

Original Research Article

Comparative study of Candidiasis in pregnant and non-Pregnant women attending Ibrahim Badamasi Babangida Specialist Hospital and General Hospital, Minna Niger state

ABSTRACT

Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast) [2]. When it affects the mouth, it is commonly called thrush. High vaginal swab (HVS) were collected from 20 pregnant and 20 non-pregnant women in General Hospital, Minna and IBB (Ibrahim Badamosi Babangida) Specialist Hospital, Minna, Niger state and screened for the presence of yeast associated with *candidiasis*. Saboroud dextrose agar (SDA) incorporated with chloramphenicol was used to isolate the *Candida* species in the HVS samples. Identification, Characterization and Biochemical test were used for confirmation of the *Candida* isolates. Thirteen (13) samples tested positive to *candida* infection. The species identified were *Candida albicans* (9), *Candida krusei* (2), *Candida tropicalis* (1), and *Candida pseudotropicalis* (1) for pregnant women in General Hospital. Ten (10) samples taken from pregnant women in Ibrahim Babangida Specialist Hospital showed positive reactions for *Candida* infection. Nine (9) out of twenty (20) samples collected from non-pregnant women in General Hospital were *Candida* infection while three (3) out of twenty (20) samples from non-pregnant women from Ibrahim Babangida tested positive to *Candida* infection. Biochemical analysis shows each *Candida* isolate's ability to utilize different sugars during Sugar Fermentation Test. Germ Tube Test was used to identify and differentiate *Candida albicans* from other species of *Candida*.

Keywords: Pregnant women, High vaginal swab (HVS), *Candida albicans*, Non-pregnant women

29 **INTRODUCTION**

30 Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast) [2]. When it affects
31 the mouth, it is commonly called thrush [2]. Signs and symptoms include white patches on the
32 tongue or other areas of the mouth and throat [3]. Other symptoms may include soreness and
33 problems swallowing.^[3] When it affects the vagina, it is commonly called a yeast infection [2].
34 Signs and symptoms include genital itching, burning, and sometimes a white "cottage cheese-
35 like" discharge from the vagina [8]. Less commonly the penis may be affected, resulting in
36 itchiness [3]. Very rarely, the infection may becoming invasive, spreading to other parts of the
37 body [9]. This may result in fevers along with other symptoms depending on the parts involved
38 [9].

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

47 Efforts to prevent infections of the mouth include the use of chlorhexidine mouth wash in those
48 with poor immune function and washing out the mouth following the use of inhaled steroids.^[5]
49 Little evidence supports probiotics for either prevention or treatment even among those with
50 frequent vaginal infections.[10][11] For infections of the mouth, treatment with topical
51 clotrimazole or nystatin is usually effective. By mouth or intravenous fluconazole, itraconazole,
52 or amphotericin B may be used if these do not work. [5] A number of topical antifungal
53 medications may be used for vaginal infections including clotrimazole. [8] In those with
54 widespread disease, an echinocandin such as caspofungin or micafungin is used. [9] A number of
55 weeks of intravenous amphotericin B may be used as an alternative [11] in certain groups at very
56 high risk, antifungal medications may be used preventatively [11].

Comment [sn1]: The literature review should not be too broad but be focused on the objectives of the study. It looks like the focus is on asymptomatic pregnancy women have this fundal infection and the hypothesis is that it may be transmitted to the newborn if born vaginally. If that is true, then we should write the intro on these areas alone.

57 Infections of the mouth occur in about 6% of babies less than a month old. About 20% of those
58 receiving chemotherapy for cancer and 20% of those with AIDS also develop the disease [6].
59 About three-quarters of women have at least one yeast infection at some time during their
60 lives.[7] Widespread disease is rare except in those who have risk factors. [10] This research
61 aimed to carry out comparative study of candidiasis between pregnant women attending General
62 Hospital and IBB Specialized Hospital, both in Minna, Niger state.

Comment [sn2]: The objective if this review should be stated here

64 MATERIALS AND METHOD

65 Material used in this research were microscope, swab stick, slide hydrogen peroxide, normal
66 saline, petri dishes, test-tubes, crystal violet, ethyl alcohol, oil immersion, safarine, SDA (
67 sabouraud dextrose agar).

