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Prevalence and Risk Factors of Vaginal Candidiasis among Pregnant Women in Owerri, Nigeria: A Focus on Trimester Distribution, Gravidity, and Antifungal Treatment

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ABSTRACT

This study examined the prevalence of vaginal candidiasis (VC) across pregnancy trimesters and its relationship with gravidity status and antifungal treatment among a population of pregnant women in Owerri, Imo State Nigeria. A total of 314 high-vaginal Swab samples were used for this study. Infection with Candida species was diagnosed by microscopy of a saline mount, gram-stained smear of material from the vagina, and colonial growth on Chrom agar pigmentation. 48% were diagnosed with VC, while 52% were uninfected. The third trimester had the highest occurrence of VC at 50%, followed by the first and second trimesters with 33% and 17% respectively. Gravidity analysis revealed that multigravida women were more prone to infection, with 67% of them affected compared to 33% of primigravida women. However, statistical analysis indicated no significant association between gravidity and VC (p-value = 0.191). Age distribution showed that the majority of participants (44%) were between 26-33 years old, with an overall VC prevalence of 48%. Regarding antifungal treatment, 80% of the participants were not on any medication, and the use of antifungal drugs showed a statistically significant effect on VC treatment (p-value = 0.018). These findings underscore the need for increased surveillance and intervention, particularly in the third trimester and among multigravida women.

Keywords: *Antifungal, Candida species, Gravidity, Microscopy, Primigravida, Trimeste.*

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Introduction

Vulvovaginal candidiasis (VVC) is a fungal infection that arises from the overgrowth of *Candida species*, an opportunistic pathogen [1]. It is considered the most common fungal infection of the vaginal flora, and is one of the most common forms of vaginitis, especially in women of reproductive age. The infection is characterized by vulvar pruritus, dysuria, dyspareunia, irritation and soreness of the vulva and swelling of the vagina with discharges. The discharge appears like curded milk and deep erythema of vulva and vagina is often seen [2,3]. The most common species of Candida is *Candida albicans* [4], and up to 85% of sexually active women have at least one symptomatic episode of vaginal candidiasis during their lifetime [5,6], with *Candida albicans* accounting for the majority ofthe cases, as reported by Jannati *et al.*(2024).Some emerging data have also suggested that vulvovaginal candidiasis during pregnancy might be associated with an increased risk of complications such as premature rupture of the fetal membranes, preterm labour, chorioamnionitis, infant mortality and up to 65% congenital cutaneous candidiasis, resulting in invasive neonatal candidiasis [7,8].

Outside pregnancy, many predisposing factors can also lead to the development of vaginal candidiasis; these include diabetes mellitus, HIV infection,contraceptive use, and use of broad-spectrum antibiotics,which disrupt the beneficial *Lactobacillus*spp microflora of the normal vaginal microbiota [9,10]. Numbers of gravida and stage of pregnancy also contribute to the development of vaginal candidiasis.

Although candidiasis is a common problem among women of the reproductive age group, the incidence of the disease is higher in pregnant women than non-pregnant women because of a number of normal and expected physiological changes that favour the growth of candidain the genitourinary tract. Also, during pregnancy the levels of reproductive hormones such as progesterone and estrogen will be elevated with its suppressive effects on the anti-candida activity of neutrophils and inhibits the activity of vaginal epithelial cells respectively [11]**.** In addition, estrogen decreases immunoglobulins secretion in the vagina resulting in an increased vulnerability of pregnant women to vaginal candidiasis [12, 13],and estrogen also helps to provide high glycogen content in the vagina which serves as a carbon source for *Candida* specie [11].

Majority of the symptomatic cases of vaginal candidiasis are caused by *Candida albicans*, however,*Candida tropicalis, Candida glabratta,* and *Candida krusei* are the most common non-albicans speciesalso implicated. Again, [13] reported that in some clinical settings, these non-albicans candida could be in a higher proportion than *C. albicans*. Surprisingly, co-infection of *C. albicans* and non-albicans have also been reported [14]. The rise in cases of candidiasis by non- albicans species is due to the extensive use of azole drugs [15]. Studies have shown that the prevalence of candidiasis signs among illiterate and low economic class women [13].

