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IN VITRO FLUCONAZOLE SUSCEPTIBILITY OF *CANDIDA* SPECIES ISOLATED FROM VULVOVAGINAL CANDIDIASIS".

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ABSTRACT **Introduction-**Vaginal candidiasis is a common problem and increasing disease in women**.** The purpose of this study was to determine the species distribution and fluconazole susceptibility of *Candida* species isolated from patients with vulvovaginal candidiasis by disk diffusion method. The non albicans *Candida* species exhibit variable susceptibilities to both new and established antifungal agents; this has made the need for prompt identification of these non albicans *Candida*.

Methods: Two vaginal swabs from each patient were processed by microscopy and culture to know the prevalence of candidial vulvovaginitis and to study their fluconazole susceptibility pattern by disk diffusion method.

Results: The most common risk factor associated with vulvovaginal candidiasis was pregnancy and the commonest species isolated was *C.albicans.* In nonalbicans candida,*C.glabrata*.was more common.By disk diffusion method 82.71% of *Candida* isolates were susceptible to fluconazole. Specieswisesusceptibility to fluconazole was *C.albicans* 72(93.51%), *C.glabrata*28(65.12%) and *C.tropicalis*10(90.91%). All (100%) *C.krusei* strains were resistant to fluconazole by disk diffusion method. The difference in susceptibility of*C.albicans* and *nonalbicans candida*to fluconazole is statistically significant (p=0.000, highly significant).

Conclusions: There is a significant increase in prevalence of infection due to *Candida* non-albicans species, particularly*, C.glabrata* and *C.tropicalis*. The presence of resistance to fluconazole among the different species of *Candida*nonalbicans emphasizes the need for early isolation, speciation and routine susceptibility testing of fungi in all mycology laboratories to control the further spread of resistance.

KEYWORDS : Antifungal susceptibility test, Disk diffusion method, Vulvovaginitis.

Key Message: The successful treatment of vulvovaginal candidiasis depends on the early identification of the species and sensitivity patterns to antifungal agents. The high growing rate of non albicans *Candida* resistant to azole confirms the importance of monitoring changes in the distribution of pathogenic *Candida* species

Introduction

Vaginal candidiasis, caused by opportunist yeast, *Candida* is a common problem and increasing disease in women.[1]

In contrast to asymptomatic colonization, VVC (Vulvovaginal candidiasis) is defined as signs and symptoms of inflammation in the presence of *Candida* species and in the absence of other infectious etiology.[2] Compared to past when most cases were caused by *C.albicans* an increasing percentage at present is caused by non-albicans *Candida* species.[3]

The disease is characterized by the presence of a thick yellow, milky discharge.[4, 5]Possible risk factors causing an increase in *Candida* infections include prior antibiotic therapy, pregnancy, diabetes mellitus (DM), oral contraceptives containing estrogen and progestin, and immunosuppressed patients (transplanted patients, cancer patients treated with chemotherapy, and HIV patients etc).[1]

Although antifungal susceptibility testing remains less well developed and utilized than antibacterial testing the specific support for its validity has benefited greatly by extrapolation from antibacterial testing. [6] Agar-based techniques have been used extensively by a few laboratories because they are simple, economical, and easy to perform simultaneously on large numbers of organisms. Despite many problems, it has been possible to develop routine testing methods that use an agar diffusion format. [7]

With this background we designed the present study to determine fluconazole susceptibility of *Candida* isolates from vulvovaginal candidiasis using disk diffusion method.

Material & Methods

The prospective study was carried out in Department of Microbiology, at Tertiary Care Hospital. Two vaginal swabs were collected from married and sexually active women between 18-45 years of age group with symptoms of vaginal discharge, genital itching/genital burning.

