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Prevalence of Vaginal Candidiasis among Pregnant Women Attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia

Khadijah Yosif Al-Aali

Assistant Professors, Microbiology Department, faculty of Medicine, Taif University, KSA

Abstract: ***This study was intended to determine the prevalence of Candida albicans causing vaginitis in pregnant women and their susceptibility to current antifungal drugs from Western Region, Taif, Saudi Arabi. A total 1207 samples of high vaginal swab were collected from pregnant women between the ages of (15-44) years attending ante-natal clinic and between 35 and 37 of weeks of gestation, from February to October 2014. Standard microbiological methods were used to isolate and identify Candida from vaginal swabs obtained from study subjects. An antimicrobial susceptibility test was performed for all Candidaisolates according to the criteria of the Clinical and laboratory Standards Institute. The result was, A total of 848 (70, 2%) isolates were obtained which comprises six different Candida species, namely Candida albicans, C. glabrata, C. tropicalis, Candida lipolytica, Candidalusitaniae, and Candida kefyr with frequency of occurrence of (848, 70, 2%), (200, 16.5%), (40, 3.3%), (32, 2.6%), (16, 1.3%), and (8, 0.0.6%) respectively. Different type of non Candida were isolated and found in very low percentage Cryptococcus laurentii, Saccharomyces cerevisiae, Stephanoascusciferrii, and Trichosporonasahii. All isolates were tested against antifungal agents, and all Candida spp., non-albicans were susceptible to ketocanazole and Fluconazole. These results were the first record of the database of VVC in pregnant women in Saudi Arabia population at western region, Taif.***

Keywords: antenatal, *Candida albicans*, *Candida*non albicans, ketoconazole, fluconazol, pregnancy, Vulvovaginal Candidiasis.

1. Introduction

Vaginal candidiasis is a fungal infection caused by overgrowth of *Candida* species affecting the genital tract as opportunistic pathogen. Vaginal candidiasis (VC) is a common type of vaginitis, a gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching.[1].Vaginal candidiasis is a common complaint among women of different age groups, regardless of their sexual activities and can be possible risk for other diseases e.g. HIV/AIDS [2-5].This infection progresses as colonization, superficial infection and hematogenous dissemination to different organ [3, 6-7].Reports show that about 75% of all women, experience at least one episode of which physician approved to be candidiasis in their lifetime [5, 8, 9].

Candida spp. are the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Vaginal candidiasis is a frequent companion of pregnancy, which greatly complicates the course of the pregnancy and threatens the health of both mother and child [10].Vaginal candidiasis (VC) is a common type of vaginitis, a gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching. The incidence of vaginal candidiasis is almost doubled (particularly in the second and third trimester) among pregnant women, due to high production or changes in the levels of sex hormones and deposition of glycogen in the vagina during pregnancy [11].Among *Candida* spp., *Candida albicans*is the most common infectious agent. Non-*C.albicans* species are emerging pathogens and can also colonize human mucocutaneous surfaces [12]. Consequently, they are also isolated in the setting of candidiasis, albeit at a lower frequency.

The pathogenesis and prognosis of candidial infections are affected by the host immune status and also differ greatly according to disease presentations. Therefore, diagnosis, management, and treatment choices vary and need to be considered in the overall setting of the affected human host. At least 75% women suffer once in their life from one episode of a candida infection [13-15].Although *Candida albicans*is the pathogen identified in most patients with vulvovaginal candidiasis, other possible pathogens include *Candida tropicalis, Candida glabrata* amongst others, which are responsible for up to 33 percent of recurrent infections [16-18].

Candida tropicalis and *glabrata* are the most important of the *non-C albicans* infections [19, 20].Although some non-*albicans* species like *Candida tropicalis*, *Candidalipolytica*, *Candida lusitaniae*, *Candidakefyr* [21].Also *Cryptococcus laurentii*, *Trichosporonasahii*, *Stephanoascus*ciferrii, *Saccharomyces cerevisiae*are recovered from infected individuals, and can cause yeast vaginitis[10, 22].Relatively higher antifungal resistance rate of non- *C albicans species* may contribute to higher rates of recurrent infections. This increase in non- *C. albicans Candida* vulvovaginitis has been attributed to overuse of antifungal therapy, which has resulted in the elimination of the more sensitive *C.albicans* and selection of other, more resistant species [23, 24]. Secretory acid proteinase production has been associated with yeast pathogenicity [25]. These enzymes are implicated in the persistence and colonization of the vaginal tract [26-28].They also facilitate tissue penetration and cleavage of immunoglobulin A (IgA), which is an important factor in

vaginal immunity [29].The lack of specificity of symptoms and signs therefore precludes a diagnosis that is based on history and physical examination without the corroborative evidence of laboratory tests (CDC, 2010) [30].

Thus, over 80% patients, referred by physicians with a putative diagnosis of VVC were found to have some other cause of vaginitis, and therefore most patients fail to respond to antifungal therapy cause of incorrect diagnosis [31] Therefore, rapid and specific detection and identification of *Candida* species will help to choose the suitable antifungal and improve patient care.

