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Research Article

Fluconazole susceptibility of *Candida* species isolated from cases of Vulvovaginal Candidiasis by using E-test method

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ABSTRACT

Candida vaginitis is one of the most frequently encountered forms of superficial candidiasis. The principal agent of vulvovaginal candidiasis is *C.albicans*, but other species known generally as *C.nonalbicans* (*C.glabrata, C.tropicalis, C.krusei, C.parapsilosis, C.lusitaniae*) are also isolated. The available therapeutic arsenal for treatment of vulvovaginal candidiasis offer few options and includes the imidazole and trizole agents, in topical and oral form. The E-test has been introduced as an easier testing procedure and an alternative for the NCCLS method. The present study determines the distribution of *Candida* species and in vitro susceptibilities of fluconazole against the *Candida* isolated from vulvovaginitis candidiasis using E-test. Methods: Two vaginal swabs from each patient were processed by microscopy and culture to know the prevalence of candidialvulvovaginitis and to study their antifungal susceptibility pattern of fluconazole by E-test. Results: Out of 300 suspected cases of vulvovaginitis, only *Candida* as a cause of vulvovaginitis was present in 133(44.33%) cases. The most common age group associated with vulvovaginal candidiasis was 20-29 years (43.61%) followed by 30-39 years (30.83%) and greater than 40 years (25.56%). The most common species isolated was *C.albicans* followed by *C.glabrata*. By E-test method 92.21% of *C.albicans*, 60.46% of *C.glabrata* and all (100%) *C.tropicalis* was susceptible, that is, showing MIC of fluconazole less than or equal to 8µg/ml and all 100% *C.krusei* was resistant (MIC≥64µg/ml) to fluconazole. Conclusion: For effective treatment of the infection, to prescribe the correct medication and to overcome recurrence, it may be advisable to identify the *Candida* species routinely from vaginal swabs of infected women. Also, the high growing rate of resistance of non-albicans*Candida*to azoles confirms the importance of monitoring changes in the distribution of pathogenic *Candida* species. The major feature of E-test method is that it can allow a quick answer concerning *Candida* resistance to antifungal agents, preventing unnecessary patients' drug abuse.

Keywords:E- test, Fluconazole, Vulvovaginitis.

Introduction

Candida vaginitis is one of the most frequently encountered forms of superficial candidiasis.[1]The vaginal carriage rate of asymotomatic non-pregnant women is around 20%. Compared to past when most cases were caused by *C.albicans* an increasing percentage at present is caused by non-albicans*Candida* species.[2] Approximately 75% of women experience vulvovaginal candidiasis during their life, and about 40% to 50% of them suffer from multiple episodes.[3-6]The principal agent of vulvovaginal candidiasis is *C.albicans*, but other species known generally as *C.nonalbicans (C.glabrata, C.tropicalis, C.krusei, C.parapsilosis, C.lusitaniae)* are also isolated, as well as, yeasts belonging to other genera such as *Saccharomyces cerevisiae, Rhodotorula*species.*C.glabrata* is the second most common yeast and its treatment is considered serious clinical challenge. The available therapeutic arsenal for treatment of vulvovaginal candidiasis offer few options and includes the imidazole and trizole agents, in topical and oral form. The azoles are the treatment of choice for vulvovaginal candidiasis in many countries;

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however the development of resistance to these drugs has been reported in yeasts isolated from vulvovaginal candidiasis, especially *C.nonalbicans* which appears to be less sensitive to azoles.[6]

The Clinical and Laboratorial Standards Institute (CLSI) approved a reference method for antifungal susceptibility testing of yeasts, the National Committee for Clinical and Laboratorial Standards (NCCLS) M-27 A2 document. The E-test has been introduced as an easier testing procedure and an alternative for the NCCLS method. The great advantage of E-test is the simplicity of the methodology. However, not all antifungal agents are available in E-test and there is a difficulty associated with endpoint interpretation, due to the growth of micro-colonies in the inhibition zone, leading to lower reproducibility when the test is performed by several technicians.[7] However, with experience and standardized techniques, the correlation between this method and the reference method has been acceptable for most *Candida* species and azoles antifungal agents.[8]

With this background we designed the present study todetermine the distribution of *Candida* species and in vitro susceptibilities of fluconazole against the *Candida* isolated from vulvovaginal candidiasis patients using E-test.

