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Occurrence and Antifungal Susceptibility of *Candida* Species Isolated from Pregnant Women in a Tertiary Hospital

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Abstract

Candida is the leading cause of infection among pregnant women. This study was aimed to determine the occurrence and antifungal susceptibility of *Candida* species isolated from apparently healthy pregnant women receiving antenatal care at Bingham University Teaching Hospital. High vaginal swab was inoculated onto Sabouraud Dextrose Agar and Gram staining was carried out. *Candida* isolates were sub- cultured on Himedia CHROM Candida differential agar and identified according to their color, morphology, and appearance. Disc antifungal susceptibility test was carried out using standard protocols. The occurrence rate of *Vulvovaginal candidiasis* (VVC) in this study was 42.5%. In this study, *Candia albicans* was the most predominant *Candida* species (51.2%) isolated. Pregnant women within the age range of 20-24 years had the highest infection occurrence of 100% while the lowest occurrence of the infection (25%) was observed among women within the age range of 40-44 years. There was a significant relationship between age and VVC infection (P>0.05). In relation to trimester, pregnant women in the second trimester had a high infection rate of 87.5% while women in the third trimester had the least occurrence rate of 10.9%. There was a significant relationship between *Vulvovaginal candidiasis* infection and trimester (P>0.05). All *Candida* species were resistant to the antifungal drugs tested except for *Candida kefyr* which was susceptible to fluconazole and voriconazole. Therefore, early diagnosis and appropriate treatment among pregnant women receiving antenatal care can reduce the infection and prevent complications during childbirth.

Keywords: *Candida*, prevalence, pregnancy, infection rate, Bingham University

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Introduction

Vulvovaginal candidiasis affects millions of women every year. It is the most frequent gynecological infection among pregnant women of childbearing age [1]. There are about 100,000 cases of this infection per year in Nigeria [2]. Vulvovaginal candidiasis (VVC), which is also known as vaginal yeast infection is a common inflammatory condition caused by vaginal overgrowth of *Candida albicans*, which is mostly observed in women in the productive age group either in the pre-pubertal or postmenopausal stage [3]. Other species such as *C. glabrata, C. kefyr, C. tropicalis, C. parapsilosis, C. krusei*can cause *Candida* infection. The second most observed cause of candidiasis is *C. glabrata* [4].

Most episodes of VVC are asymptomatic however, symptomatic VVC occur during the second and third trimesters in pregnancy. When left untreated in pregnancy, VVC could lead to chorioamnionitis, abortion, pre-term delivery and congenital infection on the neonate [5]. There are several therapeutic options available for the treatment of VVC. Azole based drugs are the most common class of antifungal drugs used to treat vaginal candidiasis due to their good bioavailability, antifungal efficacy, and relative safety. However, these treatments mainly consist of prescription oral dosage forms, or over-the-counter topical preparations, or vaginal suppositories [6].

There is paucity of data regarding the prevalence of VVC, its distribution and the in vitro antifungal susceptibility pattern of *Candida* isolates from vaginal swabs of pregnant women withVVC infection and asymptomatic carriers of Candida speciesin the study area, therefore, this study was designed to establish the prevalence of VVC, identify the most occurring species of *Candida* associated with VVC in pregnant women and determine the most effective antifungal drug for treatment.

Materials and Methods

Area of study

The study was carried out among apparently healthy pregnant women currently receiving antenatal care at Bingham University Teaching Hospital in Jos North, Plateau state, Nigeria. Plateau state is located at North Central Zone of Nigeria. It was created 3rd February, 1976 out of the Northern half of former Benue-Plateau state. It is bounded by the states of Kaduna and Bauchi on the North, Taraba on the East and Nasarawa on the South and West. It is located between latitude 08°24'N and longitude 008°32' and 010°38' East [7].

Study design

A cross sectional design was used in this study, demographic data was obtained through the administration of laid out questionnaires.

Enrollment of patients was randomized, and their consent was sought for and obtained. The study was conducted in compliance with Bingham University's ethical committee approval.

Inclusion and exclusion criteria

All apparently healthy pregnant women (in and outpatients) receiving antenatal care at the Bingham University Teaching Hospital, who were confirmed pregnant by the laboratory scientists and women within the age range of 15-44 years and those who gave their consent were recruited for the study. Non-pregnant women, pregnant women who did not fall under the age range, those who are currently taking antifungal drugs, and those who did not give consent were excluded from the study.

