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Antifungal Susceptibilities of *Candida* Species Causing Vulvovaginitis and Epidemiology of Recurrent Cases

Sandra S. Richter,[1]* Rudolph P. Galask,[2] Shawn A. Messer,[1] Richard J. Hollis,[1] Daniel J. Diekema,[1] and Michael A. Pfaller[1]

Department of Pathology[1] and Department of Obstetrics and Gynecology,[2] University of Iowa Carver College of Medicine, Iowa City, Iowa

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There are limited data regarding the antifungal susceptibility of yeast causing vulvovaginal candidiasis, since cultures are rarely performed. Susceptibility testing was performed on vaginal yeast isolates collected from January 1998 to March 2001 from 429 patients with suspected vulvovaginal candidiasis. The charts of 84 patients with multiple positive cultures were reviewed. The 593 yeast isolates were *Candida albicans* ($n = 420$), *Candida glabrata* ($n = 112$), *Candida parapsilosis* ($n = 30$), *Candida krusei* ($n = 12$), *Saccharomyces cerevisiae* ($n = 9$), *Candida tropicalis* ($n = 8$), *Candida lusitaniae* ($n = 1$), and *Trichosporon* sp. ($n = 1$). Multiple species suggesting mixed infection were isolated from 27 cultures. Resistance to fluconazole and flucytosine was observed infrequently (3.7% and 3.0%); 16.2% of isolates were resistant to itraconazole (MIC ≥ 1 μg/ml). The four imidazoles (econazole, clotrimazole, miconazole, and ketoconazole) were active: 94.3 to 98.5% were susceptible at ≤1 μg/ml. Among different species, elevated fluconazole MICs (≥16 μg/ml) were only observed in *C. glabrata* (15.2% resistant [R], 51.8% susceptible-dose dependent [S-DD]), *C. parapsilosis* (3.3% S-DD), *S. cerevisiae* (11.1% S-DD), and *C. krusei* (50% S-DD, 41.7% R, considered intrinsically fluconazole resistant). Resistance to itraconazole was observed among *C. glabrata* (74.1%), *C. krusei* (58.3%), *S. cerevisiae* (55.6%), and *C. parapsilosis* (3.4%). Among 84 patients with recurrent episodes, non-albicans species were more common (42% versus 20%). A ≥4-fold rise in fluconazole MIC was observed in only one patient with *C. parapsilosis*. These results support the use of azoles for empirical therapy of uncomplicated candidal vulvovaginitis. Recurrent episodes are more often caused by non-albicans species, for which azole agents are less likely to be effective.

Limited data addressing the incidence of vulvovaginal candidiasis suggest approximately two-thirds of women experience at least one episode during their lifetime and nearly 50% of women have multiple episodes (3, 12). The majority of cases of vulvovaginal candidiasis are caused by *Candida albicans*; however, episodes due to non-albicans species of *Candida* appear to be increasing (15, 22, 29). Most non-albicans *Candida* species have higher azole MICs, and infections they cause are often difficult to treat (10, 20, 21, 26).

A possible explanation for more frequent isolation of non-albicans species from vulvovaginitis patients may be the increased use of topical azole agents—available as over-the-counter preparations in the United States since 1992 (14, 24). Patients who see a physician usually receive empirical therapy; vaginal cultures are not routinely obtained, and susceptibility testing is rarely performed.

Surveillance programs for candidemia have demonstrated that fluconazole resistance among *C. albicans* bloodstream isolates is rare (≤1%) (16). The majority of studies analyzing yeast isolates from vulvovaginitis patients have also shown the recovery of fluconazole-resistant *C. albicans* isolates to be an unusual event (1, 7, 10, 20, 27), but they often have included a small number of isolates. The increased use of over-the-counter antifungals and prolonged therapy for recurrent candidiasis are risk factors for the emergence of azole resistance among *C. albicans* isolated from vulvovaginitis patients.

The purpose of this study was to determine the species distribution and prevalence of antifungal resistance among a large collection of yeast isolates from patients with candidal vulvovaginitis. Retrospective chart review was performed on patients with multiple vaginal yeast isolates in order to examine the epidemiology of recurrent candidal vulvovaginitis.

MATERIALS AND METHODS

Vaginal yeast cultures ($n = 564$) were collected from 429 University of Iowa vulvovaginitis clinic patients with signs and symptoms suggestive of candidal vulvovaginitis from January 1998 to March 2001. The decision to submit a culture was made by the attending gynecologist, and only the identification of yeast isolated was reported. All specimens were plated on CHROMagar (Hardy Diagnostics, Santa Maria, CA) to ensure detection of mixed infections. Cultures were incubated for 72 h at 35°C in ambient air. Identification was based on colony morphology and carbohydrate assimilation using Vitek YBC cards (bioMerieux Vitek, Inc., Hazelwood, MO). If the identity of the yeast was uncertain, isolates were plated on cornmeal agar or sent to a reference laboratory. The isolates were banked in water at room temperature.

