

Original Research Article

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Prevalence of *Non albicans Candida* Vaginal Infection among Women Attending the Obstetrics and Gynaecology Department of a Tertiary care Hospital

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ABSTRACT

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Candida species are component of normal flora of human being. They are commonly found on the skin throughout gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy. Vaginal yeast infection, also known as vaginal candidiasis, genital candidiasis, or vulvovaginal candidiasis (VVC), is an infection affecting women of all age groups. The increase in the incidence of *Candida* species over the past two decades is significant and non-albicans *Candida* species continue to replace *C. albicans* at most of the clinical sites. Diabetes, immunosuppressive disease or therapies and neutropenia are common risk factors. Other risk factors include the use of oral contraceptives, corticosteroids etc. (Chow *et al.*, 2008). *Candida* species can cause a wide spectrum of clinical disease involving mucous membrane mainly, skin and nails. The present study was undertaken to assess the prevalence of vaginal infections caused by non albicans *Candida* and to look for the antifungal susceptibility pattern of the isolates.

Introduction

Vulvovaginal Candidiasis (VVC) represents a spectrum of disease. Although it is the second most common vaginal infection, VVC is a non notifiable disease and has been excluded from the ranks of sexually transmitted diseases. For many years patients with vaginal *Candida* were categorized into two groups: Asymptomatic carriers of *Candida* (colonization) and Symptomatic disease (Vaginal Candidiasis). More recently, the concept of “VVC” has replaced these distinct categories and the “vulvar”, often dominant component of symptomatic infection.

It is classified as uncomplicated (Sporadic or infrequent VVC, Mild to moderate VVC, Likely to be caused by *Candida albicans* and in normal, non pregnant women), complicated [recurrent VVC, severe VVC commonly caused by Non albicans Candidiasis occurring in abnormal host (e.g., uncontrollable diabetes, debilitation, or immunosuppression)].

Seventy-five percent of all women develop a yeast infection at some point during their lives. The infection may be acute or chronic, superficial or deep and its clinical spectrum is

wide. It is found mainly as secondary infection in individuals with some underlying immunocompromised conditions and very rarely as the primary disease. The fungus most commonly associated with vaginal yeast infection is called *Candida albicans* which are normally found in warm and moist areas of the body. Cases of sporadic and recurrent VVC caused by nonalbicans species of *Candida* are also on the rise. The possible reason for the apparent increase in non albicans *Candida* Vulvovaginitis may be the increased use of antimycotics, which may be used inappropriately and frequently as a short, incomplete course of therapy, eliminating the more sensitive *Candida albicans* and selecting for more azole resistant non albicans *Candida* species.

The vaginal pH is typically 4 to 4.5(normal), which distinguishes candidiasis from trichomoniasis or bacterial vaginosis (where the pH is elevated), there is a thick, white, cottage cheese-like discharge associated with pruritus in cases of vulvovaginal candidiasis. Risk factors associated with vaginal candidiasis include age, race, elevated estrogen, diabetes mellitus, contraception, use of antibiotics and immune suppression. Recurring yeast infections may be a sign of a serious disease such diabetes, leukemia or AIDS.

Over 200 species of *Candida* exist in nature; so far, only a few species have been associated with the disease in humans. The medically significant *Candida* species includes -*Candida albicans* (the most common species identified), *Candida glabrata* (previously known as *Torulopsis glabrata*), *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida kefyr* (previously known as *Candida pseudotropicalis*), *Candida guilliermondii*, *Candida lusitanae*, *Candida dubliniensis*, *Candida rugosa*, *Candida viswanathii*. Among these species of genus *Candida*,

Candida albicans accounts for most cases of systemic candidiasis but infections due to *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. kefyr*, *C. guilliermondii*, *C. parapsilosis*, *C. lusitania* and *C. rugosa* are increasingly being reported (Textbook of Medical Mycology, Jagdish Chander, 3rd edition). In spite of the basic morphological similarity between various species, a number of characteristics, which are species specific, are evident on Corn Meal Agar (CMA). Sugar fermentation and assimilation are the main methods for speciation of *Candida*.