MATERIALS AND 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
- Sugar fermentation test
- Sugar assimilation test

After confirmation of *Candida* species all strains were subjected to fluconazole susceptibility test by E-test method[3]. The E-test strips were obtained from HI Media, Mumbai, India. To control the precision and accuracy of the results obtained with E-test, following quality control strains were used- *Candidaalbicans*ATCC90028, *Candidaparapsilosis* - ATCC22019, *Candidatropicalis*ATCC750 and *Candidakrusei*ATCC6258.

RESULTS

Table 1: Incidence of vulvovaginal candidiasis

Sr. No.	Etiology	Number (Percentage)
1	Vulvovaginal candidiasis	133(44.33%)
2	Vulvovaginal candidiasis associated with other STI	10(3.33%)
3	Conditions other than vulvovaginal candidiasis	157(52.34%)
	Total	300(100%)

(STI=sexually transmitted infection)

Table 1 shows the incidence of vulvovaginal candidiasis in the present study. Out of 300 suspected cases of vulvovaginitis, only *Candida* as a cause of vulvovaginitis was present in 133(44.33%) cases.

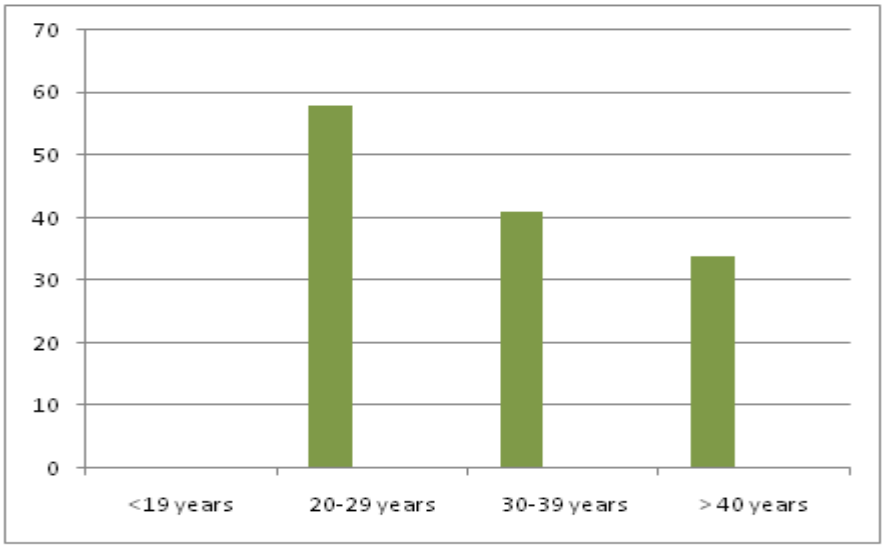

Figure 1: Age wise distribution of cases of vulvovaginalcandidiasis

Fig. 1 shows age wise distribution of cases of vulvovaginal candidiasis. Out of 133 cases, 58(43.61%) were from age group 20-29 years, 41(30.83%) belonged to age group 30-39 years and 34(25.56%) belonged to age group greater than 40 years. No case of *Candida* vulvovaginitis was seen in age group less than 19 years.

Table 2: Species distribution of *Candida* species isolated from cases of vulvovaginitis

Sr. No.	Species	No.	Percentage
1	*C. albicans*	77	57.89%
2	*C. glabrata*	43	32.34%
3	*C. tropicalis*	11	8.27%
4	*C. krusei*	2	1.50%
Total		133	100%

Table 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*. Thus, the most common species isolated from cases of vulvovaginal candidiasis was *C.albicans* followed by *C.glabrata*.

Table 3: Minimum inhibitory concentration (MIC) of fluconazole for *Candida* by E-test method

Sr. No.	Sensitivity pattern	MIC range	Number	Percentage
1	Susceptible	Less than or equal to 8µg/ml	108	81.20%
2	Susceptible dose dependent	16-32µg/ml	9	6.77%
3	Resistant	Greater than or equal to 64µg/ml	16	12.03%
	Total		133	100%

Table 3 shows that out of total 133 *Candida* isolated from vulvovaginitis cases, 108 (81.20%) were showing MIC of fluconazole less than or equal to 8µg/ml (susceptible), 9(6.77%) were showing MIC between16-32µg/ml (susceptible dose dependent) and 16(12.03%) were showing MIC greater than or equal to 64µg/ml (resistant) by E-test method.

