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PREVALENCE OF VAGINAL CANDIDA COLONIZATION AMONG WOMEN ATTENDING UNIVERSITY OF NIGERIA TEACHING HOSPITAL, ENUGU STATE, NIGERIA.

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ABSTRACT

Candida species live in a healthy vagina as normal flora and about 75% of women generally harbour these yeasts which sometimes can become pathogenic causing vaginal candidiasis. This study was undertaken to determine the prevalence of vaginal *Candida* colonization among women attending the Obstetrics and Gynecology Unit of the University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu State, Nigeria. A total of 340 high vaginal swab (HVS) specimens were collected from the women and cultured on Sabouraud Dextrose Agar supplemented with Chloramphenicol (50μg/ml.) and incubated at 25°C for 24 hours. Identification was done using standard methods which included growth on Chromogenic Candida agar. Statistical analysis was done using Chi-square test ($p<0.05$). Eighty-eight (88) *Candida* species were isolated with *Candida tropicalis* (34) being the most predominant (38.63%) followed by *Candida parapsilosis* 21 (23.86%), *Candida albicans* 20 (22.73), *Candida krusei* 7 (7.95%) and *Candida glabrata* 6 (6.82%). Women >50 years had the highest prevalence of *Candida* colonization (50%) followed by the age group 18 – 25 years (29.6%), though the difference was not statistically significant (p = 0.067). The prevalence was higher amongst the non-pregnant women (28.6%) than the pregnant women (16.0%), while women in their first trimester had the highest prevalence of 25.0% followed by those in the second trimester (11.1%) and third trimester (5.0%). Symptomatic and asymptomatic women had a prevalence of 23.3% and 29.2% respectively. This study recommends that vaginal candidiasis should not be ruled out if there are no symptoms and thus, proper diagnosis be carried out especially in pregnant women notwithstanding the absence of symptoms in order to avoid complications.

Keywords: Prevalence, *Candida* species, vaginal colonization, Enugu State.

1. INTRODUCTION

Candidiasis is a fungal infection caused by yeasts in the genus *Candida* and species in this genus can cause yeast infections in many areas of the body. The normal microbiota of an individual's mucosal cavity, gastrointestinal tract as well as vagina includes *Candida* species [1]. These *Candida* species can colonize a healthy vagina without any symptoms. However, under conditions that disrupt the vaginal microbial balance, these yeasts can cause candidal vulvovaginitis or vulvovaginal candidiasis. Candidal vulvovaginitis is responsible for one-third of cases of vulvovaginitis [2; 3]. Candidiasis in the vagina is commonly called a vaginal yeast infection or vulvovaginal candidiasis characterized by pain or discomfort when urinating and during sexual intercourse, irritation, discharge and intense itchiness of the vagina and the vulva [4].

Although some studies have shown that women normally have *Candida* vaginal colonization with absence of symptom/infection, there is a likelihood of high prevalence of vaginal yeast infection with increased rate of vaginal colonization determined by several factors such as age, pregnancy, uncontrolled diabetes mellitus, HIV/AIDS, long-term broad spectrum antibiotic treatment [5] use of contraceptives, diaphragms, orogenital sex and douching [6; 7]. High blood glucose levels do not only interfere with immune responses in the host but also promote yeast attachment and growth [8].

Reports show that about 75% of all women, experience at least one episode of which physician approved to be candidiasis in their lifetime [6]. 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 [9]. In pregnant women, it was reported to cause blood stream infections particularly in low birth weight and premature infants [10].

Probably because of the lesser percentage for the asymptomatic vaginal *Candida* colonization, much study has been targeted towards women with vaginal infection [11] but it is noteworthy that the work of [12] showed the importance of studying healthy/asymptomatic women. *Candida* species can colonize mucosal surfaces (especially vaginal) as commensals in an asymptomatic manner, but can become pathogenic owing to certain risk factors that could predispose the woman. This study was thus, carried out to determine the prevalence of vaginal *Candida* colonization among the women attending the Obstetrics and Gynecology Unit of the University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area, Specimen Collection and Processing

The study was conducted in the University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla, Enugu State, located at 6.44° N latitude and 7.50° E longitude and it is situated at elevation 192 meters above sea level in the South-East geopolitical zone of Nigeria.

An ethical clearance was obtained from the Ethical Committee of UNTH before the specimens were collected. Participation in the study was voluntary and the informed consent of each of the participants was obtained. Women of child-bearing age (18-45 years) and above attending the Obstetrics and Gynecology Unit of UNTH who gave their consent were included in the study. Also, the women may present with or without symptoms but must not be menstruating. Women attending the Obstetrics and Gynecology Unit of UNTH but did not give their consent or were menstruating or were below 18 years were excluded from the study.