Candida species are responsible for most of the frequently encountered opportunistic fungal infection. The increase in the incidence of *Candida* species over the past two decades is significant and non-albicans *Candida* species continue to replace *C.albicans* at most of the clinical sites like blood stream infections. Although *C. albicans* remains the most common causative agent of both superficial and deep fungal infection, an increase in the *Candida* non-albicans species like *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis* have been documented (Eggimann *et al.*, 2003). The ideal antifungal agents need to be effective against a broad spectrum of organisms, including all the medically important yeast of the genus *Candida*.

The most important classes of antifungal agents effective against *Candida* species are the azole and the polyene antifungal agents. Fluconazole is the most commonly used azole fungal agents and has been proven efficacious in the treatment of both superficial and systemic fungal disease.

Candida krusei, *Candida glabrata*, *Candida dubliniensis*, *Candida tropicalis* are resistant to Fluconazole. *Candida albicans*, *Candida glabrata* and *Candida krusei* are resistant to Amphotericin B.

The main aim of this study to isolate and identify *Candida* from high vaginal swabs, to identify various *Candida* species, to assess the prevalence of non albicans *Candida* Vaginal infection among symptomatic pregnant and non-pregnant women coming to the Obstetrics and Gynaecology department of SRM hospital. To determine the commonest non albicans *Candida* species causing Vaginal Candidiasis to find out the prevalence of vaginal candidiasis among various age groups. Also to find out the antifungal susceptibility test of the isolates

Materials and Methods

A prospective study was carried out from May 2010 to February 2011 to study the prevalence of *Non albicans Candida* Vaginal infection among symptomatic patients coming to the OG department of SRM hospital. A detailed history which included patients name, age, sex, date of admission, ward, a brief clinical history, the frequency of menstrual cycle, underlying diseases, antibiotic therapy, presence of any risk factors, steroid intake, pregnancy, diabetes mellitus, use of contraceptives and other symptoms suggestive of vaginal candidiasis like itching, colour and odour of vaginal discharge etc were collected from the patients. High vaginal swabs were collected aseptically from the patients by standard techniques.

Specimen processing

A direct smear for assessing of the cellularity and presence of organism was carried out in all cases. The media for the study were purchased from Himedia (Mumbai).The media and the biochemicals were prepared following standard procedures (Textbook of Medical Microbiology, Jagdish Chander, 3rd edition) (Annexure 2). Each batch of media and biochemicals were tested with suitable controls and was utilized only if it was satisfactory.

The primary isolation of the specimen was done on Sabouraud Dextrose Agar (SDA) with and without antibiotics (gentamicin) and incubated overnight at 37°C. The isolates produced cream coloured and smooth colonies on SDA. The isolates were subjected to gram stain which showed gram positive budding yeast cells. A preliminary identification of the isolates was done by performing the germ tube test and inoculating on Corn Meal Agar (CMA) which differentiated *Candida albicans* from *Candida non albicans*.

Biochemical reactions

Once presumptively identified as belonging to the genus *Candida*, the organism was subjected to further identification upto species level based on characteristic biochemical tests which includes Sugar fermentation test, Sugar assimilation test, Pellicle formation in Sabouraud Dextrose Broth (SDB), Urease test and CHROM Agar.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by NCCLS M44-A Disc diffusion method.¹⁸ Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24 h old culture of *Candida* species. Colonies were suspended in 5 ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 McFarland standard. Inoculation of test plates were done with a sterile cotton swab dipped into the suspension. The dried surface of a sterile Mueller-Hinton + GMB (glucose and methylene blue) agar plate was inoculated by evenly streaking the swab over the entire agar surface. Anti fungal disks of Fluconazole, Itraconazole and Amphotericin B were dispensed onto the surface of the inoculated agar plate. The plates were inverted and

placed in an incubator set to 35° C within 15 minutes after the anti fungal disks were applied. The inoculated plates were examined after 20 to 24 hours of incubation. The zone of inhibition was measured and the result is recorded as susceptible. The susceptible category implies that an infection due to the strain may be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species.

Susceptible-dose dependent (S-DD): The susceptible-dose dependent category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates.

Resistant (R): Resistant strains are those that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules.

Results and Discussion

The present study was carried out from May 2010 to February 2011 in the Obstetrics and Gynaecology department of SRM Medical College Hospital which is a tertiary care centre. A total of 68 samples were collected from symptomatic patients attending the outpatient department of Obstetrics and Gynaecology.

