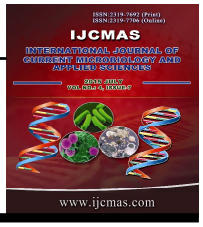
Int.J.Curr.Microbiol.App.Sci (2015) 4(8): 253-266

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 8 (2015) pp. 253-266**
http://www.ijcmas.com



Original Research Article

Prevalence of Vulvovaginal Infections and Species Specific Distribution of Vulvovaginal Candidiasis in Married Women of North India

Namarta Kalia[1], Jatinder Singh[1], Sujata Sharma[2], Sukhdev Singh Kamboj[1], Hardesh Arora[1] and Manpreet Kaur[3]*

[1]Department of Molecular Biology & Biochemistry, Guru Nanak Dev University, Amritsar, India
[2]Department of Gynaecology & Obstetrics, Bebe Nanki Mother and Child Care Centre, Government Medical College, Amritsar, India
[3]Department of Human Genetics, Guru Nanak Dev University, Amritsar, India
**Corresponding author*

Keywords

Bacterial vaginosis, Non-*albicans Candida* species, VVC, HiChrome, SEM, Trichomoniasis

A B S T R A C T

Abnormal vaginal discharge is a frequent complaint of women of child bearing age seeking gynaecological care. The frequency of different types of vulvovaginal infections (VVI) showed regional variations. Objectives of the present study were to determine the prevalence of different types of VVI and species specific distribution of vulvovaginal candidiasis (VVC) in North India. A total of 200 married women diagnosed by a gynaecologist for VVI were recruited for the present study. Vaginal swabs from these participants were processed for detection of bacterial vaginosis (BV), VVC and trichomoniasis based on European (IUSTI/WHO) guidelines on the management of vaginal discharge (2011). Species specific distribution of VVC was assessed by HiChrome *Candida* differential agar, Germ tube test as well as Scanning electron microscopy. The findings of the present study indicate that BV was the most prevalent infection (48.5%) followed by VVC (31%) and mixed infections (20.5%). However, no case of trichomoniasis was detected. *Candida albicans* was found to be the most prevalent species. Out of non-*albicans Candida* (NAC) species, *C. tropicalis* was found to be the most prevalent species. Overall distribution of *C. albicans* and NAC species indicated marginally high prevalence of NAC species (53%) than *C. albicans* (47%). When VVI were compared between pregnant and non-pregnant subjects, VVC was more prevalent in pregnant females while BV and mixed infections (MI) were more prevalent in non-pregnant females. Furthermore, in pregnant women *C. tropicalis* (42.8%) was the most prevalent species while in non-pregnant females *C. albicans* (43.9%) was the most prevalent species. In conclusion, BV was the most prevalent VVI in North India. *C. albicans* was the single most prevalent species in VVC while among NAC species, *C. tropicalis* was found to occur at highest frequency.

Introduction

Abnormal vaginal discharge is a characteristic feature of vulvovaginal infections (VVI). It occurs in 1-14% of all women of reproductive age throughout the world and its prevalence in India is estimated to be 30% (Thulkar *et al.,* 2010). Most common documented causes of symptomatic vaginal discharge includes bacterial vaginosis (BV), followed by vulvovaginal candidiasis (VVC) and trichomoniasis (Rekha and Jyothi, 2010). Earlier studies have also reported the coexistence of these VVI (French *et al.,* 2004). Epidemiologic data has demonstrated that the prevalence of various reproductive tract infections varies greatly between countries and even between regions within a country. This implies the differences in the characteristics of each pathogen and moreover other biological, behavioral, medical, social, and economic factors (Patel *et al.,* 2003).

BV and VVC involve disturbance in normal vaginal flora which is the main cause of both infections. BV is typified by decrease in the quantity and prevalence of hydrogen peroxide producing lactobacilli and overgrowth of predominantly anaerobic organisms in the vagina while VVC is caused by excessive growth of *Candida* species which is normally present in small number and is harmless (Sobel, 1988; Holland *et al.,* 2003; Larsson and Forsum, 2005). Untreated chronic BV may result in increased risk of pelvic inflammatory disease (PID), infertility, pre-term birth, premature rupture of membranes, low birth weight, intra-amniotic infections, endometritis, cervical intra-epithelial neoplasia (CIN), post-gynaecological surgery infections and increased risk of sexually transmitted diseases (STD) (Hay *et al.,* 1994; Ralph *et al.,* 1999; Atashili *et al.,* 2008). Chronic VVC can lead to severe vulvovaginal inflammation (Sobel, 1997). Trichomoniasis is a sexually transmitted disease and in untreated cases the risk of spreading other STDs increases (McClelland., 2007).

Some studies have indicated BV to be most prevalent infection, while in some other studies VVC was found to have the highest frequency (Kamara *et al.,* 2000; Gibney *et al.,* 2001; Garcia *et al.,* 2007; Gupta *et al.,* 2009; Shrestha *et al.,* 2011; Chaudhary *et al.,* 2012; Lennox *et al.,* 2013; Sivaranjini *et al.,* 2013; Mobashaeri *et al.,* 2014). *C. albicans* has been documented to be the major cause of VVC, but the proportion of non-*albicans Candida* (NAC) species appears to be increasing in last few decades (Stelzner, 1990; Spinillo *et al.,* 1997; Grigoriou *et al.,* 2006; Sobel, 2007; Guzel *et al.,* 2011; Doddaiah *et al.,* 2014; Hamad *et al.,* 2014; Hedayati *et al.,* 2015). This can be attributed to variety of interventions including single dose treatment, low-dosage azole maintenance regimens and the use of over the counter antimycotics. Studies, in the last decade suggest a NAC prevalence of 10-30% in patients with VVC (Bauters *et al.,* 2002; Corsello *et al.,* 2003; Holland *et al.,* 2003; Vermitsky *et al.,* 2008; Weissenbacher *et al.,* 2009; Zeng *et al.,* 2011). Zeng et al (2011) reported greater percentage of NAC infections than *C. albicans* infections. Similarly, Girgoriou et al (2006) reported that NAC caused more frequent vaginal soreness and dyspareunia than *C. albicans*. The predominant NAC species cited in these studies were *C. glabrata* and *C. krusei*. A single study reported in India showed that the prevalence of *C. tropicalis* was even higher than *C. albicans.*

These differences in occurrence of type of VVI and increasing prevalence of NAC

species over *C. albicans* show regional variations. In addition, these NAC species were found to be resistant to azoles (Bauters *et al.,* 2002; Singh *et al.,* 2002; Holland *et al.,* 2003; Richter *et al.,* 2005). This sought us to determine the prevalence of causative agent of VVI and species specific distribution of VVC in North India. Furthermore, the distribution of these infections was also compared in pregnant and non-pregnant women. These findings can be helpful in better management of the VVI.

