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Prevalence of *Candida albicans* Among Pregnant and Non-Pregnant Women attending a Medical Facility in Oredo, Edo State, Nigeria

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ABSTRACT: *Candida albicans* is the most prevalent cause of fungal infections worldwide as a consequence of various triggering environments. Hence, this paper evaluates the prevalence of *Candida albican* in the cultures of pregnant and non-pregnant women who attended prenatal classes at a medical facility in Oredo, Edo State, Nigeria by collecting200 vaginal swab samples for microbiological examination using standard methods. The pour plate method was used to carry out microbial isolation. Based on their cultural, morphological, and biochemical characteristics, isolated microorganisms were recognised. The agar dilution technique was used to test antifungal sensitivity. The results showed that the lowest fungal count was $0.6\pm0.48\times10^3$ cfu/g and the highest fungal count was $5.6\pm0.32\times10^3$ cfu/g. Both *Candida albicans* and non-albicans were among the fungal isolates. In comparison to non-pregnant women (18 %), pregnant women had a percentage frequency of *Candida albican* of 30.5 %. Age groups 31 to 35 and 41 to 46 showed the highest and lowest frequencies of *Candida albicans*, respectively, among the pregnant women. Additionally, the age range of 20 to 25 had the highest frequency of *Candida albicans* among the non-pregnant women, while the age range of 36 to 40 had the lowest frequency. No antifungal resistance was found in any of the *Candida albicans* isolates to the analytical grades of itraconazole or clotrimazole, respectively. Additionally, isolate proliferation was inhibited by *Ocimum gratissimum* extracts. This study revealed that pregnant women had a higher prevalence of *Candida albicans* than non-pregnant women. It is recommended that, the general public given orientation of the major health effects of vulvovaginal candidiasis during pregnancy, especially during antenatal.

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The presence of any genital tract infection during pregnancy causes significant concerns due to the risk to the mother's and child's health. Early identification is also crucial since the existence of the foetus may limit treatment options, and maternal physiological changes may make it difficult to diagnose and control the infection (Nnadi and Singh, 2017). For women, vaginal discharge is a typical sign of a genital tract infection. Finding the reason can be extremely difficult because several microorganisms may be to blame and multiple illnesses may co-exist. Inflammation of the vagina and/or vulva caused by Candida species without the presence of any additional etiological agents is known as vulvovaginal candidiasis (VVC) (Nnadi and Singh, 2017). It is a prevalent disease that has an impact on many women's quality of life. According to estimates, it follows bacterial vaginosis as the second most prevalent cause of vaginitis (Kumari *et al*., 2013). According to estimates, 75% of all women have at least one episode of VVC during their reproductive years, and nearly half of those women suffer at least one recurrence

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(Jindal *et al*., 2007). The overgrowth of yeast and its penetration into vulvovaginal epithelial cells are believed to be the root of the symptoms (Apalata *et al*., 2014). Uncomplicated VVC is characterised by a thick, cheese-like discharge, vaginal and vulvar pruritus, discomfort, burning, erythema, and/or edema. Dysuria and dyspareunia are also possible and can lead to sex and marital discord. On a moist mount, emerging yeast cells and pseudohyphae may be seen, and the vaginal pH is often normal. 10 % of women have been reported to be asymptomatic (Nnadi and Singh, 2017). *Candida albicans* is the species that most frequently causes VVC, although other Candida species, including *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*, are also developing (Apalata *et al*., 2014). When antifungal medications were developed, *C. albicans* no longer accounted for the majority of the causes of Candida infections; instead, the non-albicans species mentioned above frequently contributed to Candida infections (Nwadioha *et al*., 2010).Sexual activity, recent antibiotic use, pregnancy, and immunological suppression from illnesses like poorly treated HIV infection or diabetes are risk factors for VVC (Nwadioha *et al*., 2010). Thought to have its primary reservoir in the rectum, vaginal colonisation is also prevalent (Okonkwo and Umeanaeto, 2010). Different variables, including host vulnerability, host inflammatory responses, and Candida virulence factors, are involved in the progression from colonisation to symptomatic infection. The actual prevalence of VVC is unclear because it is not a reportable condition, is frequently diagnosed without conclusive testing, and is frequently treated empirically (Nnadi and Singh, 2017).