Although there is a lot of morbidity associated with vulvovaginal candidiasis, like mental distress and significant financial costs, there are few epidemiological data on VVC in Nigeria, especially in South-Eastern region where there is little research on the prevalence and distribution of vaginal candidiasis in pregnant women. This study therefore evaluated the prevalence and risk factors associated with vaginal candidiasis among pregnant women in a public hospital in Owerri, Imo State, Nigeria.

2.0. Materials and Method

Study Area, Population and Approach

This study was carried out at General Hospital Umuguma, Owerri-West, Local Government Area of Imo State, Nigeria.

Ethical Approval and Informed Consent

Approval of the study protocol was obtained from the Ethics Committee of the institution's Faculty Ethical Board. Patients gave their informed consent and were guaranteed complete confidentiality of their responses. For this investigation, three hundred and fourteen (314) high-vaginal swab samples in total were used.

Inclusion Criteria

Pregnant women that self-reported symptoms of abnormal vaginal discharge, itching, and genital burning or burning–micturition were included in the study.

Exclusion Criteria

Pregnant women without abnormal vaginal discharge, catheterized patients, and patients with cervical malignancies were excluded.

Specimen Collection

High vaginal swabs were collected by following aseptic precautions. The genital swabs were immediately sent to the clinical bench of the medical microbiology and parasitology laboratory of the hospital, where they were then processed according to standard procedures.

Infection with *Candida* species was diagnosed by microscopy of a saline mount, Gram-stained smear of material from the vagina, and colonial growth on Chrom agar pigmentation.

Macroscopic/physical examination

The odour, consistency and colour of each specimen discharge were observed and recorded.

Microscopic examination

Vaginal swabs were examined microscopically by 10% potassium hydroxide (KOH) wet mount and Gram staining for the presence of budding yeast and pseudo-hyphae of *Candida* species.

Wet mount preparation

Swab was rolled on clean slides, 2 drops of 10% potassium hydroxide was added and covered with cover slip, then examined under the microscope using X10 and X40 objective lenses for the presence of budding yeast and pseudo-hyphae of *Candida* species [12,15].

Gram's staining

The swab was rolled on clean slide to make a smear, then smear was left to air dry, fixed with alcohol and covered with crystal violet stain for one minute. Then washed in tap water and then covered with Lugol's iodine for one minute. Iodine was washed off and smear was thereafter decolourized with acetone-ethanol alcohol for few seconds and washed in tap water. Safranin was added for two minutes, then air dried and microscopically examined using oil immersion objective (X100) to observe the yeast cell morphology, size, Gram reaction and presence of pus cells, epithelial cells [16].

Culture

Swab was cultured on Sabouraud dextrose agar (SDA) with 0.05mg/ml Gentamicin and incubated at 37^0C for 48-72 hours.

Colonial morphology

Culture was examined for pastry, creamy and smooth white colonies.

Gram Stain

Indirect Gram's staining was performed for yeast suspected colonies.

Germ tube test (GTT)

This was done to check for yeast germination and its characteristics for the detection of *Candida albicans.* This is a rapid test for presumptive identification of *Candida albicans*. One (1 ml)milliliter of serum was added into a small Vitek tube using Pasteur pipette. A colony of yeast was touched using sterile wire loop and emulsified in the serum tube, mixed and incubated at 37^0C for 2-4 hours but not longer, a drop of serum was transferred to a slide for examination, cover slip was dropped and examined under the microscope using X40 objective.

Germ tubes are appendages half. The width is 3 to 4 times the length of the yeast cells from which they arise. There is no constriction between the yeast cell and the germination tube.

Positive test: Presence of short lateral filament (germ tube) for *Candida albicans.* Negative test: Yeast cell only for *Candida* non-albicans [16].

