline against which the general prevalence of colonization may be assessed and the relationship between previous OTC usage and resistance may be determined. More extensive population-based studies will be required to fully address this question.

Finally, the results in this study suggest that previous exposure to OTC azole-based antifungal drugs used to treat vaginal yeast infections is not a significant risk factor for evolution of clinical drug resistance in strains colonizing the genitourinary tract. However, it is noteworthy that 4 isolates (of 101) were shown either to be resistant to fluconazole (2 isolates) or to have intermediate dose-dependent susceptibility (2 isolates). Three of these isolates originated in women with a history of multiple OTC antifungal exposures. This observation, although anecdotal, is troubling, given that millions of women annually use OTC antifungal products (a market of almost $300 million), and these commensal strains may predominate if the women later become immunosuppressed.

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References

1. Sobel JD. Vaginitis. N Engl J Med **1997**; 337:1896–903.
2. Sobel JD. Vulvovaginitis in healthy women. Compr Ther **1999**; 25: 335–46.
3. Geiger AM, Foxman B, Gillespie BW. The epidemiology of vulvovaginal candidiasis among university students. Am J Public Health **1995**; 85: 1146–8.

4. Sobel JD. Vulvovaginitis due to *Candida glabrata:* an emerging problem. Mycoses **1998**; 41(Suppl 2):18–22.
5. Sobel JD. Vulvovaginitis: when *Candida* becomes a problem. Dermatol Clin **1998**; 16:763–8.
6. Sobel JD. Limitations of antifungal agents in the treatment of *Candida* vaginitis: future challenges. Drug Resist Updates **1999**; 2:148–52.
7. Vazquez JA, Sobel JD, Peng G, et al. Evolution of vaginal *Candida* species recovered from human immunodeficiency virus–infected women receiving fluconazole prophylaxis: the emergence of *Candida glabrata?* Terry Beirn Community Programs for Clinical Research in AIDS (CPCRA). Clin Infect Dis **1999**; 28:1025–31.
8. Nguyen MH, Peacock JE Jr, Morris AJ, et al. The changing face of candidemia: emergence of non–*Candida albicans* species and antifungal resistance. Am J Med **1996**; 100:617–23.
9. Pfaller MA, Messer SA, Hollis RJ, et al. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. Diagn Microbiol Infect Dis **1999**; 33: 217–22.
10. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans:* frequency of occurrence and antifungal susceptibility in the SCOPE Program. SCOPE Participant Group. Surveillance and Control of Pathogens of Epidemiologic Importance. Diagn Microbiol Infect Dis **1998**; 30(2):121–9.
11. Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000: implications for therapy and new approaches. Drugs **1997**; 54: 657–78.
12. Pfaller MA. Epidemiology of candidiasis. J Hosp Infect **1995**; 30(Suppl): 329–38.
13. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother **1995**; 39:1–8.
14. Marr KA, Lyons CN, Rustad TR, Bowden RA, White TC, Rustad T. Rapid, transient fluconazole resistance in *Candida albicans* is associated with increased mRNA levels of CDR. Antimicrob Agents Chemother **1998**; 42:2584–9.
15. Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J. Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. Antimicrob Agents Chemother **1995**; 39:2378–86.
16. Sanglard D, Ischer F, Koymans L, Bille J. Amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase (CYP51A1) from azole-resistant *Candida albicans* clinical isolates contribute to resistance to azole antifungal agents. Antimicrob Agents Chemother **1998**; 42:241–53.
17. Arias A, Arevalo MP, Andreu A, Rodriguez C, Sierra A. *Candida glabrata:* in vitro susceptibility of 84 isolates to eight antifungal agents. Chemotherapy **1996**; 42:107–11.
18. Cross EW, Park S, Perlin DS. Cross-resistance of clinical isolates of *Candida albicans* and *Candida glabrata* to over-the-counter azoles used in the treatment of vaginitis. Microb Drug Resist **2000**; 6:155–61.
19. National Committee for Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. NCCLS document M27-A. Wayne, PA: NCCLS, **1997**.
20. Sobel JD, Vazquez JA. Symptomatic vulvovaginitis due to fluconazole-resistant *Candida albicans* in a female who was not infected with human immunodeficiency virus. Clin Infect Dis **1996**; 22:726–7.
21. Lynch ME, Sobel JD, Fidel PL Jr. Role of antifungal drug resistance in the pathogenesis of recurrent vulvovaginal candidiasis. J Med Vet Mycol **1996**; 34:337–9.
22. Hitchcock CA, Pye GW, Troke PF, Johnson EM, Warnock DW. Fluconazole resistance in *Candida glabrata.* Antimicrob Agents Chemother **1993**; 37:1962–5.
23. Xu J, Boyd CM, Livingston E, Meyer W, Madden JF, Mitchell TG. Species and genotypic diversities and similarities of pathogenic yeasts colonizing women. J Clin Microbiol **1999**; 37:3835–43.
24. Xu J, Ramos AR, Vilgalys R, Mitchell TG. Clonal and spontaneous origins of fluconazole resistance in *Candida albicans.* J Clin Microbiol **2000**; 38:1214–20.
25. Goff DA, Koletar SL, Buesching WJ, Barnishan J, Fass RJ. Isolation of fluconazole-resistant *Candida albicans* from human immunodeficiency virus–negative patients never treated with azoles. Clin Infect Dis **1995**; 20:77–83.
26. Barchiesi F, Hollis RJ, Del Poeta M, et al. Transmission of fluconazole-resistant *Candida albicans* between patients with AIDS and oropharyngeal candidiasis documented by pulsed-field gel electrophoresis. Clin Infect Dis **1995**; 21:561–4.
27. Muller FM, Kasai M, Francesconi A, et al. Transmission of an azole-resistant isogenic strain of *Candida albicans* among human immunodeficiency virus–infected family members with oropharyngeal candidiasis. J Clin Microbiol **1999**; 37:3405–8.
28. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am J Obstet Gynecol **1985**; 152(7 Pt 2):924–35.