A total of 300 suspected cases of vulvovaginitis were studied.133 *Candida* strains were isolated which were positive by both microscopic examination and culture. These candidial strains were processed for further identification.Identification of *Candida* species was done by the following method-

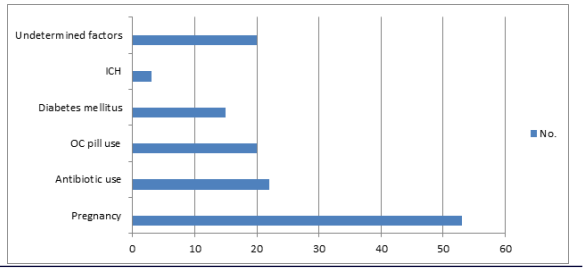
- Fungal culture- on Sabouraud's dextrose agar with antibiotics andgerm tube test
- Culture on cornmeal agar
- Culture on CHROM agar
- Sugar fermentation test
- Sugar assimilation test

After confirmation of *Candida* species all strains were subjected to fluconazole susceptibility test by disk diffusion method[8]. The flucanozole -25µg disks were obtained from Hi Media, Mumbai, India. To control the precision and accuracy of the results obtained with disk diffusion test procedure, following quality control strains were used-*Candidaalbicans* ATCC90028, *Candidaparapsilosis* ATCC22019, *Candidatropicalis* ATCC750 and *Candidakrusei* ATCC6258.

Results-

Out of 300 suspected cases of vulvovaginitis, only *Candida* as a cause of vulvovaginitis was present in 133(44.33%) cases.

Figure 1: Risk factors associated with vulvovaginal candidiasis



(OC-oral contraceptives, ICH-immunocompromised host)

Figure 1 shows risk factors associated with vulvovaginal candiidiasis found in present study. Out of total 133 candidal vulvovaginitis cases, 53(39.85%) were pregnant. The second common risk factor associated with candidal vulvovaginitis was history of antibiotic use 22(16.53%), followed by oral contraceptive pill use 20(15.04%), diabetes mellitus 15(11.28%), immunocompromised host 3(2.26%) and undetermined factors 20(15.04%). Thus, most common risk factor associated with vulvovaginal candidiasis in our study was pregnancy.

Figure 2: Species distribution of *Candida* species isolated from cases ofvulvovaginitis

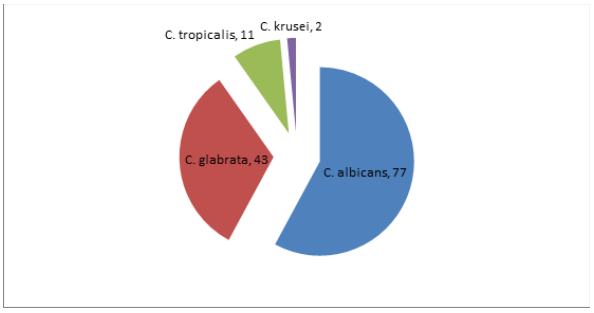


Figure 2 shows that out of 133 *Candida* species isolated from cases of vulvovaginitis, 77(57.89%) were *C.albicans*, 43(32.34%) were *C.glabrata*, 11(8.27%) were *C.tropicalis* and 2(1.50%) were *C.krusei*.

Antifungal susceptibility test of all 133 *Candida* isolates was done by disk diffusion method[8]. Of total 133 *Candida* isolates, 110(82.71%) were susceptible and 23(17.29%) were resistant to fluconazole.

Table 1: Susceptibility of different *Candida* species to fluconazole by disk diffusion method

Sr. No.	Species	Susceptible	Resistant	Total
		Number (%)	Number (%)	
1	*Candida albicans*	72(93.51%)	5(6.49%)	77
2	*Candida glabrata*	28(65.12%)	15(34.88%)	43
3	*Candida tropicalis*	10(90.91%)	1(9.09%)	11
4	*Candida krusei*	0	2(100%)	2
Total		110	23	133

Table 1 shows that out of 77 *C.albicans,* 72(93.51%) were susceptible and 5(6.49%) were resistant to fluconazole. Out of total 43 *C.glabrata,* 28(65.12%) were susceptible and 15(34.88%) were resistant to fluconazole. Of a total of 11 *C.tropicalis*, 10(90.91%) were susceptible and 1(9.09%) was resistant to fluconazole. All (100%) *C.krusei* was resistant to fluconazole.