This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids, birth control pills and hormonal drugs and nutrient deficiency [10, 32]. Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defense mechanisms [10, 33-37].The dynamic environment inside the host may include factors such as temperature, pH, and the normal flora and its metabolic products. These factors, along with the oxidative stresses associated with host defense mechanisms, require physiologic adaptation in the pathogenesis of *C. albicans*. Temperature enhances the virulence of several bacteria and influences fungal morphogenesis [38].Increased temperature, pH, and steroids have also been shown to induce the yeast-to-hyphal transformation of C. abicans, 4-6 which in turn is linked to the virulence of this organism [39].This immune imbalance is caused by a number of factors, such as excess stress, allergies, indiscriminate use of antibiotics, steroids, birth control pills and hormonal drugs and nutrient deficiency [10, 32]. Diabetes mellitus, pregnancy, and the use of tight nylon underwear also enhance overgrowth of *Candida* in a manner that cannot easily be controlled by the body's defence mechanisms [9, 34-36].

This study was intended to determine the prevalence of *Candida albicans*ca using vaginitis in pregnant women and their susceptibility to current antifungal drugs from Western Region, Taif, Saudi Arabia In the Kingdom of Saudi Arabia, there is few of information regarding *Candida albicans* causing vaginitis in pregnant women, in Ryiadh [40, 41], Makkah [42], and Al-Madinah Al-Munawarah [43, 44].This paper was the first data on incidence of *Candida albicans* causing vaginitis in in pregnant women at Western Region, Taif, Saudi Arabia.

2. Materials and Methods

2.1. Study population

A total of (1207) pregnant women attending the routine antenatal clinic of the Al-Hada Armed Forces Hospital were screened for yeast in routine examination The samples were collected during (9) months, starting from February (2014) to October (2014).The approach was based on universal screening of all pregnant women for yeast infection between 35 and 37 of weeks of gestation [11, 14].The age of the women's between 15-44 Year

2.2. Sample Collection

A total 1207 samples of high vaginal swab were collected from asymptomatic and symptomatic pregnant women between the ages of (15-44) years attending ante-natal clinic. All women who gave a history of taking anti-fungal agents during the earlier 2 weeks prior to the study were excluded from the study. A verbal consent was obtained from each participant who also completed a questionnaire with demographic data and possible risk factors such as age, pregnancy, occupational, diabetes, and history of vaginitis, abortion, antifungal use and smoking [45-49]. Collection of vaginal swab was done by exposing the posterior fornix with a sterile vaginal speculum (Coscos). All genital swabs collected in Amies transport medium received from women during the study period were included. Two vaginal swabs were collected from each patient and transpoted to the microbiology laboratory at Al-Hada Military Hospital for processing.

2.3. Macroscopic examination of samples

Each sample was examined for colour, appearance and odour and described as whitish or whitish-gray colour, cottage cheese-like discharge and odourless. The one swab was used for microscopy for wet preparation and gram stain while the other one was used for culture onto Sabouraud's dextrose agar (Saudi Prepared Media Laboratory, Saudi Arabia, Riyadh (SPML) and incubated at 37° C for 18–24 hours to see the growth of creamy, greyish moist colonies.

2.4 Microscopic examination of samples

Saline wet mount preparation of the colony was made to see budding cells and psudohyphae. The plated were read after incubation and all colonies suspected of having *Candida* morphology were gram stained.

2.5. Species identification

The species identification was based on germ tube test, sugar assimilation test, sugar fermentation test according to standard methods described earlier[50], and According to guidelines of the CDC, Sexually Transmitted Diseases Treatment Guidelines (2010) [50].Identified isolates were stored on nutrient agar slant at room temperature for subsequent susceptibility testing.

2.6. Germ-tube test

Small inoculum of suspected *Candida* cultures were inoculated into 1 ml of human serum (Sigma-Aldrich, Germany) in a small tube and incubated at 37° C for 2 hours. After incubation, a loop-full of culture was placed on a glass slide, overlaid with a cover-slip and examined microscopically for the presence or absence of germ-tubes. Formation of germ tubes was seen as long tube like projections extending from the yeast cells with no constriction or septa at the point of attachment to the yeast cells[46-49].Biochemical identifications were also performed on all isolates, and colonies were identified to species level using Vitek YBC cards (Vitek Systems, BioMerieux).

2.7 Susceptibility testing

The isolated yeasts (1207 strains) were also tested for their *in vitro* susceptibility towards fluconazole and

ketoconazole in accordance with the proposed guidelines for antifungal disk diffusion susceptibility testing of yeasts contained in the CLSI document M-44A (CLSI, 2004). A reference strain of *C. albicans* ATCC 25285 was included as control. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that yielded no bacterial growth [51].The plates were incubated at 35°C, and inhibition zone diameters were measured after 24 and 48 h especially for *C. glabrata*. The interpretive criteria for the fluconazole disk test were as those previously described by CLSI (2004): dz ≥19mm-susceptible; 15<dz<18 mm-susceptible dose dependent and dz≤14mm-resistant. As for ketoconazole: dz ≥20mm-susceptible, 20<dz<10mm-intermediae and dz<10mm-resistant [52].