Materials and Methods

Subjects

The present study enrolled 200 patients with symptoms including pruitis, burning, itching, soreness, pelvic pain, vaginal fishy smell, discharge and clinically diagnosed as VVI cases by a gynaecologist. These patients were recruited from Department of Gynaecology and Obstetrics, Bebe Nanki Mother and Child Care Centre, Government Medical College, Amritsar. The subjects with immune deficiencies, using immunosuppressive medications or chemotherapy were excluded from the study. Prior informed consent was taken from all the subjects and the study was approved by institutional ethical committee.

Swab collection and processing

Vaginal discharge samples were collected aseptically from the posterior vaginal fornix using two sterile cotton swabs, speculum and posterior vaginal wall retractor. These vaginal swabs were transferred separately to the labelled vials containing 0.5 ml of physiological saline (0.85% NaCl, pH 7). One vial was placed in ice for the diagnosis of BV and VVC while the other was kept at room temperature for the diagnosis of trichomoniasis. The material from each vial was divided further into aliquots which were subjected to various diagnostic tests according to European (IUSTI/WHO) guidelines on the management of vaginal discharge (2011).

Identification of Candidiasis

Thick vaginal discharge and vaginal pH of <4.5 were characteristics of candidiasis. The potassium hydroxide (KOH) vaginal preparation was made by mixing equal volume of vaginal solution and 20% KOH in 40% commercially available dimethyl sulphoxide (DMSO). The mixture was incubated for 5 min at 37ºC and was used for microscopic examination of hypae or pseudohypae indicative of *Candida* infection. The saline suspension of vaginal material from other aliquot was inoculated on saboraud dextrose agar (SDA) (HiMedia, India) and was incubated at 37ºC for 48-72 h. The colonies from SDA were further subtyped by using ready prepared media plates of HiChrome *Candida* differential agar (HiMedia, India). For this, isolated colonies were streaked on HiChrome agar plates and were incubated at 37ºC in dark for 48 h and the pigmented colonies were examined for species identification. Colonies suggestive of *Candida albicans* were further confirmed by germ tube test. For this 3-4 small colonies were mixed with 0.5 ml of human serum and incubated at 37ºC for 3 h. Microscopic (Olympus, India) examination was performed in oil immersion (x1000). The strains were maintained in stock media (50% glycerol) at -80ºC pending further use.

Candida species identified with HiChrome agar were further confirmed by Scanning Electron Microscopy (SEM, Zeiss EVO LS 10, Banglore, India). For this colonies from HiChrome agar plates was subcultured on

saboraud dextrose broth at 37ºC for 38 h with continuous shaking at 100 rpm in orbital shaker (Scigenics Biotech, India). The broth culture was centrifuged (Eppendorf, India) at 4,000 rpm for 10 min. The pellet thus obtained was washed quickly in five changes of sterile deionized water. The cells were fixed in 2.5% glutaraldehyde (S.d. Chem Ltd., India) at 4ºC for 12 h followed by two washings with sterile deionized water and finally dissolved in sterile distilled water. Twenty µl of this solution was transferred to a round cover slip of 0.1 mm thickness and was air dried for 5 min. Samples were dehydrated serially with various concentrations of ethanol ranging from 50–100%. The cover slips were cemented on aluminium studs with metal glue and coated first with carbon and then silver to a thickness of approximately 30 nm while spinning in vacuum of 10^{-5} torr and visualized using scanning electron microscope.

Identification of bacterial vaginosis

Vaginal samples were diagnosed for BV by Amsel's criteria (Amsel *et al.,* 1983). According to this, BV is present when three of the following four characteristics were detected i.e. thin homogeneous discharge, vaginal pH of >4.5, presence of clue cells and positive whiff test i.e., "fishy" amine odor of vaginal discharge after the addition of 20% KOH in DMSO. Furthermore, differential staining was performed with gram staining kit (K001-1KT) (HiMedia, India) according to manufacturer's instructions. Briefly, the heat fixed smear was treated with gram's crystal violet for 1min followed by gram's iodine (S013) for 1 min after washing with tap water. The smear was decolorized with gram's decolorizer (S032) until no further violet color comes off. The slides were washed with water and counter stained with 0.5% w/v saffranin for about 1 min. After washing again with water, the slides were dried and observed under oil immersion objective (x1000). All of the microbial morphotypes were interpreted and quantified by Nugent criteria (Nugent *et al.,* 1991).

Identification of trichomoniasis

The saline solution of vaginal discharge kept at room temperature was further used for identification of trichomoniasis. Thin vaginal discharge with pH > 4.5 and showing positive whiff test were indicative of trichomoniasis. Wet smear (normal saline) test was carried out for microscopic identification of trichomonds motility.

Results and Discussion

The mean age of the studied subjects was 29.5±8.38 years. Out of these, 97% of patients were of pre-menopausal status whereas 3% was of post-menopausal status (Table 1). About 59.5% patients had abnormal vaginal discharge for greater than 5 months. Only 23.5% patients were using one or the other contraceptive methods. In total, 66.5% patients had taken antibiotics during or before two weeks of sample collection and 90% of patients were having random blood sugar (RBS) < 140.