Table 2: Comparison of antifungal susceptibility of *C.albicans* and *C.nonalbicans* to fluconazole by disk diffusion method

Sr No.	Sensitivity pattern	*C.albicans*	*C.nonalbicans*
1	Susceptible	72(93.51%)	38(67.86%)
2	Resistant	5(6.49%)	18(32.14%)
	Total	77	56

(p=0.000, Highly significant)

Table 2 shows that by disk diffusion method out of 77 *C.albicans,* 72(93.51%) were susceptible to fluconazole whereas out of 56 *Candida nonalbicans,* only 38(67.86%) were susceptible to fluconazole. The difference in susceptibility of *C.albicans* and *Candida nonalbicans* to fluconazole is highly significant (p=0.000, Chi-square test) statistically.

Discussion

Most common predisposing factor associated with vulvovaginal candidiasis in present study was pregnancy accounting for 39.85% of vulvovaginal candidiasis which was in agreement with those of the studies of Nwadioha et al [9] and Bankar et al [10]. It is reported that increase in occurrence of vaginal candidiasis during pregnancy is due to increased levels of hormones such as estrogen and steroid hormones. *Candida* infection in pregnancy does not usually harm the unborn child but causes great discomfort to the mother, which includes increased discharge, redness, itching and burning sensation in the vulva area. If the disease is not treated the baby can get infected (oral thrush) at birth which can be a very serious health problem in premature babies. Also, untreated vaginal infections can lead to pelvic inflammatory disease, a condition which can scar the fallopian tube and cause infertility.[11] The next major predisposing factor was antibiotic use which accounted for 16.53% of cases (Figure 1). The use of vaginal or systemic antibiotics is found to increase the vaginal colonization rate by 10-30 %.[10] Antibiotic and vaginal douching suppresses normal bacterial flora and allow *Candida* organism to proliferate. It is commonly hypothesized that the reduction of lactobacilli in vaginal tract predisposes women to vulvovaginal candidiasis. Lactobacilli play a key role in the vaginal flora through the production of hydrogen peroxide, bacteriocin and lactic acid which protect against invasion or overgrowth of pathogenic species.[2]The present finding of association of antibiotic use with vulvovaginal candidiasis was in agreement with those of Nwadioha et al[9], Bankar et al [10] and Dressen et al [12].

In this study, we reported association of oral contraceptive with vulvovaginal candidiasis in 15.04% of women (Figure 1). High estrogen in oral contraceptive may be one of the predisposing factors as estrogen facilitates the adherence of yeast to the vaginal epithelium.[10] Our observation of association of oral contraceptive as predisposing factor with vulvovaginal candidiasis was in agreement with those of the findings of Nwadioha et al [9] and Bankar et al [10]. However in contrast, Dressen et al [12] reported most frequent predisposing factor for the vulvovaginal candidiasis as hormonal contraception in their study.

In present study, 11.28% women had diabetes mellitus, 2.26% women were immunocompromised and in 15.04% women the factors were undetermined (Figure 1).

Systemic condition such as diabetes mellitus, HIV/AIDS, organ transplant and any chronic debilitating illness can increase the women's chances of developing vulvovaginal candidiasis. Depressed cell mediated immunity provides favorable conditions for growth of *Candida* species such as HIV / AIDS, whereas dysfunction of neutrophils and monocytes favours candidal growth in diabetes mellitus .[9] Genetic factors and exposure of vaginal milieu to allergens and chemicals may act as predisposing factors. Increased prevalence of vulvovaginal candidiasis in African-American women and people with blood group ABO-Lewis non secretor phenotype suggest that there could be genetic factors that predispose individuals to colonization or vaginitis.[9,10] Chemical contact, atopy, local allergy or hypersensitivity reactions could alter the vaginal milieu and facilitates transformation from asymptomatic colonization to symptomatic vaginitis.[10]