2.8. Data analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS), Version 16 for Windows. Continuous variables were summarized using descriptive statistics in terms of means± standard deviations; 95% confidence intervals (95% CI), minimums and maximums, while a Chi-square test were used to compare categorical variables. A *p*-value < 0.05 were considered significant. The analysis was performed with Statistical Package for Social Sciences (SPSS) software version 15 (SPSS, Inc., Chicago, IL, USA).Prevalence figures were calculated for the total study population and separately by age groups. Occupation, number of antenatal clinic visit (ANC), and type of gravida also recorded. Chisquare test was used to compare results between the pregnant women with different age groups. Statistical methods, descriptive statistics included statements of frequency with percentages, means, standard deviations (SD, and differences were considered significant if p<0.05 with confidence intervals (95% CI)

3. Results

3.1. Incidence of vaginal candidiasis among pregnant women

Total of (1207) samples were collected for isolation and identification of *Candida* species and other yeast from both symptomatic and asymptomatic pregnant women. 848 of the 1207 patients examined were positive for *Candidaalbicans*, which gave an incident rate of 70.2 % (Table 1).

Candida glabrata followed in prevalence with carriage rate (200, 16.5%). Other *Candida* species were found in low percentage as follows: *Candida tropicalis* (40, 3.3%), *Candida* lipolytica (32, 2.6%), *Candida lusitaniae* (16, 1.3%), *Candida kefyr* (8, 0.6%). Different type of non *Candida* isolated from pregnant women also found in very low percentage as follows *Cryptococcus laurentii*, *Saccharomyces cerevisiae* (24, 1.9%), *Stephanoascusciferrii*, and *Trichosporonasahii* (8, 0.6%).

Table 1: Different type of *Candida* species, and non *Candida* isolated from pregnant women (*n*=1207)

Different type of Candida isolated from pregnant women		
Name	*Frequency*	*Percent (%)*
Candida albicans	848	70.2
Candida glabrata	200	16.5
Candida tropicalis	40	3.3
Candida lipolytica	32	2.6
Candida lusitaniae	16	1.3
Candida kefyr	8	0.6
Different type of non Candida isolated from pregnant women		
Name	*Frequency*	*Percent (%)*
Saccharomyces cerevisiae	24	1.9
Cryptococcus laurentii	24	1.9
Stephanoascusciferrii	8	0.6
Trichosporonasahii	8	0.6
All Total	1207	100

3.2. Socio-demographic characteristics

The socio-demographic characteristics of (1207) pregnant women screened for yeast infection are presented in Table 2.

3.2.1. Age groups in years

The highest occurrence of *Candida* species (68.3%) was recorded between ages 25-29 followed by ages 30-34 with percentage occurrence of (9.6%) while the lowest occurrence (2.4%) was recorded between age group 40-44 (table. 2).

Table 2: Socio-demographic characteristics of 1207 women attending obstetric clinic investigated for yeast, at AL-Hada Armed Forces Hospital in Taif, SA, 2014

Socio-demographic Characteristics	*Frequency*	*Percent (%)*	*P value*
1- Age groups in years			
15-19	62	5.1	
20-24	80	6.6	
25-29	825	68.3	
30-34	116	9.6	
35-39	95	7.8	
40-44	29	2.4	
Total	1207	99.8	
2-Occupation			
House wife	94	7.7	
Teacher	925	76.6	
Student	91	7.5	0.04
Nurse	57	4.7	0.01
Doctor	41	3.3	
Total	1207	100	

3.2.2. Occupation

The prevalence of *Candida* was studied in relation to Occupation. The highest occurrence of *Candida* was found in the teacher (76.6%) followed by house wife (94, 7.7%), and student (91, 7.5%) *P*0.04, while the least occurrence was found in the nurse (57, 4.7) *P* 0.01, and doctor (41, 3.3%) as shown in Table 2**.**

The present data shown, there is no statistically significant association of *Candida albicans* was observed in connection with any of the socio- demographic

characteristics of the study subjects by age groups in years. As shown in Table 2

Table 3: Variables associated/not associated with *Candida albicans* in pregnant women attending obstetric clinic investigated for yeast, at AL-Hada Armed Forces Hospital in Taif, SA, 2014

Variables	Total N=848	*Candida albicans*	Percentages (%)	P value
1- Age (in years)				
15-19	61	59	10.1	
20-24	80	71	12.2	
25-29	469	220	37.9	
30-34	115	112	19.3	
35-39	94	91	15.7	0.01
40-44	29	26	4.4	
Total	848	579	100	
2-**Occupation**				
House wife	151	127	21.9	
Teacher	301	112	19.3	
Student	181	160	27.6	
Nurses	163	151	26	0.007
Doctors	52	29	5	
Total	848	579	100	
3-Number of ANC Visit				
One time	120	118	20.3	
Two times	398	205	35.3	
Three times	203	146	25.2	
Four times	127	110	18.9	0.006
Total	848	579	100	
4-**Type of Gravida**				
Primigravida	265	198	34.1	
Multigravida	583	381	65.8	
Total	848	579	100	

The highest occurrence of *Candida* species (37.9%) was recorded between ages 25-29 followed by ages 30-34 with percentage occurrence of (19.3%) while the lowest occurrence (4.4%) was recorded between age group 40-44 (table.3).

