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RESEARCH ARTICLE

Antifungal Susceptibility Pattern and Species Distribution of *Candida* Isolates from Patients with Vulvovaginal Candidiasis

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Abstract

The objectives of this study were to determine the prevalence of Candida in patients suffering from vaginitis, to assess predisposing factors, to identify Candida species and to determine the susceptibility profile of different *Candida* species to antifungal agents. This study was carried out in Sohag University Hospital during the period from August 2013 to August 2014. High vaginal swabs (HVS) were collected from 300 women with age range from (18-50 years) clinically diagnosed as vulvovaginitis. HVS were processed by wet mount microscopy, Gram stain, culture onto Sabouraud's dextrose agar and CHROM agar plates. Isolates were identified and speciated using conventional methods, by the color of the colonies on the CHROM agar and by VITEK 2 System. Susceptibility testing to Clotrimazole, Miconazole, Fluconazole and Voriconazole by disc diffusion method was performed. Out of 300 HVS, Candida was isolated in 90 (30%) of cases. ***C.albicans*** was the predominant species isolated; 49 isolates (54.4%) followed by non albicans species like ***C. glabrata*** 22 (24.4%), ***C. tropicalis*** 13 (14.4%), ***C. famata*** 3 (3.3%), ***Candida dubliniensis***1 (1.1%), ***C. Lusitania*** 1 (1.1%) and ***C. parapsilosis*** 1 (1.1%). Mixed infection with two species of Candida was seen in 5.6% of patients. The most common mixed cause was the combination of ***Candida albicans*** and ***Candida tropicalis*** (3.3%). Recurrent vulvovagintis has been the most frequently implicated risk factor (45.56%). Susceptibility tests revealed that; highest resistance was observed against Miconazole (14.3%) among the ***Candida albicans*** isolates and was observed against Clotrimazole (19.5%) among the non albicans isolates.

INTRODUCTION

Vulvovaginal candidiasis (VVC) refers to a disorder characterized by signs and symptoms of vulvovaginal inflammation in the presence of Candida species. It is the second most common cause of vaginitis symptoms (after bacterial vaginosis) and accounts for approximately one-third of vaginitis cases ***(Workowski and Berman, 2010)***. Most cases of VVC is caused by ***Candida albicans*** (80 to 90%), while non-albicans *Candida* (NAC) including ***Candida tropicalis***, ***Candida glabrata***, ***Candida krusei*** and ***Candida parapsilosis*** account for 10 to 20% ***(Achkar and Fries ,2010)***. the virulence and antifungal drug susceptibility of *Candida* isolates differ according to the species, with ***Candida glabrata*** and ***Candida krusei*** being innately resistant to commonly prescribed antifungal drugs e.g. fluconazole ***(Farooqi et al.,2013).***The clinical diagnosis of vaginal candidiasis is unreliable, and laboratory

confirmation is needed ***(Bello et al., 2012).*** Also; the clinical manifestation caused by NAC spp. is indistinguishable from those caused by ***C.albicans*** but they differ in their susceptibility to antifungal agents and often demonstrate low sensitivity to commonly used antifungal drugs ***(Deorukhkar et al., 2012)***. On the other hand, a vaginal sample may contain mixed species of Candida and the isolation and separation process of those different species seems complicated and time consuming using the usual culture media. Identification of the infecting *Candida* to the species level is of utmost importance for clinical microbiological services for prediction of likely drug susceptibility and to guide treatment. A number of identification systems have been developed and are commercially available. Enzymatic reaction methods using chromogenic substrates in Chromogenic agar (CHROMagar) medium has high sensitivity and specificity in the differentiation of Candida species in clinical samples. Using this medium, it is possible to identify Candida species based on color and other morphologic characteristics (***Mahmoudi et al., 2011).*** VITEK 2 System is a rapid automated microbiological system used for bacteria and yeast identification, and antimicrobial susceptibility testing (AST) would allow clinical laboratories to perform both fungal identification and antifungal susceptibility testing simultaneously using a fully automated and completely standardized format ***(Pfaller et al., 2007)***. Failure to correctly diagnose and treat appropriately has led to prolonged distress in women who end up suffering from recurrent VVC defined as four or more proven infections per year ***(Sobel, 2007)***.Therefore, the aim of this study was to find out the prevalence of VVC, along with speciation of *Candida*, with special reference to its antifungal susceptibility pattern and evaluation of the risk factors responsible for VVC in patients attending our tertiary care hospital.