In our study, *C.albicans* (57.89%) was the commonest species isolated followed by *C.glabrata* (32.34%), *C.tropicalis* (8.27%) and *C.krusei* (1.50%) (Figure 2). Incidence of *C.albicans* varies from 43.1% to 87.5 % from cases of vulvovaginitis.[13] *C.glabrata* was the most commonly isolated non-albicans species reported by various authors. Sobel et al indicated that the ability of non-albicans species to cause infections is enhanced by a number of risk factors. The factors include the uncontrolled antifungal agents' use, and incomplete and prolonged use of antifungal for the prevention of *Candida* infections.[14]

In our study, 110 (82.71%) *Candida* isolates were reported susceptible and 23(17.29%) were reported resistant to fluconazole by disk diffusion method. Kikani et al [13] reported that about 91.8% of *Candida* isolated from vaginal candidiasis was susceptible to fluconazole by disk diffusion method and this finding is also near to the present finding.

In present study, out of 77 *C.albicans* isolates, 72(93.51%) were susceptible and 5(6.49%) were resistant to fluconazole by disk diffusion method (Table 1). The susceptibility pattern of *C.albicans* for fluconazole by disk diffusion method in our study was in agreement with those of the studies of Meis et al [15], Colombo et al [16], and Pfaller et al [17]. However, Capoor et al [18] reported lower susceptibility rate (78.2%) of *C.albicans,* isolated from different clinical samples, to fluconazole than present study by disk diffusion method.

Out of 43 *C.glabrata* isolates, 28(65.12%) were susceptible and 15(34.88%) were resistant to fluconazole by disk diffusion method in present study (Table 1). The susceptibility pattern of *C.glabrata* to fluconazole in our study was in agreement with those of the studies of Meis et al [15]and Pfaller et al[17]. However, Colombo et al [16], Capoor et al [18] and Zomorodian et al [19] reported higher rate of susceptibility of *C.glabrata* to fluconazole-84%, 100% and 90.5% respectively.

In present study, out of 11 *C.tropicalis* isolates, 10(90.09%) were susceptible and 1(9.09%) was resistant to fluconazole by disk diffusion method (Table 1). The present finding is also close to the findings of Colombo et al [16], Capoor et al [18] and Zomorodian et al [19]who reported susceptibility rate of *C.tropicalis* to fluconazole as 97.8%, 85.5% and 96% respectively

All (100%) *C.krusei* isolates were resistant to fluconazole by disk diffusion method in our study (Table 1). The resistance pattern of *C.krusei* to fluconazole was in agreement with those of the studies of Colombo et al [16], Capoor et al[18] and Zomorodian et al [19] who also reported all (100%) *C.krusei* in their studies as resistant to fluconazole.

The difference in susceptibility of *C.albicans* and *C.nonalbicans* to fluconazole is highly significant (p=0.000, Chi-square test) statistically(Table 2).

Conclusion

This study was carried out to determine the species distribution and fluconazole susceptibility of *Candida* species isolated from women with candidial vulvovaginitis. The difference in susceptibility pattern of *C.albicans* and *C.nonalbicans* to fluconazole is statistically significant (p=0.000, highly significant) (Table 2).Thus, high degree of resistance to fluconazole is found in nonalbicans group than in *C.albicans.* These in vitro results supports the use of alternative agents when treating vulvovaginitis caused by non-albicans species especially *C.glabrata*. Currently, in vitro antifungal susceptibility tests can allow very important guidelines for candidiasis treatment, but the standard susceptibility test (Microbroth dilution method) is not always readily available in regular laboratories and is very time consuming in opposition to the other more simple techniques such as disk diffusion. Increased use of over-the-counter antifungals and prolonged therapy for recurrent candidiasis are risk factors for the emergence of azole resistance among *Candida* species isolated from vulvovaginitis patients. This phenomenon emphasizes the importance of identification and surveillance of the *Candida* species in these cases.

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