3.2.3. Variables associated/not associated with *Candida albicans* in pregnant women

Most of women under investigation were in age25-29 (220, 37.9%).The prevalence of *Candida* was studied in relation to Occupation. The highest occurrence of *Candida* was found in the teacher (76.6%) followed by house wife (94, 7.7%), and student (91, 7.5%) *P*0.04, while the least occurrence was found in the nurse (57, 4.7) *P* 0.01, and doctor (41, 3.3%) as shown in Table 2**.** There is statistically significant association of *Candida albicans* was observed in connection with any of the socio-demographic characteristics of the study subjects by age groups in years35-39 (91, 15.7%) *P*0.01.There is statistically significant association of *Candida albicans* was observed in connection with any of the socio-demographic characteristics of the study subjects by occupation in nurses (151, 26%) *P*0.007

In contrast, there is also statistically significant association of *Candida albicans* was observed in number of ANC Visit in four times (110, 18.9%) P 0.006.Statistically no significant association of *Candida albicans* was observed in connection with any of the socio- demographic characteristics of the study subjects bytype of gravida, eitherprimigravida nor multigravida.

Table 4: Comparative susceptibilities of *Candida albicans* and non-albicans spp. against fluconazole and ketoconazole

Antifungal-agent	*Classified as*	*C. albicansn=848 (%)*	*Non-albicans n=359 (%)*
Ketoconazole	S	846 (99)	359 (100)
	I	2.0 (0.23)	0.0 (0.0)
	R	0.0 (0.0)	0.0 (0.0)
Fluconazole	S	839 (98)	348 (96.9)
	I	8.0 (0.94)	3.0 (0.83)
	R	1.0 (0.11)	8 (2.22)

Results of this study on the antifungal activity of tested agents (Table 4) indicated that all *Candida* spp. tested against ketocanazole and Fluconazole were susceptible (99%), (98%) of C. *albicans (*1207/848) strains respectively. Non-*albicans* were also susceptible to Ketoconazole and fluconazole (100%), (96.9%) respectively. Exhibited resistance to fluconazole predominantly *C. glabrata*

4. Discussion

This study investigated the prevalence of vaginal candidiasis among pregnant women attending antenatal clinic over the period of nine (9) months in Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. The study showed high prevalence (848, 70.2%) of vaginal candidiasis among pregnant women. The high prevalence of candidiasis reported was similar to the findings of [53, 54, 55] who reported a prevalence rate of 60%, 62.2% and 67% respectively among pregnant women in Jos and Enugu State, Nigeria. The finding is however in contrast to the observations of [33] who reported a frequency of 38%.

The high prevalence of vaginal candidiasis among pregnant women attending antenatal clinic in Al-Hada Military Hospital, may be due to inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits, shortage of effective treatment, increased levels of estrogens and corticoids, wearing of tight-fitting synthetic underclothing, prolonged use of antibiotics which kill the good and beneficial bacteria [33, 56, 57].This high prevalence may result into the risk of miscarriage, premature birth, fetal oxygen deficiency, low birth weight and neonate mortality, wound infection after childbirth and systemic inflammation. Other possible risks include pelvic inflammatory disease, infertility, pelvic abscess, stress, discomfort and irritation[58].In the present study the overall prevalence of *Candida albicans* among pregnant women was found to be 70.2%. Similar findings have been reported in Makkah City, Saudi Arabia *C. albicans* affecting 163 (72.4%) [42].While in Al-Madinah Al-Munawarahincidence of *C. albicans* and carnage rate higher than other cities 98 (81.7%) [43].

The highest prevalence of vaginal infections in this study was noted among the age groups 25-29 years 220 (37.9%), followed by 35–39 age group 91 (15.7%).This report agreed with [59], which reported a peak vaginal infections between age group 20 and 40. This may be due to high sexual activity, poor personal hygiene, the use of contraceptives and drug abuse among this age group. Among the 70.2% pregnant women found to be positive with vaginal candidiasis, 76% were found to be teacher (Occupation), while (91, 7.5%) was found to be Student. In contrast nurses have significant in VVS (57, 4.7%) *P* 0.01. This high prevalence among teacher may be due to the use of contraceptive and antibiotics or other reasons [60].

The data in the present study showed that, Number of ANC Visit (four time) have significant in VVS (110, 18.9%) *P* 0.006.Among the 70.2% pregnant women found to be positive with vaginal candidiasis, 34.1% were found to be primvigravidae (women with first pregnancy) while 65.85% was found to be multigravidae (women that has experience of more than one pregnancy). This high prevalence among the multigravidae may be due to the use of contraceptive and antibiotics.

This is similar to the findings of [58] who reported an occurrence of 60% among the multigravidae and 40% among privigravidae among pregnant women (patients) in Department of Pathology, AllamaIgbal Medical College, Lahore- Pakistan. *C. albicans* had the highest occurrence (70.2%), *C. glabrata* found next after *C. albicans* with 16.5% (9), *C. tropicalis* with 3.3% (6),) %), Other *Candida* species were found in low percentage. *Candida tropicalis* (40, 3.3%), *Candida* lipolytica (32, 2.6%), *Candida lusitaniae* (16, 1.3%), *Candida kefyr* (8, 0.6%) in this study which is quite comparable with the reports of [61-62]. This is similar to the reports of [63] in India and [64] in Iran.