Susceptibility testing was performed on the banked isolates using a broth microdilution method according to NCCLS guidelines (13). Antifungals and concentrations tested were fluconazole (0.12 to 128 μg/ml), flucytosine (0.06 to 64 μg/ml), nystatin (0.06 to 64 μg/ml), and itraconazole, econazole, clotrimazole, miconazole, and ketoconazole (all 0.007 to 8 μg/ml). A 0.1-ml yeast inoculum of $1.5 (\pm 1.0) \times 10^3$ cells/ml in RPMI 1640 medium was added to each microdilution well. The trays were incubated at 35°C for 46 to 50 h in ambient air. The MICs were read as the lowest antifungal concentration with substantially lower turbidity (~50%) compared to growth in the antifungal-free growth control well for all agents except nystatin. Nystatin MICs were read as the minimal antifungal concentration with complete inhibition of growth. NCCLS interpretive criteria were applied for fluconazole, itraconazole, and flucytosine. There are no NCCLS breakpoints for the other antifungals tested. Quality control was ensured by

* Corresponding author. Mailing address: Department of Pathology, University of Iowa Roy J. and Lucille A. Carver College of Medicine, 200 Hawkins Drive, C606 GH, Iowa City, IA 52242-1009. Phone: (319) 356-2990. Fax: (319) 356-4916. E-mail: sandra-richter@uiowa.edu.

TABLE 1. Species distribution of yeast isolates from 429 vulvovaginitis patients

Organism	No. (%) found among:				
	All isolates	Isolates from initial pos cx from 429 pts[a]	Isolates from 345 pts with only one pos cx[b]	Isolates from 84 pts with multiple pos cxs	
				Initial pos cx[c]	Subsequent pos cxs[d]
C. albicans	420 (70.8)	340 (75.7)	288 (80.0)	52 (58.4)	80 (55.5)
C. glabrata	112 (18.9)	70 (15.6)	44 (12.2)	26 (29.2)	42 (29.2)
C. parapsilosis	30 (5.0)	17 (3.8)	11 (3.0)	6 (6.7)	13 (9.0)
C. krusei	12 (2.0)	7 (3.8)	5 (1.4)	2 (2.2)	5 (3.5)
S. cerevisiae	9 (1.5)	8 (1.8)	6 (1.7)	2 (2.2)	1 (0.7)
C. tropicalis	8 (1.4)	5 (1.1)	4 (1.1)	1 (1.1)	3 (2.1)
C. lusitaniae	1 (0.2)	1 (0.2)	1 (0.3)	—[e]	—
Trichosporon sp.	1 (0.2)	1 (0.2)	1 (0.3)	—	—
Total	593	449	360	89	144

[a] Includes only isolates from 1st positive culture during study period for each patient; 18 specimens yielded two species ($n = 16$) or three species ($n = 2$). Pos, positive; cx, culture; pts, patients.
[b] Includes 13 cultures that yielded two species ($n = 11$) or three species ($n = 2$).
[c] Includes five cultures that yielded two species.
[d] Includes nine cultures that yielded two species.
[e] —, no isolates.

testing the NCCLS-recommended quality control strains *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258.

Patients with multiple positive cultures during January 1998 to March 2001 were considered to have recurrent candidal vulvovaginitis. A retrospective chart review was performed on this subset of patients to collect epidemiologic data that included age, symptoms, comorbid conditions, and response to therapy. Correlation of MIC with response to antifungal therapy and change in MIC among successive same-species isolates therapy were also examined. The significance of group proportion differences was assessed with Fisher's exact test.

RESULTS

A total of 593 yeast isolates were obtained from 564 vaginal specimens: 420 *C. albicans*, 112 *Candida glabrata*, 30 *C. parapsilosis*, 12 *C. krusei*, 9 *Saccharomyces cerevisiae*, 8 *Candida tropicalis*, 1 *Candida lusitaniae*, and 1 *Trichosporon* sp. (Table 1). Of the 429 women with positive yeast cultures, 84 (19.6%) had multiple positive cultures (233 isolates) during the study period. Non-albicans species were more commonly isolated ($P = 0.0001$) from the cultures of patients with multiple positive cultures (42% of initial positive cultures) than from cultures of patients with a single positive culture (20% non-albicans). The predominant non-albicans species recovered was *C. glabrata*. The prevalence of *C. glabrata* noted among patients with multiple positive cultures was significantly higher than among patients with a single positive culture (29% versus 12%; $P = 0.0002$). The species distribution of isolates collected during the 39-month period was relatively stable over time (data not shown).

Mixed infection with multiple species of yeast isolated from one specimen was observed in 27 episodes of vulvovaginitis (4.8%). The majority of these mixed cultures (21 out of 27) yielded *C. albicans* and *C. glabrata*. Three cultures yielded *C. albicans* and *C. krusei*, and the remaining three mixed cultures yielded *C. glabrata*, *C. tropicalis*, and *C. albicans*; *S. cerevisiae* and *C. glabrata*; and *C. parapsilosis* and *C. albicans*. Thirteen of the 27 mixed cultures occurred in patients with only a single positive culture during the study period. The remaining 14 mixed cultures occurred in 10 of the 84 patients with multiple positive cultures whose charts were reviewed.

