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### SPECIES DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PROFILE IN VAGINAL CANDIDIASIS

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**Abstract:** Vulvovaginal candidiasis is an important cause of morbidity in women of reproductive age. **AIM:** This study was carried out to determine the frequency of vaginal candidiasis and the susceptibility profile of the *Candida* species to antifungal agents. **MATERIALS AND METHODS:** The study was carried out prospectively in S.V Medical College over a period of one year. A total of 200 vaginal swabs were collected from women between the age group of 20-40 years with self reported symptoms of vaginal discharge and subjected to microscopy and fungal culture. Antifungal susceptibility was performed by disk diffusion method. **RESULTS:** Among 119 (59.5%) positive cultures, *Candida albicans* was the predominant species (n=57, 48%). The following non-albicans species were isolated – *Candida tropicalis* (26%), *Candida glabrata* (22%), *Candida parapsilosis* (1-7%), *Candida kefyr* (1-7 %) and *Candida krusei* (0-8%). 100% sensitivity was seen to amphotericin-B. Isolates of *Candida albicans* were susceptible to the azoles tested. Among the non-albicans, resistance to azole antifungal agents was encountered in strains of *Candida glabrata* and *Candida tropicalis*. 5 isolates (19%) of *Candida glabrata* were resistant to fluconazole and ketoconazole and 2 (7.6%) isolates were resistant to itraconazole whereas 1(3.2%) isolate of *Candida tropicalis* is resistant to itraconazole and ketoconazole. **CONCLUSION:** The frequency with which non-albicans *Candida* species were isolated and their reduced susceptibility to the azole agents emphasizes the need for species identification and antifungal susceptibility as a part of laboratory diagnosis of vaginal candidiasis.

**Keywords:** Vulvovaginal candidiasis, leucorrhoea, *Candida* species, Antifungal susceptibility



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## INTRODUCTION

Candidal vulvovaginitis is a common female infection, primarily during the fecund period. It is the most frequent vaginal infection, depending on geographical area<sup>1</sup>. An estimated 75% of women experience at least one episode of vulvovaginal candidiasis during their lifetimes, with some experiencing two or more episodes<sup>2</sup>. *Candida albicans* is the commonest cause of vaginal candidiasis; however, episodes due to non-*albicans* species of *Candida* appear to be increasing these non-*albicans* *Candida* species have higher minimum inhibitory concentrations to the azole antifungal agents, and infections they cause are refractory to treatment. This phenomenon emphasizes the importance of identification, surveillance and antifungal susceptibility of the *Candida* species in the clinical settings<sup>3</sup>.

Various authors have studied many aspects of the candidial vulvovaginitis and their response to antifungal agents, there by the need to evaluate the incidence of Candidal infections and their response to antifungal agents and to establish its properties is necessary.

Hence the present study was undertaken to determine the species prevalence and antifungal susceptibility among yeast isolates from women with candidial vulvovaginitis. Such a study was considered might be helpful in elucidating the role of this organism in the causation of vaginitis and its effect on the patient and society in large, especially in this era of antibiotics and immunodeficiency.

## MATERIALS AND METHODS

This prospective study was conducted over a period of one year in Microbiology department in S.V Medical College Tirupati. A total of 200 married sexually active non-pregnant women between the age group of 20-40 years with the self reported symptoms of vaginal discharge and other complaints of vulvovaginitis attending outpatient clinic of Government Maternity hospital, Tirupati were included in this study.