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committees of Bingham University Teaching Hospital, with reference numbers (NHREC/21/05/2005/00767). The samples were obtained with the informed consent of the women.

Specimen collection

Two hundred high vaginal swabs were collected by a gynecologist from pregnant women within the age range from 15-44yrs, who were receiving antenatal care in the hospital. A sterile vaginal speculum was inserted, and a swab was then rotated to collect the secretions, removed, and placed in the swab container [8].

Sample processing

A sterile swab stick was used to make a smear on a grease free slide, a drop of 10% potassium hydroxide (KOH) solution was added and viewed under the microscope using x10 and x40 objective lenses for pseudohyphae, hyphae, budding yeast cells and spores [9]. The collected vaginal swab specimens were inoculated on Sabouraud Dextrose Agar and incubated at 37°C for 24 h. Colonies on SDA plates were identified according to colony morphology and Gram staining for yeast-like cells. *Candida* isolates were sub-cultured on Himedia CHROM Candida differential agar, incubated for 48 h at 37ºC and classified according to their color, morphology, and appearance.

*Candida*cells were subjected to germ tube test. Colonies of *Candida* were inoculated into 0.5 ml of human serum and incubated at 37ºC for 3 h. On a clean grease free slide, a drop of the properly mixed suspension was added and covered with a cover slip and observed under the microscope with x40 objective lens. Lateral tube without septum and constriction at initiating site were considered as germ tubes.

Antifungal testing by disk diffusion method was performed according to standard microbiological procedures. Muller-Hinton agar was prepared and supplemented with freshly prepared 2% glucose and 0.5 µg of methylene blue dye. A colony of the *Candida* species was picked with a sterile wire loop and inoculated on the agar plate. The following antifungal disks nystatin (100 µg), fluconazole (50 µg) and voriconazole (100 µg) was used. It was placed on the already inoculated agar and incubated at 37ºC for 24 h to observe for zones of inhibition which was interpreted as resistant, intermediate, or susceptible to the antifungal drug. The zone of inhibition was determined by measuring the diameter of zone inhibition for each antifungal disk using a ruler as describe by the NCCLS, 2000 document [10].

The data was analyzed using statistical package SPSS version 22. The relationships between the variables were determined using Chi-square test. AP-value less than 0.05 was considered statistically significant. Categorical data variables were statistically described in the form of frequencies and percentages.

Results and Discussion

Two hundred (200) pregnant women receiving antenatal care in Bingham University Teaching Hospital were recruited for this study; one hundred and twenty (120) women gave their consent and were successfully screened for VVC infection. Out of these, 51 pregnant women were positive and 69 were negative (Fig. 1). An occurrence rate of 42.5% was recorded in this study.

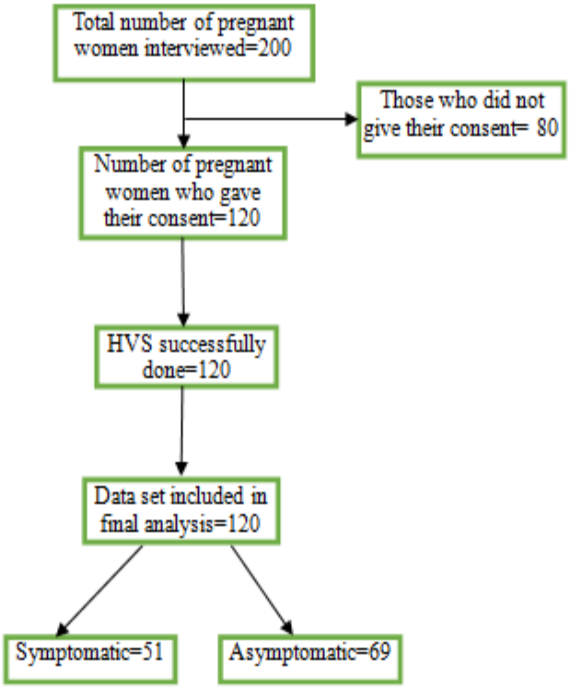

Figure 1: Study participant flow

Table 1: Distribution of *Candida* species in the study area

Candida species	Frequency	Percentage (%)
C. glabrata	17	41.5
C. parapsilosis	1	2.4
C. kefyr	2	4.9
C. albicans	21	51.2
Total	**51**	**100**

Table 2: Occurrence of *Vulvovaginal candidiasis* among pregnant women in the study area

Results	Number Screened	Occurrence (%)
Positive	51	42.5
Negative	69	57.5
Total	**120**	**100**

Table 1 shows the distribution of *Candida* species isolated from the study. These species include *Candida albicans* (51.2%), *Candida glabrata* (41.5%), *Candida krusei*, *Candida kefyr* (4.9%), *Candida parapsilosis* (2.4%). The occurrence of vulvovaginal candidiasis (42.5%) among pregnant women in the study area is shown on Table 2.