A total of 340 high vaginal swab (HVS) specimens were collected from the women attending the Obstetrics and Gynecology Unit of the University of Nigeria Teaching Hospital (UNTH) Ituku/Ozalla, Enugu State, and were qualified for the study. Each HVS specimen was collected by a medical personnel using a sterile swab stick with the aid of a sterile speculum. Structured questionnaires were used to gather some relevant information from the participants after obtaining their consent. The collected specimens were labeled appropriately and then transported to the Department of Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria for processing.

Each swab was used to inoculate directly unto already prepared plates of Sabouraud dextrose agar (SDA)(Hi Media Laboratory, Pvt. Ltd, India) supplemented with Chloramphenicol (50μg/ml). The plates were then incubated aerobically at room temperature for 24-48 hours [13]. Yeast colonies were subcultured for purification and pure cultures were then stored in slants of SDA supplemented with Chloramphenicol (50μg/ml.) for further use.

2.2 Identification of the Yeast Isolates

All the isolates were identified based on their macroscopic, microscopic, physiological as well as biochemical characteristics which included growth on cornmeal agar as described by [14], germ tube test as described by [15] and growth on Chromogenic Candida agar. These characteristics were compared with photomacrographs and photomicrographs in Medical Mycology [16] and the Colour Atlas of Pathogenic Fungi [17]. The yeast isolates were also sent to the yeast collection centre in Netherlands, Centraal Bureau Voors Schimmel culture (CBS) for identification by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

2.3 Statistical Analysis

Demographic characteristics of the respondents were extracted from the questionnaire and statistical analysis and data presentation were performed using Statistical Package for Social Sciences (SPSS) Version 22.0. Chi- square Test was used to compare the differences between the effects of various parameters on the prevalence of vaginal *Candida* colonization. Values of $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Occurrence of the *Candida* species in the High Vaginal Swab Specimens

Out of the 340 HVS specimens collected, 4 were regarded invalid (the questionnaires were not completed) while 83 yielded growth of yeasts giving a prevalence of vaginal *Candida* colonization of 24.7%. Among the yeasts isolated, *Candida tropicalis* was the most predominant with a distribution frequency of 34 (38.63%) followed by *Candida parapsilosis* 21 (23.86%), *Candida albicans* 20 (22.73), *Candida krusei* (*Issatchenkia orientalis*) 7 (7.95%) and *Candida glabrata* 6 (6.82%). Out of the 83 specimens that were positive for *Candida*, 2 species each were isolated from 5 specimens and single species from 78 specimens giving a total of 88 species. A combination of *Candida albicans* and *Candida tropicalis* was isolated from 2 specimens, a combination of *Candida albicans* and *Candida krusei* was isolated from 1 specimen, a combination of *Candida albicans* and *C. parapsilosis* was isolated from 1 specimen and finally, a combination of *Candida tropicalis and Candida parapsilosis* was isolated from 1 specimen. The overall occurrence of non-albicans species was 61.37% (Table 1). This result agrees with that of [18] in which *Candida tropicalis* was the most predominant species constituting 38.2%. The results of [19] also indicated *Candida tropicalis* as the most commonly isolated yeast species from patients being treated at the University Hospital of the Federal University at Grande Dourados, Central-western Brazil. Also, in a research carried out by [20], in Madhya Pradesh, India, *Candida albicans* was the third most predominant species isolated constituting 21.1% which is very close to the 22.73% recorded in the present study. This result, however doesn't agree with those of many other researchers who reported *Candida albicans* as the most predominant species isolated [21; 22; 23; 24; 25; 26]. In a similar study by [27], in some seven cities in Nigeria, *Candida parapsilosis* had the highest frequency of occurrence (33.7%) while *Candida glabrata* had the least (5%). This also disagrees with the present study. A possible explanation for these differences in the result of the present study and those of similar studies is the changes that occur in the study populations vis-à-vis menopausal and postmenopausal women and those that might have some underlying disorders like diabetes. Thus, there can be changes in patient physiology, hormone balance, and decrease in immune function [28]. One reason why *Candida albicans* predominates non-albicans *Candida* (NAC) species especially from vaginal specimens may be due to the fact that *C. albicans* adheres to vaginal epithelial cells in significantly higher numbers than do other *Candida* species [29] resulting to the relative infrequency of NAC in vaginal candidiasis. Also, the rising trend in the isolation of NAC from vaginal specimens might be attributed to factors such as former exposure to polyenes and azoles, geographical regions as well as the improved biochemical and molecular diagnostic methods in laboratories [30; 31].

