

Original Research Article

A study of isolation and identification of non-albicans Candida species from clinically suspected cases of vulvovaginitis

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ABSTRACT

Keywords

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fluconazole,
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Vulvovaginal Candidiasis is an important cause of morbidity in women of reproductive age group. Approximately 75% women suffer from at least one episode of vulvovaginal candidiasis during their life time. The majority of cases of vulvovaginal candidiasis are caused by *Candida albicans*; however episodes due to non-albicans *Candida* species are increasing. Present study undertaken over a period of one year included 100 suspected cases of VVC. Of the 100 samples, 63 samples were culture positive for *Candida* species. *Candida non albicans* isolated were *Candida glabrata* (25.3%), *Candida tropicalis* (22.2%), *Candida parapsilosis* (3.17%), *Candida krusei* (1.58%) and *Candida albicans* (47.6%). There is a significant increase in prevalence of infection caused by *Candida non-albicans* species, particularly, *Candida glabrata* and *Candida tropicalis*. This increasing detection of non albicans *Candida* species is probably related to the wide spread and inappropriate use of antifungal treatment. The incidence of fluconazole resistant *Candida non-albicans* species in our study was 30.3% (10 out of 33 isolates). Susceptibility test revealed that there was no in-vitro resistance to voriconazole among all the isolates. The presence of resistance to first line antifungal agents among the different species of *Candida* emphasizes the need for early isolation, speciation and routine susceptibility testing of fungi in all mycology laboratories of the country.

Introduction

The list of opportunistic fungi causing serious life threatening infection increases every year, and without question the single most important cause of opportunistic mycoses worldwide remains *Candida* species¹. The *Candida* species are yeast-like fungi, ubiquitous in nature and are the component of normal flora of human beings. Candidiasis is the most common fungal infection affecting man, caused by a member of the genus *Candida*. Infections

due to *Candida* species have increased dramatically in recent years and are of particular importance because of the rising number of immunocompromised patients, as it causes opportunistic infections in them. Although *Candida albicans* remains the most common cause of human Candidiasis, the frequency of infection attributed to other members of the genus is increasing. This is primarily due to increase in number of at-risk individuals, particularly those with impaired immunity such as transplant

recipients, cancer patients receiving chemotherapy and human immunodeficiency virus infected patients². Widespread and indiscriminate use of broad spectrum antibiotics, use of therapeutic modalities for advanced life support, implantation of prosthetic devices and the emergence of resistance to antifungal agents have continued to be important in expanding the incidence of candidial infections.

The clinical manifestations of the disease are extremely varied ranging from acute, subacute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs or the gastrointestinal tract or may become systemic as in septicemia, endocarditis and meningitis. The pathological processes evoked are also diverse and vary from irritation and inflammation to chronic and acute suppuration or granulomatous response.

There are about 163 anamorphic species of the genus *Candida* with teleomorphs in at least thirteen genera, nearly 20 of these species are considered to be pathogenic causing various infections in humans. Some of these are *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Candida guilliermondii*, *Candida kefyr*, *Candida lusitanae*, *Candida dubliniensis* and *Candida viswanathii*³. *Candida albicans* is the most common and clinically relevant species; however recent studies suggest that there is an increasing incidence of isolation of non albicans species such as *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*. 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 candidal yeasts from clinical material much more compelling and necessary².

Vulvovaginitis is characterized by the presence of a yellowish white, milky or curdy discharge and patches of greyish white pseudomembrane are often seen on the vaginal mucosa. The lesions vary from a slight eczematoid reaction with minimal erythema to a severe disease process with excoriations and ulcers. The whole area is greatly inflamed and pruritis is usually intense. The condition may extend to involve the perineum, the vulva and sometimes the external genitalia⁴. Candidial vulvovaginitis is frequently associated with recurrence. Several factors predispose women to recurrence including genetic factors⁵, pregnancy, uncontrolled diabetes mellitus, and use of high oestrogen contraceptives, steroids and antibiotics. Chronic, recurrent infections and their refractoriness to the treatment pose a medical problem.⁶

Most non albicans *Candida* species have higher minimum inhibitory concentrations to the azole antifungal agents and infections they cause are often difficult to treat. This phenomenon emphasizes the importance of identification, surveillance and antifungal susceptibility testing of *Candida* species in the clinical setting⁷. Recent research has linked vulvovaginal candidiasis to medical, gynaecological and obstetrical complications, highlighting the importance of accurate detection, diagnosis and effective treatment. Hence, this present study was undertaken to determine the species prevalence and antifungal susceptibility amongst yeast isolates from women with suspected cases of vulvovaginal candidiasis.