Table 3: Association between socio-demographic factors and VVC infection

Variable	Number examine/ prevalence (%)	Number positive/ prevalence (%)	P-value
Age range			
20-24	8(6.7)	8(100)	
25-29	30(15.0)	9(30)	0.02
30-34	47(39.2)	21(44.7)	
35-39	27(22.5)	11(40.7)	
40-44	8(6.7)	2(25.0)	
Trimester			
First	16(8.0)	9(56.2)	0.01
Second	40(20.0)	35(87.5)	
Third	64(32.0)	7(10.9)	
Marital status			
Single	20(16.7)	9(45.0)	0.50
Married	100(50.0)	42(42.0)	
Educational status			
Primary	1(0.8)	1(100)	
Secondary	38(31.7)	22(57.9)	0.64
Tertiary	71(59.2)	41(57.7)	
Informal	10(8.3)	6(60.0)	
HIV status			
Positive	9(7.5)	5(55.5)	0.00
Negative	111(92.5)	46(41.4)	
Antibiotic usage			
Yes	13(10.8)	10(76.9)	0.00
No	107(89.2)	41(38.3)	
Number of children			
One-three	97(48.5)	43(44.3)	0.46
Four-six	18(9.0)	6(33.3)	
Seven-nine	5(2.5)	2(40.0)	

Table 4: Distribution of signs and symptoms among pregnant women

Symptoms	Number Screened (%)	Number positive/ prevalence (%)	Number negative (%)	Prevalence (%)
Burning sensation and discharge only	9(7.5)	69(67.0)	3(33.3)	67.0
Burning sensation and irritation only	5(4.2)	2(40.0)	3(60.0)	40.0
Itching and discharge only	18(15.0)	14(78.0)	4(22.2)	78.0
Burning sensation only	10(8.3)	1(10.0)	9(90.0)	10.0
Itching only	8(6.7)	6(75.0)	2(25.0)	75.0
Discharge only	27(22.5)	7(26.0)	20(74.1)	26.0
All symptoms	4(3.3)	1(25.0)	3(75.0)	25.0
None	39(32.5)	14(36.0)	25(64.1)	36.0
Total	**120**	**51(42.5)**	**69(57.5)**	**42.5**

Table 3 shows the relationship between socio-demographic factors and VVC infection. The mean age of the pregnant women was 32 years. Pregnant women within the age range of 20-24 years had the highest infection occurrence of 100%. In relation to marital status, the single women had the highest prevalence rate of 45.0%. Women in the second trimester had the highest prevalence rate by 87.5% while the lowest prevalence rate of 10.9% was observed in the third trimester. Statistical analysis reveals a significant relationship between trimester and VVC infection (P>0.05).

Pregnant women who had obtained secondary 57.9% and tertiary 57.7% education had the highest prevalence while the lowest prevalence 40% was observed among women with for informal education. Statistical analysis shows significant relationship between educational status and VVC. Pregnant women who were recently administered antibiotics had a higher prevalence rate of 76.9% in comparison to those who were not administered antibiotics (38.3%). Statistical analysis shows a significant relationship between the antibiotics and the infection (P>0.05).

The distribution of signs and symptoms among pregnant women reveals that women who had itching and discharge only had a higher prevalence of 78% as shown in Table 4. The susceptibility tests revealed that all the *Candida* isolates were resistant to nystatin, fluconazole and voriconazole except *C. kefyr* which revealed 19.6% and 9.8% susceptibility to fluconazole and voriconazole (Table 5).