According to the association between pregnancy and VVC, an increase in gestational hormones causes the pH of the vagina to change, improving a woman's chance of having VVC. High oestrogen levels cause vaginal secretions to have more glycogen, which serves as a food source for Candida fungi (Jindal *et al*., 2007). Both vaginal proliferation and symptomatic vaginitis are more common as a result of the vagina's greater sensitivity to infection by Candida species. Additionally, it is said that oestrogen promotes yeast cells' adhesion to the vaginal mucosa (Nnadi and Singh, 2017). As a result, VVC might persist longer and be linked to worse symptoms during pregnancy, and longer therapeutic regimens are often needed to alleviate symptoms. Unfortunately, pregnant women are only advised to use topical azoles (Molgaard-Nielsen *et al*., 2013). Since oral fluconazole may raise the foetus's chance of developing tetralogy of Fallot, it is often avoided (Molgaard-Nielsen *et al*., 2013). Chorioamniotic disease, abortion, premature birth, and congenital infection in the newborn are among the risks associated with untreated VVC during pregnancy. Menstrual abnormalities, infertility, pelvic abscess, and pelvic inflammatory disease are other issues that might affect non-pregnant women (Vijaya *et al*., 2014). It has been observed that Northern Nigeria had a prevalence rate of 31.5 %, 41 %, and 56.3 % among pregnant women with vaginal discharge (Nwadioha *et al*., 2010; Ibrahim *et al*., 2013; Nwosu and Djieyep, 2007). To our knowledge, however, there have been no prior investigations on the prevalence of vaginal candidiasis among pregnant women in this locality. Therefore, the purpose of this study is to ascertain the prevalence of *Candida albicans* in the cultures of pregnant and non-pregnant women who attended prenatal classes at a medical facility in Oredo, Edo State, Nigeria.

MATERIALS AND METHODS

Collection of Sample: A total of two hundred (200) female subjects were collected. Hundred (100) pregnant women and hundred (100) non-pregnant female subjects who visited Central hospital in Oredo local government area, Edo State, Nigeria. Sterile swab sticks were used to swab the posterior vaginal fornix and the lateral vaginal wall of the two hundred female subjects. Thereafter, the swab sticks were put back into their respective containers with 2 mls of normal saline added, sealed, and immediately transported to the laboratory for processing or kept in refrigerator within 24 hour prior processing. Samples were collected within a period of 6 months. The samples were labelled appropriately and taken to the laboratory immediately for analysis.

Wet mount preparation: Samples were mixed in their swab stick vial with normal saline, two drops of the sample mixtures were placed into a test tube. Two drops of 10 % KOH were add to the tube, mixed and was allowed to sit for 5 minutes until the material has cleared. One drop from the test tube was placed on the slide and examine microscopically for the presence of budding yeast and pseudohyphae.

Germ Tube Test: Germ tube experiment was used as a rapid tool for identification of *Candida albicans*. Using a sterile loop, a small portion of a pure colony of *C. albicans* was harvested and inoculated in to sterile test tubes containing 0.5 ml of human serum. The resulting suspension was incubated aerobically at 37 ^{o}C for 3 hrs. A drop of the yeast-serum suspension was placed on a clean microscope slide, covered with a cover slip and examined microscopically, using the x10 and x40 objective lenses. The appearance of small, sprouting tube-like outgrowths or filaments

projecting from the cell surface confirmed production of germ tubes (Elmer *et al*., 1992).

