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## Isolation and diagnosis of vaginal candida spp. and the effect of some anti-fungal drugs in pregnant women with and without diabetes

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### ABSTRACT

This study aims to evaluate the effect of antifungal drug treatment on Vulvo-Vaginal Candidiasis (VVC) in pregnant women which is the most common yeast infection in females otherwise worldwide. The secondary objective was to see effects if any between age, gravidity, pregnancy age of the subjects and VVC. This observational study was conducted at our fertility centre during one year period from the first of October 2017 to the beginning of March 2018. The 209 sterile cotton swab vaginal discharge samples were examined by cell detection & optical microscopy to observe the presence of causative yeast. These samples were collected from pregnant women, who, after being suspected to have VVC infection, were diagnosed for the same by a physician. Sabouraud & chrome agar cultures were prepared for positive samples to test the sensitivity of the isolates against antifungal drugs. The results of this study showed that *Candida albicans* was predominating among other types of *Candida* spp. (93.75%) As causative of VVC. It also reported that women ageing 30 to 40 years, multigravidas & pregnant women in their 3<sup>rd</sup> trimester were potentially more susceptible to VVC. Statistically stating, stated age group was found to be more affected than others (51.33%) for *C. albicans* (70%) & while women with multigravidas (95.33%) than primigravida women. The sensitivity test showed that the antifungal drug Ketoconazole & Fluconazole seems to have the highest effect against *C. albicans* while Miconazole against *C. krusei*. This study concluded that *Candida albicans* is the most common species responsible for causing VVC. Pregnant women ageing between 30-40 years, those in the 3<sup>rd</sup> trimester and multigravidas are more affected with VVC. Ketoconazole and fluconazole have a comparable effect against *Candida albicans*. Fluconazole does not affect *Candida Cruisii* while Miconazole had.



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### INTRODUCTION

Though many species of *Candida*, a genus of yeast, are usually harmless commensals of humans, yet in immunocompromised hosts, they can cause diseases, ranging from superficial skin infections to systemic infections (Kourkoumpetis *et al.*, 2011). Vulvovaginal candidiasis (VVC) is a common gynaecological ailment caused by *Candida*, a yeast infection, affecting 3 out of 4 women in their lifetime (Das *et al.*, 2008). 75% of them have had at least one occurrence of vulvovaginal candidiasis while 40 to 45% will have two or more (Corsello *et al.*, 2003). It is quite common in pregnant women. The reason for this is altered vaginal PH along with an increased level of estrogen. Both of these leads to

the production of more glycogen which further causes candida to grow faster in the walls of the vagina (Monif *et al.*, 2003). The most common species are *C. albicans*, *C. krusei*, *C. fam* etc. (Kim *et al.*, 2011). Amongst all these species of the Candida genus, *Candida albicans*, though part of commensal flora in > 50% of the healthy population, is interestingly the most common cause affecting almost 50-90% of reported cases of candidiasis in humans.

Its colonization otherwise proves to be beneficial to the host, as it limits the growth of many other pathogenic fungi, along with promoting the immune system functions (Brunke *et al.*, 2013).

It has been researched that candida is not capable of causing any disease unless the host is in the immune incompetent state either by the altered cellular defence, the body physiology, or displacement of other normal flora. Such host state enables a perfect environment for this yeast infectious growth (Geider *et al.*, 1995).

With pregnancy, changes in the host vaginal environment, regarding pH further precipitate and inculcate pathological effects of the causative organism (Sobel, 2007). Though not a life-threatening condition, The symptoms of VVC are unpleasant and problematic causing Symptoms like itching and whitish discharge.

The lab diagnosis of Candida is usually done by microscopy, yeast culture or antigen detection assays. Budding yeasts cells grow well on routine culture media while on gram-stain, they show gram-positive and are particularly oval. Germ tube test, economic indicator agar preparations (Chrome Agar) or multiparameter kits are used for categorising the speciations of candida (Kennedy *et al.*, 2017).