The high occurrence rate (72.2%) of *C. albicans* observed in this study as shown in figure 2 is an indication that it is a leading causative agent of the reproductive tract yeast infections in women of child bearing age as also observed by [65].This may be due to its virulent factors which include dimorphism and phenotypic switching. *Candida albicans* produces protease and phosphatase which enhance its attachment to human epithelium.

It can also be deduced that the high incidence rate of *C. albicans* could be due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favor its growth during pregnancy. However, Wise *et al*. [66] and Trofa*et al.* [67] reported a low occurrence of *C. albicans*in New York. The low occurrence of *C. albicans* reported by Wise *et al*. [66] and Trofa*et al.* [67] may be as a result of good personal hygiene, appropriate nutrition, adequate diagnostic facilities and treatment. Furthermore, our data on the antifungal activity of fluconazole against *C. albicans*, revealed that 98% of tested strains were susceptible. This sensitivity rate is more or less comparable with those reported by [68-70].

In agreement with the study of [71], most of the detected resistant strains belong to *C. glabrata*, emphasizing, and its greatest potential to acquire resistance to fluconazole. Resistance to fluconazole in clinical isolates of *C. tropicalis* has increased [72-74].The precise mechanisms responsible for drug resistance in *Candida* species have been described [75].*Saccharomyces cerevisiae*, the baker's yeast, causes disseminated infections in immunocompromised hosts [76, 77].VVC is infrequently caused by *C. tropicalis*, and *Candida lipolytica*, *Candida lusitaniae*, *Candida kefyr*, although most species of *Candida* have been associated with condition [78, 5]. VVC induced by NCAC is clinically indistinguishable from the caused by *C. albicans*; moreover, such species are often more resistant to treatment [79].

5. Conclusion

The results of this study showed a high occurrence of *Candida* among pregnant women attending Al-Hada Military Hospital, Western Region, Taif, Saudia Arabia.*C. albicans* was the most prevalent among the isolates with high prevalence of vaginal candidiasis. The overall results of this study emphasize the importance of the different risk factors that play a role in *Candida*-colonization. The culture of vaginal discharge should be warranted because culture technique is more sensitive than direct smear. These findings may help in drawing strategies in preventing and controlling vulvovaginal candidiasis that may affect women and their newborns. Use of fluconazole should not be continued in patients with recurrent *Candida* infections.

6. Recommendations

1. Rapid species identification from clinical specimens and standard drug susceptibility testing would be an effective approach for controlling nosocomial outbreaks caused by C. tropicalis or CNA species in different clinical settings.
2. Early diagnosis and adequate treatment of vaginal candidiasis should be practiced.
3. Consistent application of standard techniques, guidelines for the use of antifungal agents and control measures for predisposing factors or risk factors may potentially reduce the risk of drug-resistant life-threatening infections.
4. The need for regular check-up at different gestation period should be encouraged.
5. Drug abuse among pregnant women should be discouraged
6. Application of standardized technology and its consistent use in diagnostics and research still remains a major global challenge.

References

[1] Richards, M. J., J. R. Edwards, D. H. Culver, and R. P. Gaynes. ACHKAR AND FRIES CLIN. MICROBIOL. REV. Nosocomial infections in combined medical-surgical intensive care units in the