Sugar Tests: One gram (1 g) each of sugars galactose, glucose, maltose, lactose and sucrose were measured into 1ml of double distilled water in different test tubes and allowed to dissolve. The solution was then incubated with the Candida isolates and then stained with phenol red, an indicator and incubated for 48 hrs at 30 ^{o}C after which they were observed for sugar fermentation (change in colour).

Determination of the minimum inhibitory concentration (MIC) of analytical grade of itraconazole on Candida albicans: The minimum inhibitory concentration (MIC) of itraconazole for *Candida albicans* was determined using the Agar dilution method (Afolayan and Meyer 1997). The least concentration that prevented the visible growth of *Candida albicans* was recorded as the minimum inhibitory concentration. 0.1 g of 1traconazole was measured into test tube and 1ml of Dimethyl sulfoxide (50 %) was added to give 100 mg. Since the targeted dilution concentration is < 25 mg, to achieve this (i.e < 25 mg). 1ml of distilled water was added to 1 ml DMSO (100 mg), the concentration becomes 50 mg. To the solution, another 1ml of distilled water, the concentration becomes 25 mg. same procedure was taken to get 12.5 mg and 6.25 mg (since I needed three concentrations to work on). 1 ml was taken from 25mg and add to 20 ml molten PDA to give a concentration of 1.25 mg i.e 1/20 x 25 = 1.25 mg, same is done for 12.5 mg and 6.25 mg, 1/20 x 12.5 = 0.625 mg,1/20 x 6.25 = 0.3125 mg. The petri dishes were labelled respectively, i.e, 1.25 mg, 0.625 mg and 0.3125 mg. The plates sterilize for few minutes, using a wire loop, the broth were inoculated onto the plates. They were incubated at 37 ^{o}C for 48 hours.

Determination of the minimum inhibitory concentration (MIC) of Ocimum gratissimum on Candida albicans: The minimum inhibitory concentration (MIC) of ocimum gratissismum for *Candida albicans* was determined using the Agar dilution method (Afolayan and Meyer 1997). The least concentration that prevented the visible growth of *Candida albicans* was recorded as the minimum inhibitory concentration.

Determination of the minimum fungicidal concentration (MFC) of analytical grade of itraconazole on *Candida albicans:*The minimum fungicidal concentration (MFC) of itraconazole for *Candida albicans* was determined using the Agar dilution method (Afolayan and Meyer 1997). One millilitre (1ml) of broth from the test tubes, which showed no growth was inoculated into potato dextrose agar plate. The plates were incubated for 48 hours at 37 ^{o}C. The least concentration of the analytical grade in which no growth was observed on the potato dextrose agar media, after 48 hours was recorded as the minimum fungicidal concentration of itraconazole.

Determination of the minimum fungicidal concentration (MFC) of clomatrizole on Candida albicans: The minimum fungicidal concentration (MFC) of *Ocimum gratissimum* for *Candida albicans* was determined using Agar dilution method (Afolayan and Meyer 1997). One millilitre (1 ml) of agar extract from the test tubes that showed no growth was inoculated into potato dextrose agar plate. The plates were incubated for 48 hours at 37 ^{o}C. The least concentration of volatile oil of *Ocimum gratissimum* in which no growth was observed on the potato dextrose agar media after 48 hours, was recorded as the minimum fungicidal concentration of volatile oil of *Ocimum gratissimum*.

Thirty-nine grams (39 g) of potato dextrose agar (PDA) powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The medium was cooled to 45-50 ^{o}C and then dispensed aseptically into sterile Petri dishes.