Analysis of vaginal swabs had indicated the presence of BV and VVC while no case of trichomoniasis was detected. BV was found to be the most prevalent infection (48.5%) followed by VVC (31%) and MI (20.5%) (Fig. 1). All of the bacterial morphotypes (Fig. 2A-C) were interpreted and quantified by Nugent criteria (Nugent *et al.,* 1991). Candidiasis was indicated by creamy white smooth colonies in SDA culture (Fig. 3i). For identification of *Candida* species the cases positive for VVC including MI (N=103) were further examined by HiChrome *Candida* differential agar. The

HiChrome agar showed the presence of five *Candida* species (Fig. 3ii) i.e., *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. dubeligensis*. Out of these, *C. albicans* was the most prevalent species which was further confirmed by germ tube test (Fig. 3iii). Out of NAC species *C. tropicalis* was the most prevalent species (Fig. 4). These species were further confirmed by SEM based on morphological characteristics (Fig. 5A-E) as described previously (Joshi *et al.,* 1973; Ferreira *et al.,* 2009; Bandara *et al.,* 2010; Thibane *et al.,* 2010; Singhai *et al.,* 2012). Overall distribution of *C. albicans* and NAC species has indicated marginally high prevalence of NAC species (53%) in comparison to *C. albicans* (47%).

When only VVC cases (N = 62) were further explored, it was found that 40.3% patients were infected with *C. albicans* and similar percentage were infected with single NAC species. However, 19.3% cases were found to be infected with mixed species of *Candida* (Table 2). Similarly, the vaginal sample from patients with MI (N = 41) were further investigated for co-distribution of *Candida* species with BV (Table 3). Out of these, 48.7% cases were found to have co-infection of *C. albicans* whereas 46.3% patients were found to be coinfected with one or other single NAC species except *C. dubeligensis*. Prevalence of NAC species was found to be marginally higher when coinfected with bacteria.

In pregnant and non-pregnant women, the pattern of VVI was similar i.e., BV was the most prevalent infection followed by VVC and MI. However, when pregnant females were compared with non-pregnant females, VVC was more prevalent in pregnant females while BV and MI were more prevalent in non-pregnant females (Fig. 6). When these study groups were compared for only *Candida* species. *C. tropicalis* (42.8%) was the most prevalent species in pregnant women, followed by *C. albicans* (33.3%). However, in non-pregnant women, *C. albicans* was the most prevalent species (43.9%) while very low prevalence of *C. tropicalis* (14.6%) was observed (Table 2).

BV, VVC and trichomoniasis have been documented to account for about 90% cases of VVI (Luglio-Agosto, 2005). The present study reported BV to be the most prevalent infection among VVI in North India followed by VVC and MI. This finding is in consonance with various earlier studies which have indicated BV to be the most prevalent cause of VVI (Kamara *et al.,* 2000; Gibney *et al.,* 2001; Garcia *et al.,* 2007; Gupta *et al.,* 2009; Shrestha *et al.,* 2011; Chaudhary *et al.,* 2012; Sivaranjini *et al.,* 2013; Mobashaeri *et al.,* 2014). However, percentage prevalence of BV and VVC in the present study was found to be comparatively higher as reported in earlier studies from India and some other countries (Kamara *et al.,* 2000; Gibney *et al.,* 2001; Garcia *et al.,* 2007; Gupta *et al.,* 2009; Shrestha *et al.,* 2011; Chaudhary *et al.,* 2012; Lennox *et al.,* 2013; Sivaranjini *et al.,* 2013). No case of trichomoniasis was found in the studied group. This finding is in agreement with some earlier reports which have documented very low prevalence of trichomoniasis (Shrestha *et al.,* 2011; Chaudhary *et al.,* 2012). However, some earlier studies in India and some other countries have indicated relatively high prevalence of trichomoniaisis (Kamara *et al.,* 2000; Gibney *et al.,* 2001; Garcia *et al.,* 2007; Gupta *et al.,* 2009; Fule *et al.,* 2012). These differences in the prevalence of different types of VVI can be attributed to geographical variations (Patel *et al.,* 2003).

C. albicans was found to be the single most prevalent species in VVC. This finding of the present study was similar to the studies

conducted in Nigeria, USA, Kenya, India, UAE, Iran (Alli *et al.,* 2011; Mintz and Martens, 2013; Nelson *et al.,* 2013; Doddaiah *et al.,* 2014; Hamad *et al.,* 2014; Hedayati *et al.,* 2015). However, this is in contrast to a single study reported in India where *C. tropicalis* was found to be more prevalent than *C. albicans* (Sharma and Solanki, 2014). However, in the present study the overall prevalence of NAC species was found to be marginally higher than *C. albicans*. Among NAC species, *C. tropicalis* was the most prevalent species followed by *C. glabrata*, *C. krusei and C. dubeligensis*. This pattern was not similar when compared to the studies conducted in some other countries including Kenya and USA where *C. glabrata* was found to be the most prevalent species. However present as well as earlier studies have reported very low prevalence of *C. krusei* (Leon *et al.,* 2002; Nelson *et al.,* 2013). During the last few years the reports regarding the increased frequency of NAC species in VVC have emerged (Spinillo *et al.,* 1997; Grigoriou *et al.,* 2006; Sobel, 2007; Guzel *et al.,* 2011; Vijaya *et al.,* 2014; Hamad *et al.,* 2014; Hedayati *et al.,* 2015). This may be due to the reason that most of the researchers have not preceded the Candida species differentiation.

Table.1 Clinical characteristics of patients with vulvovaginal infections

Characteristics	Patients with VVI	
	N= 200	
	No. of patients	%
Menopausal status		
Pre-menopausal status	194	97
Post-menopausal status	6	3
Patients having discharge duration for		
1 – 5 months	81	40.5
> 5 months	119	59.5
• 5 months -1 y	36	18
• >1 – 3 y	24	12
• >3 – 5 y	5	2.5
• >5 – 10 y	13	6.5
• >10 y	41	20.5
Patients using birth control methods		
OCP	9	4.5
IUCD	11	5.5
Tubectomy	11	5.5
Condom	16	8
Patients taken antibiotics in last two weeks	133	66.5
Random blood glucose (RBS)		
RBS >140	20	10
RBS <140	180	90

OCP: Oral contraceptive pills, IUCD: Intra uterine contraceptive device.