United States. Infect. Control Hosp. Epidemiol. 21: 510–515. 2000.
[2] Irving G, Miller D, Robinson A, Reynolds S, Copas AJ. Psychological factors associated with recurrent vaginal candidiasis: a preliminary study**.** Sex TransmInf; 74: 334–338. 1998
[3] Lindau ST, Mendoza K, Surawska H, Jordan JA. Vaginal Swab Measurement of Candidiasis in Wave I of the National Social Life, Health & Aging Project (NSHAP), NORC and the University of Chicago. 2007.
[4] Nwadioha SI, Egah DZ, Alao OO, Iheanacho E. Risk factors for vaginal candidiasis among women attending primary health care centers of Jos, Nigeria. *Nigeria J Clin Med Res*;2 (7): 110-113. 2010
[5] Center for Disease Control and Prevention, CDC. Genital / Vulvovaginal Candidiasis (VVC) online at http: //www. cdc. gov/fungal/candidiasis/genital. 2012.
[6] Arora DR, Arora B. *Textbook of Microbiology*. 3rdEdn. CBS, India, pp. 653-675. 2008.
[7] Nviriesy P. Yeast Infection. *Infect Dis Clin North Am* 22: 637-652. 2008
[8] White D, Robertson C. United Kingdom National Guideline on the Management of Vulvovaginal Candidiasis Clinical Effectiveness Group British Association of Sexual Health and HIV, 1-17. 2008.
[9] Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. *Nigeria J Med Medical Sci*1 (10): 447-452. 2010
[10] Giraldo PC, A raújo ED, Junior JE, Amaral RLG, Passos MRL, Gonçalves AK. The prevalence of urogenital infections in pregnant women experiencing preterm and full-term labor. *Inf Dis ObsGynecol*1-4. 2012
[11] Mikolajczyk K, Zimmer M, Tomialowicz M, Fuchs T. *MikologiaLekarska* 13 (3): 175-179. 2006
[12] Sobel, J. D. The emergence of non-albicans*Candida* species as causes of invasive candidiasis and candidemia. *Curr. Infect. Dis. Rep*. 8: 427–433. 2006.
[13] Weissenbacher T. M, Witkin S. S, Gingelmaier A, et al. Relationship between recurrent vulvivaginalcandidosis and immune mediators in vaginal fluid. *Eur J of Obs&Gyn and Rep Biol* 144: 59-63. 2009
[14] Mardh P. A, Rodrigues A. G, Genc M, Novikova N, Martinez-de-Oliviera J, GuaschinoS. Facts and myths on recurrent vulvovaginalcandidosis – a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. *Int STD AIDS*. 13 (8): 522-39. 2002
[15] Nyirjesy P. Chronic vulvovaginal candidiasis. *Am FamPhysician* 63 (4): 697-702. 2001
[16] Nyirjesy P, Seeney SM, Grody MH, Jordan CA, Buckley HR. Chronic fungal vaginitis: the value of cultures. *Am J ObstetGynecol* 173: 820-3. 1995
[17] Holland J. , Young, M. L. , Lee, O. and Chen, S, C-A. Vulvovaginal carriage of yeasts other than *Candida albicans*. *Sexual TransmInfect* 79: 249-250. 2003
[18] Onifade, A. K. and Olorunfemi, O. B. Epidemiology of vulvo-vaginal candidiasis in female patients in Ondo State Government Hospitals. *J Food, Agric and Environ*. 3: 118-199. 2005
[19] Spinillo A, Capuzzo E, Gulminetti R, Marone P, Colonna L, Piazza G. Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. *Am J Obst Gynecol*. 176: 138-41. 1997
[20] Neerja, J. , Aruna, A. and Paramjeet, G. Significances of *Candida* culture in women with vulvovaginal symptoms. *J obstetGynecolIndia* 56: 139-141. 2006
[21] Saleh S. A. Al-Hedaithy. Spectrum and proteinase production of yeasts causing vaginitis in Saudi Arabian women. *Med SciMonit* 8 (7): 498-501. 2002
[22] AnaulKabirM. , Mohammad AsifHussain, and Zulfiqar Ahmad. *Candida albicans*: A Model. Organism for Studying Fungal Pathogens. *International Scholarly Research Network*. Volume 2012, Article ID 538694, 15 pages. 2012
[23] Denning DW: Can we prevent azole resistance in fungi? Lancet, 346: 454-455. 1995
[24] Sobel JD, Faro S, Force RW et al: Vulvovaginal Candidiasis: Epidemiologic, diagnostic and therapeutic considerations. *Am J ObstetGynecol*, 178: 203-211. 1998;
[25] Ruchel R: A variety of Candida proteinase and their possible targets of proteolytic attachments in the host. Zentralbl. BakteriolMikrobiolHyg [A], 257: 266-274. 1984;
[26] Odds FC: Candidosis of the genitalia, In: Odds FC, editor. *Candida* and Candidosis 2nd eds: London: Bailliere Tindal 124-135. 1988;
[27] Ruchel R: Properties of a purified proteinase from the yeast *Candida albicans*. *BiochimBiophysActa* 659: 99-113. 1981
[28] Cassone A, De Bernardis F, Mondello F et al: Evidence for a correlation between proteinase secretion and vulvovaginalcandidosis. *J Infect Dis* 156: 777-783. 1987;
[29] Ruchel R, Uhlemann K, Boning B: Secretion of acid proteinases by different species of genus *Candida*. ZblBaktHyg, *I AbtOrig* A, 255: 537-548. 1983;
[30] CDC, Centers for Disease Control and Prevention. *Sexually Transmitted Diseases Treatment Guidelines*, 59 (RR12): 1-110. (2010).
[31] Jindal N, Gill P, Aggarwal A. An epidemiological study of vulvovaginal candidiasis in women of childbearing age. *Indian J. Med. Microbiol*. 25 (2): 175-176. (2007).
[32] Williams OT, Joy OA, Ugonnal NP, Chukwuemeka IJ, Kester UE. *International Journal of Pharmaceutical Research and Development* 4 (3): 323-331. 2012
[33] Parveen N, Munir AA, Din I, Majeed R. Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic**.** *Journal of Coll Physicians*SurgPak 18 (3): 154-157. 2008
[34] Moore D, Ahmadjian V, Alexopoulos CJ. Fungus. Encyclopaedia Britannica Student and Home Edition. Chicago Encyclopædia Britannica. 2010.
[35] Akpan UP, Ekpenyong CE, Ibu JE, Ibu JO. Incidence of vulvovaginal candidiasis among Nigeria women in tight fitting underwears: The need for counseling and health education. *Journal of Public Health and Epidemiology* 3 (10): 478-481. 2011

[36] Roberts CL, Rickard K, Kotsiou G, Morris JM. (2011). Pregnancy and Childbirth, 11: 18. Ochei JO, Kolhatkar AA. Medical Laboratory Science Theory and Practice. 6th Edn. Tata McGraw-Hill, New Delhi, pp. 1072 - 1078. 2007.