Positive cultures noted during chart review obtained before and after the January 1998 to March 20001 study period are included in Table 2 to present a more comprehensive picture of the pattern of yeast isolation for patients with recurrent vulvovaginitis. The cultures of 52 patients yielded the same species repeatedly, while multiple species (different species over time or multiple species from a single specimen) were isolated from the other 32 patients. The age of patients at their first vulvovaginitis clinic visit ranged from 18 to 78 years. Among 18 to 59 year olds, a single species (*C. albicans*) was the most common culture result (34 of 67 patients; 50.7%). The majority of patients age 60 or greater (70.6%) had cultures that yielded different species over time or multiple species from a single specimen.

Every patient with multiple yeast isolates reported at least one symptom consistent with, but not specific for, candidal vulvovaginitis. These symptoms included vulvovaginal itching, vulvovaginal burning, vulvovaginal pain, vaginal discharge, and dyspareunia.

Every patient with recurrent candidal vulvovaginitis had at least one of 40 medical conditions listed in Table 3 noted in their medical history or occurring while they were followed at the vulvovaginitis clinic. A large portion of the 84 patients (46.4%) had received recent antibiotic therapy. Many patients had lower genital tract conditions, including vulvar contact dermatitis (60.7%), vulvar vestibulitis (25%), vulvar intraepithelial neoplasia (23.8%), and lichen simplex chronicus (21.4%). Other predominant medical conditions noted among these patients were diabetes (15.5%), depression (10.7%), and postmenopausal symptoms treated with hormone replacement therapy (11.9%).

Susceptibility test results for the 593 isolates revealed that resistance to fluconazole (3.7%) or flucytosine (3.0%) was infrequent. Resistance to itraconazole occurred among 16.2% of the isolates. The imidazoles (econazole, clotrimazole, miconazole, and ketoconazole) were active against 94.3 to 98.5% of the isolates (MIC $\leq$ 1 μg/ml). Nystatin MICs ranged from 1 to 16 μg/ml, with a MIC inhibiting 90% of isolates (MIC_{90}) of 4 μg/ml.

The susceptibility test results for each species are presented

TABLE 2. Yeast isolates and age[a] distribution of 84 patients with recurrent vulvovaginitis[b]

Yeast organism(s) isolated	No. of patients in age group with species						
	All ages	18–29 yrs	30–39 yrs	40–49 yrs	50–59 yrs	60–69 yrs	70–79 yrs
One species	52	13	7	12	15	2	3
C. albicans	36	13	6	5	10	1	1
C. glabrata	10	—[g]	1	3	3	1	2
C. parapsilosis	3	—	—	1	2	—	—
C. krusei	1	—	—	1	—	—	—
C. tropicalis	1	—	—	1	—	—	—
S. cerevisiae	1	—	—	1	—	—	—
Multiple species	32	4	4	6	6	7	5
Nonconsecutive[c] *C. albicans*, *C. glabrata*	13	—	3	2	1	5	2
C. glabrata, then *C. albicans*	8	—	1	—	5	2	—
At least three different species[c]	3	1[d]	—	2[e,f]	—	—	—
C. albicans, then *C. glabrata*	2	—	—	1	—	—	1
C. albicans and *C. parapsilosis*	2	—	—	—	—	—	2
C. albicans and *C. krusei*[c]	2	1	—	1	—	—	—
C. albicans and *Saccharomyces* sp.[c]	1	1	—	—	—	—	—
C. lusitaniae, then *C. albicans*	1	1	—	—	—	—	—
All patients (%), by age	84	17 (20.2)	11 (13.1)	18 (21.4)	21 (25.0)	9 (10.7)	8 (9.5)

[a] Age at first visit to vulvovaginitis clinic.
[b] Recurrent vulvovaginitis, multiple vaginal yeast cultures positive during 1/98 to 3/01 and clinical signs or symptoms consistent with candidal vulvovaginitis.
[c] In some cases, multiple species were isolated from one culture.
[d] *C. glabrata*, then *Saccharomyces* sp., then *C. parapsilosis* twice, then *Saccharomyces* sp.
[e] *C. parapsolisis* three times, then *C. krusei*, then *C. albicans*, then *C. parapsilosis*, then *C. tropicalis*.
[f] *Saccharomyces* sp. plus *C. glabrata*, then *C. glabrata* twice, then *C. albicans* plus *C. glabrata*.
[g] —, no patients.

in Table 4. Fluconazole resistance (MIC $\geq$ 64 μg/ml) was observed only among *C. glabrata* isolates (15.2%) and *C. krusei* isolates (41.7%; all *C. krusei* isolates should be reported as fluconazole resistant per NCCLS guidelines). Fluconazole susceptible-dose dependent (MIC, 16 to 32 μg/ml) isolates were found among *C. glabrata* (51.8%), *C. krusei* (50%), *S. cerevisiae* (11.1%), and *C. parapsilosis* (3.3%). Itraconazole resistance was observed among *C. glabrata* (74.1%), *C. parapsilosis* (3.3%), *C. krusei* (58.3%), and *S. cerevisiae* (55.6%) isolates. Flucytosine resistance was noted among *C. albicans* (3.3%), *C. krusei* (8.3%), *C. tropicalis* (12.5%), *C. lusitaniae* (100%), and *Trichosporon* sp. (100%).