PATIENTS AND METHODS

- **Study design and patients**

This study was carried out in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Sohag University during the period from August 2013 to August 2014**.** A total of 300 women attending the out-patient clinic of the Department of Obstetrics and Gynaecology; clinically diagnosed as vulvovaginitis cases were included in this study. The study was approved by the ethical committee of Sohag Faculty of Medicine.

- ***Inclusion criteria:*** women between 18- 50 years of age who presented with self-reported symptoms of vaginal discharge, pruritis, irritation, soreness, dysuria, and/or dyspareunia, not using any vaginal medication in the previous 48 hrs.
- ***Exclusion criteria:*** Women of age group below 18 and above 50 years, catheterized patients, patients with genital neoplasms, and Post menopausal women.
- **Collection of High Vaginal Swabs (HVS)**

Two sterile, cotton tipped swabs were collected from each patient according to the laboratory procedures of the **WHO (2009)**; a sterile vaginal speculum was inserted into the vagina to examine and appreciate the state of the cervix. A sterile cotton tipped swab was then inserted 20-30 mm into the endocervical canal and gently rotated against the endocervical wall to pick a high vaginal swab. The swab was immediately replaced in its casing and labeled appropriately with the patient's information. In addition to the vaginal discharge sampling, participants were asked to complete a questionnaire with information covering complaints, nature of vaginal discharge, personal history, marital history, and predisposing factors for VVC.

- **Processing of samples**

  - One swab was used for microscopy: Direct wet mount examination with normal saline solution **(Figure 1)** and Gram stained film examination **(Figure 2)**. Presence of psuedohyphae was specially examined because it is an indicator for invasion and active infection. Demonstration of pseudohyphae along with yeast cells **(Figure 1 & 2)** is an important diagnostic feature to distinguish infection from normal colonization ***(Segal and Elad, 2005***).

  - The second swab was inoculated onto Sabouraud's dextrose agar (SDA) ***(Oxoid, UK)*** and incubated at 37°C for 48 h. It permits the growth of *Candida* and suppresses the growth of many but not all bacteria due to its low pH. As some strains of ***C. krusei***, ***C. tropicalis*** and ***C. parapsilosis*** are sensitive to cycloheximide, we used SDA without cycloheximide ***(Segal and Elad, 2005)***. The colonies of candida are opaque white to creamy on SDA plates. **(Figure 3)**

- HVS also; was inoculated onto CHROM agar plates ***(BBL™ CHROMagar™ Candida; Becton Dickinson GmbH)*** and incubated at 37°C for 48 h. These media contains chromogenic substrates that react with enzymes secreted by yeast cells, resulting in various pigmentations. These enzymes are species specific and allow species identification on the basis of colony color and characteristics ***(Horvath et al., 2003)***. **(Figure 4)**

- **Species identification procedures**

Candida species was identified based on colony morphology and pigmentations on CHROM agar ***(Nadeem et al., 2010)***, germ tube testing, and VITEK 2 Compact system results ***(bioMerieux, Marcy l' Etoile, France)***.

Germ Tube Test:

A rapid method for identifying ***C.albicans*** and ***C. dubliniensis*** by its ability to produce short, slender, tube like structures called germ tubes when it is incubated in serum at 37°C for 2 hours ***(Deorukhkar and Saini, 2014).*** In this test we must be able to differentiate between germ tubes and pseudohyphae. The elongated daughter cells from the mother cell without constriction at their origin are referred to as germ tubes **(Figure 5)** whereas constriction at the origin of mother cells is called pseudohyphae **(Figure 1)** ***(Kim et al., 2002)***. A criterion for germ tube positivity is observation of minimum five germ tubes in entire wet mount preparation. Negative results are confirmed by examining at least 10 high power fields for the presence of germ tubes ***(Deorukhkar et al., 2012)***.