[37] Larsen B: *Normal genital microflora*. In Keith LG, Berger GS, Edelman DA (eds): Infections in ReproductiveHealth. Vol 1. Common Infections. Hingham, MA: MTP Press Limited, pp 3-32, 1985.

[38] Lathigra RB, Butcher PD, Garbe TR, Young DB: Heat shock proteins as virulence factors of pathogens. Curr Topics MicrobiolImmunol 167: 125-140, 1991.

[39] Kohler JR, Fink GR: Candida albicansstriins heterozygous and homozygous for mutations in mitogenactivated protein kinase signaling components have defects in hyphal development. ProcNatlAcadSci USA 93: 13223-13228, 1996.

[40] Nermin H. Ibrahim. , Nahla A. Melake. , Ali M. Somily. , Azza S. Zakaria. , ManalM. Baddour. , Amany. Z. Mahmoud. The effect of antifungal combination on transcripts of a subset of drug-resistance genes in clinical isolates of *Candida* species induced biofilms. *Saudi Pharmaceutical Journal* 23, 55–66. 2015.

[41] Al-Hedaithy SS. Spectrum and proteinase production of yeasts causing vaginitis in Saudi Arabian women. *Med. Sci. Monit*. 8 (7): 498-501. 2002.

[42] Faidah, H. S Vulvovaginal Candidiasis among Women in Makkah City, Saudi Arabia. *Research Journal of Medical Sciences*. Vol: 7. 4: 86-89. 2013

[43] Raid A. Al-akeel, Talat A. El-kersh, Yazeed A. Al-Sheikh, and ZiabZakey Al-Ahmadey Prevalence and comparison for detection methods of *Candida* species in vaginal specimens from pregnant and non pregnant Saudi women. *African Journal of Microbiology Research* Vol. 7 (1), pp. 56-65, 1 January, 2013

[44] Al-ZahraaKaram El-Din. , FawziaHabib. , NajwaAbd-Allah. , OmaymaKhorshid, MycoticVulvoVaginitis: Epidemiology, Pathogenesis and Profile of Antifungal Agents. *Journal of Taibah University Medical Sciences*. Volume 4, Issue 2, Pages 123–136. 2009.

[45] Neerja, J. , Aruna, A. and Paramjeet, G. Significances of Candida culture in women with vulvovaginal symptoms. *J obstetGynecolIndia* 56: 139-141. 2006.

[46] Capoor, M. R. , Nair, D. , Deb, Monorama. , Verma, P. K. , Srivastava, L. and Aggarival, P. Emergence of Non-*albicans Candida species* and antifungal Resistance in a tertiary careHospital. *Jpn. J. infect. Dis.* 58: 344-348. 2005

[47] Magill, S. S. , C. Shields, C. L. Sears, M. Choti, and W. G. Merz. Triazole cross-resistance among Candida spp.: Case report, occurrence among bloodstream isolates, and implications for antifungal therapy. *J ClinMicrobiol* 44: 529-535. 2006

[48] Bhavan, PS. ; Rajkumar, R. ; Radhakrishnan, S. ; Seenivasan, C. and Kannan, S. Culture and *Candida albicans* from Vaginal Ulcer an lase on SDS-PAGE. *Interna J Biol* 2: 84 (2010)

[49] Chander, j. Candidiasis. A text Book of *Medical Microbilogy*. 2nd Edn, Interprint, new Delhi, pp: 212-230. 2002

[50] Center for Disease Control and Prevention, *Sexually Transmitted Diseases Treatment Guidelines*. 59: (12). 2010.

[51] CLSI, Clinical and Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing of yeasts: Approved guideline M44-A, *Clinical and Laboratory Standards Institute*, Wayne, PA, USA. pp. 65-74. (2004).

[52] Ng KP, Saw TL, Na SL, Soo-Hoo TS. Systemic *Candida* infection of University Hospital 1997-1999: the distribution of Candida biotypes and antifungal susceptibility patterns. *Mycopathologia* 149 (3): 141-146. 2000.

[53] Nwadioha SI, Egah DZ, Alao OO, Iheanacho E. Nigeria Risk factors for vaginal candidiasis among women attending primary health care centers of Jos, Nigeria. *J Clin Med Res* 2 (7): 110-113. 2010

[54] Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. *Nigeria J Med Medical Sci* 1 (10): 447-452. 2010

[55] Oyewole OA, Okoliegbe IN, Alkhalil S, and 1Isah P. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Federal University of Technology, Minna, Nigeria, Bosso Clinic. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 4: 1. 113. 2013.

[56] Mikolajczyk K, Zimmer M, Tomialowicz M, Fuchs T. Congenital candidiasis as a subject of research in medicine and human ecology. *MikologiaLekarska* 13 (3): 175-179. 2006.