Table 1: Occurrence of the *Candida* species in the High Vaginal Swab Specimens.

Species	Frequency	Percentage (%)
Candida tropicalis	34	38.63
Candida parapsilosis	21	23.86
Candida albicans	20	22.73
Candida krusei	7	7.95
Candida glabrata	6	6.82
Total	**88**	**100**

3.2 Prevalence of Vaginal *Candida* Colonization According to Age Group

The prevalence of vaginal *Candida* colonization according to age group is presented in Table 2. It can be observed from the table that women of aged 50 years and above recorded the highest prevalence (6, 50%), followed by women in the age groups 18 – 25 years (40, 29.6%), 42 - 49 years (6, 23.1%) and 34 – 41 years (12, 19.4%). Women in the age bracket 26 – 33 years had the least prevalence (19, 18.8%). The difference in the prevalence of vaginal *Candida* colonization among the age groups was not statistically significant ($p>0.05$). This agrees with the findings of [27] who reported that the age bracket 21 – 25 ranked second (28%) in the frequency of *Candida* positive samples, in their research carried out in seven cities in the Southern part of Nigeria. Also, [12] reported a prevalence rate of 30.2% within the age group 21 – 25 years among female students in Bingham University, Nasarawa State. The result of the present study, however, contradicts that of [21]. In their work, the highest frequency of *Candida* species was seen amongst the age bracket 26 - 35 years (57.4%), followed by the age group 16 - 25 years (29.6%) while ages 46 and above recorded the lowest (1.0%). Results of similar work by some other researches also disagree with that of the present study; the highest prevalence of vaginal *Candida* species was recorded in age groups 26 – 30 (35%), 15 – 20 (25.6%), 16 – 20 (15.6%) and 21 – 30 (46.8%) by [27], [22], [12] and [32] respectively. This can be attributed to the fact that women aged 16 to 30 years are very much sexually active and more often engage in sexual promiscuity, drug abuse and the use of contraceptives making them more vulnerable.

Contrary to the result of the present study, [27], [22], [33] and [32] reported the least prevalence of vaginal *Candida* isolates within the age groups 41years and above (1.2%), 51 years and above (2.6%), 40 – 49 (2%) and above 41 years (4.3%) respectively. The high prevalence (50%) of vaginal *Candida* colonization seen in the women aged 50 years and above as observed in this study, may be

because the women had reached menopause. The usual age range is 45 – 55 years. At menopause, the vaginal walls become thinner, dryer, less elastic, and possibly irritated. This increases the risk of vaginal yeast infections [34; 35; 36]. Also, it has been reported that postmenopausal women taking hormone replacement therapy (HRT) are significantly more prone to develop vulvovaginal candidiasis (VVC) than women who are not and those with VVC are likely to have been susceptible to it before menopause [37].

3.3 Prevalence of Vaginal *Candida* Colonization amongst the Women In Relation to Pregnancy

The prevalence of vaginal *Candida* colonization was higher amongst the non-pregnant women (28.6%) than the pregnant women (16.0%) though the difference was statistically not significant (p> 0.05). The prevalence amongst the women who were not sure of their pregnancy status was 0% while those who never responded vis-à-vis their pregnancy status had a prevalence of 30.8% (Table 3). The prevalence of vaginal *Candida* colonization was higher amongst the non-pregnant women (28.6%) than the pregnant women (16.0%) though the difference was statistically not significant (p> 0.05) (Table 3). This agrees with the findings of [38] who reported a higher prevalence of *Candida albicans* in non-pregnant women (76.8%) than in the pregnant women (23.2%) but differs in that the difference was statistically significant. They suggested the higher prevalence in non-pregnant women than their pregnant counterparts could be as a result of the fact that the number of pregnant women whose data were reviewed was much higher when compared with the number of non-pregnant women. The result of the present study disagrees with that of [33] and [18] who demonstrated a higher prevalence of vaginal *Candida* colonization in the pregnant women (40% and 36.8%) than in their non-pregnant counterparts (17% and 21.7%) respectively. **[39]** also reported a higher prevalence rate of vulvovaginal candidiasis amongst pregnant women (55.4%) than amongst non-pregnant women (35.4%) in Maroua, Far-North, Cameroon. [39] attributed the higher prevalence in the pregnant women to changes in the levels of female sex hormones, such as estrogen and progesterone [40] during pregnancy. However, pregnancy is not the only predisposing factor of vaginal candidiasis in women. The higher prevalence in the non-pregnant women as observed in the present study can possibly be explained by differences in the population types; the non-pregnant women would have had other more predisposing factors. In their study, [41] reported that diabetes mellitus was the major predisposing factor causing candidiasis constituting 33% followed by pregnancy (22.3%), It is likely that most of the non-pregnant women were not married and being pregnant while still single is considered a taboo in this part of the world. Thus, these women would have been engaged in various forms of practices including douching and the use of contraceptives to prevent pregnancy. Frequent douching with antiseptics can alter the microbial flora of the vagina thus exposing it to *Candida* infection [39]. [42] showed there was a significant difference in the rate of colonization by *Candida* between women who used contraceptives (58.3%) and those who never used contraceptives (35.3%). Also, [43] showed that women who used contraceptive had a prevalence of 51.5% of vaginal colonization compared to 40.6% for non-contraceptive users in Edo State, Nigeria.