RESULTS AND DISCUSSION

Table 1 shows the total fungi count from the female subject, among the pregnant women it was observed that age group 26 – 30 ($5.6\pm0.32\times10^3$ cfu/g) had the highest growth, followed by 31 – 35 ($5.4\pm0.14\times10^3$ cfu/g), 36 – 40 ($3.4\pm0.15\times10^3$ cfu/g), 20 – 25 ($1.8\pm0.15\times10^3$ cfu/g) and the lowest was observed in 41 – 46 ($1.1\pm0.12\times10^3$ cfu/g). However, for non – pregnant women, the highest growth was observed in age group 26 – 30 ($4.1\pm0.16\times10^3$ cfu/g), followed by 36 – 40 ($3.5\pm3.33\times10^3$ cfu/g), 31 – 35 ($2.6\pm2.15\times10^3$ cfu/g), while the lowest was recorded in 20 – 25 ($0.6\pm0.48\times10^3$ cfu/g) but no growth was observed in age group 41 – 46.

Table 1: Total fungi counts from female subject

Age group	Pregnant women	Non-Pregnant women
20 – 25	$1.8\pm0.15\times10^3$ cfu/g	$0.6\pm0.48\times10^3$ cfu/g
26 – 30	$5.6\pm0.32\times10^3$ cfu/g	$4.1\pm0.16\times10^3$ cfu/g
31 – 35	$5.4\pm0.14\times10^3$ cfu/g	$2.6\pm2.15\times10^3$ cfu/g
36 – 40	$3.4\pm0.15\times10^3$ cfu/g	$3.5\pm3.33\times10^3$ cfu/g
41 – 46	$1.1\pm0.12\times10^3$ cfu/g	$0.0\pm0.00\times10^3$ cfu/g

Candida albicans was the most prevalent species isolated in 30.50 % of pregnant women while 18 %

was isolated from non-pregnant women as shown in Figure 1.

Figure 2 shows the percentage frequency of *Candida albicans* among pregnant and non-pregnant women of different age groups. Among the pregnant women, *C. albicans* was most frequent in the age group 31–35 with 12% and less frequent (0.5%) in the 41–46 age groups. However, *C. albicans* was more frequent (6.5%) in adults aged 20–25 but was least frequent (3%) in this age group (36–40).

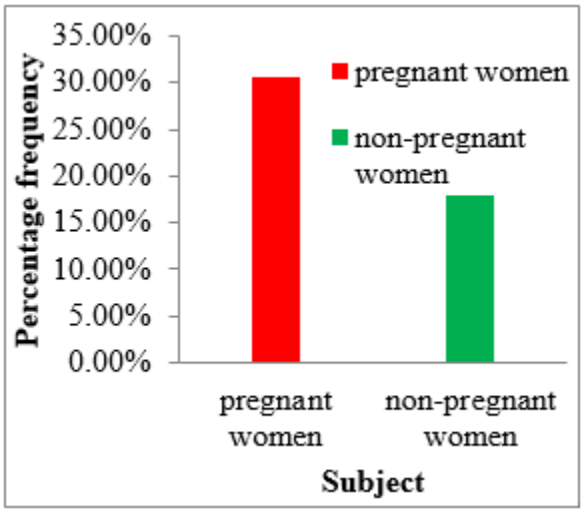

Fig 1: Percentage frequency of *Candida albicans* isolated from pregnant and non-pregnant women.

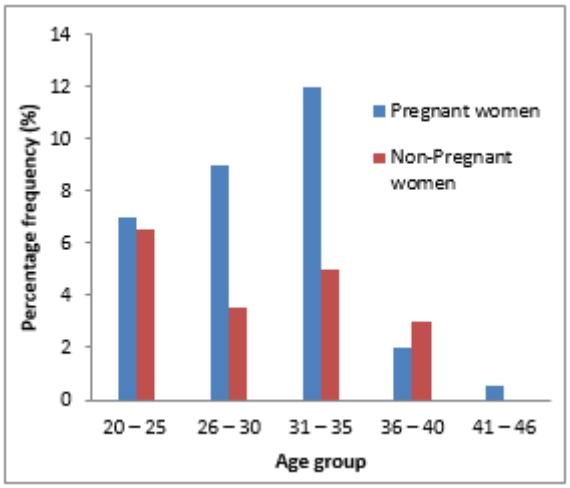

Fig 2: Percentage frequency of *Candida albicans* among pregnant and non-pregnant women of different age groups.