Response to antifungal therapy among patients with multiple positive cultures is compared to the MIC in Table 5. The most common antifungal prescribed was fluconazole, with a typical course of oral therapy 200 mg every other day for three doses. If *C. glabrata* was suspected, boric acid was prescribed (600-mg capsule inserted intravaginally once daily for 2 weeks). A few patients received intravaginal econazole or clotrimazole once or twice daily for 14 days. Data regarding compliance with prescribed therapy were not collected.

A majority (61.5%) of the patients with 78 evaluable episodes of *C. albicans* vulvovaginitis treated with fluconazole reported improvement in symptoms at the next clinic visit (Table 5). All of the *C. albicans* isolates were well below the fluconazole susceptibility breakpoint of $\leq$8 μg/ml, and there was no difference in outcome associated with the MIC. A minority of patients treated with fluconazole for all but one species of non-albicans vulvovaginitis reported improvement. The exception was two episodes of *C. krusei* vulvovaginitis with symptomatic improvement after fluconazole therapy despite MICs of 32 and 128 μg/ml.

Patients reported improvement for only 48.6% of the 70 evaluable episodes of *C. glabrata* vulvovaginitis treated with boric acid therapy. There was no attempt to measure in vitro boric acid activity in this study, since there are no standardized methods for testing this agent.

Four of the 7 evaluable episodes (57.1%) treated with clotrimazole and 4 of 10 vulvovaginitis episodes treated with econazole were associated with clinical improvement. All of the isolates treated with clotrimazole or econazole had MICs generally considered susceptible ($\leq$1 μg/ml).

The MICs of isolates (same species) from patients with multiple positive cultures were relatively stable. A fourfold or greater rise in fluconazole, econazole, or clotrimazole MIC within a species was observed in only two patients (Table 6). The fluconazole MIC of *C. parapsilosis* isolates from one patient increased sixfold (0.5 μg/ml to 32 μg/ml) after three courses of fluconazole. The econazole MICs for successive *C. glabrata* isolates increased fourfold in a second patient, but there was no record of the patient receiving this antifungal. There were five instances of a threefold MIC increase without evidence the patients had received the antifungal.

DISCUSSION

In this study, *C. albicans* was the most common species associated with vulvovaginitis (76%), followed by *C. glabrata* (16%). The overall percentage of non-albicans vaginitis (24%) was higher than in two previous reports (9, 29). Spinillo et al. reported 17% of 209 isolates from symptomatic patients referred to an Italian vulvovaginitis clinic were non-albicans species in 1995 (29). An Australian study found vulvovaginal yeast carriage among 21% of 5,802 women receiving a pelvic exam in a primary care setting; non-albicans species were isolated in only 11% of the positive cultures (9). This difference likely

TABLE 3. Medical history of 84 patients with recurrent candidal vulvovaginitis

Medical condition[f]	No. of patients in age group with condition						
	All ages	18–29 yrs	30–39 yrs	40–49 yrs	50–59 yrs	60–69 yrs	70–79 yrs
Recent antibiotic therapy for:							
Urinary tract infections	21	4	4	3	7	1	2
Bacterial vaginosis	6	4	1	1	—[g]	—	—
Chronic sinusitis	5	—	1	2	1	1	—
Acne	2	2	—	—	—	—	—
Chlamydia infection	2	1	—	1	—	—	—
Other[a]	3	1	1	—	1	—	—
Lower genital tract conditions							
Vulvar contact dermatitis	51	11	8	12	13	4	3
Vulvar vestibulitis	21	9	2	6	4	—	—
VIN	20	4	1	4	5	3	3
Lichen simplex chronicus	18	5	2	1	8	—	2
Lichen sclerosis	8	—	1	2	—	3	2
Lichen planus	6	—	—	—	4	1	1
Genital warts	6	—	1	3	1	1	—
Atrophic vaginitis	3	—	—	—	1	1	1
CIN I	2	—	—	1	1	—	—
Other[b]	2	—	1	—	—	1	—
Other conditions							
Diabetes	13	3	2	2	3	2	1
Hormone replacement therapy	10	—	—	—	4	2	4
Depression	9	1	4	1	1	2	—
Irritable bowel syndrome	6	2	—	1	2	—	1
Asthma	4	—	—	1	1	2	—
Fibromyalgia	3	—	—	—	3	—	—
Urinary incontinence	3	—	—	1	—	1	1
Immunodeficiency	2	—	—	1[c]	1[d]	—	—
Obesity	2	—	—	1	—	—	1
Other[e]	12	1	3	4	2	1	1

[a] Bronchitis (age 18–29), *Helicobacter pylori* (age 30–39); *Trichomonas* (age 50–59).
[b] Genital herpes (age 30–39), vulvar squamous cell carcinoma s/p vulvectomy (age 60–69).
[c] Immunodeficiency with low-titer antibody responses to pneumococcal and tetanus vaccines.
[d] Probable immunodeficiency, anergic to delayed-type hypersensitivity skin testing (PPD, *Candida*, *Trichophyton*, streptokinase).
[e] Anorexia nervosa (age 30–39), COPD (age 60–69), Crohn's disease (age 30–39), eczema (age 40–49), interstitial cystitis (age 50–59), oral candidiasis (age 30–39), polycystic ovary syndrome (age 18–29), rheumatoid arthritis (age 40–49), cutaneous lupus erythematosus (age 40–49), melanoma (age 70–79), papillary thyroid carcinoma (age 40–49), multiple basal cell caricinomas (age 50–59).
[f] VIN, vulvar intraepithelial neoplasia; CIN I, cervical intraepithelial neoplasia stage I.
[g] —, no patients.

reflects a bias towards more complicated patients in the current study who are symptomatic and had prior episodes before being referred to a vulvovaginitis clinic. Nyirjesy et al. (15) reported a higher rate of non-albicans species than the current study: 32% of 77 isolates from 74 patients with chronic vulvovaginitis.