Table 2: Prevalence of Vaginal *Candida* Colonization According to Age Group

Age group (in years)	Number sampled	Number of *Candida* positive specimens	Percentage of *Candida* positive Women (%)
18 – 25	135	40	29.6
26 – 33	101	19	18.8
34 – 41	62	12	19.4
42 – 49	26	6	23.1
>50	12	6	50
Total	**336**	**83**	**24.7**

p – value = 0.067 (p> 0.05); df = 4

Table 3: Prevalence of Vaginal *Candida* Colonization amongst the Women in Relation to Pregnancy

Status	Number sampled	Number of *Candida* positive specimens	Percentage of *Candida* positive Women (%)
Pregnant	100	16	16.0
Non-pregnant	220	63	28.6
Not sure	3	0	0.0
No response	13	4	30.8
Total	**336**	**83**	**24.7**

p – value = 0.067 (p> 0.05); df = 3

3.4 Prevalence of Vaginal *Candida* Colonization amongst the Women According to Trimester

Table 4 shows the prevalence of vaginal *Candida* colonization amongst the women according to trimester. Women in their first trimester had the highest prevalence of 25.0% followed by those in the second trimester and third trimester with prevalence of 11.1% and 5.0% respectively. Statistically, there was no significant difference in the effect of trimester on the prevalence of vaginal *Candida* colonization. This finding is in agreement with that of [44] who reported that the highest frequency of vaginal candidiasis was among those in first trimester of pregnancy (37%) followed by those in second trimester (34%) and the lowest among those in 3rd trimester (29%). [42] reported that the least prevalence of *Candida* species was also recorded in the third trimester, although the prevalence (30.6%) was much higher than the 5.0% recorded in the present study. [45] showed that first and second trimester pregnant women were at a higher risk of getting vaginal candidiasis when compared to those in the third trimester. However, the findings of [42] contradicts that of the present study in that they recorded the highest occurrence (68.8%) of *Candida* species amongst women in their second trimester, followed by those in their first trimester (33.3%). Amutaigwe *et al*. (2007), in a similar study observed the highest prevalence (43.2%) of vaginal *Candida* colonization amongst women in their second trimester of pregnancy followed by those in their third trimester (35.3%) while the women in their first trimester of pregnancy had zero (0%) prevalence. [33], working on prevalence of *Candida albicans* among women attending Federal Medical Centre Asaba, South-South, Nigeria, recorded the highest prevalence of 47% amongst women in their third year of pregnancy. During pregnancy, there is an increase in the level of estrogens. As the pregnancy ages, the level of this hormone increases providing an increased amount of glycogen in the vagina. Glycogen provides a ready source of utilizable sugar that favours the growth of yeasts like *Candida* species and thus a higher prevalence in the third and second trimester. However, the least prevalence and highest prevalence respectively observed in the third and first trimester can be explained in relation to attendance to antenatal clinics. The pregnant women may have begun treatment after diagnosis in the first trimester and thus, the least prevalence observed in the third trimester [44].

3.5 Prevalence of Vaginal *Candida* Colonization amongst the Women According to Marital Status.

As can be seen in Table 5, the single women had the highest prevalence (26.0%) of vaginal *Candida* colonization when compared to that of the married women (23.8%) and the divorcees (0.0%). However, of the 4 women who never responded vis-à-vis their marital status, 2 were positive for vaginal *Candida* colonization giving a prevalence of 50%. There was statistically, no significant difference in the prevalence of vaginal *Candida* colonization between the married and single women ($p> 0.05$). In a retrospective study to determine the prevalence and distribution of *Candida* vaginitis in women of reproductive age in Onitsha metropolis and its environs, [38] also recorded that single women had a higher prevalence (57.2%) than the married women (42,8%). The result of [43] contradicts that of the present study in that they reported a higher prevalence (50.9%) of vaginal candidiasis amongst married women than the single women (48.4%).