Table 4: Minimum inhibitory concentration (MIC) of fluconazole for different *Candida* species by E-test method

Sr. No.	Species	Susceptible	Susceptible Dose Dependant	Resistant	
		Number (%)	Number (%)	Number (%)	Total
1	*Candida albicans*	71(92.21%)		6(7.79%)	77
2	*Candida glabrata*	26(60.46%)	9(20.93%)	8(18.61%)	43
3	*Candida tropicalis*	11(100%)			11
4	*Candida krusei*			2(100%)	2
Total		108	9	16	133

Table 4 shows that out of 77 *C.albicans*, 71(92.21%) were susceptible (MIC≤8µg/ml) and 6(7.79%) were resistant (MIC ≥ 64µg/ml) to fluconazole by Etest method. Of a total 43 *C.glabrata,* 26(60.46%) were susceptible (MIC≤8µ/ml), 9(20.93%) were susceptible dose dependent (MIC=16-32µg/ml) and 8(18.61%) were resistant (MIC≥64µg/ml) to fluconazole by E-test method. All (100%) *C.tropicalis* were susceptible (MIC≤8µg/ml) and all (100%) *C.krusei* was resistant (MIC≥64µg/ml) to fluconazole by E-test method.

DISCUSSION

In present study, out of 300 suspected cases of vulvovaginitis, 133 (44.33%) had vulvovaginal candidiasis which correlated with the studies of Raoet al[9] and Khan et al[5] who reported 40% and 38.02% vulvovaginal candidiasis. In present study, the peak incidence of vulvovaginal candidiasis was in 20-29 years age group which corresponds to 43.61%, followed by 30-39 years age group (30.83%), followed by >40 years of age group(25.56%) (Fig 1).The age decade 21-30 years is the most sexually active age group with highest risk of pregnancy, indulgence in family planning pills and immunosuppression due to HIV/AIDS.[10] Ovarian activity as well as sexual activity is maximum in women of 20-30 years age. During this period, the ovary produces adequate amount of estrogen, which favours the *Candida* growth by maintaining the acidic pH and enhancing the yeast adherence to vaginal epithelial cells.[11]Finding of present study that the peak incidence of vulvovaginal candidiasis was in age group 20-29 years (43.61%) correlated with those of the studies ofOviasogie et al (2009)[12]. Present study reports *C.albicans* (57.89%) was the commonest species isolated followed by *C.glabrata*(32.34%), *C.tropicalis*(8.27%) and *C.krusei*(1.50%) (Table2). The present finding correlated with those of the studies of Asticcioliet al[13]and Mahmoudi Rad et al[14] in their studies *C.albicans* accounted for 61.2% and 65.1% respectively. However Holland etal[15] reportedhigher rate of isolation of *C.albicans* -89%, and 80.95% respectively but Mohantyet al[4] reported *C.glabrata* (50.4%) as the most common species followed by *C.albicans*(35.1%).

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 as incomplete or prolonged use for prevention of *Candida*infections [16].

In the present study, *C.glabrata* accounted for 32.34% of total *Candida* species isolated (Table 2) which correlated with the studies of Vergheseet al [17](38.09%) and Asticcioliet al[13](28.8%). However, Holland et *a*l[15]&Yusuf et al[18] reported lower isolation rate of*C.glabratai.e*.7.28% & 16.9%.

Present study reported*C.tropicalis*in8.27% which correlated with Mohantyetal[4] (10.8%) while Vergheseet al[17]&Khan et al[5]reported higher isolation rate of*C.tropicalis*(14.28% and 21% respectively).

Isolation rate of *C.krusei*(1.50%) of total *Candida*species (Table 2) correlated with studies of Mohantyet al[4] and Asticcioliet al[13] (2.7% and 1.8% respectively).