[57] Limia OF, Lantero DMI. Lantero, A. Betancourt, E. deArmas and A. Villoch, 2004. Prevalance of *Candida albicans* and Trichomonasvaginals in pregnant women in Havana City by an immunololgic latex agglutination. *Medscape Geral Medicine* 6 (4): 50. 2004

[58] Aslam M, Hafeez R, Ijaz S, Tahir M. Vulvovaginal Candidiasis in Pregnancy. *Biomedica* 24: 54-56. 2008

[59] Willacy H, Jackson C. Vaginal and Vulval Candidiasis. Online at Patient. co. uk/doctor/Vaginal-and-Vulval-Candidiasis. 2011.

[60] Nwadioha SI, Egah DZ, Alao OO, Iheanacho E. Risk factors for vaginal candidiasis among women attending primary health care centers of Jos, Nigeria. *Nigeria J Clin Med Res* 2 (7): 110-113. 2010

[61] Esmaeilzadeh S, Omran SM, Rahmani Z. Frequency and etiology of vulvovaginal candidiasis in women referred to gynecological center in Babol. *Intl J Fertility Sterility* 3 (2): 74-77. 2009.

[62] Holland J. , Young, M. L. , Lee, O. and Chen, S, C-A. Vulvovaginal carriage of yeasts other than *Candida albicans*. *Sexual Transm Infect* 2003; 79: 249-250.

[63] Onifade, A. K. and Olorunfemi, O. B. Epidemiology of vulvo-vaginal candidiasis in female patients in Ondo State Government Hospitals. J Food, Agric and Environ. 3: 118-199. 2005.

[64] Nucci, M. & Colombo, A. Candidemia due to Candida tropicalis: clinical, epidemiologic, and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. *DiagnMicrobiol Infect Dis* 58, 77–82. 2007.

[65] Zainab A. Abbas, Salman Aziz, Abbas Abdul-Mueed. Epidemiological and Molecular Study for *Candida*spp in Vagina. *Medical Journal of Babylon* 11: 1, 2014.
[66] Wise MG, Healy M, Reece K, Smith R, Walton D, Dutch W, Renwick A, Huong J, Young S, Tarrand J, Kontoyiannis D. J Med Microbiol 56 (6): 778-787. 2007.
[67] Mardh P. A, Rodrigues A. G, Genc M, Novikova N, Martinez-de-Oliviera J, Guaschino S. Facts and myths on recurrent vulvovaginalcandidosis – a review onepidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. *Int J STD AIDS*. 13 (8): 522-39. 2002.
[68] Citak S, Ozçelik B, Cesur S, Abbasoğlu U *In vitro* susceptibility of *Candida* isolated from blood culture to some antifungal agents. *Jpn. J. Infect. Dis*. 58 (1): 44-46. 2005.
[69] Teseng YH, Lee WT, Kuo. TC *In vitro* susceptibility of Fluconazole and Amphotericin B against *Candida* Isolates from women with vaginal candidiasis in Taiwan. *J. Food. Drug Anal*. 13: 12-16. 2005.
[70] Badiee P, Alborzi A Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J. Microbiol*. 3 (4): 183-188. 2011.
[71] Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, Loebenberg D, Black TA, McNicholasPM. *In vitro* activities of Posaconazole, Fluconazole, Itraconazole, Voriconazole, and Amphotericin B against a large collection of clinically important moulds and yeasts. *Antimicrob. Agents Chemother*. 50 (6): 2009-2015. 2006.
[72] Tortorano, A. M. , Rigoni, A. L. , Biraghi, E. , Prigitano, A. , Viviani, M. A. & FIMUA-ECMM Candidemia Study Group. The European Confederation of Medical Mycology (ECMM) survey of candidemia in Italy: antifungal susceptibility patterns of 261 non-albicans Candida isolates from blood. *J AntimicrobChemother*52, 679–682. 2003.
[73] Yang, Y. L. , Ho, Y. A. , Cheng, H. H. , Ho, M. & Lo, H. J. (2004). Susceptibilities of Candida species to amphotericin B and fluconazole: the emergence of fluconazole resistance in Candida tropicalis. Infect Control HospEpidemiol 25, 60–64.
[74] Myoken, Y. , Kyo, T. , Fujihara, M. , Sugata, T. &Mikami, Y. Clinical significance of breakthrough fungemia caused by azoleresistant Candida tropicalis in patients with hematologic malignancies. Haematologica 89, 378–380. 2004.
[75] Sanglard, D. & Odds, F. C. Resistance of Candida species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2, 73–85. 2002.
[76] Cherifi S, Robberecht J, Miendje Y. *Saccharomyces cerevisiae*fungemia in an elderly patient with *Clostridium difficile*colitis. *ActaClin Belg*. 59: 223-224. 2004.
[77] Enache-Angoulvant A, Hennequin C. Invasive *Saccharomyces* infection: a comprehensive review. *Clin Infect Dis*. 41: 1559-1568. 2005.
[78] ShinghS, Sobel JD, Bhargava P, Boika D, Vazquez JA, 2002. Vaginitis due to Candida krusei, epidemiology, clinical aspects and therapy. *Clin Infect Dis*, 35 (9): 1066-1070.
[79] Erdem H, Getin M, Timuroglu T, Cetin A, Yanar O, Pahsa A, Identification of yeasts in public hospital primary care patients with or without clinical vaginitis. *Aust N Z J ObstetGynaecol*, 43 (4): 312-316. 2003