VITEK 2 Compact System:

Fresh subcultures of each of the *Candida* isolates were obtained for identification using the VITEK 2 Compact ID YST cards as per manufacturer's instructions. Prior to testing, a suspension of each isolate was inoculated at least twice onto chromogenic agar plates and onto Sabouraud dextrose agar slants to ensure the purity and the viability of the cultures. The inoculum suspensions for the VITEK 2 were prepared in sterile saline at turbidity equal to a 2.0 McFarland standard. The individual test cards were automatically filled with the prepared culture suspension, sealed, and incubated by the VITEK 2 instrument. The cards were incubated at 35.5 °C for 18 h, and optical density readings were taken automatically every 15 min. The final profile results were compared with the database, and the identification of the unknown organism was obtained. A final identification of "excellent," "very good," "good," "acceptable," or "low-discrimination" was considered to be correct. Both the conventional tests and the tests performed using the VITEK 2 was considered to correctly identify the clinical isolates at the species level.

- **Antifungal susceptibility testing:**

All isolates were subjected to antifungal susceptibility testing for Clotrimazole (50 µg), Miconazole (10 µg), Fluconazole (25µg), and Voriconazole (1 µg) ***(Liofilchem diagnostic, Italy)*** by disc diffusion method according to M44-A Clinical and Laboratory Standards Institute(CLSI) guidelines ***(Clinical and Laboratory Standards Institute, 2009)***.

Disk diffusion method:

Mueller Hinton Agar (MHA) ***(Oxoid, UK)*** supplemented with 2% glucose and 0.5 µg/ ml methylene blue dye was used. The agar surface was inoculated by using a swab dipped in a *Candida* cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Antifungal discs were dispensed on agar surface and the plates were incubated aerobically at 35°C for 24 h. Zone diameter endpoints were read manually with calipers and interpreted according to standard sizes. The interpretive categories include susceptible (S), resistant (R) and susceptible dose-dependent (SDD). By maintaining blood levels with higher doses of antifungal, an isolate with an SDD endpoint may be successfully treated with a given azole ***(Wayne, 2004)***. Criteria for susceptibility to used antifungal drugs have been summarized in **Table 1**. ***(Gianni, 2010)*** & ***(Al-mamari et al., 2014)***.

Statistical Analysis

Data was analyzed using STATA intercooled version 9.2. Quantitative data was analyzed using *analysis of variance* (ANOVA). Qualitative data was compared using either Chi square test or fisher exact test. . ***P** values* < 0.05 were considered significant. ***P** values* < 0.01 and ***P** values* < 0.001 were considered highly significant.

RESULTS

Of the 300 symptomatic women studied, vaginal candidiasis was found in 90 (30%) patients. In the present study we were able to characterize seven species of *Candida* by conventional, CHROM agar and by the VITEK 2 system method. No isolates failed to grow in the VITEK 2 system. ***C. albicans*** (apple green colonies on CHROM agar) 49 (54.4%) was the predominant species isolated followed by non albicans species 41, with an overall rate of (45.6%): ***C.glabrata*** (pale pink to violet colonies on CHROM agar) 22(24.4%), ***C.tropicalis*** (dull blue colonies on CHROM agar) 13(14.4%), ***C.famata*** (White to light pink colonies on CHROM agar) 3(3.3%) and one strain (1.11%) of each of ***C. Lusitania*** (pink gray purple on CHROM agar)**,** *and* ***C. parapsilosis*** (white to pale pink colonies on CHROM agar) were isolated ***(Nadeem et al., 2010)***. The seventh species which is the ***Candida dubliniensis*** was identified by the VITEK 2 system. Mixed infection with two species of Candida was seen in 5 (5.6%) of patients. The most common mixed cause was the combination of ***Candida albicans*** and ***Candida tropicalis*** in 3 (3.3%) cases followed by ***Candida albicans*** and ***Candida glabrata*** in 2 (2.2%) cases. **(Table 2)**