Out of 133 *Candida* isolates, 108(81.20%) were susceptible to fluconazole by E-test method, that

is, showing MIC of fluconazole less than or equal to 8µg/ml in our study (Table 3) which is near to the finding of Madhavan et al (2010)[19] who reported that 71% of *Candida* isolated from different clinical isolates as susceptible to fluconazole by E-test.In contrast, Tseng et al (2005)[3] and Ooga et al (2011)[20] reported lower susceptibility rate of *Candida* to fluconazole by E-test-63.4% and 56% respectively. Also the susceptibility pattern of *Candida* to fluconazole by E-test method in present study correlated with the susceptibility pattern of *Candida* to fluconazole by microbroth dilution method of Dota et al (2011)[6]who reported that about 83.9% of vaginal *Candida* isolates as susceptible to fluconazole**.** In present study, out of 77 *C.albicans* isolates, 71(92.21%) were susceptible (MIC≤8µg/ml) to fluconazole by E-test method (Table 4) which correlated with those of the findings of Badieeetal (2011)[21]whoreported about 89.5% of *C.albicans* as susceptible to fluconazole by E-test method. This finding is also close to the findings ofTseng et al (2005)[3] andMadhavan et al (2010)[19]. Tseng et al (2005)[3] reported that 80.9% of vaginal *C.albicans*as susceptible to fluconazole by E-test method.Madhavan et al (2010)[19] reported 100% of *C.albicans* isolated from different clinical samples as susceptible to fluconazole by E-test.

The susceptibility rate of*C.glabrata* in present study to fluconazole by E-test method is 60.46% which is in contrast to the findingofMadhavan et al (2010)[19]whoreported lower susceptibility rate (33%) of*C.glabrata* isolated from different clinical samples to fluconazole by E-test method. In present study 20.93% of*C.glabrata* was susceptible dose dependent to fluconazole by E-test method (Table 4) which is close to the finding of Tseng et al (2005)[3]who reported 32% of vaginal *C.glabrata* isolates as susceptible dose dependent to fluconazole by E-test method. 18.61% of *C.glabrata* was resistant to fluconazole by E-test method in present study (Table 4). This finding is in contrast to the finding of Tseng et al (2005)[3] who reported higher resistant rate of vaginal *C.glabrata* (64%) to fluconazole by E-test method. However the resistance rate of *C.glabrata* to fluconazole by E-test method in present study correlated with the resistance rate of *C.glabrata* to fluconazole by microbroth dilution method of Richter et al (2005)[22]who reported that about 15.2% of vaginal *C.glabrata* isolates as resistant to fluconazole**.**

In present study, all (100%) *C.tropicalis* were susceptible to fluconazole by E-test method, that is, showing MIC of fluconazole less than or equal to 8µg/ml (Table 4) which correlated with those of the studies of Tseng et al (2005)[3]and Madhvan et al (2010)[19]who reported 100% of *C.tropicalis* isolated in their studies as susceptible to fluconazole by E-test.

All(100%) of *C.krusei* were resistant (MIC≥64µg/ml) to fluconazole by E-test method in present study (Table 4) which correlated with the study of Madhavan et al (2010)[19]who also reported 100% of *C. krusei* in their study as resistant to fluconazole.

CONCLUSION

The candidiasis is the most common condition associated with vulvovaginitis in this study. Vulvovaginal candidiasis (VVC) cannot be definitely identified by clinical criteria alone. It requires culture for *Candida* species and its correlation to vulvovaginal symptoms. Culture is valuable not only for the accurate diagnosis of VVC but also to avoid indiscriminate use of antifungal agents, which may ultimately decrease the incidence of VVC caused by resistant and non-albicans*Candida* species. This study has demonstrated the need to emphasize the importance of accurate diagnosis in patients presenting with common complaint of vulvovaginitis. This study provides information on fluconazole susceptibility of vaginal *Candida* isolates in our area. As majority of *Candida* isolated from vulvovaginal candidiasis remains *C.albicans* as reported by various studies, which shows higher susceptibility to fluconazole, so its use has been continued for empirical therapy of uncomplicated *Candida*vulvovaginitis in the community. This may have resulted in colonization and subsequent infection of female vaginal tract with fluconazole resistant *Candida* species especially *C.glabrata*resulting in complicated vulvovaginal candidiasis. Therefore knowledge of local antifungal susceptibility pattern, in conjunction with knowledge of local prevalence or identification of more resistant species can greatly aid in the selection of effective antifungal agents for empirical use.

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