Chrom agar pigmentation

Chromogenic media was prepared according to the manufacturer's instruction and the organism inoculated in the press, then incubated at 37^{0}C for 48 hours.The growth of *Candida* species was observed by the change in the colour of the colonies according to the pigment as a result of the reaction between chromogenic substrate and enzymes that were secreted by different *Candida* species, thus, allowing organism to be identified to the species level by their colour and colony characteristics. Chrom agar has been shown to allow differentiation of *Candida* yeast by colour and morphology. The result was as follows: the product identifies *Candida albicans* by growth as light to medium green and wet colonies, *Candida glabrata* dark pink and wet colonies, *Candida krusei*as white pink and dry colonies and other *Candida* species that grow as white colour colonies [17]

Chlamydospore formation

By using a sterile inoculating needle or loop, the appropriate yeast colony was touched and immediately scraped or cut "X" through prepared corn meal agar (CMA) in the middle of one half of the agar plate, the anus of the "X" should be about 2 cm long. This procedure was repeated making a duplicate "X" in the middle of the other half of the agar plate. Using sterile forceps, sterile cover slip was centered over the cross of one of the "X" patterns. The plate was inverted and incubated for up to 3 days (72 hours) at 25 ± 2^{0}C. Plates were examined daily for the development of chlamydospores with the aid of dissecting or stage microscope. The "X" without cover slip serves as a growth control. The result seen by microscopic examination of the yeast under the cover slip revealed chlamydospore that appear as terminal double walled spheres on the pseudohyphae which indicated positive result [17]

Zymogram (Carbohydrate fermentation tests)

Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol (the production of gas rather than a pH shift is indicative of fermentation). Dextrose, maltose, sucrose, lactose, galactose, and trehalose were used in the test. The five (5 ml) milliliters of carbohydrate (pH 7.4) containing 1% peptone, 1% sugar, 0.3 beef extract, 0.5% NaCl, 0.2% anhydrides in distilled water medium was dispensed in sterilized Durham's tube and 0.2ml of saline suspension of the test organism was added and incubated at 37^{0}C for 48-72 hours up to 10 days and the fermentation pattern.

3.0 Results

The occurrence of vaginal candidiasis per trimester among the study population is shown in Table 1. A total of 151 women representing 48% across all the 3 trimesters of the study population had vaginal candidiasis, while 163 (52%) of the study population were not infected. Women in their third trimester had the highest percentage of vaginal candidiasis at 50%, while second and first trimesters had 33% and 17% respectively.

Table 1: Vaginal Candidiasis Infection Per Trimester among the Study Population

	Trimester (%)			
Category	**1st**	**2nd**	**3rd**	**Total**
Infected	50 (33)	25 (17)	76 (50)	151 (48)
Non-infected	7 (4)	44 (27)	112 (69)	163(52)
Total	**57 (18)**	**69 (22)**	**188 (60)**	**314 (100)**

The relationship between vaginal candidiasis and gravidity status among pregnant women in the study category is presented below in Table 2. Most of the pregnant women studied were multigravida 189(60%), as against 125(40%) recorded for primigravida. Multigravida was more infected than primigravida with prevalence of 101 (67%) and 50 (33%) respectively. Similar trend was also observed among the non-infected pregnant women. Statistical analysis showed that there is no significant association (p-value = 0.191) between vaginal candidiasis and gravidity.

Table 2: Vaginal Candidiasis and Gravidity Status Among Pregnant Women

	Gravida (%)		
Category	**Primigravida**	**Multigravida**	**Total (%)**
Infected	50 (33)	101 (67)	151 (48)
Non-Infected	75 (46)	88 (54)	163 (52)
Total	**125 (40)**	**189 (60)**	**314 (100)**
P value = 0.091 Sig value = P≤0.05			

The distributions of vaginal candidiasis among the various age ranges are presented in Table 3. Result shows that 44% of the study populations were between the agerangesof 26-33 years, followed by 18-25 years, with 40% of the population, while age range of 31-41 years (16%) had the least number of participants. The overall prevalence of vaginal candidiasis among the different age ranges from this study was 48%.

Table 3: Vaginal Candidiasis Among the Different Age Ranges

	Age Range (%)			
Category	**18-25**	**26-33**	**34-41**	**Total**
Infected	75 (50)	56 (37)	20 (13)	151 (48)
Non-infected	50 (31)	82 (50)	31 (19)	163(52)
Total	**125 (40)**	**138 (44)**	**51 (16)**	**314 (100)**
P value = 0.001 Sig. value = P≤0.05				

The result of the use of antifungal drugs on vaginal candidiasis is shown in Table 4. Eighty-four percent (84 %)of the participants were not on any antifungal drug.