The demographic profile of the study subjects showed (Figure 1) symptomatic patients were common in the reproductive age group and least among the adolescent and premenopausal age group. Of the total 68 samples collected, majority of the patients (n=51) were between 21 to 40 years of age.

The distribution of *Candida* species (Figure 2) showed a majority of *Candida non albicans* which was 71% (n=17) and *Candida albicans* constitute only 29% (n=7)

The distribution of non *albicans Candida* among pregnant and non pregnant women (Table 1). Among the pregnant women (n=25) *Candida albicans* were isolated from 4 samples and *Candida non albicans* from 6 samples. Likewise in non pregnant women (n=43) only 3 samples grew *Candida albicans* and *Candida non albicans* was isolated from 11 samples.

Figure 3 depicts the distribution of species among *Candida non albicans*. Among the *Candida non albicans* (n=17), *Candida parapsilosis* was the commonest isolate 41% (n=7), followed by *Candida krusei* 23% (n=4), *Candida glabrata* 18% (n=3), *Candida kefyr* 12% (n=2) and *Candida tropicalis* 6% (n=1).

Figure 4 depicts the percentage of diabetic and non diabetic patients. Among the total of 68 symptomatic patients who attended the obstetrics and gynaecology department of SRM hospital 1.5% (n=1) was diabetic whereas 98.5% (n=67) were non diabetic patients.

Figure 5 depicts the percentage of antibiotic user and nonuser. Among the total of 68 symptomatic patients who attended the obstetrics and gynaecology department of SRM hospital 6% (n=4) were antibiotic users whereas 94% (n=64) were antibiotic nonusers.

Table 2 shows the distribution of *non albicans Candida* isolates based on sugar fermentation. In the present study, out of the 24 positive samples, glucose was found to be fermented by all the species of *Candida*, maltose was fermented by *Candida albicans*, *Candida kefyr* and *Candida tropicalis* and sucrose was fermented by both *Candida kefyr* and *Candida tropicalis*.

Table 3 shows the distribution based on sugar assimilation. *Candida parapsilosis* (n=7) did

not assimilate lactose and dulcitol, on the other hand Glucose and xylose were assimilated by *Candida krusei* (n=4) and *Candida glabrata* (n=3) assimilated glucose, xylose and dulcitol. *Candida kefir* (n=2) did not assimilate sucrose and dulcitol; likewise dulcitol and lactose were not assimilated by *Candida tropicalis* (n=1).

Growth on CHROM agar

Among the 24 isolates grown on CHROM agar *Candida albicans* (n=7) produced light green coloured colonies, whereas *Candida parapsilosis* (n=7) showed cream to pale pink coloured colonies. Likewise *Candida krusei*

(n=4) and *Candida glabrata* (n=3) produced pink and pink to purple coloured colonies. *Candida kefir* (n=2) and *Candida tropicalis* (n=1) produced pink or purple coloured and dark blue coloured colonies.

Table 4 shows susceptibility pattern of *Candida* species to Fluconazole, Itraconazole and Amphotericine B. Out of all species of *Candida* NON ALBICANS, resistance to Fluconazole, Itraconazole and Amphotericine B in 5, 5 and 1 case respectively. Resistance to Fluconazole and Itraconazole were found maximum in *Candida krusei* followed by *Candida parapsilosis*.

Table.1 Prevalent of *C. albicans* and Non albicans *Candida*

	Pregnant	Non pregnant
Non albicans <i>Candida</i>	6	11
<i>Candida albicans</i>	4	3