Fig.1 Prevalence of vulvovaginal infections

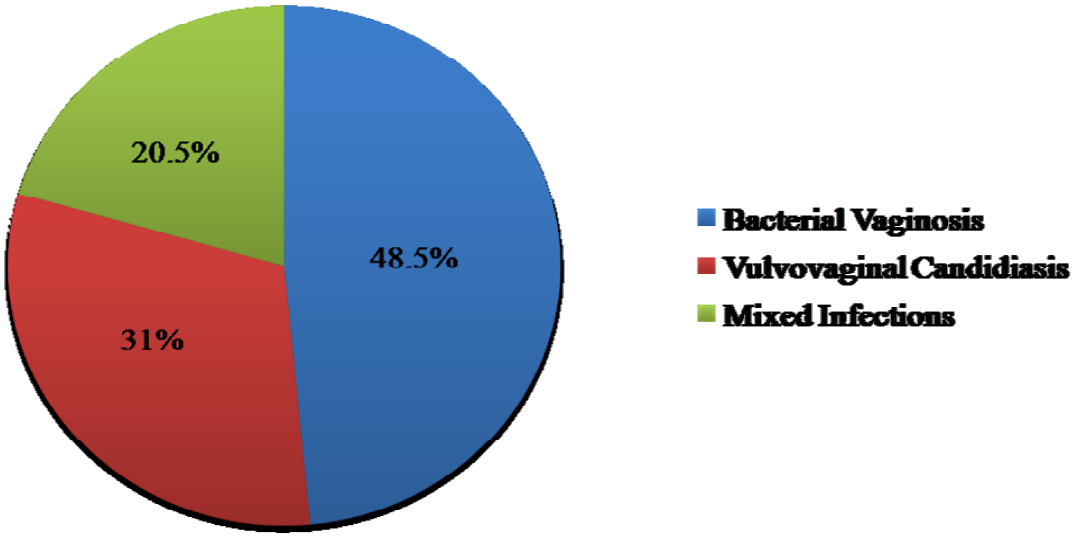
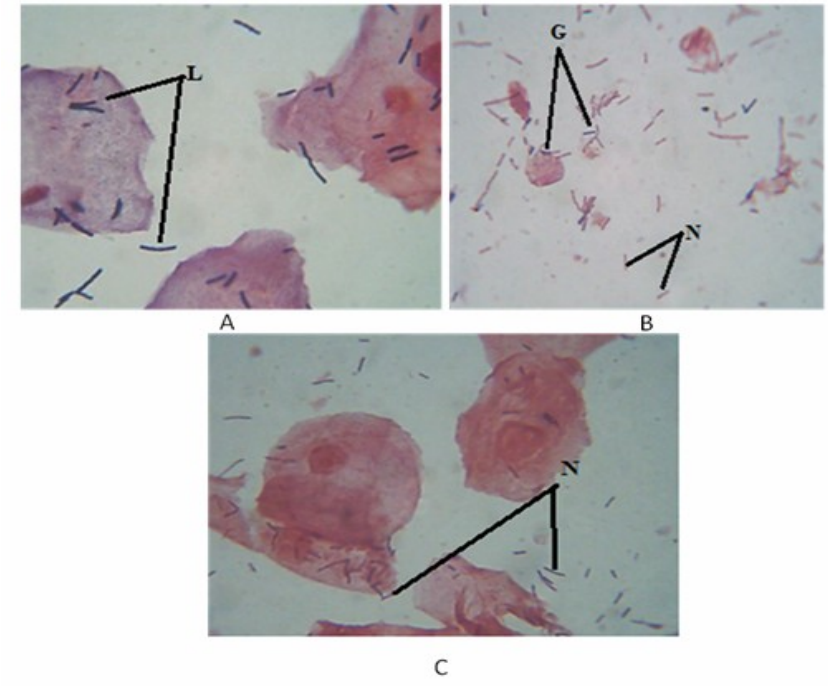


Fig.2 Gram staining of vaginal fluid smears: L- Lactobacillus morphotype; G- *Gardnerella* morphotype; N- small Gram-negative rods



(A) Vaginal squamous epithelial cells and only 3+ large gram positive rods (*Lactobacillus* morphotype), clinical examination was normal. (B) Mixed vaginal flora including 2+ small gram-positive rods (*Gardnerella* morphotype) and 4+ large gram-negative rods. No *Lactobacillus* morphotype was present, clinical diagnosis was BV (C) Vaginal squamous epithelial cells with many 4+ large gram negative rods only, clinical diagnosis was BV.

Fig.3 (i) Saboraud Dextrose Agar (SDA) showing creamy white smooth colonies of *Candida*. (ii) Colored streaks of *Candida* colonies on HiCrome *Candida* differential agar showing differential *Candida* species Section A: *C. albicans*; B: *C. dubeligensis*; C: *C. glabrata*; D: *C. krusei*; E: *C. tropicalis* (iii) Arrow showing germ tube, a characteristic feature of *C. albicans*