Table 4: Use of Antifungal Drugs on Vaginal Candidiasis

	Response (%)		
Category	Yes	No	Total (%)
Infected	38 (25)	113 (75)	151 (48)
Non-Infected	13 (8)	150 (92)	163 (52)
Total	**51 (16)**	**263 (84)**	**314 (100)**
P value = 0.081 Sig value = P≤0.05			

4.0 Discussion

Vaginal candidiasis is a common condition among women of various age groups, regardless of sexual activity, and is often neglected in pregnant women in many countries[18,11]. The health of the mother during pregnancy is crucial to the birth of a healthy baby.In this study, the prevalence of vaginal candidiasis among pregnant women 48%, was higher thanthe prevalence rate of 25-31.5% reported for Nigerian by [19] in their systematic review and meta-analysis of the vulvovaginal candidiasis prevalence among pregnant women in Africa, and 25% reported by the rate was however lower than 55.4%and 90.38% reported in studies carried out in Cameron and Kenya respectively [20, 21].The observed variations could be attributed to the study design, as the present study was restricted to women with obvious symptoms of vaginitis.

In this study also, women in their third trimester recorded the highest prevalence rate (50%),followed by the first (33%) and second trimester (17%). This result corroborates the reports by previous authors that women in their third trimester had the highest infection rate, though there were variations in prevalence rates reported. For instance, [22] reported a prevalence rate of 30.0%, at the third trimester, as against 50% observed in our study. Similarly, a study in Kenya recorded 68.09%, 21.28% and 10.63%, for third, second and first trimester respectively [21,23]. However, a study by reported a higher occurrence in second trimester (61%) of pregnancy, followed by third (21.4%) and first (16.7%) trimester respectively. The observed differences could be due to the sample size and the inclusion of only pregnant women with symptoms in our study.

This study also showed that multigravida women were generally more affected (67%) than primigravidae women (33%). This observation was also in agreement with the report by previous authors [24]. This observation could be attributed to the reported increase in rate of infection with the number of pregnancy(3rd > 2nd > 1st), and that the frequency of pregnancy reduces immunity; hence candida colonization might be intense. There was however no significant association between vaginal candidiasis and gravidity in our study.

Regarding age distribution, this study observed that the age range of 18-25 years recorded the highest prevalence of 50%. The result also lent credence to the reports [25-27], where the highest prevalence was observed among 17-23 years and 22-26 year age groups respectively. This age range is sexually active and this may have contributed significantly in the spread of the candidiasis. It has also been reported that women within this age range secrete high concentration of reproductive hormones which can suppress the immune system and creates favorable condition for candida colonization [28]. A study conducted in Ethiopia highlighted that younger women may have higher exposure risks due to physiological factors such as increased vaginal epithelial cell adhesion during pregnancy, which promotes *Candida* colonization[20]. However, other studies indicate that women aged 26-35 years have higher infection rates, suggesting that age distribution may vary regionally [22].

In this study, it was observed that most of the infected pregnant women (75%) were not on any antifungal agent. however, treatment resistance, especially among non-albicans *Candida* species, is emerging as a significant concern. Antifungal-resistant strains, such as *Candida glabrata* and *Candida krusei*, are increasingly identified in pregnant women [29,30]. These strains are less sensitive to conventional azole treatments, leading to recurrent infections, which poses challenges for managing vulvovaginal candidiasis.

Conclusion

Findings from this study suggest that vaginal candidiasis remains a significant health issue in pregnant women, and*Candida albicans*is still the mainculprit. There is increasedrisk primarily in the third trimester and among multigravida women. Younger women arealsoexperiencinghigh rates ofthe infection. Therefore,the high prevalence of vulvovaginal candidiasis in Owerri, Imo State should be a course for concern in view of the possible neonatal infection and the attendant consequences. Timely diagnosis and appropriate therapeuticinterventions prior to the time of delivery should be encouraged to protecting maternal and neonatal health.

Conflict of interests

The authors declare no conflict of interest.

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