Materials and Methods

The present study was carried out in the Department of Microbiology, Vydehi institute of medical science and research centre, white field, Bangalore. The patients for this study were chosen from the

outpatient clinic of Gynaecology department. This prospective study was undertaken from September 2012 to July 2013

Sample size consisted of 100 women, who were clinically suspected cases of vulvovaginitis suffering from pruritis and thick curdy white discharge per vaginum. The women belonged to the reproductive age group of 16- 45 years of age and they were selected randomly from the outpatient department. Married and non-pregnant women with suspected cases of vulvovaginitis were included in this study whereas unmarried, pregnant or post-menopausal women were excluded from this study. History from the patients was taken to determine any predisposing or underlying conditions like diabetes mellitus, prolonged antibiotic or antifungal therapy, usage of oral contraceptive pills etc. Vaginal discharge was collected from the patients with a sterile swab. Two swabs were collected from each patient.

Isolation and culture

One swab was used for the preparation of a smear and it was stained by Grams method and it was examined for budding yeast cells with or without pseudohyphae, pus cells, epithelial cells and other bacterial flora. The second swab was inoculated immediately onto Sabouraud dextrose agar slant with 0.05% chloramphenicol and incubated at 37°C for 24-48 hours. When growth was observed on inoculated SDA slopes, the colony morphology was noted and a Gram stained smear from the colony was prepared and observed under oil immersion objective for Gram positive budding yeast cells and pseudohyphae. If the smear showed the above mentioned features, the colony was further tested for the identification of the species and antifungal susceptibility testing of the isolate was carried out. If there was no

growth after 72 hours of incubation it was treated as negative.

Speciation of candida

Germ tube test: - 0.5ml of human serum was taken in a clean test tube. 2-3 colonies were lightly touched with a straight wire and then inoculated into the human serum. It was incubated for 2 hours at 37°C in a water bath. After 2 hours of incubation, one loopful of the serum was taken on a dry, clean glass slide and cover slip was applied over the drop. The preparation was first observed under low power and then under high power objective for the germ tubes. If the test was positive within 2 hours of incubation at 37°C, it was considered as a presumptive identification of *Candida albicans*. This was later confirmed by other tests.

Microscopic morphology on Cornmeal agar (Dalmau plate culture)^{2 and 9}: - Yeast like colonies were picked up with a sterile straight wire. With this straight wire, three parallel cuts at 1 cm intervals were made in agar by holding the wire at a 45° angle. Alcohol sterilized dried cover slip was laid on the surface of the agar, covering a portion of inoculated streaks. The inoculated plates were incubated at 25°C for 24-48 hours. They were examined under a microscope, with 10x and 40x objectives by keeping the whole plate on the microscope stage and observed growth through the cover slip. Presence of pseudo hyphae or true hyphae, blastoconidia, chlamyospore and their arrangement⁹ were used to speciate candida.

Carbohydrate fermentation tests⁸: - A loopful of culture was suspended in sterile distilled water. 0.2ml of this suspension was then added to 2% sugar fermentation media. Glucose, sucrose, lactose, maltose and trehalose were the sugars tested. The tubes

were incubated at 30°C for 48 to 72 hours. The ability to ferment a sugar was shown by the presence of acid (colour change) and gas trapped in Durham's tube.

Carbohydrate assimilation tests: - Carbohydrate assimilation tests were performed to speciate isolated candida. The test was performed by Auxanographic plate method.

