



Comparative Study of Vaginal Candidiasis in Pregnant and Non-Pregnant Women Attending Ibrahim Badamasi Babangida Specialist Hospital and General Hospital, Minna Niger State, Nigeria

K. T. Mumuney^{1*} and M. E. Abalaka¹

¹*Department of Microbiology, Faculty of Science, Federal University of Technology Minna, Niger State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between the authors. Author KTM carried out the sample collection, designed the study template, managed the literature searches and prepared the manuscripts for publication. Author MEA carried out the biochemical analysis, statistical analysis, and discussion of results. The authors read and approved the final manuscript.

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ABSTRACT

Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast). Vaginal candidiasis is common during pregnancy and may impact negatively on the health of the woman. The aim of this study is to determine and compare the prevalence of vaginal candidiasis among pregnant and non-pregnant women attending Ibrahim Badamasi Babangida (IBB) Specialist Hospital and General Hospital in Minna, Niger state, Nigeria were screened for vaginal candidiasis. High vaginal swab (HVS) were each collected from 20 pregnant and 20 non-pregnant women attending General Hospital, Minna also 20 pregnant women and 20 non pregnant women at IBB (Ibrahim Badamasi

*Corresponding author: E-mail: temitopekafilat@gmail.com;

Babangida) Specialist Hospital, Minna, Niger state, Nigeria and screened for vaginal candidiasis. Sabouraud Dextrose Agar (SDA) incorporated with chloramphenicol was used to isolate the *Candida* species in the HVS samples. *Candida* isolates were identified and characterized using standard methods including: colonial morphology (macroscopic characteristics), microscopy (Sugar fermentation) tests. Confirmation of *Candida albicans* was done with the Germ Tube Test. (Gram staining and Lactophenol cotton blue staining) and biochemical (Sugar fermentation) tests. Thirteen samples tested showed positive for *candida* species. The species identified were *Candida albicans* (Nine), *Candida krusei* (two), *Candida tropicalis* (One), and *Candida pseudotropicalis* (one) for pregnant women in General Hospital. Ten samples taken from pregnant women in Ibrahim Badamosi Babangida Specialist Hospital were positive reactions for *Candida* species. Nine out of twenty samples collected from non-pregnant women in General Hospital were *Candida* species while three out of twenty samples from non-pregnant women from Ibrahim Babangida Specialist Hospital tested positive to *Candida* species. Within the samples space 45% and 25% of the pregnant women considered in the study in General Hospital and IBB Specialist Hospital respectively had *Candida albicans*, while amongst the non-pregnant sample space, the infection rate was 10% for both study locations.

This result showed vaginal candidiasis is more prevalent in pregnant women than non-pregnant women which may lead to pregnancy complications like abortions, premature birth, low birth weight and other morbidities. Screening protocol incorporated with routine ante-natal checkup for early diagnosis of Candidiasis and its treatment is hereby recommended.

Keywords: *Pregnancy; High Vaginal Swab (HVS); Candida species; vaginal candidiasis; SDA.*

1. AIM OF THE STUDY

To carryout comparative study on the prevalence of vaginal Candidiasis between Pregnant and Non pregnant women attending General Hospital Minna and IBB specialist Hospital Minna Niger-State.

2. INTRODUCTION

Candidiasis is a fungal infection (mycosis) of any of the *Candida* species (all yeast) of which *Candida albicans* is the most common [1]. When it affects the mouth, it is commonly called thrush [2]. Although commonly referred to as yeast infection, Candidiasis is the technically known as Candidiosis, Moniliasis and oidiomycosis. Signs and symptoms include white patches on the tongue or other areas of the mouth and throat [3]. Other symptoms may include soreness and problems swallowing [3]. When it affects the vagina, it is commonly called a yeast infection [3]. Signs and symptoms include genital itching, burning, and sometimes a white "cottage cheese-like" discharge from the vagina [3]. Less commonly the penis may be affected, resulting in itchiness [3]. Very rarely, the infection may become invasive, spreading to other parts of the body. This may result in fevers along with other symptoms depending on the parts involved [3].

More than 20 types of *Candida* species can cause infection, with *Candida albicans* being the

most common [3]. Infections of the mouth are most common among children less than one month old, the elderly, and those with weak immune systems. Conditions that result in a weak immune system include HIV/AIDS, the medications used after organ transplantation, diabetes, and the use of corticosteroids. Other risks factors include dentures and antibiotic therapy [4]. Vaginal infections occur more commonly during pregnancy, in those with weak immune systems, and following antibiotic use [4]. Risk factors for invasive candidiasis include being in an intensive care unit, following surgery, low birth weight infants, and those with weak immune systems [5].