The antifungal agents use for VVC includes imidazole antifungals like Clotrimazole, Ketoconazole, Miconazole, and Tioconazole OR Triazole antifungals like Fluconazole & Itraconazole OR Polyene antifungals like Nystatin (NYS) (Soong and Einarson, 2009). According to the safety data collected from various research studies, topical formulations of imidazole & Triazole are preferred during pregnancy due to minimal risk to the foetus (Soong and Einarson, 2009; Owen and Clenney, 2004)

### Sample

The 209 pregnant subjects at our teaching centre & hospital, attending the facility from October 1, 2017, to March 1, 2018, were recruited as samples. Sterile cotton swabs were used to collect vaginal discharge samples. The subjects were diagnosed for VVC, by the physician as per clinical symptoms. Data collection for these cases, name, age, gravidity, the age of pregnancy and clinical manifestation

were documented. The inclusion criteria held patients from any ingestion of any medication three days prior to sample collection.

### Identification of Candida species

The swap samples were cultured immediately on sabouraud dextrose agar (SDA) & incubated aerobically at 35-37° C for up to 48 hours, the agar plates were examined for visible growth. The Candida identification was made according to colonial morphology microscopic examination to find hypha and pseudohyphae by Germ TT. A specific colour for each spp. appeared on the chromagar identification of Colonies.

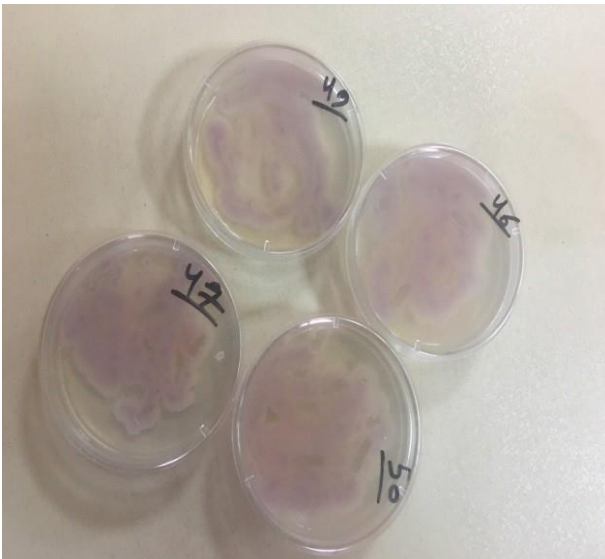
The /L dextrose, 20/L peptone, 20 g/L agar at PH 5.6 was the preferred composition of sabouraud agar which was prepared by suspending 65g in 1L water, boiled and autoclaved for 15 minutes at 120C. After that, it was left to dry in petri dish & solidification started. Vaginal swap sample was inoculated & incubated for 24 hours at 37C. White to creamy, smooth, glabrous colonies appeared as shown in the picture.



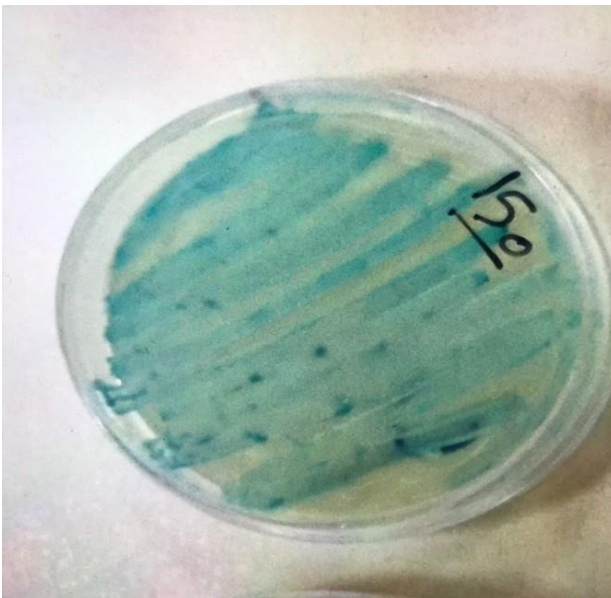
**Figure 1: Candida spp. on sabouraud agar**

The chrome agar used was powder of 15.0 g/L, Peptone 10.2 g/L, Chloramphenicol 0.5 g/L, Chromogenic mix 22.0 g/L. 47 g of this base was mixed with 1L water & boiled (100°C) while swirling. After cooling it was left to solidify. In the inoculation process, samples were streaked on the plates with the help of sterile loops and incubated at 30-37°C for 48 hours.