Table.2 Distribution of isolates based on sugar fermentation

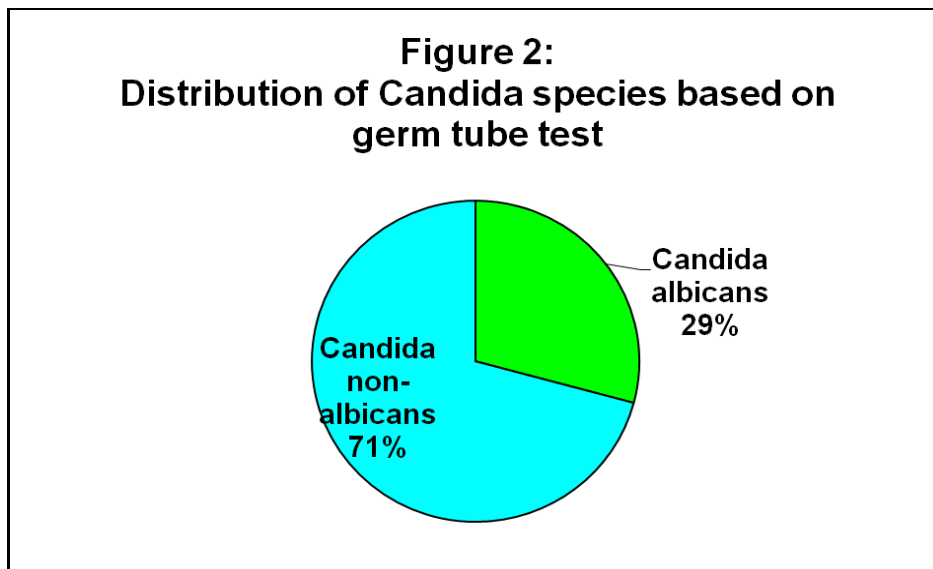
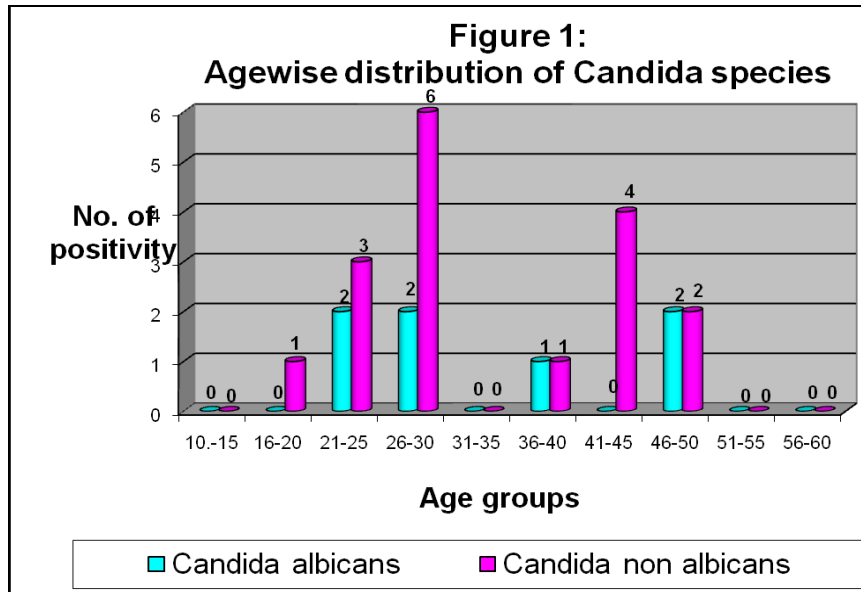
Isolate	Total	Glucose	Maltose	Sucrose	Lactose
<i>C. parapsilosis</i>	7	7	-	-	-
<i>C. krusei</i>	4	4	-	-	-
<i>C. glabrata</i>	3	3	-	-	-
<i>C. kefir</i>	2	2	2	2	-
<i>C. tropicalis</i>	1	1	1	1	-