Vaginal candidiasis is usually treated with antifungal medications [5]. For most infections, the treatment is an antifungal applied inside the vagina, or a single dose of fluconazole administered orally. For more severe infections, infections that don't get better, or keep recurring, other treatments might be needed. These treatments include more doses of fluconazole or other medicines applied inside the vagina such as boric acid, nystatin, or flucytosine. Little evidence supports probiotics for either prevention or treatment even among those with frequent vaginal infections [6,7]. Vaginal candidiasis is common, though more research is needed to ascertain the population of women affected. Women who are more likely to get vaginal candidiasis include those who are pregnant, use

hormonal contraceptives (for example, birth control pills), have diabetes, have a weakened immune system (for example, due to HIV infection or medicines that weaken the immune system, such as steroids and chemotherapy), are taking or have recently taken antibiotics. About three-quarters of women have at least one yeast infection at some time during their lives [7]. Widespread disease is rare except in those who have risk factors [7]. This research aimed to carry out comparative study of Prevalence of vaginal candidiasis between pregnant women and non-pregnant attending General Hospital and IBB Specialist Hospital, both in Minna, Niger state, Nigeria.

3. AREA OF STUDY

The study areas of this research were General hospital Minna and IBB Specialist Hospital, co-located in Minna, Niger State. Minna is a city (estimated population 299373 in 2018) in west-central Nigeria. It is the capital of Niger State, one of Nigeria's 36 federal states. It consists of 2 major ethnic groups: the Nupe and the Gbagyi. Minna has the Latitude 9.61389 and Longitude of 6.55694.

3.1 Study Population

A total of 80 women: 40 pregnant women and 40 non-pregnant women were screened for vaginal Candidiasis using standard methods including: colonial morphology (macroscopic characteristics), microscopy (Gram staining and Lactophenol cotton blue staining) and biochemical (Sugar fermentation) tests. Confirmation of *Candida albicans* was done with the Germ Tube Test. The ages of the subjects were between 15 and 50 years.

3.2 Media Preparation

The media used, Sabouraud dextrose agar (SDA) was prepared in line with manufacturer's instruction.

3.3 Media Composition

65 grams of SDA suspended in 1 litre distilled water, 0.5 grams of Chloramphenicol powder.

The SDA suspension was sterilized by autoclaving at 121°C for 15 minutes. 110ml of the medium was then dispensed into Petri dishes after cooling.

3.4 Sample Collection and Processing

The study sample space comprising eighty subjects without vaginal disorder were studied: 20 pregnant women and 20 non pregnant in each hospital, constituting a total of eighty subjects. The cervixes were opened with sterile unlubricated bivalve. Vaginal spectrum specimen of vaginal discharge was collected from each posterior and lateral fornix. The samples were transported immediately to the laboratory and inoculated into freshly-prepared SDA and kept on the incubating hood at 28°C for 48 hours. Growths having milk to white colour and palm wine smell were picked for further identification and characterization.

3.5 Identification and Characterisation of *Candida* Isolate

Growths of yeast were seen on the petri dishes after 48 hours of incubation at 28°C on the SDA medium. Colonies were counted using colony counter. Smear preparation was made on a clean slide and gram-stained to use the morphological characteristics of the organism.

3.6 Gram Staining

Suspected *Candida* colonies on any of the agar plates were emulsified in Normal saline on the slide to form a smear. The smear was allowed to air dry completely. The slide, (with the smear uppermost) was fixed by rapidly passing through flame of a Bunsen burner. The smear was allowed to cool. The fixed smear was covered with crystal violet stain for 30 seconds – 1 minute. The stain was rapidly washed off with clean running tap water. The smear was again covered with Lugol's iodine for another 30 seconds – 1 minute and washed off with clean tap water and was rapidly decolourized (few seconds) with acetone-alcohol. This was washed immediately with clean tap water. The smear was then covered with neutral red stain for 2 minutes and washed off with clean tap water. The stained slide was placed in a draining rack and the smear allowed to air dry. The smear was examined microscopically using oil immersion objective lens (×100). *Candida* species were Gram positive yeast like cell under the microscope.

3.7 Lactose Phenol Cotton Blue Stain Procedure

A glass slide was cleared with clean cotton wool and a drop of lactose phenol cotton blue was

dropped on the slide after which a small portion of the pure isolate was picked and smeared with the lactose phenol on the slide and covered with clean cover slide mounted. It was viewed under a microscope at 10X magnification. The chlamyospores of the organism were clearly visible.