Yeast suspension was prepared by emulsifying colonies from a 24 hour old culture in 6ml of distilled water. The turbidity adjusted to McFarland 0.5 standard. Two sterile glass petri dishes labelled with the isolate number and the names of the sugars were used. The Yeast suspension was poured equally into both the glass petridishes. 15 ml of molten yeast nitrogen base cooled to 50°C was added to the yeast suspension and mixed well and allowed to set at room temperature. Carbohydrate disks of 4% concentration

Antifungal agent	Disk content	Resistant mm or less	Sensitive mm
Fluconazole	25 µgm	14	19
Voriconazole	1 µgm	13	17

(Glucose, sucrose, lactose, maltose, cellobiose, raffinose, and trehalose) were evenly spaced in the designated area on the plates. The plates were incubated at 25°C for 24-48 hours. The plates were observed for growth around the disks. Those isolates which assimilated a particular carbohydrate grow well around the disk. A negative assimilation test was reported when there was no opacity around the carbohydrate disc⁹.

Antifungal susceptibility testing¹⁰:-

Antifungal susceptibility testing was done as

per CLSI –M44-A guidelines. The disk diffusion method described in this document has been standardized for Candida species. The media used was Mueller Hinton Agar + 2% glucose medium and 0.5 µ gm/ml methylene blue dye. Fluconazole and voriconazole drugs are tested.

Procedure: - Inoculum was prepared by picking 5 colonies of approximately 1 mm in diameter from 24-h-old culture of Candida isolate. Colonies were suspended in 5 ml of sterile saline, and its turbidity was adjusted visually with the transmittance to that produced by 0.5 McFarland standards. Lawn culture was made with the inoculum on the Mueller Hinton Agar +2% glucose medium and 0.5 µgm/ml methylene blue dye. With the help of sterile forceps antifungal disks were placed at a distance of 24mm from centre to centre. The plates were incubated at 35°C for 24 hours. Plates were examined after 24 hr and the measurements of the zone of inhibition were taken.

Test for fluconazole: -Mueller Hinton Agar with added 2%glucose +0.5 µgm/ml methylene blue dye was prepared. The sterilized molten medium was cooled to 50°C and poured on to sterilized dry Petri plates on a levelled surface to a depth of 4 mm and allowed to solidify. Inoculum was prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on SDA. The turbidity should be adjusted visually, with transmittance to that of McFarland 0.5 standard. The prepared Mueller Hinton agar plate was taken; a sterile non toxic cotton swab on a wooden applicator was dipped into the standardized inoculum, and rotated firmly against the upper inside wall of the tube to express excess fluid. Then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking. The inoculum

was then allowed to dry for 10- 15 minutes with the lid in place. Using an applicator, the E-strip for the antifungal drug Fluconazole was then placed on the agar plate swabbed with the test culture. Within 60 second this E-strip got firmly adhered to the agar surface. The E-strip was not repositioned or adjusted once placed. The plates were then incubated at 35⁰C for 24 hours. MIC was read where the eclipse intersects the MIC scale on the strip. On satisfactorily streaking, the resultant zones of inhibition will be uniform and there will be semi confluent lawn of growth. The test is read at 48 hours, only when insufficient growth is observed after 24 hours of incubation.

Following is the interpretive criteria for susceptibility categorization:

When testing	Incubation	<susceptible	Susceptible – dose dependent	>resistant
Candida species	35-37*c for 24 hrs	8	16-32	64

#- isolates of *C.krusei* are assumed to be intrinsically resistant to fluconazole. The results of fluconazole susceptible testing should not be interpreted using these criteria for this species.

Candida species are usually component of normal flora of the vagina. Changes in the normal bacterial flora of vagina, acidity of the vaginal fluid and hormonal variation are generally necessary for candida to induce pathological changes associated with clinical symptoms.

Vulvovaginal candidiasis is one of the most common presenting complaints in child bearing age and sexually active period. It

affects the patient’s physical and mental health and may cause marital disharmony. At least 70 - 75% of the women will develop one or more infections during their life time with 5 - 8% of these individuals developing recurrent infections¹¹.

The rise in vulvovaginal candidiasis infection could be due to several factors like increase in use of over the counter antibiotics, oral contraceptive pills, antifungal drugs, HIV, diabetes mellitus and STD.

The majority of the cases of vulvovaginal candidiasis are caused by *Candida albicans*, however infection due to non- albicans species of *Candida* appear to be increasing, which have higher azole MICs, and are refractory to treatment. This highlights the importance of identifying *Candida* species within the clinical samples in order to provide physician with information concerning the proper treatment for their patients.¹²

This present study was undertaken to determine the prevalence, species distribution and antifungal susceptibility pattern in isolated *Candida* from the suspected cases of VVC.