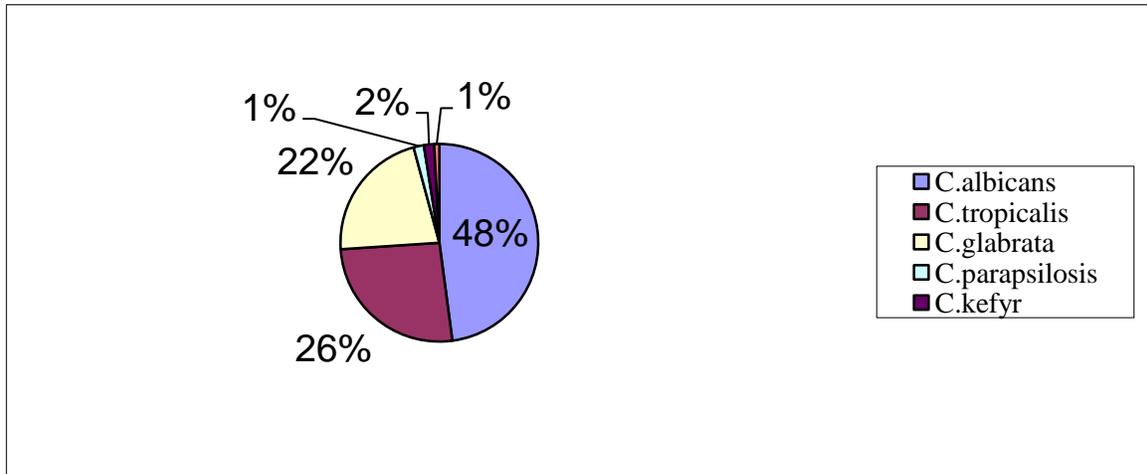
Vaginal swabs were collected and subjected to microscopy and fungal culture on Sabouraud's dextrose agar. *Candida* species were identified by germ tube test<sup>4</sup>, characteristic morphology on corn meal agar<sup>5</sup>, carbohydrate fermentation<sup>4</sup> and carbohydrate assimilation tests<sup>5</sup>. Antifungal susceptibility was performed by disk diffusion method and response of the *Candida* strains to amphotericin-B, fluconazole, itraconazole and ketoconazole was evaluated and compared with the standard strain (*Candida parapsilosis* 22019)<sup>6</sup>.

## RESULTS

The present study included 200 female patients who presented with genital manifestations, suspicious of candidial infections. The study revealed an overall isolation rate of (119/200) candidiasis from vaginal swabs. All the 119 positive isolates of *Candida* species were processed for speciation and Antifungal sensitivity testing.

In the present study six species of *Candida* were characterized. *Candida albicans* was the predominant species (48%). The following non-albicans species were isolated – *Candida tropicalis* (26%), *Candida glabrata* (22%), *Candida parapsilosis* (1.7%), *Candida kefyr* (1.7 %) and *Candida krusei* (0.8%). The overall isolation of non- albicans species was 52% (Figure -1).

**Figure 1: Species distribution of *Candida*.**



Antifungal Susceptibility testing was performed by disk diffusion method and it revealed that none of the isolates were resistant to amphotericin-B. Isolates of *Candida albicans* were susceptible to the azoles tested. Among the non-albicans, resistance to azole antifungal agents was encountered in strains of *Candida glabrata* and *Candida tropicalis*. 5 isolates (19%) of *Candida glabrata* were resistant to fluconazole and ketoconazole and 2 (7.6%) isolates were resistant to itraconazole whereas 1(3.2%) isolate of *Candida tropicalis* was resistant to itraconazole and ketoconazole (Table-3).

**Table 1: Susceptibility pattern of the isolates**

Isolates	Amphotericin B			Fluconazole			Ketoconazole			Itraconazole		
	S (%)	SDD(%)	R (%)	S (%)	SDD(%)	R (%)	S (%)	SDD(%)	R (%)	S (%)	SDD(%)	R (%)
<i>C. albicans</i> (n=57)	98.2	1.7	0	96.4	3.5	0	93	5.2	1.7	96.5	3.5	0
<i>C. tropicalis</i> (n=31)	93.5	2	0	90.3	9.6	0	84	13	3.2	87	9.6	3.2
<i>C. glabrata</i> (n=26)	84.6	15.3	0	81	0	19	65.3	15.3	19.2	77	15.3	7.6

C.	100	0	0	100	0	0	100	0	0	100	0	0
Parapsilos--is (n=2)												
C.kefyr (n=2)	50	50	0	50	50	0	50	50	0	100	0	0
C. krusei (n=1)	100	0	0	0	0	100	100	0	0	0	100	0

## DISCUSSION

Candida may be either a commensal or a pathogen of the vagina, a fact which indicates, that changes in the vaginal microenvironment are generally necessary for Candida to induce pathological changes associated with clinical symptoms.