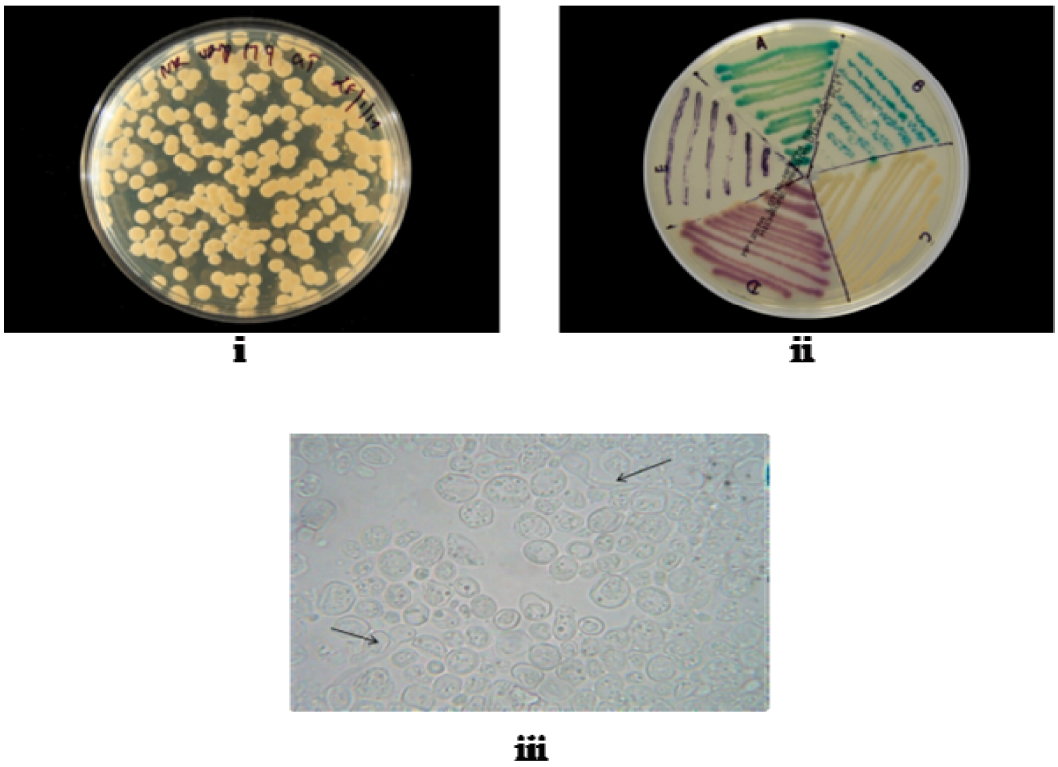


Fig.4 Overall distribution of VVC in vulvovaginal samples

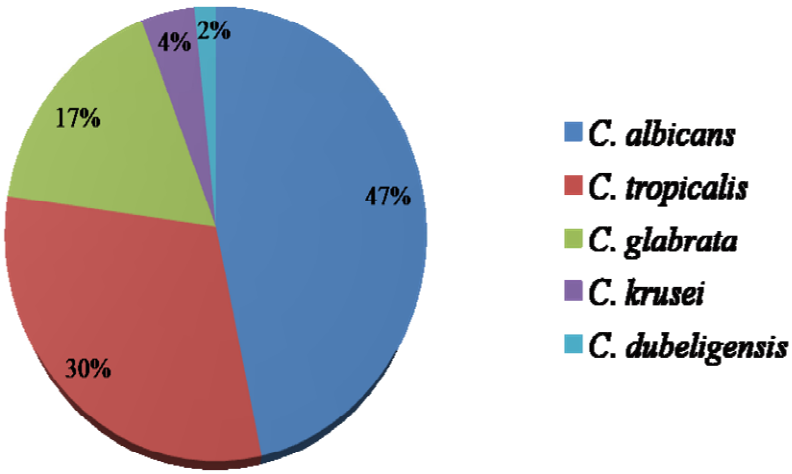


Fig.5 Scanning electron micrographs showing various *Candida* species. (A) *C. albicans* colonies with smooth round spores (Length × Breath = 4.507×3.373 µm). A large arrow showing bud scar (B) *C. glabrata* colonies having spherical shape with rough sides and pseudohypae, smaller than *C. albicans* (2.961×2.130 µm) (C) *C. krusei* colonies having oval to elongated spores with convoluted, rough, irregular and elevated surfaces (3.988×2.275 µm) (D) *C. dubeligensis* spores having extensively rough convoluted surface (5.049×2.613 µm). (E) *C. tropicalis* colonies showing oval spores with some pseudohypae. Smallest of all species studied (1.741×622.9 nm). Scale marker 2µm is used to view each sample