Table 2: Antifungal sensitivity on *Candida albicans* from pregnant and non-pregnant women

Analytical grades	Minimum inhibitory concentration(MIC mg/ml)	Minimum fungicidal concentration (MFC mg/ml)
Itraconazole	0.125 mg/ml	0.25 mg/ml
Clomatrizole	0.125 mg/ml	0.25 mg/ml
Ocimum gratissimum	0.65 mg/ml	1.25mg/ml

Table 2 shows the antifungal sensitivity to *Candida albicans* in pregnant and non-pregnant women. The MIC and MFC of analytical grade itraconale against *Candida albicans* isolated from vaginals of pregnant and non-pregnant women were 0.125 mg/ml and 0.25 mg/ml, respectively. The MIC and MFC of the analytical grade of Clomatrizole against *Candida albicans* were 0.125 mg/ml and 0.25 mg/ml, respectively. However, the MIC and MFC of *Ocimum gratissimum* against *Candida albicans* were at 0.65 mg/mL and 1.25 mg/mL, respectively.

This study seeks to evaluate the antifungal activity of *Ocimum gratissimum*, Itraconazole, and Clomatrizole in the treatment of vulvovaginal candidiasis in pregnant and non-pregnant women in Benin City.
The prevalence of *Candida albicans* among pregnant women in this study was 30.50 %. This is lower than the 56.3% reported by Okonkwo and Umeanaeto (2010), 41% observed by Ibrahim *et al*. (2013) from studies from North-Eastern Nigeria, and slightly lower than the 30% reported by Nwosu and Djieyep (2007) from South-Eastern Nigeria. The prevalence of *Candida albicans* among pregnant women varies among different countries. Reports from several countries indicate the prevalence hovers around 20% (Lisiak *et al*., 2000; Nwosu and Djieyep, 2007).

The age group of the pregnant women in this study was 20 - 46 years. The age range of 31–35 years was more affected by 24 %. This is lower than the report of Nnadi and Singh (2017). This age group represents the active reproductive period and the peak of childbearing in Nigerian society (Ugwa, 2015). There is intense sexual activity and thus a high tendency to acquire some sexually transmitted infections which may subsequently predispose the woman to *C. albicans*. Similarly, it has been observed that there is a high concentration of reproductive hormones during pregnancy which provides a favourable environment for the growth of Candida species (Okonkwo and Umeanaeto, 2010).

The increasing incidence of synthetic antibiotic resistance against Candida species is of public health importance. The urge for alternative sources of natural antifungals with therapeutical effects has led to the search for plants or herbs as unorthodox medicine. The antifungal study of the volatile oil of *Ocimum gratissimum* at varying concentrations of low to high (0.313 mg/ml – 1.25 mg/ml) revealed significant inhibition (antifungal effect) against *Candida albicans*

isolated from the vaginals of pregnant and non-pregnant women of different ages, at low concentration of 0.6mg/ml, while the minimum antifungal concentration was at the value of 1.25 mg. The result of this study revealed that the volatile oil of *Ocimum gratissimum* may be used as an effective natural healing means antifungal agent in the medical field and pharmaceutical industries. Conversely, the analytical grade of itraconazole used in this study revealed a significant inhibition effect against *Candida albicans*. The result revealed that *Candida albicans* isolated from pregnant and non-pregnant women were inhibited at a low concentration of 0.125 mg/ml and 0.25 mg/ml for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) respectively.

Conclusion: This study revealed that *Candida albicans* was frequently encountered among pregnant women as compared to those detected among non-pregnant women. It is therefore imperative that there should be public health awareness about *vulvovaginal candidiasis* especially in ante-natal classes, on the health implication when left untreated during pregnancy.

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