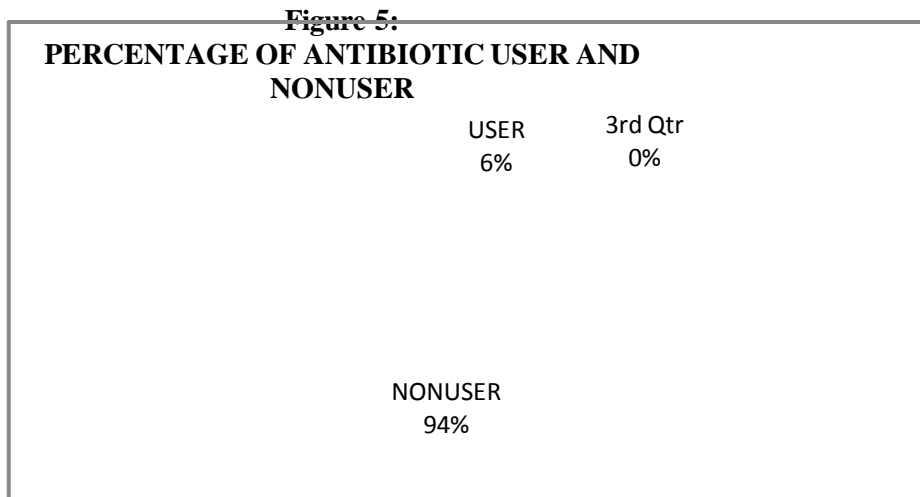
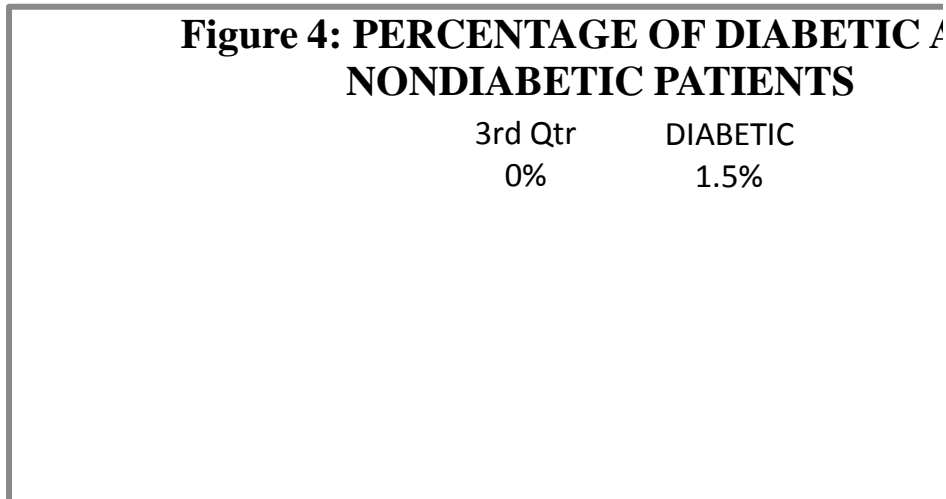
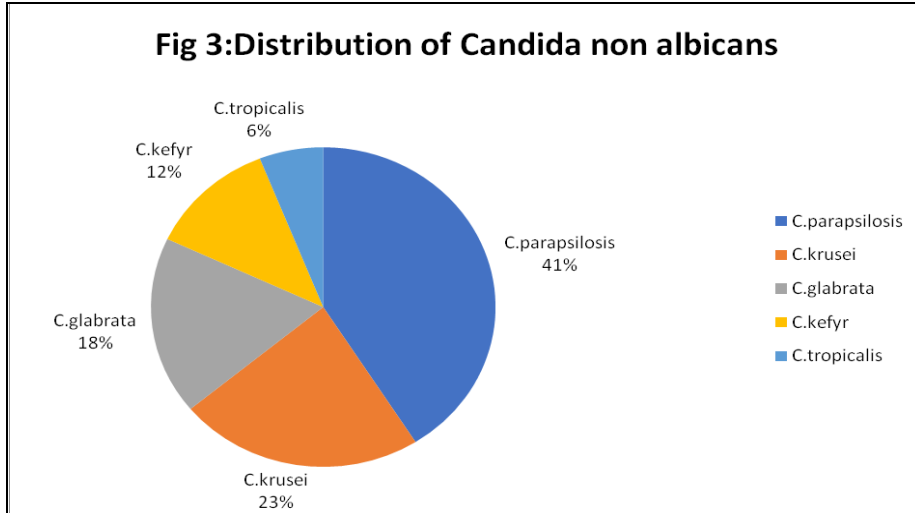
Table.3 Distribution of non albicans candida isolates based on sugar assimilation

Isolate	Total	Glu	Mal	Suc	Lac	Xyl	Dul
<i>C. parapsilosis</i>	7	+	+	+	-	+	-
<i>C. krusei</i>	4	+	-	-	-	+	-
<i>C. glabrata</i>	3	+	-	-	-	+	+
<i>C. kefir</i>	2	+	-	+	+	+	-
<i>C. tropicalis</i>	1	+	+	+	-	+	-

Table.4 Susceptibility pattern of nonalbicans *Candida*

Isolates	No: of isolates	Fluconazole		Itraconazole		Amphotericin B	
		S	R	S	R	S	R
<i>C.parapsilosis</i>	7	5(80%)	2(20%)	6(87%)	1(13%)	7(100%)	0%
<i>C.krusei</i>	4	3(88%)	1(12%)	2(80%)	2(20%)	3(88%)	1(12%)
<i>C.glabrata</i>	3	2(88%)	1(12%)	2(88%)	1(12%)	3(100%)	0%
<i>C.kefyr</i>	2	1(50%)	1(50%)	1(50%)	1(50%)	2(100%)	0%
<i>C.tropicalis</i>	1	1(100%)	0%	1(100%)	0%	1(100%)	0%