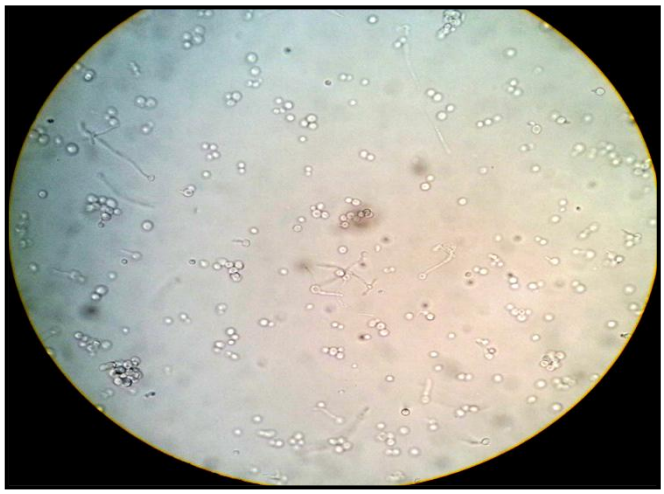


Figure 1: Wet smear showing oval budding yeast cells with pseudohyphae (*Candida* spp.).

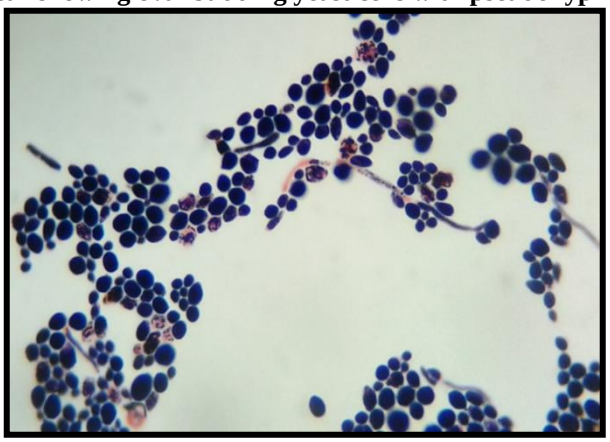


Figure 2: Gram stained smear showing Gram positive oval budding yeast cells with pseudohyphae (*Candida* spp.).

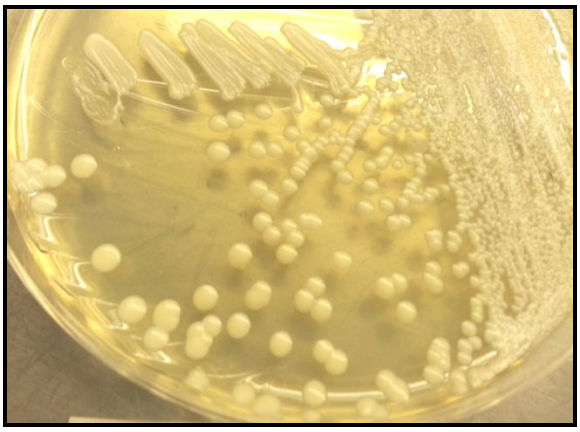
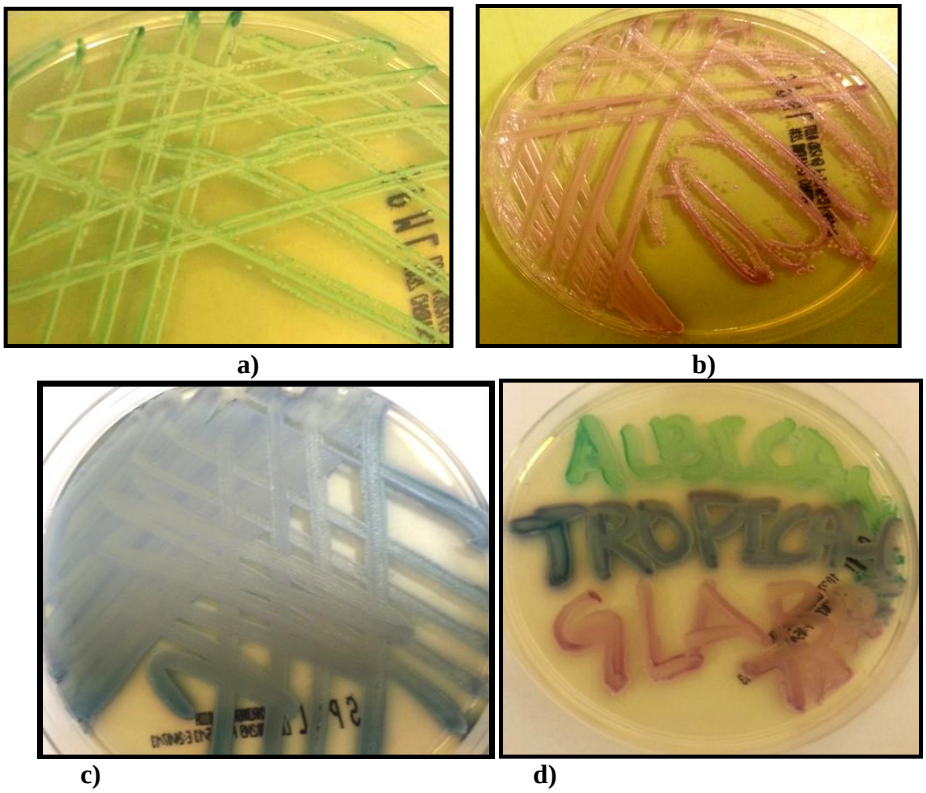


