

## Original Research Article

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2 **Comparative study of Vaginal Candidiasis in pregnant and non-Pregnant women attending**  
3 **Ibrahim Badamasi Babangida Specialist Hospital and General Hospital, Minna Niger state,**  
4 **Nigeria.**

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### 9 **AIM OF THE STUDY**

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

### 13 **ABSTRACT**

14 Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast). **Vaginal**  
15 **candidiasis is common during pregnancy and may impact negatively on the health of the woman.**

16 **The aim of this study is to determine and compare the prevalence of vaginal candidiasis among**  
17 **pregnant and non-pregnant women attending Ibrahim Badamasi Babangida (IBB) Specialist**  
18 **Hospital and General Hospital in Minna, Niger state, Nigeria.** High vaginal swab (HVS) were

19 each collected from **20** pregnant and **20** non-pregnant women attending General Hospital, Minna  
20 also **20** pregnant women and **20** non pregnant women at IBB (Ibrahim Badamosi Babangida)  
21 Specialist Hospital, Minna, Niger state, Nigeria and screened for vaginal candidiasis. **Sabouraud**

22 Dextrose Agar (SDA) incorporated with chloramphenicol was used to isolate the *Candida*  
23 species in the HVS samples. **Candida isolates were identified and characterized using standard**

24 **methods including: colonial morphology (macroscopic characteristics), microscopy (Sugar**  
25 **fermentation) tests. Confirmation of *Candida albicans* was done with the Germ Tube Test.**

26 **(Gram staining and Lactophenol cotton blue staining) and biochemical (Sugar fermentation)**  
27 **tests. Confirmation of *Candida albicans* was done with the Germ Tube Test.** Thirteen samples

28 tested positive for *candida* species. The species identified were *Candida albicans* **(Nine),**  
29 *Candida krusei* (2), *Candida tropicalis* **(One),** and *Candida pseudotropicalis* **(one)** for pregnant

30 women in General Hospital. Ten samples taken from pregnant women in Ibrahim Babangida  
31 Specialist Hospital were positive reactions for *Candida* species. Nine out of twenty samples

32 collected from non-pregnant women in General Hospital were *Candida* species while three out  
33 of twenty samples from non-pregnant women from Ibrahim Babangida tested positive to  
34 *Candida* species. Within the samples space 45% and 25% of the pregnant women considered in  
35 the study in General Hospital and IBB Specialist Hospital respectively had *Candida albicans*,  
36 while amongst the non-pregnant sample space, the infection rate was 10% for both study  
37 locations.

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

42 **Keywords:** Pregnancy, High vaginal swab (HVS), *Candida species*, Vaginal candidiasis, SDA

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## 57 INTRODUCTION

58 Candidiasis is a fungal infection (mycosis) of any of the *Candida* species (all yeast) of which  
59 *Candida albicans* is the most common [2]. When it affects the mouth, it is commonly called  
60 thrush [2]. Although commonly referred to as yeast infection, Candidiasis is the technically  
61 known as Candidiosis, Moniliasis and oidiomycosis. Signs and symptoms include white patches  
62 on the tongue or other areas of the mouth and throat [3]. Other symptoms may include soreness  
63 and problems swallowing [3]. When it affects the vagina, it is commonly called a yeast infection  
64 [2]. Signs and symptoms include genital itching, burning, and sometimes a white "cottage  
65 cheese-like" discharge from the vagina [8]. Less commonly the penis may be affected, resulting  
66 in itchiness [3]. Very rarely, the infection may becoming invasive, spreading to other parts of the  
67 body. This may result in fevers along with other symptoms depending on the parts involved [3]

68 More than 20 types of *Candida* species can cause infection, with *Candida albicans* being the  
69 most common [2]. Infections of the mouth are most common among children less than one  
70 month old, the elderly, and those with weak immune systems. Conditions that result in a weak  
71 immune system include HIV/AIDS, the medications used after organ transplantation, diabetes,  
72 and the use of corticosteroids. Other risks factors include dentures and antibiotic therapy [4]  
73 .Vaginal infections occur more commonly during pregnancy, in those with weak immune  
74 systems, and following antibiotic use [10]. Risk factors for invasive candidiasis include being in  
75 an intensive care unit, following surgery, low birth weight infants, and those with weak immune  
76 systems. [11]

77 Vaginal candidiasis is usually treated with antifungal medications [3]. For most infections, the  
78 treatment is an antifungal applied inside the vagina, or a single dose of fluconazole administered  
79 orally. For more severe infections, infections that don't get better, or keep recurring, other  
80 treatments might be needed. These treatments include more doses of fluconazole or other  
81 medicines applied inside the vagina such as boric acid, nystatin, or flucytosine. Little evidence  
82 supports probiotics for either prevention or treatment even among those with frequent vaginal  
83 infections. [10][11] Vaginal candidiasis is common, though more research is needed to ascertain  
84 the population of women affected. Women who are more likely to get vaginal candidiasis  
85 include those who are pregnant, use hormonal contraceptives (for example, birth control pills),  
86 have diabetes, have a weakened immune system (for example, due to HIV infection or medicines

87 that weaken the immune system, such as steroids and chemotherapy), are taking or have recently  
88 taken antibiotics . About three-quarters of women have at least one yeast infection at some time  
89 during their lives.[7] Widespread disease is rare except in those who have risk factors. [10] This  
90 research aimed to carry out comparative study of Prevalence of vaginal candidiasis between  
91 pregnant women attending General Hospital and IBB Specialist Hospital, both in Minna, Niger  
92 state.