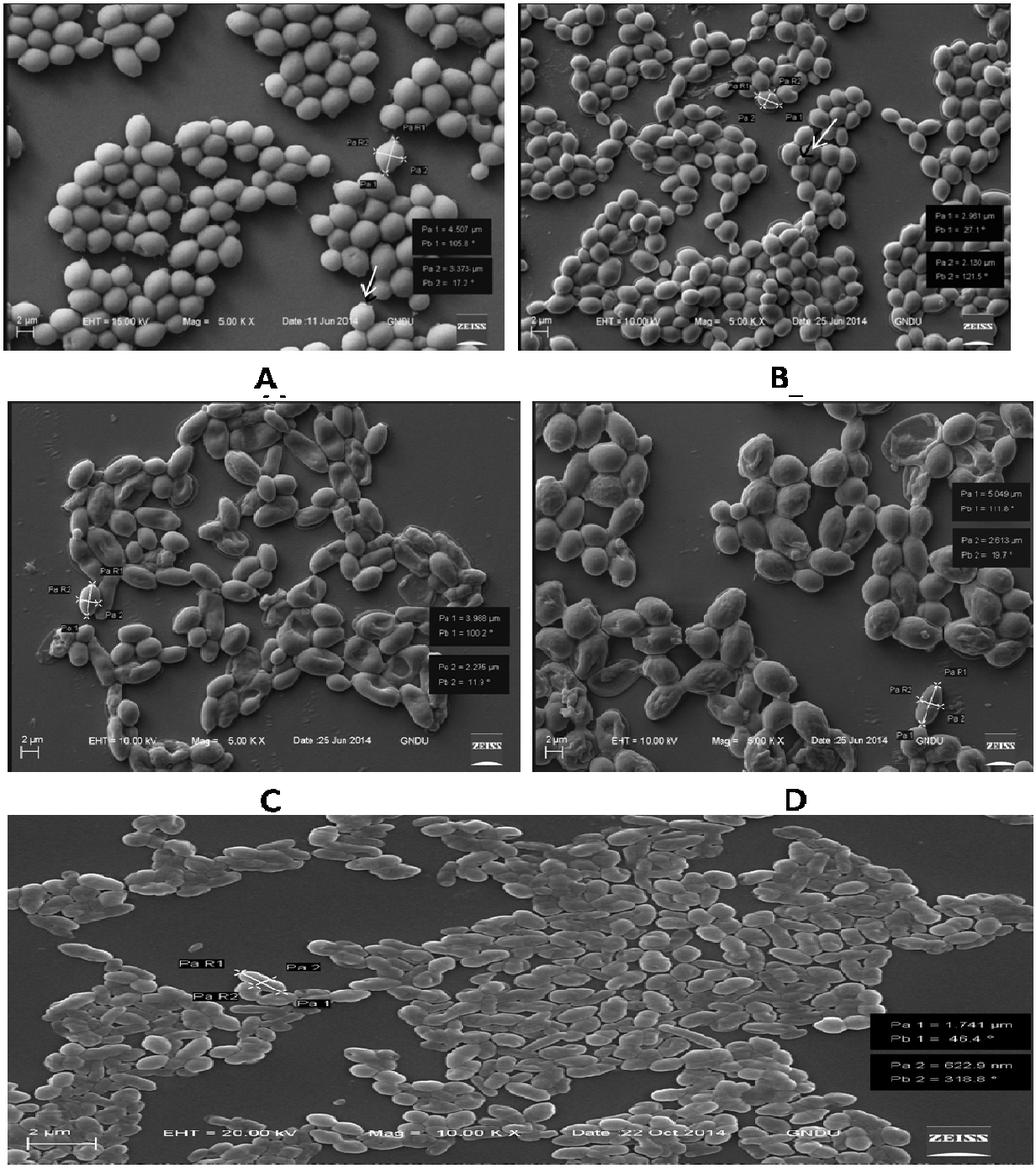


Fig.6 Prevalence of vaginal infections in pregnant and non-pregnant women

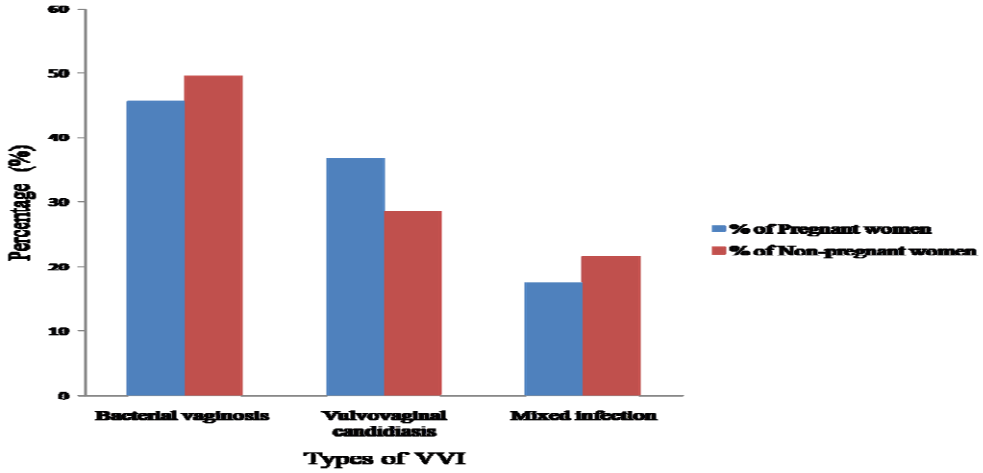


Table.2 Distribution of *Candida* species in vulvovaginal candidiasis patients

Candida species	Patients	Pregnant women	Non-pregnant women
	N = 62	N = 21 (33.8)	N = 41 (66.1)
C. albicans	25 (40.3)	7 (33.3)	18 (43.9)
C. tropicalis	15 (24.1)	9 (42.8)	6 (14.6)
C. glabrata	7 (11.2)	1 (4.7)	6 (14.6)
C. krusei	2 (3.2)	1 (4.7)	1 (2.4)
C. dubliniensis	1 (1.6)	0 (0)	1 (2.4)
C. albicans, C.tropicalis	5 (8.0)	2 (9.5)	3 (7.3)
C. albicans, C.glabrata	3 (4.8)	1 (4.7)	2 (4.8)
C. albicans, C. glabrata, C. tropicalis	1 (1.6)	0 (0)	1 (2.4)
C. krusei, C. tropicalis	1 (1.6)	0 (0)	1 (2.4)
C. dubliniensis, C. glabrata	1 (1.6)	0 (0)	1 (2.4)
C. glabrata, C. tropicalis	1 (1.6)	0 (0)	1 (2.4)

Figures in parenthesis represent percentages

Table.3 Distribution of *Candida* species in patients with mixed infections

Candida species	Patients	Pregnant women	Non-pregnant women
	N = 41	N = 10 (24.3)	N = 31 (75.6)
C. albicans	20 (48.7)	5 (50)	15 (48.3)
C. tropicalis	13 (31.7)	2 (20)	11 (35.4)
C. glabrata	5 (12.1)	2 (20)	3 (9.6)
C. krusei	1 (2.4)	1 (10)	0 (0)
C. albicans, C. glabrata	1 (2.4)	0 (0)	1 (3.2)
C. glabrata, C. krusei	1 (2.4)	0 (0)	1 (3.2)

Figures in parenthesis represent percentages

When patients were segregated into pregnant and non-pregnant categories, the overall pattern of VVI was found to be same that is BV was the most prevalent VVI followed by VVC and MI. These findings in case of pregnant women are in consonance with previous studies (Shrestha *et al.,* 2011; Olowe *et al.,* 2014). However, comparison of type of VVI between pregnant and non-pregnant women indicated that VVC was more prevalent in pregnant women than non-pregnant women whereas prevalence of BV and MI was higher in non-pregnant women. Till date no study is available to compare prevalence of VVI in pregnant and non-pregnant women. Furthermore, in case of VVC, *C. tropicalis* was found to be the most prevalent species in case of pregnant women while *C. albicans* was the most prevalent species in non-pregnant women. The finding in case of pregnant women was found to be similar to an earlier study conducted in India (Sharma and Solanki, 2014), whereas it is in contrast to the study conducted in Kenya where *C. albicans* was the most prevalent species in pregnant women followed by *C. glabrata, C. tropicalis, C. krusei* and *C. parapsilosis* (Nelson *et al.,* 2013).

The present study indicated BV to be the most common cause of VVI in North India. *C. albicans* was the most prevalent species and out of non-*albicans Candida* (NAC) species, *C. tropicalis* was found to be the most prevalent species. Furthermore, VVC was more prevalent in pregnant females while BV and mixed infections (MI) were more prevalent in non-pregnant females. High prevalence of NAC species in pregnant women indicates their emergence as opportunistic pathogens in immunocompromised conditions. Therefore, screening of pregnant women for differential *Candida* species can be helpful in better diagnosis of VVI and in providing better antenatal care.

