

Prevalence & susceptibility to fluconazole of *Candida* species causing vulvovaginitis

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Background & objectives: Vulvovaginal candidiasis is an important cause of morbidity in women of reproductive age. This study was carried out to determine the species prevalence and susceptibility pattern to fluconazole of yeasts isolated from the vagina of symptomatic women.

Methods: This prospective study was conducted in a rural primary health care center of north India from May 2003 to April 2004 and included 601 married, sexually active women (18-49 yr) with the self reported symptoms of vaginal discharge and/or genital itching and/or genital burning. Specific aetiology of the genitourinary symptoms including candidal infection were determined. Specimens from the lateral wall of vagina were subjected to direct wet mount microscopy and fungal culture on Sabouraud's dextrose agar. Susceptibility testing to fluconazole was carried out using broth microdilution method.

Results: Yeasts were isolated in 111 (18.5%) women and these consisted of *Candida glabrata* (56, 50.4%), *C. albicans* (39, 35.1%), *C. tropicalis* (12, 10.8%), *C. krusei* (3, 2.7%) and *C. parapsilosis* (1, 0.9%). Susceptibility testing carried out on 30 representative isolates (15 *C. glabrata*, 10 *C. albicans*, 4 *C. tropicalis* and 1 *C. parapsilosis*) revealed that 21 isolates (70%) were susceptible (MIC, $\leq 8 \mu\text{g/ml}$) to fluconazole while 9 (30%) were susceptible-dose dependent (S-DD, MIC 16-32 $\mu\text{g/ml}$).

Interpretation & conclusion: Our findings suggest a low prevalence of fluconazole resistance in vaginal candida isolates in our population. However, a high prevalence of non-albicans candida species and increased dose-dependent resistance in these isolates necessitates vigilance since this may warrant a change in the optimal therapy of non-albicans candida vaginitis.

Key words Antifungal susceptibility - *Candida* - candida vaginitis - fluconazole - non-albicans species

Approximately three-quarters of all women experience at least one episode of vulvovaginal candidiasis during their lifetime and nearly half of them suffer from multiple episode¹. In about 5 per cent of cases, the disease has a chronic course, showing frequent

and refractory episodes^{1,2}. 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^{1,3}. Most non-albicans *Candida* species have higher minimum

inhibitory concentrations (MICs) to the azole antifungal agents, and infections they cause are often difficult to treat^{3,4}. This phenomenon emphasizes the importance of identification and surveillance of the *Candida* species in the clinical settings. This study was carried out to determine the species prevalence and fluconazole susceptibility among yeast isolates from women with candidal vulvovaginitis.

Material & Methods

This prospective study was conducted from May 2003 to April 2004 at a rural primary health care center at Ballabgarh, Haryana, a rural field practice area in north India, under the All India Institute of Medical Sciences (AIIMS), New Delhi. It was done as part of a larger study of evaluation of the use of syndromic management of reproductive tract infections. The ethical review committees of AIIMS, New Delhi, Thomas Jefferson University, Global Network for Perinatal and Reproductive Health (GNPRH), USA, and Population Council approved the protocol.

Patient population: The inclusion criteria for the study were married and sexually active women between 18-49 yr of age who presented to the health care center with self reported symptoms of vaginal discharge and/or genital itching and/or genital burning during the study period. Pregnant women, women with severe medical disorders requiring immediate referral to higher level of healthcare, women who were currently menstruating, never been sexually active, who had a hysterectomy, had taken a course of antibiotics within preceding three weeks and who had been previously enrolled in this study were excluded. Written informed consent was obtained from each participant woman.

An attempt was made to determine the specific aetiology of the genitourinary symptoms and determination of candidal infection was one of them. Two sterile, cotton tipped swabs were used to collect specimens from lateral wall of vagina of each woman. One of the two swabs was used to determine the presence of yeast by direct wet-mount microscopy using a drop of 10 per cent potassium hydroxide solution. The other swab was used for fungal culture on Sabouraud's dextrose agar (Hi-Media, Mumbai, India) supplemented with 0.06 µg/ml gentamicin, with and without cycloheximide (0.5%)^{5,6}.

Identification: Species identification of yeast isolates was done by standard procedures including morphology, germ tube test, cornmeal agar test (Hi-Media, Mumbai, India), triphenyl tetrazolium chloride reduction (Hi-

Media Mumbai, India), assimilation of various sugars, and growth in presence of actidione^{5,6}.

Antifungal susceptibility testing: Susceptibility testing to fluconazole was performed using a broth microdilution method (M27-A2) according to the Clinical Laboratory Standards Institute (CLSI) guidelines⁷. The microtitre plates were incubated at 35°C for 24-48 h. The amount of growth in a well containing the antifungal agent was compared with the amount of growth in an antifungal-free growth control well. The minimum inhibitory concentration (MIC) was read as the lowest concentration of antifungal that inhibited 50 per cent growth of the organism detected visually. Quality control was ensured by testing the CLSI recommended quality control strains *Candida parapsilopsis* ATCC 22019 (MIC range 2-8 µg/ml) and *Candida krusei* ATCC 6258 (MIC range 16-64 µg/ml).

Isolates with MIC ≤ 8 µg/ml were considered to be susceptible to fluconazole, whereas isolates with MIC ≥ 64 µg/ml were considered to be resistant⁷. Isolates with MICs between 16-32 µg/ml were fluconazole susceptible-dose dependant (S-DD)⁷.