The peak age for vaginal candidiasis in this study was between 32 – 40 years. Similar findings were reported by Dharmik P.G et al in 2012¹³, Jackson et al in 2005¹⁴ and Deoki Nandam et al in 2007¹⁵ where in the age group commonly affected by vaginal candidiasis was between 26 – 35 years which corresponds to the fertility period.

Study of various factors responsible for vaginal candidiasis showed that the prevalence of *Candida* was significantly related to the increase in parity. N. Jindal et al in a study in 2007¹⁶ observed that women

with parity of more than two showed significantly higher rate of *Candida* culture positivity than women with parity one or nulliparous. Similar finding was also reported by Leela Vyas et al 2007¹⁷. In the current study the prevalence was higher among women with parity two and above when compared to the nulliparous women. High levels of reproductive hormones present during this phase and age, provides an excellent environment for the growth of *Candida* organisms.

Many investigators identify the use of oral contraceptive pills (OCP) as one of the predisposing factors for vulvovaginal candidiasis and this might be because of similarity between the mechanism operating during pregnancy and high oestrogen in OCP¹⁸. This increased yeast carriage is thought to result from the glycogen and other substrates available to the microorganisms as well as the direct effect of OCP on the yeast virulence.

Prevalence of vaginal candidiasis and its association with contraceptive methods was studied by Goldacre et al in 1979¹⁹ and they found that the OCP enables *Candida* to assume a pathogenic role in a small proportion of women. Joharah M et al in 2000¹⁸ observed that use of OCPs led to increased colonization by *Candida*, the commonest isolate being *Candida albicans* (38%). In their study the infection rate was 53.5% among OCP users. Similar findings were also reported by Jindal N et al 2007¹⁶, where in prevalence of vaginal candidiasis was 36.9% among OCP users. In the present study there were 10 cultures positive among 34 OCP users, *C. albicans* 03(30%), *C. glabrata* 03(30%), *C. tropicalis* 03(30%) and *C. parapsilosis* 01(10%). *Candida albicans* being the commonest isolate, which correlated well with the previous authors.

Indiscriminate use of antibiotics influences the increase in the incidence of vulvovaginal candidiasis as was observed in the present study. Jindal N et al in a study in 2006²⁰ found a prevalence of 42.8% among antibiotic users. Deepa B et al²¹ in 2013 found a prevalence of 19.83% amongst the antibiotic users. In the present study the prevalence of candidiasis was 35% by culture. (Out of 20 antibiotic users 7 are culture positive. On speciation 42.8% were *C. albicans*, 28.2% were *C. glabrata* and 28.2% were *C. tropicalis*). Antibiotics suppress the bacterial flora, which allows the colonization by *Candida* species, there by the risk of candidial infection increases with the duration of treatment.

In the present study vaginal swabs were collected from 100 symptomatic women with complaints suggestive of vulvovaginal Candidiasis, of which 63 were culture positive accounting to a prevalence of 63%. Of the various symptoms a triad of vaginal discharge, pain abdomen and pruritis was present significantly in women who were culture positive. M.J. Goldacre¹⁹ reported vulval itch as the most frequent clinical manifestation of vulvovaginal candidiasis followed by backache and vaginal discharge. Similar finding were reported by Jindal N et al in a study in 2006²⁰ where in 87% of the patients with pruritis were culture positive for *Candida*. In a similar study by S T Jackson et al¹⁴ in 2005 the culture positivity was 32% among all the patients attending the Obstetrics and Gynaecology outpatient clinic with a clinical diagnosis of vulvovaginitis. It was noted that the prevalence of vaginal candidiasis varied from 23% - 73% among various authors, which could be explained by the different epidemiological factors and criteria for the selection of study population.

In the present study, the 63 culture positive cases were subjected to further tests for the characterization of the species. This revealed that *Candida albicans* species was the predominant etiological agent which accounted for 47.6% and non *albicans* species accounted for 52.4%. This finding is in accordance with the studies by Verghese S²² et al in 2001 where in *Candida albicans* was isolated from 40.4% of the cases, Sandra S Richter et al²³ (71%), Jindal Neeraja et al²⁰ (69%) and Srujana Mohanty et al (35%).