Figure 3: Creamy white opaque colonies of *Candida albicans* on SDA.



a) **b)**

c) **d)**

Figure 4: Differentiation of *Candida* spp. on CHROM agar candida medium showing: a) Apple green Colonies of *candida albicans*, b) Violet colonies of *Candida glabrata* and c) Dull blue colonies of *Candida tropicalis*. d) Greenish, Bluish, and violet colonies of *candida albicans*, *tropicalis*, and *glabrata* respectively.

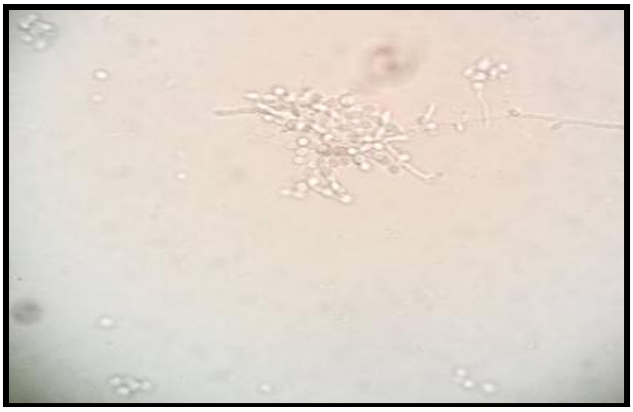


Figure 5: Germ tube formation by *Candida albicans*.

Table 1: Criteria of Susceptibility and Resistance of Antifungal Drugs.

	Zone Diameter in mm		
	Sensitive	**Dose Dependant**	**Resistant**
Clotrimazole (50 µg) CLO	**≥ 20**	**12-19**	**≤ 11**
Miconazole (10 µg) MCL	**≥ 20**	**12-19**	**≤ 11**
Fluconazole (25 µg) FLU	**≥ 19**	**15-18**	**≤ 14**
Voriconazole (1µg) VO	**≥ 17**	**14-16**	**≤ 13**

Table 2: Species distribution of candida isolates.

Species of candida	No.	Percent %
C. albicans	**49**	**54.44**
C. glabrata	**22**	**24.44**
C. tropicalis	**13**	**14.44**
C. famata	**3**	**3.33**
C. dubliniensis	**1**	**1.11**
C. lusitaniae	**1**	**1.11**
C. parapsilosis	**1**	**1.11**
Total	**90**	**100**

Age wise distribution showing that; the highest prevalence of vaginal candidiasis (43.3%) was in the age group of (36-45 years). **(Table 3)**

Table 3: Distribution of candida species in different age groups.

Age range	*Candida albicans*	*Candida glabarata*	*Candida tropicalis*	*Candida famata*	*Candida parapsilosis*	*Candida dubliniensis*	*Candida lusitaniae*	NO.	%
18-25	**4**	**0**	**2**	**0**	**0**	**0**	**0**	**6**	**6.7**
26-35	**12**	**7**	**3**	**0**	**0**	**0**	**0**	**22**	**24.4**
36-45	**20**	**10**	**6**	**2**	**0**	**0**	**1**	**39**	**43.3**
45-50	**13**	**5**	**2**	**1**	**1**	**1**	**0**	**23**	**25.6**
total	**49**	**22**	**13**	**3**	**1**	**1**	**1**	**90**	**100**