The green colour showed *Candida albicans* colonies while pink, fussy colour showed *Candida krusei* colonies.



**Figure 2: Candida krusei on chrom agar**



**Figure 3: Candida albicans on chrom agar**

Crystal violet, gram iodine, ethanol 70% and safranin stain were used for staining purpose. The standard Germ tube screening test was used to isolate *C. albicans*.

#### Susceptibility for some antifungal drugs

Of the many agar media available, the subcommittee considers supplemented Mueller-Hinton agar to be a good choice for routine susceptibility testing of yeasts as its readily available & shows acceptable reproducibility.

#### Disk diffusion method

The susceptibility testing by modified disc diffusion method was done for Anti-fungal susceptibility which was done by using 3 antifungal discs. Inoculate of the pure culture were taken from a nutrient slant to Mueller Hinton agar plates and streaked on the surface of the medium many times. After that antifungal disks were applied to each

plate and were secured by sterile forceps. They were gently pressed to entrust even contact with the medium. Plates were later incubated at 37°C for 18 hours.

#### Statistical Analysis

Statistical Package For Social Science version 21 for Windows Software and Microsoft Excel 2010 was used for the statistical analysis of data obtained from this study. For the assessment of the association of the variables, Chi-square test & one way ANOVA with were used. A value of  $P < 0.05$  was considered statistically significant.

#### RESULT AND DISCUSSION

The study showed that VVC was presented in 77.29% out of the total 209 samples. The analysis showed that VVC is commonly diagnosed in pregnant women with significantly high association with pregnancy. The results have been well supported by Xu & Sobel, 2004 (Xu and Sobel, 2004).

**Table 1: Percentage of Candida spp**

Samples	Percentage
Positive sample	77.25%
Negative sample	22.66%
P value	<0.001

When the causes of VVC were isolated and referenced, Spinillo *et al.*, reported the same causes of candidiasis in pregnancy as did our study (Scudamore *et al.*, 1994).

**Table 2: Percentage of Candida spp.**

Candida species	Percentage
<i>C.albicans</i>	93.73
<i>C.Krusei</i>	6.20
P value	<0.0001

\*significant association

Regarding the Relationship between patient's age and VVC, the results of this study showed no significant association. For *C.albicans* the decreasing order of groups according to age as of 30-40 years with 51.33%, then 18-30 years with 32.66%, and above 40 years with 16% patients. It was 30-40 years 70 %, then 18-30 years) with 30 % and above 40 years 0% patients for *C.Krusei*. Though Isibor *et al* disagree with the results as they found out that 26 to 30 years age group women are more frequent a target for VVC but at the same time, these results were supported by Bushra *et al.*, who also concluded that women in 31 to 40 years age group were more affected by VVC (Isibor *et al.*, 2011; Bushra *et al.*, 2016).

The possible reason for increased VVC in pregnant women (30-40 years) may be because of the lack of cell-mediated immunity. This cell-mediated immunity is supposed to encourage *Candida* colonization and serve as a risk factor as reported by

Ueda and Cengiz (Ueda and Cengiz, 2007). Fidel *et al.*, (2000) also found out that pregnant women with this age have decreased the ability of vaginal epithelial cells which can inhibit the growth of *Candida albicans* during pregnancy (Fidel *et al.*, 2000). Over 40 years' age women are nearing to menopause and are not sexually active. There are also less chances of misuse of drugs like contraceptives or otherwise. This might be the reason of least occurrence of VVC. They also have reduced levels of estrogen and corticoids. All these factors collectively make them resistant to *Candida* infection as concluded by Okungbowa, *et al.*, (2003) This correspond with the results of our study.