The subset of isolates from patients with multiple positive cultures during the study period included a higher percentage of non-albicans species (42%) than the isolates from patients with only one positive culture (20% non-albicans). The marked difference in species distribution between these two groups may be the result of a higher level of antifungal exposure among patients with multiple positive cultures providing selective pressure for non-albicans species that are more likely to be azole resistant. An alternative explanation is that patients who develop vulvovaginitis from a non-albicans species are more likely to fail therapy and have multiple positive cultures.

The prevalence of *C. albicans* noted among the subset of patients with multiple positive cultures in the current study (58%) was similar to the prevalence of *C. albicans* reported by *Candida* bloodstream infection surveillance studies (52 to 56%) (18). The prevalence of *C. glabrata* (29%) among patients with multiple positive vaginal cultures was higher than that reported among bloodstream isolates (8 to 18%) (18).

The fluconazole susceptibility of all 420 *C. albicans* vaginal isolates in the current study is consistent with other reports. A recent U.S. study found no fluconazole resistance among 401 *C. albicans* isolates recovered from women with recurrent vulvovaginitis (27). In an earlier U.S. study, all 100 *C. albicans* isolates from vulvovaginitis clinic patients were also fluconazole susceptible (10). No fluconazole resistance was identified among 75 *C. albicans* vaginal isolates from symptomatic women in England (7). A study in Brazil reported no fluconazole resistance among 56 *C. albicans* vaginal isolates (12 isolates were from asymptomatic women, suggesting colonization rather than infection) (20). An Italian study with only 19 *C. albicans* vaginal isolates (13 from asymptomatic women) reported no fluconazole resistance (1).

There was only one case report of a fluconazole-resistant (MIC, ≥64 μg/ml) *C. albicans* vaginal isolate causing vulvovaginitis (collected in 1995) until recently (25). A fluconazole-resistant *C. albicans* isolate from a vulvovaginitis patient in the