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## 94 MATERIALS AND METHOD

95 Material used in this research were microscope, swab stick, slide hydrogen peroxide, normal  
96 saline, petri dishes, test-tubes, crystal violet, ethyl alcohol, oil immersion, safarine, SDA (   
97 sabouraud Dextrose Agar).

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## 99 AREA OF STUDY

100 The study areas of this research were General hospital Minna and IBB Specialist Hospital, co-  
101 located in Minna, Niger State. Minna is a city (estimated population 299373 in 2018) in west-  
102 central Nigeria. It is the capital of Niger State, one of Nigeria's 36 federal states. It consists of 2  
103 major ethnic groups: the Nupe and the Gbagyi. Minna has the Latitude 9.61389 and Longitude of  
104 6.55694.

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## 106 STUDY POPULATION

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

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115 **MEDIA PREPARATON**

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

118 **MEDIA COMPOSITION**

119 65 grams of SDA suspended in 1litre distilled water

120 0.5grams of Chloramphenicol powder.

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

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124 **SAMPLE COLLECTION**

125 **High vaginal swab was collected in Batches using sterile swab stick by a Nurse in each Hospital,**  
126 **stored and was immediately transported inside an Ice Box to the research laboratory where the analyses**  
127 **was carried out at the Microbiology Departments Research Laboratory Federal University of Technology**  
128 **Minna, Niger-State. The study sample space consisted of eighty subjects without vaginal disorder**  
129 **were studied 20 pregnant women and 20 non pregnant in each Hospital constituting a total**  
130 **number of eighty subjects. The cervix was opened with sterile unlubricated bivalve. Vaginal**  
131 **spectrum specimen of vaginal discharge was collected from the posterior and lateral fornices.** The  
132 sample was then transported immediately to the laboratory and inoculated into freshly-prepared  
133 SDA and kept on the incubating hood at 28<sup>0</sup>C for 48 hours. Growths having milk to white colour  
134 and palm wine smell were picked for further identification and characterization.

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136 **IDENTIFICATION AND CHARACTERISATION OF *Candida* ISOLATE**

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

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## 142 **GRAM STAINING**

143 The Gram staining was performed as described previously by [12]. The following reagents were  
144 used for the staining: Crystal violet stain, Lugol's iodine, Acetone-alcohol decolorizer and  
145 Neutral red. The reagents were prepared according to manufacturer's instructions. Suspected  
146 *Candida* colonies on any of the agar plates were emulsified in Normal saline on the slide to form  
147 a smear. The smear was allowed to air dry completely. The slide, (with the smear uppermost)  
148 was fixed by rapidly passing through flame of a Bunsen burner. The smear was allowed to cool.  
149 The fixed smear was covered with crystal violet stain for 30 seconds – 1 minute. The stain was  
150 rapidly washed off with clean running tap water. The smear was again covered with Lugol's  
151 iodine for another 30 seconds – 1 minute and washed off with clean tap water and was rapidly  
152 decolourized (few seconds) with acetone-alcohol. This was washed immediately with clean tap  
153 water. The smear was then covered with neutral red stain for 2 minutes and washed off with  
154 clean tap water. The stained slide was placed in a draining rack and the smear allowed to air dry.  
155 The smear was examined microscopically using oil immersion objective lens ( $\times 100$ ). *Candida*  
156 species were Gram positive yeast like cell under the microscope

## 157 **LACTOSE PHENOL COTTON BLUE STAIN PROCEDURE**

158 A glass slide was cleared with clean cotton wool and a drop of lactose phenol cotton blue was  
159 dropped on the slide after which a small portion of the pure isolate was picked and smeared with  
160 the lactose phenol on the slide and covered with clean cover slide mounted. It was viewed under  
161 a microscope at 10X magnification. The chlamydospores of the organism were clearly visible.

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## 165 **BIOCHEMICAL (SUGAR FERMENTATION) TEST**

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

### 169 **Procedure**

170 Two grams of each sugar were weighed into different beakers. One gram of peptone was added  
171 to each along with phenol red indicator powder. 100ml of distilled water was added to each  
172 beaker and shaken till it dissolved. 10ml of the sugar solution was dispensed inside test tube that  
173 had been labelled accordingly and covered with the cotton wrapped with foil paper and then  
174 autoclaved at 121<sup>0</sup>C for 15minutes. It was allowed to cool after which small quantity of the pure  
175 yeast isolated was inoculated inside each test and incubated in the inoculating hood at the room  
176 temperature for 3 days. The result of color change was observed and recorded appropriately.