Table 4: Prevalence of Vaginal *Candida* Colonization amongst the Women According to Trimester

Trimester	Number sampled (%)	*Candida* positive	*Candida* negative	Percentage of *Candida* positive Women (%)
First	44	116	33	25.0
Second	36	4	32	11.1
Third	20	1	19	5.0
Non-pregnant	236	67	169	28.46
Total	**336**	**83**	**253**	

p – value = 0.024 (p> 0.05); df = 3

Table 5: Prevalence of Vaginal *Candida* Colonization amongst the Women in Relation to Marital Status

Marital Status	Number sampled	Number of *Candida* positive specimens	Percentage of *Candida* positive Women (%)
Married	181	43	23.8
Single	146	38	26.0
Divorced	5	0	0.0
No response	4	2	50.0
Total	**336**	**83**	**24.7**

p – value = 0.356 (p> 0.05); df = 3

3.6 Prevalence of Vaginal *Candida* Colonization in Symptomatic and Asymptomatic Women

In Table 6 is presented the prevalence of vaginal *Candida* colonization in symptomatic and asymptomatic women. It can be observed that out of the 254 symptomatic women who took part in the study, 59 were positive for vaginal *Candida* colonization giving a prevalence of 23.3% while out of the 82 asymptomatic women, 24 were positive for vaginal *Candida* colonization giving a prevalence of 29.2%. Thus, the prevalence was higher in asymptomatic women than in the symptomatic women. However, the difference was not significant statistically. This result disagrees with those of similar researches in which there was a higher prevalence of vaginal *Candida* colonization in the symptomatic women than their asymptomatic counterparts [18; 47]. Also, [46] recorded a higher prevalence of *Candida albicans* in symptomatic women (26.7%) compared to the asymptomatic women (21.7%) though; the difference was statistically not significant. The higher prevalence among the asymptomatic women in the present study suggests that vaginal candidiasis should not be ruled out if there are no symptoms. This is because infections by *Candida* species that do not present with symptoms could likely lead to other severe complications [46]. Although the evidence may be incomplete, some emerging data suggests that candidiasis in pregnancy may be associated with increased risk of pregnancy complications, such as premature rupture of membranes, chorioamnionitis, preterm labor and congenital cutaneous candidiasis [48]. Thus, it is of utmost importance that diagnosis be carried out especially in pregnant women notwithstanding the absence of symptoms in order to avoid such possible complications. Though there was a higher prevalence in symptomatic women than their asymptomatic counterparts in the research carried out by some other researchers [46; 47; 18], it is worthy to note that vaginal candidiasis should not be diagnosed based on clinical features only. This is because an appreciable number of women may present with symptoms as a result of some other conditions like allergies [6].

Table 6: Prevalence of Vaginal *Candida* Colonization in Symptomatic and Asymptomatic Women

Clinical Manifestation	Number sampled (%)	*Candida* positive	*Candida* negative	Percentage of *Candida* positive Women (%)
Symptomatic	254	59	195	23.2
Asymptomatic	82	24	59	29.3
Total	**336 (100%)**	**83 (24.7**	**253 (75.3%)**	

p – value = 0.305 (p> 0.05); df = 4

4. CONCLUSION

In the present study, a prevalence of vaginal *Candida* colonization of 24.7% was observed. Women of ages 50 years and above recorded the highest prevalence of vaginal *Candida* colonization, followed by women in the age groups 18 – 25 years. The prevalence of vaginal *Candida* colonization was higher amongst the non-pregnant women (28.6%) than the pregnant women (16.0%) though the difference was statistically not significant (p> 0.05). Also, amongst the pregnant women, the highest prevalence (25%) of vaginal *Candida* colonization was observed in the first trimester. The prevalence was higher in asymptomatic women than in the symptomatic women though statistically, the difference was not significant. *Candida tropicalis* was the most predominant species isolated from the HVS specimens followed by *Candida parapsilosis*.

The high prevalence of non- albicans *Candida* (NAC) from vaginal specimens as observed in this study, suggests that proper diagnosis and identification of causal agents be carried out to avoid misuse of drugs. Also indiscriminate use of antifungal drugs should be avoided. In the present study, there was a higher prevalence of vaginal *Candida* colonization among the asymptomatic women than the symptomatic women which suggests that vaginal candidiasis should not be ruled out if there are no symptoms. It is thus, recommended that diagnosis be carried out especially in pregnant women notwithstanding the absence of symptoms in order to avoid complications. This also implies that vaginal candidiasis should not be diagnosed based on clinical features only since an appreciable number of women may present with symptoms as a result of some other conditions like allergies.

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