TABLE 4. Antifungal susceptibilities of 593 vaginal yeast isolates

Antifungal agent	Cumulative % of isolates with MIC (μg/ml) of:																	S-DD[a] (%)	R[b] (%)
	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8	16	32	64	128	>128		
C. albicans (n = 420)																			
Fluconazole	—[d]	—	—	—	7.6	91.2	98.3	99.3	99.8	100	—	—	—	—	—	—	—	—	—
Itraconazole	—	1	37.4	90.7	99.5	100	—	—	—	—	—	—	—	—	—	—	—	0.5	—
Flucytosine	—	—	—	4.8	41.2	53.6	65.5	93.3	96.7	—	—	—	—	—	—	—	100	—	3.3
Econazole	14.3	72.1	94.5	99	99.5	99.8	—	—	—	—	—	100	—	—	—	—	—	—	—
Clotrimazole	2.1	24	83.8	99.5	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	10.7	73.3	97.4	99.8	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—
Ketoconazole	92.1	99.3	99.8	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	0.2	12.9	99.5	100	—	—	—	—	—	—	—	—
C. glabrata (n = 112)																			
Fluconazole	—	—	—	—	—	—	—	—	4.5	10.7	33	—	74.1	84.8	90.2	100	—	51.8	15.2
Itraconazole	—	—	—	—	—	6.3	25.9	36.6	88.4	94.6	95.5	—	100	—	—	—	—	25.9	74.1
Flucytosine	—	—	—	77.7	99.1	—	—	—	100	—	—	—	—	—	—	—	—	—	—
Econazole	0.9	7.1	12.5	42	66.1	83	90.2	97.3	100	—	—	—	—	—	—	—	—	—	—
Clotrimazole	—	—	—	0.9	3.6	23.2	41.1	69.6	85.7	94.6	100	—	—	—	—	—	—	—	—
Miconazole	—	2.7	6.3	24.1	55.4	76.8	87.5	94.6	100	—	—	—	—	—	—	—	—	—	—
Ketoconazole	—	—	—	4.5	12.5	48.2	76.8	92	100	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	2.7	59.8	98.2	99.1	—	100	—	—	—	—	—	—
C. parapsilosis (n = 30)																			
Fluconazole	—	—	—	—	—	3.3	33.3	73.3	86.7	93.3	96.7	—	—	100	—	—	—	3.3	—
Itraconazole	—	—	—	16.7	36.7	86.7	96.7	100	—	—	—	—	—	—	—	—	—	60	3.3
Flucytosine	—	—	—	36.7	76.7	96.7	100	—	—	—	—	—	—	—	—	—	—	—	—
Econazole	—	—	3.3	—	—	23.3	43.3	46.7	83.3	93.3	100	—	—	—	—	—	—	—	—
Clotrimazole	—	—	10	56.7	80	96.7	100	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	3.3	—	—	10	33.3	46.7	83.3	96.7	100	—	—	—	—	—	—	—	—	—
Ketoconazole	6.7	26.7	46.7	76.7	83.3	96.7	100	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	—	3.3	96.7	100	—	—	—	—	—	—	—	—
C. krusei (n = 12)																			
Fluconazole	—	—	—	—	—	—	—	8.3	—	—	—	—	—	58.3	75	100	—	50[c]	41.7[c]
Itraconazole	—	—	—	—	8.3	—	41.7	—	100	—	—	—	—	—	—	—	—	33.3	58.3
Flucytosine	—	—	—	—	—	8.3	—	—	—	16.7	33.3	—	91.7	100	—	—	—	75	8.3
Econazole	—	—	—	—	—	—	—	8.3	33.3	91.7	100	—	—	—	—	—	—	—	—
Clotrimazole	—	—	—	—	16.7	58.3	91.7	100	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	—	—	—	—	—	—	8.3	58.3	100	—	—	—	—	—	—	—	—	—
Ketoconazole	—	—	—	—	8.3	—	41.7	100	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	—	8.3	91.7	100	—	—	—	—	—	—	—	—
S. cerevisiae (n = 9)																			
Fluconazole	—	—	—	—	—	—	—	—	44.4	66.7	88.9	—	100	—	—	—	—	11.1	—
Itraconazole	—	—	—	—	—	22.2	44.4	—	100	—	—	—	—	—	—	—	—	44.4	55.6
Flucytosine	—	—	—	66.7	77.8	100	—	—	—	—	—	—	—	—	—	—	—	—	—
Econazole	—	11.1	44.4	77.8	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clotrimazole	—	—	—	22.2	33.3	100	—	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	—	22.2	55.6	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ketoconazole	—	—	—	—	44.4	88.9	100	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	11.1	66.7	100	—	—	—	—	—	—	—	—	—
C. tropicalis (n = 8)																			
Fluconazole	—	—	—	—	—	12.5	37.5	87.5	100	—	—	—	—	—	—	—	—	—	—
Itraconazole	—	—	12.5	37.5	62.5	100	—	—	—	—	—	—	—	—	—	—	—	37.5	—
Flucytosine	—	—	—	—	—	50	87.5	—	—	—	—	—	—	—	—	—	100	—	12.5
Econazole	—	12.5	—	25	50	62.5	75	—	87.5	100	—	—	—	—	—	—	—	—	—
Clotrimazole	—	—	12.5	50	75	100	—	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	12.5	—	25	50	75	—	87.5	100	—	—	—	—	—	—	—	—	—	—
Ketoconazole	25	75	87.5	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	—	12.5	100	—	—	—	—	—	—	—	—	—
C. lusitaniae (n = 1)																			
Fluconazole	—	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—
Itraconazole	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	100	—
Flucytosine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	100	—	—	—	100
Econazole	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clotrimazole	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ketoconazole	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—
Trichosporon spp. (n = 1)																			
Fluconazole	—	—	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—
Itraconazole	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	100	—
Flucytosine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	100	—	100
Econazole	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clotrimazole	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miconazole	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—
Ketoconazole	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nystatin	—	—	—	—	—	—	—	—	—	100	—	—	—	—	—	—	—	—	—

[a] S-DD, susceptible-dose dependent (fluconazole, 16–32 μg/ml; itraconazole, 0.25–0.5 μg/ml) or intermediate (flucytosine, 8–16 μg/ml) per NCCLS interpretive criteria.

[b] R, resistant according to NCCLS interpretive criteria for fluconazole (≥64 μg/ml), itraconazole (≥1 μg/ml), and flucytosine (≥32 μg/ml).

[c] *C. krusei* is considered to have intrinsic fluconazole resistance.

[d] —, no isolates.

TABLE 5. Correlation of response to antifungal therapy with MICs for patients with recurrent vulvovaginitis[a]

Antifungal and organism	Antifungal MIC (μg/ml)	No. of isolates[a]	No. of patients (%) reporting clinical outcome at next clinic visit of:			
			Worse	Same	Better	Not evaluable[b]
Fluconazole						
C. albicans	0.125	7	1 (14.3)	1 (14.3)	2 (28.6)	3 (42.9)
	0.25	79	5 (6.3)	22 (27.8)	39 (49.4)	13 (16.5)
	0.5	8	1 (12.5)	—	6 (75.0)	1 (12.5)
	1	1	—[d]	—	1 (100)	—
C. parapsilosis	0.5	3	1 (33.3)	2 (66.7)	—	—
	2	1	—	1 (100)	—	—
	4	1	—	—	1 (100)	—
	8	1	—	—	1 (100)	—
C. tropicalis	0.25	1	—	—	1 (100)	—
	0.5	2	1 (50.0)	1 (50.0)	—	—
	1	1	—	—	—	1 (100)
C. krusei	32	1	—	—	1 (100)	—
	128	1	—	—	1 (100)	—
C. glabrata	4	1	—	1 (100)	—	—
S. cerevisiae	8	1	1 (100)	—	—	—
Boric acid						
C. glabrata	ND[c]	73	4 (5.5)	32 (43.8)	34 (46.6)	3 (4.1)
C. parapsilosis	ND	3	—	3 (100)	—	—
Clotrimazole						
C. albicans	0.007	1	—	1 (100)	—	—
	0.03	4	—	—	2 (50.0)	2 (50.0)
	0.06	1	—	—	—	1 (100)
C. parapsilosis	0.06	1	1 (100)	—	—	—
	0.25	3	1 (33.3)	—	2 (66.7)	—
Econazole						
C. albicans	0.015	4	2 (50.0)	1 (25.0)	1 (25.0)	—
	0.03	1	—	—	1 (100)	—
C. glabrata	0.06	2	1 (50.0)	—	1 (50.0)	—
	0.12	1	—	1 (100)	—	—
C. krusei	1	1	—	1 (100)	—	—
S. cerevisiae	0.06	1	—	—	1 (100)	—