- **Risk factors associated with candida vulvovaginitis among the study group:**

Recurrent vulvovagintis has been the most frequently associated risk factor (45.56%) followed by pregnancy (25.56%), use of antibiotics (24.44%), the use of Intrauterine devices (IUD) (24.44%), then the use oral contraceptive pills (OCPs) (23.33%), and lastly the presence of associated diabetes mellitus (18.89%). There was a highly statistically significant association between all the studied risk factors and occurrence of candida vulvovaginitis in the study group (***p*** value < 0.001). **(Table 4)**

Table 4: The association between vulvovaginal candidiasis and identified risk factors among the study participants

Variable	Cases positive for Candida N= 90	Cases negative for Candida N=210	*P* value
Recurrent vulvovaginitis			
No	**49 (54.44%)**	**190 (90.48%)**	**<0.001***
Yes	**41 (45.56%)**	**20 (9.52%)**	
Pregnancy			
No	**67 (74.44%)**	**200 (95.24%)**	**<0.001***
Yes	**23 (25.56%)**	**10 (4.76%)**	
Use of antibiotic			
No	**68 (75.56%)**	**190 (90.48%)**	**<0.001***
Yes	**22 (24.44%)**	**20 (9.52%)**	
IUD			
No	**68 (75.56%)**	**195 (92.86%)**	**<0.001***
Yes	**22 (24.44%)**	**15 (7.14%)**	
OCP			
No	**69 (76.67%)**	**195 (92.86%)**	**<0.001***
Yes	**21 (23.33%)**	**15 (7.14%)**	
Diabetes			
No	**73 (81.11%)**	**202 (96.19%)**	**<0.001***
Yes	**17 (18.89%)**	**8 (3.81%)**	

*** Highly significant *p*<0.001**

- **Antifungal susceptibility pattern of candida isolates:**

C. albicans were (100%) susceptible to Voriconazole; (91.8%) susceptible to Fluconazole, (89.8%) susceptible to Clotrimazole, and (85.7%) were susceptible to Miconazole. As regards the non-albicans *Candida*; (95.1%) were susceptible to voriconazole; (87.8%) susceptible to Fluconazole, (80.5%) susceptible to Clotrimazole, and (90.2%) were susceptible to Miconazole. In spite of the higher resistance rates in non-albicans candida compared to ***Candida albicans*** isolates to antifungal drugs tested (except for Miconazole) the difference was only statistically significant with ***p*** value < 0.05 for Voriconazole and Clotrimazole but was not statistically significant (***p*** value > 0.05) for Fluconazole and Miconazole. **(Table 5)**

All NAC isolates were (100%) susceptible to Voriconazole except ***C. glabrata*** isolates where (9%) of them were resistant to Voriconazole **(Table 6)**.

Table 5: Antifungal susceptibility of candida isolates.

Antifungal Drug	*Candida albicans* N=49		Non-albicans candida N= 41		*P*- value
	S	R	S	R	
Voriconazole	**49 (100%)**	**- (0%)**	**39 (95.1%)**	**2 (4.9%)**	**0.02**

Fluconazole	**45 (91.8%)**	**4 (8.2%)**	**36 (87.8%)**	**5 (12.2%)**	**0.35**
Clotrimazole	**44 (89.8%)**	**5 (10.2%)**	**33 (80.5%)**	**8 (19.5%)**	**0.04**
Miconazole	**42 (85.7%)**	**7 (14.3%)**	**37 (90.2%)**	**4 (9.8%)**	**0.38**

Table 6: Antifungal susceptibility pattern of *Candida* species

Variable	*C. albicans* N=49	*C. tropicalis* N=13	*C. glabrata* N=22	Others N=6
Voriconazole				
Susceptible	**49 (100%)**	**13 (100%)**	**20 (91%)**	**6 (100%)**
Resistant	**0**	**0**	**2 (9%)**	**0**
Fluconazole				
Susceptible	**45 (91.8%)**	**10 (76.9%)**	**21 (95.5%)**	**5 (83.3%)**
Resistant	**4 (8.2%)**	**3(23.1%)**	**1 (4.5%)**	**1 (16.7%)**
Clotrimazole				
Susceptible	**44 (89.8%)**	**11(84.6%)**	**17(77.27%)**	**5 (83.3%)**
Resistant	**5 (10.2%)**	**2(15.4%)**	**5(22.73%)**	**1(16.7%)**
Miconazole				
Susceptible	**42 (85.7%)**	**12 (92.3%)**	**20 (91%)**	**5 (83.3%)**
Resistant	**7 (14.3%)**	**1(7.7%)**	**2 (9%)**	**1(16.7%)**