In our study, among the non *albicans* species *Candida glabrata* was most frequent isolate (25.3%) followed by *Candida tropicalis* (22.2%) *Candida parapsilosis* (3.17%) & *Candida krusei* (1.58%). The fact that *Candida glabrata* was more frequently isolated among non *albicans* species had been observed in studies conducted by Verghese S et al²² (38%), Sandra S Richter et al²³ (19%), Srujana Mohanty et al⁶ (50.4%).

In our present study *Candida tropicalis* was isolated from 22.2% of the cases which corresponded with the studies by Jackson et al¹⁴ (10%), Somansu Basu et al²⁴ (33%) and Srujana Mohanty et al (10.8%).

Candida parapsilosis was isolated by Sandra S Richter et al²³ (5%), Jindal Neeraja et al²⁰ (4.3%) and Srujana Mohanty et al⁶ (1%) from the cases of vaginal candidiasis and in the present study 2 (3.17%) cases of *Candida parapsilosis* were isolated.

In our present study, *Candida krusei* was isolated from 1.58% of the cases as was also observed by Sandra S Richter et al²³ (2%), Srujana Mohanty et al⁶ (2.7%).

These non *albicans* *Candida* are relatively non pathogenic but ultimately get selected

and start appearing more frequently because of the widespread use of over the counter antifungal drugs and long term maintenance regimens of oral azoles. *Candida albicans* eradication by these means causes a positive selection of non *albicans* that are resistant to commonly used first line drugs. Therefore culture is valuable for identifying the microbiology of vulvovaginal candidiasis which is essential for the complete and appropriate treatment.

Antifungal Susceptibility of *Candida non albicans* in current study showed that out of 33 *Candida non albicans* isolates tested, none of these isolates were resistant to voriconazole.

The susceptibility pattern of the non-*albicans* isolates to fluconazole in the present study showed that 69.69% are sensitive and 30.3% are resistant. The susceptibility pattern of fluconazole is in accordance with the data obtained by Verghese S et al²² and srujana m et al⁶ where in the sensitivity was 83.3% and 70% respectively.

There is a significant increase in the infections caused by non *albicans* species of *Candida*, particularly, *Candida glabrata* and *Candida tropicalis*. This increase is probably related to the wide spread and inappropriate use of antifungal agents and over the counter use of antibacterial drugs. *Candida albicans* eradication by these means will lead to resistance or a shift towards intrinsically resistant non-*albicans* *Candida* species¹⁰.

Vulvovaginal candidiasis should not be diagnosed only on the basis of clinical criteria alone. Appropriate culture, characterization of *Candidal* species followed by antifungal susceptibility testing should be routinely used for accurate diagnosis and treatment of vulvovaginitis as

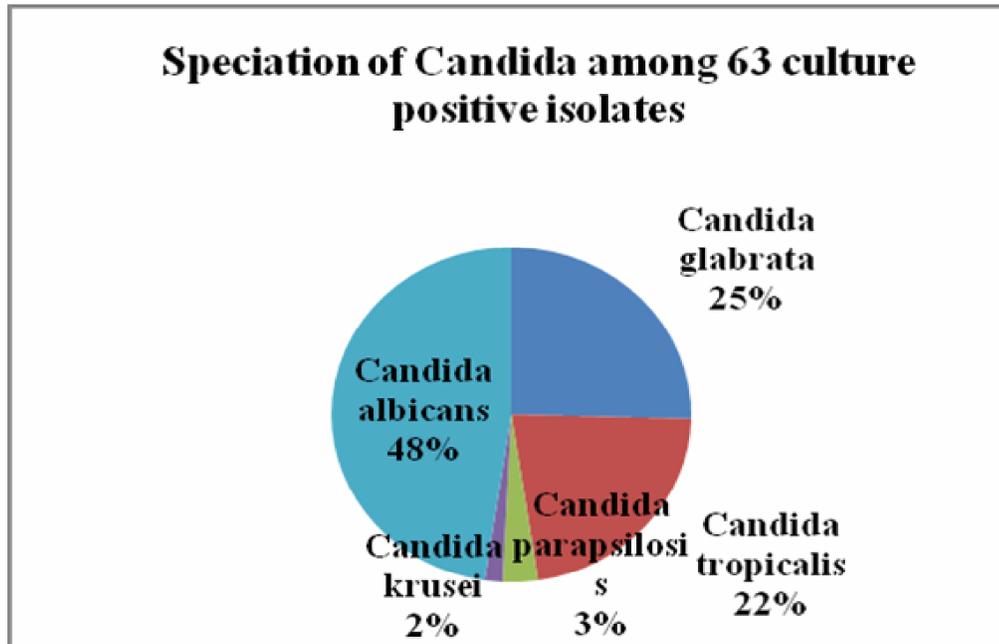
this avoids indiscriminate and over use of antifungal agents and may ultimately decrease the incidence of vulvovaginal candidiasis caused by non-albicans Candida species.