Acknowledgments

The authors wish to thank Mr. Kanwaljit Kumar, Department of Emerging Life Sciences, Guru Nanak Dev University, Amritsar for SEM work. One of the authors, Namarta Kalia, is thankful to University Grants Commission, New Delhi for providing research fellowship.

References

Alli, J.A.O., Okonko, I.O., Odu, N.N., Kolade, A.F., Nwanze, J.C. 2011. Detection and prevalence of *Candida* isolates among patients in Ibadan, Southwestern. *J. Microbiol. Biotech. Res.,* 1: 176–184.

Amsel, R., Totten, P.A., Spiegel, C.A., Chen, K.C., Eschenbach, D., Holmes, K.K. 1983. Nonspecific vaginitis: Diagnostic criteria and microbial and epidemiological associations. *Am. J. Med.,* 74: 14–22.

Atashili, J., Poole, C., Ndumbe, PM., Adimora, A.A., Smith, J.S. 2008. Bacterial vaginosis and HIV acquisition: a meta analysis of published studies. *AIDS,* 22: 1493–1501.

Bandara, H.M., Yau, J.Y., Watt, R.M., Jin, L.J., Samaranayake, L.P. 2010. *Pseudomonas aeruginosa* inhibits in-vitro *Candida* biofilm development. *BMC Microbiol.,* 10: 125–133.

Bauters, T.G., Dhont, M.A., Temmerman, M.I., Nelis, H.J. 2002. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. *Am. J. Obstet. Gynecol.,* 3: 569–574.

Chaudhary, V., Prakesh, V., Agarwal, K.,

Agrawal, V., Singh, A., Pandey, A. 2012. Clinicomicrobiological profile of women with vaginal discharge in a Tertiary care hospital. *Int. J. Med. Sci. Public Health*, 1: 75–80.

Corsello, S., Spinillo, A., Osnengo, G., Penna, C., Guaschino, S., Beltrame, A., Blasi, N., Festa, A. 2003. An epidemiological survey of vulvovaginal candidiasis in Italy. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 110: 66–72.

Ferreira, J.A., Carr, J.H., Starling, C.E., de Resende, M.A., Donlan, R.M. 2009. Biofilm formation and effect of caspofungin on biofilm structure of *Candida* species bloodstream isolates. *Antimicrob. Agents Chemother*., 53: 4377–4384.

French, L., Horton, J., Matousek, M. 2004. Abnormal vaginal discharge: what does and does not work in treating underlying causes. *J. Fam. Pract.*, 53: 805–814.

Fule, S.R., Fule, R.P., Tankhiwale, N.S. 2012. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J. Med. Microbiol.*, 30: 314–316.

García, P.J., Carcamo, C.P., Chiappe, M., Holmes, K.K. 2007. Sexually transmitted and reproductive tract infections in symptomatic clients of pharmacies in Lima, Peru. *Sex Transm. Infect.*, 83: 142–146.

Gibney, L., Macaluso, M., Kirk, K., Hassan, MS., Schwebke, J., Vermund, S.H., Choudhury, P. 2001. Prevalence of infectious diseases in Bangladeshi women living adjacent to a truck stand. *Sex Transm. Infect.*, 77: 344–350.

Grigoriou, O., Baka, S., Makrakis, E., Hassiakos, D., Kapparos, G., Kouskouni, E. 2006. Prevalence of clinical vaginal candidiasis in a university hospital and possible risk factors. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1: 121–125.

Gupta, V., Gupta, P., Chatterjee, B., Bansal, R. 2009. Clinicomicrobiological profile of women with vaginal discharge. *J. Indian Med. Assoc.* 107: 164–166.

Guzel, A.B., Ilkit, M., Akar, T., Burgut, R., Demir, S.C. 2011. Evaluation of risk factors in patients with vulvovaginal candidiasis and the value of chromID *Candida* agar versus CHROMagar *Candida* for recovery and presumptive identification of vaginal yeast species. *Med. Mycol.*, 1: 16–25.

Hamad, M., Kazandji, N., Awadallah, S., Allam, H. 2014. Prevalence and epidemiological characteristics of vaginal candidiasis in the UAE. *Mycoses*, 57: 184–190.

Hay, P.E., Lamont, R.F., Taylor-Robinson, D., Morgan, D.J., Ison, C., Pearson, J. 1994. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ*, 308: 295–298.

Hedayati, MT., Taheri, Z., Galinimoghadam, T., Aghili, SR., Yazdani Cherati, J., Mosayebi, E. 2014. Isolation of different species of *Candida* in patients with vulvovaginal candidiasis from Sari, Iran. *Jundishapur J. Microbiol.*, 8: 1–5

Holland, J., Young, M.L., Lee, O., Chen, S.C.A. 2003. Vulvovaginal carriage of yeasts other than *Candida albicans*. *Eur. J. Obstet. Gynecol. Reprod. Biol*., 79: 249–250.

Joshi, K.R., Wheeler, E.E., Gavin, J.B. 1973. Scanning electron microscopy of colonies of six species of *Candida*. *J. Bacteriol.*, 115: 341–348.

Kamara, P., Hylton-Kong, T., Brathwaite, A., Del Rosario, G.R., Kristensen, S., Patrick, N., Weiss, H., Figueroa, P.J., Vermund, S.H., Jolly, P.E. 2000. Vaginal infections in pregnant women in Jamaica: prevalence and risk factors. *Int. J. STD AIDS,* 11: 516–520.

Larsson, P.G., Forsum, U. 2005. Bacterial vaginosis--a disturbed bacterial flora and treatment enigma. *APMIS,* 113: 305–16.

Lennox, J.A., Abbey, S.D., Udiba, D., Mboto, C.I., Ikpoh, I.S., Akubuenyi, F.C. 2013. Prevalence of vaginitis and vaginosis among University of Calabar female students. *J. Public Health Epidemiol.,* 5: 167–172.

Leon, M.D., Jacober, S.J., Sobel, J.D., Foxman, B. 2002. Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *BMC Infect Dis.,* 2: 1471–2334.