DISCUSSION

Vulvovaginal candidiasis is a common fungal infection that affects healthy women of all ages. At least 75% of women develop one or more infections once during their lifetime with 5% of those developing recurrent type ***(Consolaro et al., 2004)***. In the present study, vaginal candidiasis was found in 90 (30%) of symptomatic women with a higher prevalence rate than that reported by other studies as; ***Bauters et al***., ***2002***; who reported a prevalence of 20.1% and by ***Ragunathan et al., 2014*** who reported 22.2% prevalence of vulvovaginal candidiasis in their study.74.4% of our patients were in the age group of 18-45 years. Our results were in consistent with other studies done by various authors saying that; the incidence of reproductive tract infections in women is highest in the age group of 15-45 years and followed by a decline. The reason for the high incidence in this age group includes low levels of protective cervical antibodies, increased sexual activity, and new influence of reproductive hormones that may lead to increased susceptibility to reproductive tract infections ***(Sobel, 1993)***. In addition, many related problems are associated with Candida vaginitis such as; reduced immunity, prolonged antibiotic therapy, use of contraceptives, malnutrition, pregnancy, diabetes, obesity, use of immunosuppressive agents and neutropenia ***(Babin et al.,2013)***. In our study; we analyzed the predisposing factors associated with vaginal candidiasis. Recurrent vulvovagintis has been the most frequently associated risk factor (45.56%); followed by pregnancy (25.56%). We found a highly statistically significant difference in incidence of VVC between pregnant and non pregnant women. This may be probably due to high level of reproductive hormones during pregnancy which provides an excellent carbon source for growth of *Candida*, inducing higher glycogen contents in the vaginal epithelial cells and also some studies say that estrogens have a direct effect on the growth of *Candida* and its adherence to the vaginal epithelium ***(Lakshmi and Lakshmi, 2014)***. Our results agreed with those of ***Ragunathan et al., (2014)*** where there was highly statistically significant difference in incidence of VVC between pregnant and non pregnant women; and 55% of the females who presented with the complaints were pregnant. Also; we observed highly statistically significant difference in incidence of VVC between patients used OCPs (23.33%) and those did not. Many investigators continue to identify OCPs as a predisposing factor. This might be because of similarity between the mechanism operating in pregnancy and high estrogen OCP in increasing vaginal colonization of *Candida* ***(Neerja et al., 2006).*** In addition, we reported highly statistically significant influence during use of broad spectrum antibiotics by increasing the incidence of VVC (24.44%). VVC is a common occurrence after systemic use of broad spectrum antibiotics. Antibiotic usage will cause the elimination and alteration of normal bacterial flora and allows the colonization of *Candida* ***(Ahmad and Khan, 2009)***. Antibiotic agents are thought to act by eliminating lactobacilli, thereby facilitating *Candida* to grow, adhere and germinate. The concept of interaction between lactobacilli and