3.8 Biochemical (Sugar Fermentation) Test

The biochemical test for *Candida* isolate is sugar fermentation, this test shows how each organism ferments, or utilizes, different types of sugar (glucose, lactose, maltose, galactose, fructose, and sucrose).

Procedure: Two grams of each sugar were weighed into different beakers. One gram of peptone was added to each along with phenol red indicator powder. 100ml of distilled water was added to each beaker and shaken till it dissolved. 10ml of the sugar solution was dispensed inside test tube that had been labelled accordingly and covered with the cotton wrapped with foil paper and then autoclaved at 121°C for 15minutes. It was allowed to cool after which small quantity of the pure yeast isolated was inoculated inside each test and incubated in the inoculating hood at the room temperature for 3 days. The result of color change was observed and recorded appropriately.

3.9 (Germ Tube Test) of *Candida albicans*

This is presumptive test a for the presence of *Candida albicans* in a sample.

Procedure: A mammalian serum was obtained. 0.5ml of the serum was added in a clean sterile container. Small colony of the pure isolated *Candida* was emulsified with a sterile wire loop and incubated for 3 hours, after which it was smeared onto a clean slide, covered with a slide and viewed under the microscope using X10 objectives lenses. The spore and hyphae of the organism were visible, establishing a positive result for *Candida species* only.

4. RESULTS

4.1 Candidiasis in Relation to Age

The result revealed that of the 20 sample screened in pregnant women in General

Hospital, Minna, 13 had *Candida*, with the remaining 7 samples testing negative. 10 of the 20 samples taken from pregnant women in IBB Specialist Hospital Minna tested positive for *Candida* this is shown in Table 1.

Amongst the non-pregnant women study group, 5 of 20 screened at the General Hospital tested positive, with 15 testing negative while the study group of IBB Specialist Hospital tested positive to *Candida* in 3 cases, with 17 testing negative. The age range mostly affected in non-pregnant women in both hospitals was 21 – 30 years as shown in all the tables. The result of which is given in Table 2.

The identification and frequency of occurrence of *Candida* isolates identified in this research are shown in Table 3. These are *Candida albicans*, *Candida krusei*, *Candida pseudotropicalis* and *Candida tropicalis*. *Candida albicans* had the highest frequency occurrence, closely followed by *Candida krusei*. The rest two isolate *Candida pseudotropicalis* and *Candida tropicalis* had the least frequency of occurrence in both hospitals.

Table 4 shows the results of the biochemical test (fermentation of sugars) and morphological characteristics as viewed under a microscope, of the *Candida* species isolated during the research work. *Candida albicans* was able to utilize glucose, maltose, lactose, galactose, fructose, and sucrose.

The chlamyospore of *Candida albicans* are row-like round ovals budding yeast that form smooth, creamy, and numerous colonies. Pseudomycelia are also numerous.

Candida krusei has no chlamyospore, but are elongated budding cells with occasional pseudohyphae, forming whitish growth, flat, dry, and often small semi-glossy wrinkled colonies. It is capable of utilizing any of the sugars as source of carbon.

Candida tropicalis possesses no chlamyospore, and is characteristically exhibits flat growth with smooth margin. It utilizes only Fructose, glucose, Maltose, and Sucrose as carbon source.

Candida pseudotropicalis has no chlamyospore, and exhibits moist, creamy, round smooth-walled colonies. It utilizes all the sugars with the exception of maltose.

5. DISCUSSION

The result showed that 45% and 25% of the pregnant women considered in the study in General Hospital and IBB Specialist Hospital respectively had *Candida albicans*, while amongst the non-pregnant sample space, the infection rate was 10% for both study locations. This represents a high prevalence of *Candida* infections in pregnant women relative to non-pregnant women our founding similar to the observations of [8] who reported a frequency of 48% and 15%. Feyi [9] in Tanzania and Menza et al in Kenya also in agreement with our result which they reported that, vaginal candidiasis in among pregnant women were 42.9%, 18.7% respectively [10].

The high prevalence rate of infection amongst the study subjects in General Hospital compared to IBB Specialist Hospital may be attributed to the higher hygiene awareness among users of the Specialist Hospital; this may be premised on the differentials in the financial disposition of the two classes of subjects.

Hormonal changes contribute to a high rate of Candidiasis in pregnant women, and up to 90% of women in their third trimester are mostly involved aside from the extreme discomfort of the symptoms compared to non-pregnant women [11].

Inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits also contributed in high prevalence vaginal candidiasis [12,13].