Table 5: *In vitro* susceptibility of *Candida* species

Drugs	*Candida* species	S No(%)	SDD No(%)	R No (%)	Total
Nystatin	*C. glabrata*	0(0.0)	0(0.0)	0.(0.0)	0(0.0)
	C. parapsilosis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	C. kefyr	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	C. albicans	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Fluconazole	*C. glabrata*	0(0.0)	0(0.0)	0.0(0.0)	0(0.0)
	C. parapsilosis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	C. kefyr	10(19.6)	0(0.0)	0(0.0)	10 (19.6)
	C. albicans	0(0.0)	0(0.0)	0(0.0)	0(100.0)
Voriconazole	*C. glabrata*	0(0.0)	0(0.0)	0.0(0.00)	0(0.0)
	C. parapsilosis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	C. kefyr	5(9.8)	0(0.0)	0(0.0)	5(9.8)
	C. albicans	0(0.0)	0(0.0)	0(0.0)	0(0.0)

S: Susceptible; **SDD:** Susceptible Dose Dependent; **R:** Resistant
Data is presented as frequency and percentage in parenthesis.

The study revealed a high prevalence of 42.5% VVC infection among pregnant women in the study area. This occurrence rate may be attributed to the number of women who received antenatal care during the study period and gave their consent for enrollment. In North-western Nigeria, a prevalence rate was of 60.8% was reported among pregnant women receiving health care in a tertiary facility in North-western Nigeria [11]. Reports from Libya, Tanzania, Turkey, and Ghana recorded prevalence rate of 43.8, 42.9, 42.7, 35 and 30.7% in similar studies [12, 13, 14, 4].

Candida albicans was found to be the most predominant species (51.2%) and this may be due to its ability to adhere to the epithelial cells of the vagina. Previous studies have reported the high distribution of *C. albicans* with prevalence rate of 53.9, and 58.6% [12, 15]. However, lower prevalence rate of *C. albicans* was reported 25.9, 41.7, 43.23 and 44.21% were reported by Nigeria, Ghana, Pakistan, and Iran, respectively [16, 4, 17, 18].

Women who were currently on antibiotics had a higher occurrence of 76.9%, and this agrees with previous reports that antibiotic usage/prolonged antibiotic usage predisposes individuals to VVC infection similarly, constant abuse or self-administration of drugs may lead to recurrent VVC.

The highest infection rate of 87.5% was observed in the second trimester and this may be attributed to hormonal changes in the second trimester. This agrees with studies carried out by some scholars [19, 20] who observed high prevalence rates of 55 and 56.7% in the second trimester but contradicts the finding of Bamniya *et al.* [21] who reported the highest prevalence rate of 63.5% among women in the third semester.

The highest prevalence rate (100%) was observed among those with primary education by 100% and this agrees with the report of [22] who observed the highest infection rate of 50 and 31.5% among women with primary education. However, in this study, only one woman was screened and diagnosed with VVC infection in this category. Women who had tertiary and secondary education had an occurrence rate of 57%. Studies conducted by some workers [23, 4, 24, 15] had lower prevalence rates of 45, 52.3, 27.3 and 13.6% among women with tertiary and secondary education.

Asymptomatic women had a higher infection rate of 57.5% and this may be attributed to unawareness of the infection, hence a higher infection rate. This agrees with the separate studies of some workers [25, 26, 27, 28] who reported 61.9, 67.9, 68.2, and 70.74% asymptomatic colonization among pregnant women in Argentina, Nepal, Ghana, and Burkina Faso, respectively.

The susceptibility test revealed that *C. glabrata*, *C. albicans* and *C. parapsilosis* were (100%) resistant to nystatin, fluconazole and voriconazole. This high resistance may be attributed to continuous use and abuse of these antifungal agents as these drugs are sold over the counter in pharmacies or probable mutation in the fungal genome. This report is in contrast with the findings of some studies [29, 30, 31] that observed susceptibility of these *Candida* isolates to these drugs. *Candida kefyr* was reported to have 19.6 and 9.8% susceptibility to fluconazole and voriconazole which validates it efficacy and suggest its continuous usage against *Candida kefyr*.

Conclusion

This research reveals a high occurrence of vulvovaginal candidiasis among pregnant women receiving antenatal care in the study area. *Candida albicans* was more predominant species isolated and C. *glabrata* as the most frequent non-*Candida albicans* isolate causing the infection. The high resistance to the three antifungal drugs observed in this study strongly suggests the need for screening and diagnosis of VVC before the onset of therapy.

Conflicts of interest: The authors declare there is no conflict of interest in this article.

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