Presence of inherent and acquired 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. CLSI M44-A disk diffusion testing can be suitably standardized in all laboratories. And E- test can be considered as a convenient and compatible method for antifungal susceptibility testing of non

albicans Candida.

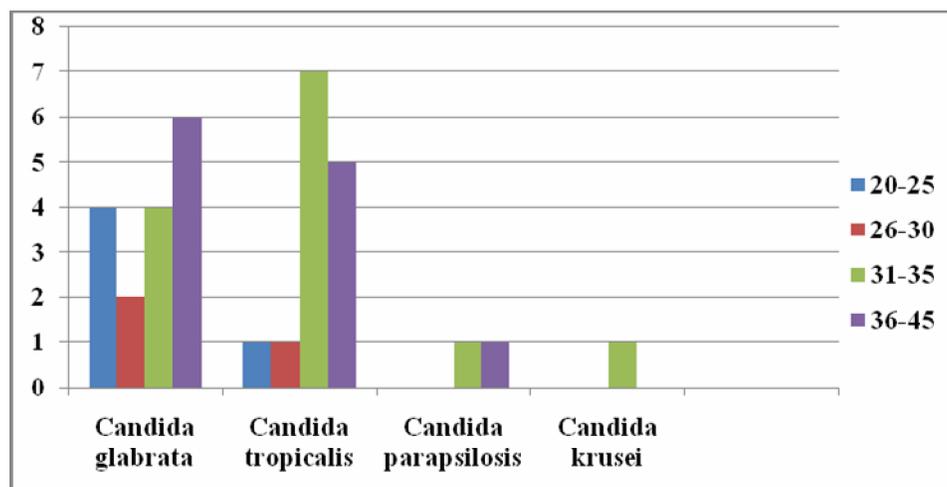
In conclusion, we studied various aspects of vaginal candidiasis. The importance of isolation and speciation of Candidal species and antifungal susceptibility testing was reaffirmed as need of the hour, as there has been a significant trend towards the emergence of non albicans species like Candida glabrata, Candida tropicalis and Candida parapsilosis. Hence the differentiation of diverse species of Candida in the laboratories seems necessary so as to prevent inappropriate use of antifungal drugs so that the emergence of resistance can be avoided amongst the Candida non-albicans.

Graph.1 Speciation of Candidal growth



Candida non-albicans species isolated were Candida glabrata 25.39% (16), Candida tropicalis 22.20% (14), Candida parapsilosis 3.17% (2) and Candida krusei 1.58% (1). Candida albicans 48% (30) were isolated.

Graph.2 Prevalence of non-albicans Candida species according to age



In the present study prevalence of Candida non albicans causing VVC in relation to age was as follows-