Luglio-Agosto, 2005. Vaginal infections: epidemiology and risk factors *Giorn. It. Ost. Gin.,* 28: 263–265.

McClelland, R.S., Sangare, L., Hassan, W.M., Lavreys, L., Mandaliya, K., Kiarie, J., Ndinya-Achola, J., Jaoko, W., Baeten, J.M. 2007. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. *J. Infect. Dis.,* 195: 698–702.

Mintz1, J.D., Martens, M.G. 2013. Prevalence of non-*albicans Candida* infections in women with recurrent vulvovaginal symptomatology. *Adv. Infect. Dis.,* 3: 238–242.

Mobashaeri, M., Varnamkhast, N.S., Karimini, A., Banaeiyan, S. 2014. Prevalence study of genital tract infections in pregnant women referred to health centers in Iran. *Turk. J. Med. Sci.,* 44: 232–236.

Nelson, K.M., Wanjiru, W., Margaret, M.W. 2013. Prevalence of vaginal candidiasis and determination of the occurrence of *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital. *Open J. Med. Microbiol.,* 3: 264–272.

Nugent, R.P., Krohn, M.A., Hillier, S.L. 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J. Clin. Microbiol.,* 29: 297–301.

Olowe, O.A., Makanjuola, O.B., Olowe, R., Adekanle, D.A. 2014. Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. *Eur. J. Microbiol. Immunol.,* 4: 193–197.

Patel, D.A., Burnett, N.M., Curtis, K.M. 2003. Reproductive tract infections. *Reprod. Health Epidemiol. Series Module,* 3: 1–83.

Ralph, S.G., Rutherford, A.J., Wilson, J.D. 1999. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ,* 319: 220–223.

Rekha, S., Jyothi, S. 2010. Comparison of visual, clinical and microbiological diagnosis of symptomatic vaginal discharge in the reproductive age group. *Int. J. Pharm. Biomed. Res.,* 1: 144–148.

Richter, S.S., Galask, R.P., Messer, S.A., Hollis, R.J., Diekema, D.J., Pfaller, M.A. 2005. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J. Clin. Microbiol.,* 43: 2155–2162.

Sharma, M., Solanki, A. 2014. Prevalence of *Candida* infection in pregnant

women with and without diabetes. *Int. J. Curr. Microbiol. App. Sci.*, 3: 605–610.

Sherrard, J., Donders, G., White, D., Jensen, J.S. 2011. European (IUSTI/WHO) Guideline on the management of vaginal discharge. *Int. J. STD AIDS*, 22: 421–429.

Shrestha, S., Tuladhar, N.R., Basnyat, S., Acharya, G.P., Shrestha, P., Kumar, P. 2011. Prevalence of vaginitis among pregnant women attending Paropakar Maternity and Women's Hospital. *Med. Coll. J.*, 13: 293–296.

Singh, S., Sobel, J.D., Bhargava, P., Boikov, D., Vasquez, J.A. 2002. Vaginitis due to *Candida krusei*: epidemiology, clinical aspects, and therapy. *Clin. Infect. Dis.*, 35: 1066–1070.

Singhai, M., Malik, A., Shahid, M., Malik, M.A., Goyal, R. 2012. Characterization of fungal biofilm-based catheter-related sepsis. *Chron. Young Sci.*, 3: 48–52.

Sivaranjini, R., Jaisankar, T.J., Thappa, D.M., Kumari, R., Chandrasekhar, L., Malathi, M., Parija, S.C., Habeebullah, S. 2013. Spectrum of vaginal discharge in a tertiary care setting. *Trop. Parasitol.*, 3: 135–139.

Sobel, J.D. 1988. Pathogenesis and epidemiology of vulvovaginal candidiasis. *Ann. NY. Acad. Sci*., 544: 547–557.

Sobel, J.D. 1997. Vaginitis. *N. Engl. J. Med.*, 337: 1896–1903.

Sobel, JD. 2007. Vulvovaginal candidosis. *Lancet*, 369: 1961–1971.

Spinillo, A., Capuzzo, E., Gulminetti, R., Marone, R., Colonna, L., Piazzi, G. 1977. Prevalence and risk factors for fungal vaginitis caused by non-*albicans* species. *Am. J. Obstet. Gynecol.*, 1: 138–141.

Stelzner, A. 1990. F.C. Odds, *Candida* and Candidosis: A review and bibliography. *J. Basic Microbiol.*, 30: 382–383.

Thibane, V.S., Kock, J.L., Ells, R., van Wyk, P.W., Pohl, C.H. 2010. Effect of marine polyunsaturated fatty acids on biofilm formation of *Candida albicans* and *Candida dubliniensis*. *Mar. Drugs*, 8: 2597–2604

Thulkar, J., Kriplani, A., Agarwal, N., Vishnubhatla, S. 2010. Aetiology & risk factors of recurrent vaginitis & its association with various contraceptive methods. *Indian J. Med. Res.*, 131: 83–87.

Vermitsky, J.P., Self, M.J., Chadwick, S.G., Trama, J.P., Adelson, M.E., Mordechai, E., Gygax, S.E. 2008. Survey of vaginal flora *Candida* species isolates from women of different age groups by use of species-specific PCR detection. *J. Clin. Microbiol.*, 46: 1501–1503.

Vijaya, D., Dhanalakshmi, T.A., Kulkarni, S. 2014. Changing trends of vulvovaginal candidiasis. *J. Lab. Physicians*, 6: 28–30.

Weissenbacher, T., Witkin, S.S., Ledger, W.J., Tolbert, V., Gingelmaier, A., Scholz, C., Weissenbacher, E.R., Friese, K., Mylonas, I. 2009. Relationship between clinical diagnosis of recurrent vulvovaginal candidiasis and detection of *Candida* species by culture and polymerase chain reaction. *Arch. Gynecol. Obste.*, 279: 125–129.

Zeng, J., Zong, L.L., Mao, T., Huang, Y.X., Xu, Z.M. 2011. Distribution of *Candida albicans* genotype and *Candida* species is associated with the severity of vulvovaginal candidiasis. *J. South Med. Univ.*, 10: 1649–1653.