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### 178 **(GERM TUBE TEST) OF *Candida albicans***

179 This is presumptive test a for the presence of *candida albicans* in a sample

### 180 **PROCEDURE**

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

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## 191 RESULTS

192 *Candidiasis* in relation to age

193 The result revealed that of the 20 sample screened in pregnant women in General Hospital,  
194 Minna, 13 had *Candida*, with the remaining 7 samples testing negative. 10 of the 20 samples  
195 taken from pregnant women in IBB Specialist Hospital Minna tested positive for *Candida* this is  
196 shown in Table 1.

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

202 The identification and frequency of occurrence of *Candida* isolates identified in this research are  
203 shown in Table 4. These are *Candida albicans*, *Candida krusei*, *Candida pseudotropicalis* and  
204 *Candida tropicalis*. *Candida albicans* had the highest frequency occurrence, closely followed by  
205 *Candida krusei*. The rest two isolate *Candida pseudotropicalis* and *Candida tropicalis* had the  
206 least frequency of occurrence in both hospitals.

207 Table 5 shows the results of the biochemical test (fermentation of sugars) and morphological  
208 characteristics as viewed under a microscope, of the *Candida* species isolated during the research  
209 work. *Candida albicans* was able to utilize glucose, maltose, lactose, galactose, fructose, and  
210 sucrose.

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

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

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



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

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UNDER PEER REVIEW

240 **Table 1.** Number of HVS positive cases of *Candida* for pregnant women in General Hospital and  
241 IBB Specialist Hospital Minna, Niger State.

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General Hospital				IBB specialist Hospital		
Age group(years)	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

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

General Hospital				IBB specialist Hospital		
Age group(years)	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

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270 **Table 3.** Frequency of occurrence of *Candida* species percentage in pregnant and non-pregnant  
 271 women attending General Hospital and IBB Specialist Hospital Minna, Niger- State.

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<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

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Table 4. Morphological and Biochemical (Sugar Fermentation) Characteristics of the *Candida* isolates

<i>Candida</i> Isolates	Morphological Characteristics	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	+	-	+	+	+	+

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## 292 DISCUSSION

293 The result showed that 45% and 25% of the pregnant women considered in the study in General  
294 Hospital and IBB Specialist Hospital respectively had *candida albicans*, while amongst the non-  
295 pregnant sample space, the infection rate was 10% for both study locations. This represents a  
296 high prevalence of *candida* infections in pregnant women relative to non-pregnant women our  
297 founding similar to the observations of [13] who reported a frequency of 48% and 15%. Feyi  
298 [14] in Tanzania and Menza et al in Kenya also in agreement with our result which they reported  
299 that, vaginal candidiasis in among pregnant women were 42.9%, 18.7% respectively. [15]

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

304 Hormonal changes contribute to a high rate of Candidiasis in pregnant women, and up to 90% of  
305 women in their third trimester are mostly involved aside from the extreme discomfort of the  
306 symptoms compared to non-pregnant women [16].

307 Inadequate knowledge, poor personal hygiene, limited diagnostic facilities, poor dietary habits  
308 also contributed in high prevalence vaginal candidiasis. [17, 18].

309 *Candida albicans* poses a threat to newborns, and neonatal thrushes are traced to contact with the  
310 mother vagina during birth in infected pregnant women [17]. There also seems to be a trend for  
311 re-occurrence during pregnancy as a result of the increased level of estrogens and corticoid, the  
312 vaginal defence mechanism against such opportunistic infection of *Candida* [17] This  
313 distribution studies showed that are the vast majority of the sufferers in pregnant and non-  
314 pregnant women lie between the ages of 21 to 30 years and 31 to 40years. Pregnancy is  
315 uncommon in females younger than 15 years or older than 40 years. Most pregnancy occurs  
316 between 20 and 35years of age [19]

317 The *Candida* species isolated were *C. albicans*, *C. tropicalis*, *C. krusei* and *C pseudotropicalis*.  
318 One or more of these were isolated from each positive case of the infection, meaning that were  
319 may be multiple etiology. However, [3] have been reported in cases of mycosis reported that *C.*  
320 *pseudotropicalis* causes oral or genital thrush but rarely meningitis or encephalitis.

321 The current findings however contradicts the earlier report by Okungbowa *et al.* who reported  
322 *Candida glabrata* as the most common *Candida species* among the symptomatic pregnant  
323 women in Nigeria cities. [20] Virulent factors of *Candida albicans* like dimorphism, phenotypic  
324 switching, protease and phosphatase which enhance its attachment to human epithelium play an  
325 importance role in there highly occurrence. High incidence rate also may due to increased  
326 physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby  
327 providing an adequate supply of utilizable sugar that favor *Candida albicas* growth during  
328 pregnancy.

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## 330 CONCLUSION

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

### 338 **Disclaimer regarding Consent/Ethical Approval:**

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

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