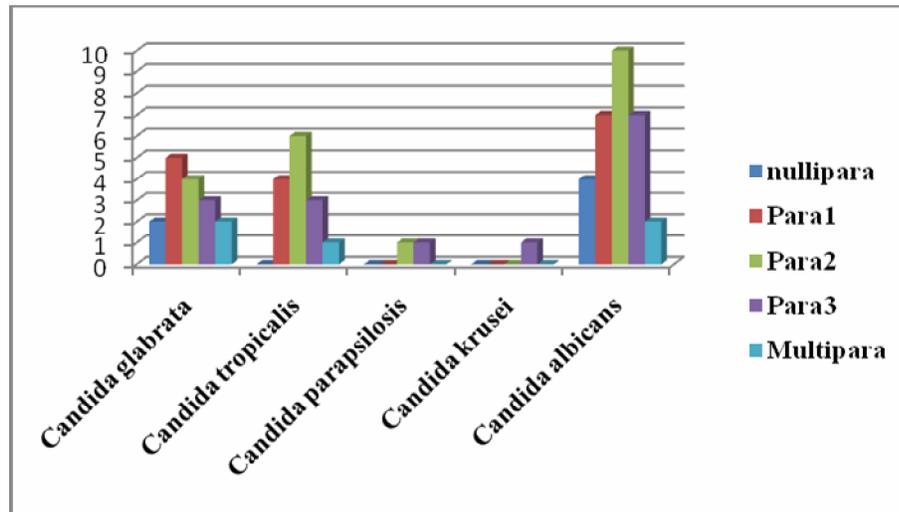
Prevalence of non-albicans species between age group 31-35 yrs, 36-45 yrs both showed high prevalence of 36.36% each, between 20-25 yrs prevalence was 15.15% and 26-30 yrs it was 9%.

Table.1 Prevalence of non-albicans Candida in relation to parity

Parity	Candida glabrata n=16 (%)	Candida tropicalis n=14 (%)	Candida parapsilosis n=2 (%)	Candida krusei n=1 (%)
Nulli para	2(12.5%)	0	0	0
Para 1	5(31.2%)	4(28.5%)	0	0
Para 2	4(25%)	6(42.8%)	1(50%)	0
Para 3	3(18.5%)	3(21.4%)	1(50%)	1(100%)
Multipara	2(12.5%)	1(7.1%)	0	0

In the present study prevalence of non albicans Candida causing VVC in relation to parity was highest in women having parity-2, and least prevalence was seen amongst the nullipara women.

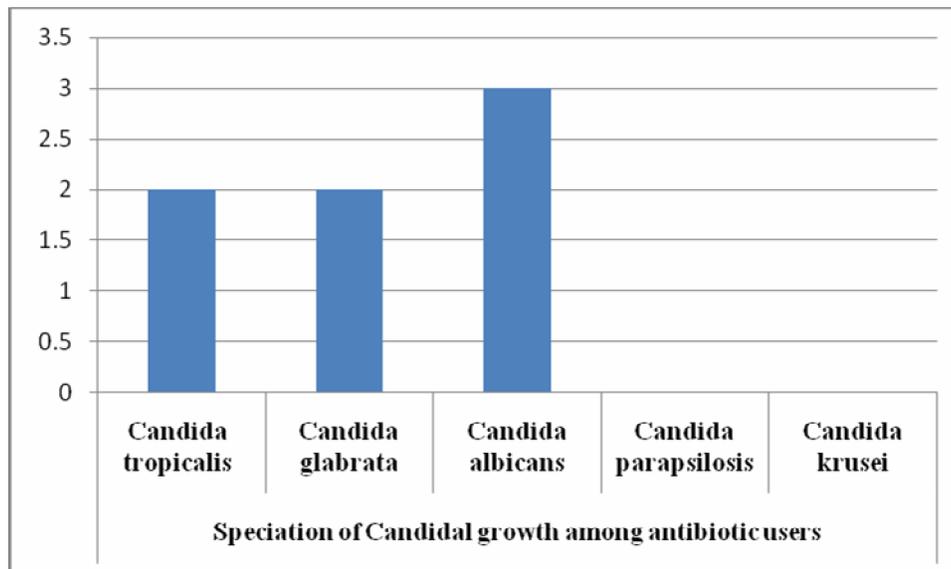
Graph.3 Prevalence of Candidial growth in relation to parity



In the present study prevalence of non albicans Candida causing VVC in relation to parity was as follows

Prevalence of non-albicans isolates was 33.3% among women with parity-2, 27.27% among para-1, and 24.24% isolated in para-3 women, in multipara prevalence was 9% and in nulliparous was 6%. Prevalence of non albicans Candida infection is highest amongst women belonging to parity 2.

Graph.4 Speciation of Candidal growth among antibiotic users



Amongst 20 patients who had taken antibiotics, 7 (35%) were culture positive for Candida species. On speciation of the 7 culture positive Candida, 28.2% were Candida glabrata and 28.2% were Candida tropicalis. (42.8%) were candida albicans.