Candida includes competition for nutrients and stearic interference of adherence to vaginal epithelial cells ***(Mohanty et al., 2007).*** In our study; out of 90 *candida* species isolated, ***C.albicans*** accounted for 54.4%, followed by ***C.glabrata*** in 24.5%, then ***C. tropicalis*** in 14.4%, and ***C. famata*** in 3.3%. Non albicans species were found to present in 45.5% of infections. Recently several authors have also reported an increase in the incidence of VVC caused by nonalbicans species of *candida* ***(Bankar et al., 2012)***. Our results agreed with those of different studies conducted in various countries revealed ***C. albicans*** to be the most common species in women with VVC (76 to 89%), followed by ***C. glabrata*** (7 to 16%) ***(Corsello et al., 2003)***. The percentage of non ***C. albicans*** species associated with VVC in these countries ranged from 11% to 24%. ***Candida glabrata*** was the second most common isolated species (24.5%) in our study, which was consistent with the results of prior studies with a prevalence of 18–37% ***(Mahmoudi et al., 2011)*** & ***(Ahmad and Khan ,2009)***. However in the study of ***Mohanty et al., (2007)***; ***C. glabrata*** was the most common species among the vaginal isolates (50.4%) followed by ***C. albicans*** (35.1%). Thus, the prevalence of nonalbicans species (especially ***Candida glabrata***) seems to increase steadily and that raises the concern regarding the increase of antifungal drug resistance. We speculated this increasing detection of non-albicans species are probably related to the widespread and inappropriate use of antimycotic treatments (self medication, topical use, and long-term treatments). Hence, the reliable and rapid identification method of *Candida* species is a fundamental goal of microbiology laboratories. In the present study, our results were obtained regarding speciation of *Candida* by conventional and CHROM agar methods. CHROM agar has the advantage of being rapid, simple and cost effective as compared to conventional methods which are slow, technically demanding and expensive ***(Vijaya et al., 2011).*** However, one of the NAC species; ***Candida dubliniensis*** we could not be able to identify it by CHROM agar methods only and it was identified by the VITEK 2 system. VITEK 2 system correctly identified all of the 90 isolates at the species level. In fact, the VITEK 2 system performed satisfactorily in the identification of non-*Candida* yeast species. In addition, we confirmed a clinically important advantage of the VITEK 2 system over conventional identification methods: species were identified within 18 h compared to 48-72 h for the other methods. There is an important point which must be taken into consideration that; with the increasing rate of infections due to non-*albicans Candida* and varying susceptibilities to commonly used empirical antifungal agents like fluconazole, early and accurate species identification would help the clinician in befitting therapeutic management ***(Tellapragada et al.,2014)***. For example; ***Candida glabrata*** is known to have acquired resistance to fluconazole and other azole drugs ***(Melhem et al., 2013)***. So, in the present study we performed antifungal susceptibility testing by CLSI M44-A disc diffusion method with glucose methylene blue Muller Hinton agar for Clotrimazole, Miconazole, Fluconazole, and Voriconazole. It was easy to perform and inexpensive for routine laboratories. Previously; it was stated that, given the rarity of VVC caused by resistant ***C.albicans*** strains, susceptibility testing was rarely indicated ***(Nabhan, 2006)***; but in the present study highest resistance was observed against Miconazole (14.3%), followed by Clotrimazole (10.2%) and Fluconazole (8.2%). The resistance of ***C.albicans*** to azoles has also been reported by others ***(Zomorodian et al., 2011)***. Most non-albicans *Candida* species in our study had higher azole resistance: (4.5%) of ***C. glabrata*** isolates were resistant to fluconazole while; (23.1%) of ***C. tropicalis*** isolates were resistant to fluconazole. Our results agreed with those of ***Babin et al., (2013)*** who reported a higher resistance rates. In our study, ***C.albicans*** and majority of NAC species were found susceptible to voriconazole as compared to fluconazole. So, Voriconazole can be preferred over fluconazole as it has broad spectrum activity against *Candida* species which are inherently resistant to fluconazole. Therefore vaginal culture is valuable not only for identifying the species of vaginal *candida* but also for monitoring the changing trends in the microbiology of vulvovaginal candidiasis which is essential for the complete and prolonged treatment. As without rapid and accurate diagnostic tools, safe and effective drug treatment, prevention of resistance to antimicrobial therapy, and monitoring of resistance are not possible.

CONCLUSION

VVC cannot be definitely identified by clinical criteria alone. Culture is valuable not only for the accurate diagnosis of VVC but also to avoid indiscriminate use of antifungal agents. In our setting we found an increase in non albicans *Candida* infections and the emergence of azole resistant ***C.albicans*** and non albicans species in vaginal candidiasis cases. Resistance to various commonly used antifungal agents among non-*albicans Candida* suggests the need for species identification in routine laboratories for initiation of appropriate antifungal therapy. Surveillance of antifungal susceptibility profiles provides a useful tool for hospitals to validate empiric treatment regimens.

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