[a] Cultures with more than one species were excluded. With the exception of *C. glabrata* treated with boric acid, only isolates with MICs of the antifungal used were included in the table.
[b] Clinical outcome not available (no follow-up or no documentation).
[c] ND, not determined.
[d] —, no patients.

United Kingdom was reported in 2002 (6). A U.S. study reported fluconazole resistance in 14 of 393 (3.6%) *C. albicans* isolates collected from complicated vaginitis patients prior to 2001 (28). In New York, 2 of 93 (2.1%) *C. albicans* vaginal isolates from 1999 were fluconazole resistant—only 1 was from a symptomatic patient (11). A 2002 Belgium study found an astonishing 21% of 84 vaginal *C. albicans* isolates to have fluconazole MICs of ≥64 μg/ml by NCCLS methods, but the 8 fluconazole-resistant isolates that were available for retesting were susceptible by the EUCAST method (2). This illustrates the difficulty that can be encountered in interpreting the trailing growth of static azole agents and the importance of sending isolates with unexpected susceptibility test results to established reference laboratories for retesting.

Previous studies correlating susceptibility test results of *Candida* species for azoles with clinical outcome support the "90-60 rule" established for bacterial infections: approximately 90% of infections due to susceptible isolates respond, while infections due to resistant isolates respond approximately 60% of the time (19). Demonstration of a correlation between clinical response and MIC in the current study was hindered by (i) the lack of in vitro azole resistance detected among the predominant organism isolated (*C. albicans*) and (ii) the small number of episodes due to non-albicans species treated with azoles (i.e., the most common non-albicans species, *C. glabrata*, was usually treated with boric acid). Among patients with multiple positive cultures, only 62% of the evaluable episodes of *C. albicans* vulvovaginitis treated with fluconazole showed clinical improvement at the next clinic visit—a lower response rate than predicted by the 90-60 rule.

As expected, higher azole MICs were found among non-albicans *Candida* species. A larger portion of the *C. glabrata* vaginal isolates in the current study was fluconazole nonsusceptible (67% [51.8% susceptible-dose dependent, 15.2% resistant]) than has been reported for bloodstream infection isolates (43% [36% susceptible-dose dependent, 7% resistant]) (17). These in vitro results support the use of alternative agents when treating vulvovaginitis caused by non-albicans species (especially *C. glabrata* or *C. krusei*).

Among patients with recurrent positive cultures, 34 of 70 evaluable episodes of *C. glabrata* vulvovaginitis treated with boric acid (49%) were reported as improved at the next clinic

TABLE 6. Correlation of intraspecies MIC variation with antifungal therapy for yeast isolates from patients with recurrent vulvovaginitis

Organism (no. of patients with multiple isolates)	Antifungal	No. of patients who received antifungal[a]	No. of patients with change in MIC of:							
			No change (± 1 dilution)	Dilutional increase in MIC of:			Dilutional decrease in MIC of:			
				+2	+3	+4	+6	−2	−3	−4	−5
C. albicans (n = 46)	Fluconazole	41[b]	46	—[n]	—	—	—	—	—	—	—
	Econazole	3	45	—	—	—	—	1[c]	—	—	—
	Clotrimazole	4	44	1[c]	—	—	—	1[c]	—	—	—
C. glabrata (n = 22)	Fluconazole	4[d]	15	1[e]	1[c]	—	—	3[f]	—	1[c]	—
									1[c]		
	Econazole	3[g]	13	4[h]	1[c]	1[c]	—	2[c]	—	1[c]	—
	Clotrimazole	1	13	3[c]	1[c]	—	—	4[i]	—	—	1[c]
C. parapsilosis (n = 5)	Fluconazole	4[j]	4	—	—	—	1[k]	—	—	—	—
	Econazole	1	3	—	1[c]	—	—	1[c]	—	—	—
	Clotrimazole	2	4	—	1[c]	—	—	—	—	—	—
C. krusei (n = 2)	Fluconazole	2	1	1[l]	—	—	—	—	—	—	—
C. tropicalis (n = 1)	Fluconazole	1[m]	1	—	—	—	—	—	—	—	—