Table.2 Speciation of Candidal growth among OCP users

Speciation of Candidial growth among OCP users					
Culture positive	Candida tropicalis	Candida glabrata	Candida albicans	Candida parapsilosis	Candida krusei
10	3	3	3	1	0

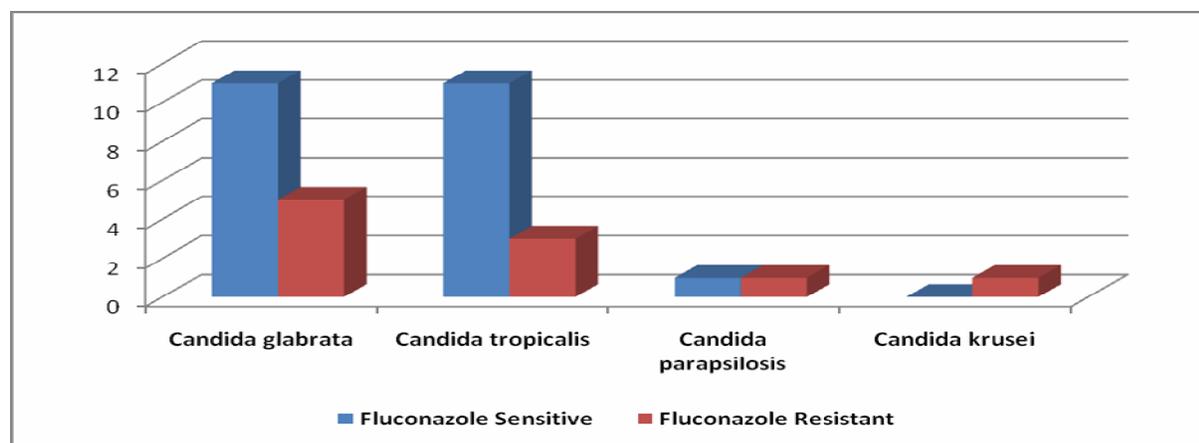
A total of 10 culture positive isolates were detected among the 34 OCP users. The non-albicans isolated were Candida glabrata 3(30%), Candida tropicalis 3(30%) and Candida parapsilosis 1(10%).

Table.3 Results of antifungal susceptibility test by Voriconazole disk

Isolates	Number of growth	Voriconazole	
		Sensitive (%)	Resistant (%)
Candida glabrata	16	16 (100%)	0
Candida tropicalis	14	14 (100%)	0
Candida parapsilosis	2	2 (100%)	0
Candida krusei	1	1 (100%)	0

All the non-albicans Candida isolates tested by Disk diffusion method were sensitive to Voriconazole. Sensitivity to voriconazole was 100%.

Graph.5 Results of Antifungal susceptibility test by disc method



The susceptibility pattern of the isolated Candida non-albicans to fluconazole in the present study showed that 69.69% are sensitive and 30.3% are resistant.

Of the isolated non-albicans Candida, Candida krusei was 100% resistant, 50% of the isolates of Candida parapsilosis were resistant, 31.25% of Candida glabrata and 21.42% of Candida tropicalis were resistant.

Table.4 Minimal inhibitory concentration (MIC) of Fluconazole by E-test

Isolated Species	No. of isolates	Geometric Mean MIC after 24 hours	Mean range($\mu\text{gm/ml}$)
Candida glabrata	11	2.23 $\mu\text{gm/ml}$	0.25-8 $\mu\text{gm/ml}$
Candida tropicalis	11	1.92 $\mu\text{gm/ml}$	0.25-8 $\mu\text{gm/ml}$
Candida parapsilosis	1	2.0 $\mu\text{gm/ml}$	2 $\mu\text{gm/ml}$

The geometric mean MIC of *Candida glabrata* was detected as 2.23 $\mu\text{gm/ml}$ (range of MIC 0.25-8 $\mu\text{gm/ml}$), 1.92 $\mu\text{gm/ml}$ for *Candida tropicalis* (range 0.25-8 $\mu\text{gm/ml}$) and 2.0 $\mu\text{gm/ml}$ for *Candida parapsilosis*.

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