**Table 3: Percentage of VVC according to the age of the patient**

Age of the patient	C. Albicans (in %)	C. Krusei (in %)
18-30	32.64	31
30-40	51.32	69
Above 40	15.99	0
P value	0.323	

No significant association has thus been found between age of the pregnant women and the occurrence of VVC. Other than these, the gravidity was also studied about its relation if any, with the occurrence of VVC. The results showed that the increased number of VVC infections were associated with an increased number of gravidity. For *Candida albicans*, 95.33% of samples were multigravida, and 4.66% were primigravida while for *Candida krusei*, multigravidas were 80% and in primigravida were 10%. These results were statistically significant at P value 0.041. In commotion with the results of our study, Omar, also reported that the multigravidae suffered more from VVC more than primigravidae (Omar, 2001). The longer sexual history and use of contraceptives in multigravidae are an important risk factor associated with VVC as mentioned by Sobel (Sobel, 2002).

**Table 4: Percentage of VVC according to the gravidity**

Gravidity	C. Albicans (in%)	C. Krusei (in %)
Primigravidae	4.66	10
Multigravidae	95	80
P value	0.041	

Significant association at  $p < 0.05$

The Relationship between pregnancy age and VVC was shown significantly different as in different trimesters of pregnancy. According to the Observation of this study, the 3<sup>rd</sup> trimester had the highest prevalence rate, for *C. albicans* with 67.33%, and *C. krusei* with 50% patients. In the 2<sup>nd</sup> trimester, *C. albicans* was reported in 31.33% and *C. krusei* in

30% of subjects while in the 1<sup>st</sup> trimester the occurrence of VVC showed minimum rates. It was 1.33 % for *C. albicans* and 20% for *C. krusei*.

Sobel, (1997) explain the possible cause for this as a suppressed immune system in 3<sup>rd</sup> then 2<sup>nd</sup> and 1<sup>st</sup> trimesters (Sobel J. (1997). The increased levels of estrogen & corticoids compromise vaginal defence mechanisms which leads to VVC. The results are also in communion with (Sobel, 2000), who reported a rate of 67% in the 3<sup>rd</sup> trimester of pregnancy (Sobel, 2000). There are other studies compatible with this study like Xu & Sobel (Xu and Sobel, 2004).

**Table 5: Percentage of VVC & pregnancy age**

Trimester	<i>C. Albicans</i> (in %)	<i>C. Krusei</i> (in %)
1 <sup>st</sup> trimester	1.32	20
2 <sup>nd</sup> trimester	31.34	30
3 <sup>rd</sup> trimester	67.32	50
P value	0.001	

Significant association at  $p < 0.0$

### The effect of antifungal drugs

In vitro, *Candida albicans* and *Candida krusei* isolates have undergone susceptibility testing as per CLSI 2017 guidelines using 3 antifungals (Fluconazole, Ketoconazole, and Miconazole). It was found that Fluconazole and Ketoconazole have the most effect against *Candida albicans* while the other antifungal drug (Miconazole) is found to be most effective than *C. Krusei*. These findings were supported by (Rodriguez-Tudela *et al.*, 1995).

Both Miconazole & Ketoconazole showed a significant effect against *C. krusei* compared with Fluconazole which does not affect at all. Bennett *et al.* also supported these results by concluding that *C. krusei* are resistant to fluconazole (Bennett *et al.*, 2004). The possible explanation for this resistance may be the loss of affinity of fluconazole for its target enzyme (Erg11p) as reported by (Morio *et al.*, 2010).

**Table 6: The effect of antifungal drugs**

Antifungal Drug	<i>C. Albicans</i>	<i>C. Krusei</i>
Fluconazole	30.50±0.13	0±0
Ketoconazole	31.15±0.14	13.3±0.33
Miconazole	20.03±0.08	18.7±0.95

### CONCLUSIONS

On the basis of the results of this study, it can be concluded that VVC is the most common vaginal infection during pregnancy which is proved to be caused commonly by *Candida Albicans*. The pregnant women ageing between 30 to 40 years, multigravidas and those in their 3<sup>rd</sup> trimester of pregnancy are more often affected. Among the available antifungal drugs, Ketoconazole & fluconazole has a



comparable effect against *C. albicans*, but Fluconazole does not have any effect against *C. krusei*.

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