

Original Research Article

1
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.**

5 6 **AIM OF THE STUDY**

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

10 **ABSTRACT**

11 Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast). **Vaginal**
12 **candidiasis is common during pregnancy and may impact negatively on the health of the woman.**
13 **The aim of this study is to determine and compare the prevalence of vaginal candidiasis among**
14 **pregnant and non-pregnant women attending Ibrahim Badamasi Babangida (IBB) Specialist**
15 **Hospital and General Hospital in Minna, Niger state, Nigeria** were screened for vaginal
16 candidiasis. High vaginal swab (HVS) were each collected from 20 pregnant and 20 non-
17 pregnant women attending General Hospital, Minna also 20 pregnant women and 20 non
18 pregnant women at IBB (Ibrahim Badamosi Babangida) Specialist Hospital, Minna, Niger state,
19 Nigeria and screened for vaginal candidiasis. Sabouraud Dextrose Agar (SDA) incorporated with
20 chloramphenicol was used to isolate the *Candida* species in the HVS samples. . **Candida isolates**
21 **were identified and characterized using standard methods including: colonial morphology**
22 **(macroscopic characteristics), microscopy (Sugar fermentation) tests. Confirmation of *Candida***
23 ***albicans* was done with the Germ Tube Test. (Gram staining and Lactophenol cotton blue**
24 **staining) and biochemical (Sugar fermentation) tests.** Thirteen samples tested showed positive
25 for *candida* species. The species identified were *Candida albicans* (Nine), *Candida krusei* (two),
26 *Candida tropicalis* (One), and *Candida pseudotropicalis* (one) for pregnant women in General
27 Hospital. Ten samples taken from pregnant women in Ibrahim Badamosi Babangida Specialist
28 Hospital were positive reactions for *Candida* species. Nine out of twenty samples collected from
29 non-pregnant women in General Hospital were *Candida* species while three out of twenty
30 samples from non-pregnant women from Ibrahim Babangida Specialist Hospital tested positive

31 to *Candida* species. Within the samples space 45% and 25% of the pregnant women considered
32 in the study in General Hospital and IBB Specialist Hospital respectively had *Candida albicans*,
33 while amongst the non-pregnant sample space, the infection rate was 10% for both study
34 locations.

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

39 **Keywords:** Pregnancy, High vaginal swab (HVS), *Candida species*, vaginal candidiasis, SDA s

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55 1.0 INTRODUCTION

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

66 More than 20 types of *Candida* species can cause infection, with *Candida albicans* being the
67 most common [3]. Infections of the mouth are most common among children less than one
68 month old, the elderly, and those with weak immune systems. Conditions that result in a weak
69 immune system include HIV/AIDS, the medications used after organ transplantation, diabetes,
70 and the use of corticosteroids. Other risks factors include dentures and antibiotic therapy [4]
71 .Vaginal infections occur more commonly during pregnancy, in those with weak immune
72 systems, and following antibiotic use [4]. Risk factors for invasive candidiasis include being in
73 an intensive care unit, following surgery, low birth weight infants, and those with weak immune
74 systems. [10]

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

85 that weaken the immune system, such as steroids and chemotherapy), are taking or have recently
86 taken antibiotics. About three-quarters of women have at least one yeast infection at some time
87 during their lives.[11] Widespread disease is rare except in those who have risk factors. [11] This
88 research aimed to carry out comparative study of Prevalence of vaginal candidiasis between
89 pregnant women and non-pregnant attending General Hospital and IBB Specialist Hospital, both
90 in Minna, Niger state, Nigeria.

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92 2.0 AREA OF STUDY

93 The study areas of this research were General hospital Minna and IBB Specialist Hospital, co-
94 located in Minna, Niger State. Minna is a city (estimated population 299373 in 2018) in west-
95 central Nigeria. It is the capital of Niger State, one of Nigeria's 36 federal states. It consists of 2
96 major ethnic groups: the Nupe and the Gbagyi. Minna has the Latitude 9.61389 and Longitude of
97 6.55694.

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99 2.1 STUDY POPULATION

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

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106 2.2 MEDIA PREPARATON

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

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110 2.3 MEDIA COMPOSITION

111 65 grams of SDA suspended in 1litre distilled water

112 0.5grams of Chloramphenicol powder.

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

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116 **2.4 SAMPLE COLLECTION AND PROCESSING**

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

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125 **2.5 IDENTIFICATION AND CHARACTERISATION OF *Candida* ISOLATE**

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

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130 **2.6 GRAM STAINING**

131 Suspected *Candida* colonies on any of the agar plates were emulsified in Normal saline on the
132 slide to form a smear. The smear was allowed to air dry completely. The slide, (with the smear
133 uppermost) was fixed by rapidly passing through flame of a Bunsen burner. The smear was
134 allowed to cool. The fixed smear was covered with crystal violet stain for 30 seconds – 1 minute.
135 The stain was rapidly washed off with clean running tap water. The smear was again covered
136 with Lugol's iodine for another 30 seconds – 1 minute and washed off with clean tap water and

137 was rapidly decolourized (few seconds) with acetone-alcohol. This was washed immediately
138 with clean tap water. The smear was then covered with neutral red stain for 2 minutes and
139 washed off with clean tap water. The stained slide was placed in a draining rack and the smear
140 allowed to air dry. The smear was examined microscopically using oil immersion objective lens
141 ($\times 100$). *Candida* species were Gram positive yeast like cell under the microscope

142 **2.7 LACTOSE PHENOL COTTON BLUE STAIN PROCEDURE**

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

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148 **2.8 BIOCHEMICAL (SUGAR FERMENTATION) TEST**

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

152 **PROCEDURE**

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

160 **2.9 (GERM TUBE TEST) OF *Candida albicans***

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

162 **PROCEDURE**

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

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185 **3.0 RESULTS**

186 **3.1 Candidiasis** in relation to age

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

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

196 The identification and frequency of occurrence of *Candida* isolates identified in this research are
197 shown in Table 4. These are *Candida albicans*, *Candida krusei*, *Candida pseudotropicalis* and
198 *Candida tropicalis*. *Candida albicans* had the highest frequency occurrence, closely followed by
199 *Candida krusei*. The rest two isolate *Candida pseudotropicalis* and *Candida tropicalis* had the
200 least frequency of occurrence in both hospitals.

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

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

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

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

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

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

UNDER PEER REVIEW

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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249 **3.3 Table 2.** HVS positive cases of *Candida* for non-pregnant women attending in General
 250 Hospital and IBB Specialist Hospital Minna, Niger state, Nigeria.

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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264 **3.4 Table 3.** Frequency of occurrence of *Candida* species percentage in pregnant and non-
 265 pregnant women attending General Hospital and IBB Specialist Hospital Minna, Niger- State,
 266 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

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278 **3.5**Table 4. Morphological and Biochemical (Sugar Fermentation) Characteristics of the *Candida*

279 isolates

<i>Candida</i>	Morphological	Sugar Fermentation Test
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Isolates	Characteristics as seen microscope	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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288 **4.1 DISCUSSION**

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

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

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

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

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

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

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

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326 4.2 CONCLUSION

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

334 **Disclaimer regarding Consent/Ethical Approval:**

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

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