Vaginal candidiasis is a common problem throughout the world and is a frequent disorder in Obstetrics and Gynaecology. Yeast may be present in the vagina despite the absence of clinical symptoms in up to 30% of patients. It has been estimated that about ¾ of all women suffer from at least one attack of this disease (Daniela *et al.*, 1997).

In the present study, among the 68 samples collected 35% (n=24) were found to grow *Candida*. In a study done by Jindal *et al.*, 2006, 23.4% (n=82 of 350 women) was found to be positive for *Candida* species.

In the study, *Candida albicans* was isolated at the rate of 29% (n=7) and *Candida non albicans* 71% (n=17). Daniela Arzeni *et al.*, 1997 reported that the *Candida non albicans* were more prevalent than the *Candida albicans* in the symptomatic women with Jindal *et al.*, 2006 reported 74% *Candida albicans* and only 26% to be *Candida non albicans*. Likewise Alireza Khosravi *et al.*, 2010 and Richter *et al.*, 2005 reported 65% and 71% of *Candida albicans* in high vaginal swabs.

In the present study VVC was observed more in patients belonging to the age group 21 to 30 years. Similarly Alireza Khosravi *et al.*, 2010 has also reported maximum prevalence in patients from 20 to 40 years of age. Sandra Richter *et al.*, 2005 reported maximum prevalence of VVC in patients from the age group of 50 to 59 years.

In the present study, VVC was observed to be more in non pregnant women 63% (n=43) than in the pregnant women 37% (n=25). Likewise David Trofa *et al.*, 2008 also observed higher incidences of VVC among non pregnant women. Whereas Jindal *et al.*, 2006 observed that the prevalence of VVC was more in pregnant women than in non pregnant women.

In the present study regarding the distribution of *Candida non albicans* species *Candida parapsilosis* 41%(n=7) was found to be the most prevalent species followed by *Candida krusei* 23%(n=4), *Candida glabrata* 18%(n=3), *Candida kefyr* 12%(n=2) and *Candida tropicalis* 6%(n=1). David Trofa *et al.*, 2008 observed that among the *Candida non albicans* species *Candida parapsilosis* though rarely isolated in earlier days have found to be seen in increasing numbers now a days. Alireza Khosravi *et al.*, 2010 observed that among the *Candida non albicans* species *Candida tropicalis* was found to be more prevalent followed by *Candida glabrata*, *Candida guilliermondii*, *Candida parapsilosis* and *Candida krusei*.

Jindal *et al.*, 2006 observed that out of the *Candida non albicans* *Candida glabrata* was found to be more prevalent followed by *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis* and *Candida guilliermondii*. Most non-*albicans* *Candida* species have higher azole MICs and infections they cause are often difficult to treat. In present study also higher resistance was observed. One of the possible explanations for more frequent isolation of non-*albicans* species may be the increased use of topical azole agents. The extended prophylactic use of fluconazole in suspected cases would be a probable cause of high resistance pattern to fluconazole in our institute. Another established fact is that antifungal drug response in vitro may be dose dependent which is expressed as susceptible dose dependent (SDD), that is, although susceptible in vitro but resistance failure may be seen in vivo at the usual dose. In such situations, increase in dose of drug above the usual dose often results in clinical cure. But Indian studies shown a very high resistance to fluconazole for all candidal isolates although the amphotericin B susceptibility is high, Adhikary (2011).

In conclusion, vaginal Candidiasis affects about 50 to 72% of women, 40 to 50% having recurrent episodes. The majority of episodes of VVC are uncomplicated. Many women in whom recurrent yeast infections are diagnosed have been misdiagnosed. Recurrent episodes require clinical examination, culture of swabs, and consideration of underlying disease. Azoles can be used for empirical therapy of uncomplicated candidiasis as most of the isolates were found susceptible. However, culture should be done to detect non-albicans species and antifungal susceptibility testing is essential in recurrent cases of candidiasis.

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