Candida albicans poses a threat to newborns, and neonatal thrushes are traced to contact with the mother vagina during birth in infected pregnant women [14]. There also seems to be a trend for re-occurrence during pregnancy as a result of the increased level of estrogens and corticoid, the vaginal defense mechanism against such opportunistic infection of *Candida* [14] This distribution studies showed that are the vast majority of the sufferers in pregnant and non-pregnant women lie between the ages of 21 to 30 years and 31 to 40 years. Pregnancy is uncommon in females younger than 15 years or older than 40 years. Most pregnancy occurs between 20 and 35 years of age [15].

The *Candida* species isolated were *C. albicans*, *C. tropicalis*, *C. krusei* and *C pseudotropicalis*. One or more of these were isolated from each positive case of the infection, meaning that were may be multiple etiology. However, [16] have been reported in cases of mycosis reported that *C. pseudotropicalis* causes oral or genital thrush but rarely meningitis or encephalitis.

The current findings however contradicts the earlier report by Okungbowa et al. who reported *Candida glabrata* as the most common *Candida* species among the symptomatic pregnant women in Nigeria cities [17]. Virulent factors of *Candida albicans* like dimorphism, phenotypic switching, protease and phosphatase which enhance its attachment to human epithelium play an importance role in there highly occurrence. High incidence rate also may due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favor *Candida albicans* growth during pregnancy.

Table 1. Number of HVS positive cases of *Candida* for pregnant women in general hospital and IBB specialist hospital Minna, Niger State, Nigeria

Age group (years)	General hospital			IBB specialist hospital		
	No of samples collected	No of positive samples	No of negatives Samples	No of samples collected	No of positive samples	No of negatives Samples
11-20	2	1	1	2	0	2
21-30	10	8	2	10	7	3
31-40	7	4	3	7	3	4
41-50	1	0	1	1	0	1
Total	20	13	7	20	10	10

Table 2. HVS positive cases of *Candida* for non-pregnant women attending in General Hospital and IBB Specialist Hospital Minna, Niger state, Nigeria

Age group (years)	General hospital			IBB specialist hospital		
	No of samples collected	No of positive samples	No of negatives Samples	No of samples collected	No of positive samples	No of negatives Samples
11-20	2	0	2	2	0	2
21-30	10	3	7	10	2	8
31-40	7	2	5	7	1	6
41-50	1	0	1	1	0	1
Total	20	5	15	20	3	17

Table 3. Frequency of occurrence of *Candida* species percentage in pregnant and non-pregnant women attending general hospital and IBB specialist hospital Minna, Niger- State, Nigeria

<i>Candida</i> spp	General hospital				IBB specialist			
	No of positive isolates in pregnant women	No of positive isolates in non-pregnant women	% in pregnant women	% in non-pregnant women	No of positive isolates in Pregnant women	No of positive isolates in non-pregnant women	% in pregnant women	% in non-pregnant women
<i>C. albicans</i>	9	2	45	10	5	2	25	10
<i>C. krusei</i>	2	1	10	5	3	1	15	5
<i>C. tropicalis</i>	1	1	5	5	1	0	5	0
<i>C. pseudotropicalis</i>	1	1	5	5	1	0	5	0

Table 4. Morphological and biochemical (sugar fermentation) characteristics of the *Candida* isolates

<i>Candida</i> isolates	Morphological characteristics as seen microscope	Sugar fermentation test					
		Glucose	Maltose	Lactose	Galactose	Fructose	Sucrose
<i>C. albicans</i>	Chlamydospore:round oval in row, oval budding yeast that forms smooth, creamy, and numerous colonies, Pseudomycelium: numerous	+	+	+	+	+	+
<i>C. krusei</i>	No chlamydospore. Elongated budding cells with occasional pseudohyphae. whitish growth, flat, dry and often with semi-glossy wrinkled small colonies	-	-	-	-	-	-
<i>C. tropicalis</i>	No chlamydospore. flat growth with smooth margin	+	+	-	+	+	-
<i>C. pseudotropicalis</i>	No chlamydospore. Moist and creamy, round, smooth-walled colonies	+	-	+	+	+	+

6. CONCLUSION

The study has revealed different *Candida* species; *C. albicans*, *C. krusei*, *C. tropicalis*, *C. pseudotropicalis*. *C. albicans* had the most frequency in the positive cases in pregnant and non-pregnant women. This suggests an etiology. The higher prevalence of Candidiasis in pregnant women is due primarily to hormonal changes which is markedly higher during pregnancy. This may lead to pregnancy complications like abortions, premature birth, low birth weight and other morbidities. Screening protocol incorporated with routine ante-natal checkup for early diagnosis of Candidiasis in pregnant women is highly recommended.

CONSENT AND ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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