[a] One of two courses of therapy unless otherwise specified. A typical course of fluconazole therapy was 200 mg every other day for three doses.
[b] Fluconazole: one course in 28 patients, two courses in 8 patients, three courses in 2 patients, and 3 patients with four, five, and eight courses.
[c] There was no record of the patient receiving the antifungal during the time period for which the MIC changed >1 dilution.
[d] Fluconazole: one course in two patients, two patients with seven and eight courses.
[e] Patient received eight courses of fluconazole for concomitant *C. albicans* during the 27-month time period when the fluconazole MIC increased.
[f] Only one of the three patients received fluconazole (seven courses) during the 20-month time period when the fluconazole MIC decreased.
[g] Econazole: three patients with one, two, and five courses.
[h] Only one of the four patients received econazole (five courses) during the 30-month time period when the econazole MIC increased.
[i] Only one of the four patients received clotrimazole therapy (one course); 3 months later, the clotrimazole MIC was two-fold lower.
[j] Fluconazole: one course in three patients, three courses in one patient.
[k] Received three courses of fluconazole during the 7-month period when the fluconazole MIC increased six-fold.
[l] Patient received two courses of fluconazole for concomitant *C. albicans* during the time period of MIC increase.
[m] Patient received three courses of fluconazole therapy.
[n] —, no patients.

visit. A higher 81% clinical improvement rate was noted in a retrospective study of 26 episodes of *C. glabrata* vulvovaginitis treated with boric acid (22). Another retrospective study of boric acid therapy reported successful outcome for 64% of 73 patients in Michigan and 71% of 38 patients in Israel with *C. glabrata* vulvovaginitis (23). There are case reports of boric acid poisoning associated with accidental ingestion and open wound absorption, but a 1974 study found no detectable levels of boric acid in the serum of 20 women after 2 weeks of therapy (600-mg capsule inserted intravaginally twice daily) (30). A lower daily dose of boric acid (600 mg once daily for 2 weeks) was prescribed to patients in the current study and is recommended by other studies (22, 23). There is a recent report of successful therapy with topical flucytosine among 90% of 27 women with *C. glabrata* vulvovaginitis who had failed azole and boric acid therapy (23). The in vitro activity of flucytosine was superior to all other agents tested against *C. glabrata* in the current study.

There are few studies reporting in vitro susceptibility data using the NCCLS method for the polyene nystatin. Carrillo-Munoz reported the nystatin MIC_{90} values for 55 *C. albicans* and 11 *C. glabrata* clinical isolates as 2 μg/ml and 4 μg/ml, respectively (5). The nystatin MICs for 589 oral yeast isolates from South African human immunodeficiency virus patients and healthy individuals ranged from 2 to 16 μg/ml with an MIC_{90} of 8 μg/ml for 466 *C. albicans* isolates (4). These values are similar to the nystatin MIC_{90} of 4 μg/ml for the isolates tested in the current study. The clustering of nystatin MICs at 2 to 4 μg/ml and the use of other agents to treat patients prevent any meaningful interpretation of the nystatin in vitro results in this study.

In view of the overall high level of susceptibility to antifungals, the small number of patients with substantial increases in antifungal MICs (Table 6) was not surprising. The highest rise in fluconazole MIC (from 0.5 μg/ml to 32 μg/ml after three courses of fluconazole over a 7-month period) occurred in a middle-aged woman with a history of chronic sinusitis, urinary tract infections, and vaginal yeast infections who was referred to an immunologist for further evaluation. She had low-titer antibody responses to pneumococcal and tetanus vaccines, but normal immunoglobulin G levels. Every vaginal culture collected from this patient during the study period yielded *C. parapsilosis*. In addition to fluconazole, therapy included boric acid, nystatin, econazole, itraconazole, gentian violet, topical amphotericin B, and topical flucytosine. This patient was still symptomatic with yeast seen on wet mount 3 years after her first vulvovaginitis clinic visit and is an unfortunate example of the difficult challenge treatment of these patients may present.

The conclusions that can be drawn from the epidemiologic data from the current study are limited because of the retrospective collection of information and the lack of a control group. Fidel and Sobel presented a complex model for the immunopathogenesis of recurrent candidal vulvovaginitis and concluded recurrent candidal vulvovaginitis is not caused by impaired systemic cell-mediated immunity, but they postulated a host defect in local vaginal mucosa immunity (8). Factors known to impact the vaginal environment and contribute to vulvovaginal candidiasis (antibiotic therapy, diabetes, and hormone therapy) were present in a minority of the patients with multiple positive cultures in the current study, and only two patients had an immunodeficiency diagnosis. A notable portion of patients in the current study had multiple yeast species

isolated from a single culture and changes in species isolated over time. The paucity of predisposing conditions, the diversity among infecting yeast, and the predominance of antifungal-susceptible *C. albicans* isolates support a host defect in vaginal mucosal immunity as a key factor in the pathogenesis of recurrent candidal vulvovaginitis. However, the increased prevalence of non-albicans species (42%) with higher azole MICs among patients with multiple positive cultures in the current study suggests antifungal resistance may also be an important factor for some patients.

The high frequency with which *C. albicans* was recovered in this study and its azole susceptibility support the continued use of azole agents for empirical therapy of uncomplicated candidal vulvovaginitis. Cultures from patients with recurrent candidal vulvovaginitis should be obtained to detect non-albicans species that are less likely to respond to an azole agent. Prospective studies with control groups are needed to determine the optimal therapy for candidal vulvovaginitis caused by non-albicans species.

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