Vulvovaginal candidiasis is a common problem in women and may affect their physical and emotional health, and may cause marital disharmony.

The risk factors for vaginal candidiasis in this study were reproductive age group, usage of oral contraceptive devices, insertion of intrauterine contraceptive devices, antibiotics and illiteracy

In the present study vaginal swabs were collected from 200 symptomatic women with complaints suggestive of vaginal candidiasis of which 119 were culture positive accounting to a prevalence of 59%.

119 culture positive cases, were subjected to further tests for the characterization of the species which revealed that Candida albicans was the most frequent etiological agent which accounted for 48% of the isolates (Figure -1), similar findings have been reported by Verghese S et al<sup>7</sup> where in Candida albicans was isolated from 40.4% of the cases, Jindal Neeraja et al<sup>8</sup> (69%) and Srujana Mohanty et al<sup>3</sup> (35%).

Our study showed the increase in frequency of non albicans species as potential cause of vaginal candidiasis which accounted for 52% (Candida tropicalis 26%, Candida glabrata 22%, Candida parapsilosis 1.7%, Candida kefyr 1.7% and Candida krusei 0.8%) (Figure-1). Similar observations have been made by Jackson et al<sup>9</sup> and Somansu Basu et al<sup>10</sup>. These non albicans yeasts are relatively non pathogenic but ultimately get selected and start appearing more frequently because of the widespread abuse of over the counter antifungals, use of single dose oral or topical azole regimens and long term maintenance regimens of oral azoles. Candida albicans eradication by these means causes a selection of non albicans that are resistant to commonly used drugs. Therefore vaginal culture is valuable for identifying the species of Candida and to monitor the changing trends in the microbiology of vulvovaginal candidiasis

which is essential for the complete and prolonged treatment of the patients of vulvovaginal candidiasis.

Antifungal susceptibility in the present study showed that out of 119 *Candida* isolates none were resistant to amphotericin B. For fluconazole the overall susceptibility rate for *Candida albicans* was 96.4%, susceptible dose dependent 3.5% and none were resistant followed by ketoconazole where resistance was shown by 1.7% of the isolates, however no resistance was shown for itraconazole.

None of the *Candida tropicalis* isolates were resistant to fluconazole, however 3.2% resistance was seen for ketoconazole and itraconazole. Azole resistance was seen mainly by *Candida glabrata* strains where in 19% isolates were resistant to fluconazole and ketoconazole and 7.6% were resistant to itraconazole. None of the isolates of *Candida parapsilosis* and *Candida kefyr* exhibited resistance to the tested drugs. *Candida krusei* is intrinsically resistant to fluconazole as was seen in this study (Table -3). These findings correlated with the studies done by Verghese S et al<sup>11</sup> Rubia Andreia et al<sup>12</sup> and Sandra S. Richter et al<sup>13</sup>.

Azole resistance in *Candida* species is of concern because these drugs are frequently used as therapeutic alternatives to amphotericin B. Azole antifungals are easy for administration and less toxic, these have high bioavailability, good water solubility, wide volume of distribution into tissues and body fluids and long half life<sup>14</sup>.

## CONCLUSIONS

The high frequency with which *Candida albicans* was recovered in this study and its azole susceptibility support the continued use of azole agents for the empirical therapy of candidal vulvovaginitis. There was a significant increase in infection caused by non *albicans* species of *Candida*, particularly, *Candida glabrata* and *Candida tropicalis* which is attributed to widespread and inappropriate use of antimycotic treatment. The presence of resistance to various antifungal agents among the different species of *Candida* emphasizes the need for routine susceptibility testing of fungi in all mycology laboratories of the country. Disk diffusion method could be used for preliminary screening of antifungal susceptibility. This simple disk diffusion method can be suitably standardized in the laboratories as it shows good correlation with the reproducible broth dilution methods.

In the study it was interesting to note the importance of early diagnosis and appropriate use of antimycotic agents in the patients presenting with the commonest complaint of vaginitis which could go a long way in relieving women from the distress of vaginal candidiasis.

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