Results & Discussion

A total of 710 women were screened, of whom only 611 were found eligible for enrollment. Ten women declined internal examination. Thus, a total of 601 women were included in the study and yeasts were isolated in 111 (18.5%) of them. *Candida glabrata* was the most common species among the isolates (56, 50.4%) followed by *C. albicans* (39, 35.1%). Other *Candida* species isolated were *C. tropicalis* (12, 10.8%), *C. krusei* (3, 2.7%) and *C. parapsilosis* (1, 0.9%). Thus, the overall prevalence of non-albicans candida species was 64.8 per cent (72/111). Susceptibility testing to fluconazole was carried out on 30 representative isolates (15 *C. glabrata*, 10 *C. albicans*, 4 *C. tropicalis* and 1 *C. parapsilosis*). Of these, 21 isolates (70%) were susceptible to fluconazole while 9 (30%) were susceptible-dose dependent (Table). Complete resistance was not detected in any of the isolates tested. Of the 9 isolates with elevated MICs, 7 were *C. glabrata*, 1 was *C. tropicalis* and 1 was *C. albicans*.

In this study, the overall prevalence of vulvovaginal candidiasis in a community setting was found to be 18.5 per cent which is similar to studies from India^{8,9} and elsewhere^{10,11} with rates ranging from 20.8 - 23 per cent. However, the overall percentage of non-albicans vaginitis (64.8%) was much higher than in previous reports^{3,4,10,11}. In a study conducted on 1498 women who

Table. Susceptibility to fluconazole of vaginal *Candida* isolates

MIC (µg/ml)	Number of isolates (%)				Total
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>C. parapsilosis</i>	
0.25	1 (10)	0	1 (25)	0	2 (6.7)
0.5	4 (40)	0	0	0	4 (13.3)
1	2 (20)	2 (13.3)	0	0	4 (13.3)
2	1 (10)	0	1 (25)	1 (100)	3 (10)
4	0	3 (20)	0	0	3 (10)
8	1 (10)	3 (20)	1 (25)	0	5 (16.7)
16	1 (10)	2 (13.3)	0	0	3 (10)
32	0	5 (33.3)	1 (25)	0	6 (20)
Total	10	15	4	1	30

MIC, minimum inhibitory concentration

attended the Edinburgh Family Planning Centre over a 18 month period¹⁰, fungi other than *C. albicans* comprised 27.7 per cent of all fungal species obtained. An Australian study found vulvovaginal yeast carriage among 21 per cent of 5,802 women in a primary care setting; non-albicans species were isolated in only 11 per cent of the positive cultures¹¹. Spinillo *et al*³ reported that 17 per cent of 209 isolates from symptomatic patients referred to an Italian vulvovaginitis clinic were non-albicans species. Richter *et al*⁴ reported an overall percentage of 24 per cent of non-albicans vaginitis among 564 vaginal yeast cultures collected from 429 symptomatic candidal vulvovaginitis patients.

Antifungal susceptibility testing in our study revealed that none of the *Candida* isolates tested were resistant to fluconazole, though 9 of 30 (30%) isolates were fluconazole S-DD and maximum number of these S-DD isolates were *C. glabrata*. However, we could not perform susceptibility testing for *C. krusei* which is intrinsically resistant to fluconazole. Fluconazole resistance in vaginal *C. albicans* isolates is an uncommon occurrence¹²⁻¹⁴. A recent US study found no fluconazole resistance among 401 *C. albicans* isolates recovered from women with recurrent vulvovaginitis¹². No fluconazole resistance was identified among 75 *C. albicans* vaginal isolates from symptomatic women in England¹³. A study in Brazil also reported no fluconazole resistance among 56 *C. albicans* vaginal isolates¹⁴. However, in another US study, fluconazole resistance has been reported in 14 of 393 (3.6 %) *C. albicans* isolates collected from complicated vaginitis patients prior to 2001¹⁵. As regards the antifungal susceptibility of non-albicans *Candida* species in a study by Richter *et al*⁴, a large proportion (67%) of the *C. glabrata* vaginal isolates was non-susceptible 51.8% susceptible-dose dependent, 15.2% resistant).

Thus in recent years, there has been a significant increase in infections caused by non-albicans species of *Candida*, particularly, *C. glabrata* and *C. tropicalis*. We speculate this increasing detection of non-albicans *Candida* species is probably related to the widespread and inappropriate use of antimycotic treatments (self-medication, long-term maintenance treatments and repeated treatments for candidosis episodes)¹. *C. albicans* eradication by these means causes a selection of species (such as *C. glabrata*) that are resistant to commonly used agents¹.

In conclusion, our study provides information on antifungal susceptibility of vaginal yeast isolates in a rural community in India. Since the majority of *C. albicans* isolates were susceptible to fluconazole, its use may be continued for empirical therapy of uncomplicated candidal vulvovaginitis in the community. Use of alternative agents (like boric acid, flucytosine)¹⁶ may be considered when treating vulvovaginitis caused by non-albicans species (especially *C. glabrata* and *C. krusei*). As only a limited number of *Candida* isolates could be tested in this study, further clinical studies need to be performed involving more number of isolates to confirm the findings.

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