Comment [sn3]:

Comment [sn4]: The specific objectives should be listed here.
Then state what is to be done (methods)- recruit subjects from antenatal clini0 state the inclusion and exclusion criteria; sampling method-convenience (they have their own limitations>
The next part is the method used in isolating the fungus (make reference to standard method is applicable

69 AREA OF STUDY

70 The studies areas of this research were General hospital Minna and IBB Specialist Hospital, co-
71 located in Minna, Niger State.

Comment [sn5]: Locate the clinic-pregnant women from Ensure inclusion and exclusion criteria are stated

73 STUDY POPULATION

74 A total of eighty (80) women were studied: 20 pregnant and 20 non-pregnant from each data
75 source. The ages of the subjects were between 15 and 50 years.

Comment [sn6]: Total of subjects evaluated in only 40?

77 MEDIA PREPARATON

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

Comment [sn7]: Better to describe the lab isolation in narrative with references as applicable

81 MEDIA COMPOSITION

82 65 grams of SDA suspended in 1litre distilled water

83 0.5grams of Chloramphenicol powder.

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

86

87 **SAMPLE COLLECTION**

88 High vaginal swab was collected using sterile swab stick. The study sample space consisted of
89 eighty subjects without vaginal disorder were studied. The cervix was opened with sterile
90 unlubricated bivalve. Vaginal spectrum specimen of vaginal discharge was collected from the
91 posterior and lateral fornices. The sample was then transported immediately to the laboratory and
92 inoculated into freshly-prepared SDA and kept on the incubating hood at 28⁰C for 48 hours.
93 Growths having milk to white colour and palm wine smell were picked for further identification
94 and characterization.

95

96 **IDENTIFICATION AND CHARACTERISATION OF *Candida* ISOLATE**

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

100

101 **GRAM STAINING**

102 The Gram staining was performed as described previously by (Cheesbrough, 2008, Oyeleke and
103 Manga, 2008) .The following reagents were used for the staining: Crystal violet stain, Lugol's
104 iodine, Acetone-alcohol decolorizer and Neutral red. The reagents were prepared according to
105 manufacturer's instructions. Suspected *Listeria monocytogenes* bacteria colonies on any of the
106 agar plates were emulsified in Normal saline on the slide to form a smear. The smear was
107 allowed to air dry completely. The slide, (with the smear uppermost) was fixed by rapidly

Comment [sn8]: Pregnancy stage –
gestation age; any one with previos infection,
gestational diabetes , hiv ?

108 passing through flame of a Bunsen burner. The smear was allowed to cool. The fixed smear was
109 covered with crystal violet stain for 30 seconds – 1 minute. The stain was rapidly washed off
110 with clean running tap water. The smear was again covered with Lugol's iodine for another 30
111 seconds – 1 minute and washed off with clean tap water and was rapidly decolorized (few
112 seconds) with acetone-alcohol. This was washed immediately with clean tap water. The smear
113 was then covered with neutral red stain for 2 minutes and washed off with clean tap water. The
114 stained slide was placed in a draining rack and the smear allowed to air dry. The smear was
115 examined microscopically using oil immersion objective lens ($\times 100$).

116 **BIOCHEMICAL TEST (FERMENTATION OF SUGAR)**

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

120 **Procedure**

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

128

129 **LACTOSE PHENOL COTTON BLUE STAIN PROCEDURE**

130 A glass slide was cleared with clean cotton wool and a drop of lactose phenol cotton blue was
131 dropped on the slide after which a small portion of the pure isolate was picked and smeared with

132 the lactose phenol on the slide and covered with clean cover slide mounted. It was viewed under
133 a microscope at 10X magnification. The chlamydo spores of the organism were clearly visible.

134

135 **CONFIRMATION OF *Candida albicans***

136 This is a confirmatory test for the presence of *Candida albicans* in a sample

137 **PROCEDURE**

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

143

144 **RESULTS**

145 *Candidiasis* in relation to age

146 The result revealed that of the 20 sample screened in pregnant women in General Hospital,
147 Minna, 13 had *Candida*, with the remaining 7 samples testing negative. This is shown in Table 1.
148 Ten of the 20 samples taken from pregnant women in IBB Specialist Hospital Minna tested
149 positive for *Candida*, shown in Table 2.

150 Amongst the non-pregnant women study group, 5 of 20 screened at the General Hospital tested
151 positive, with 15 testing negative the result of which is given in Table 3. The study group of IBB
152 Specialist Hospital tested positive to *Candida* in three cases, with 17 testing negative. This is
153 shown in Table 4. The age range mostly affected in non-pregnant women in both hospitals was
154 21 – 30 years as shown in all the tables.

155 The identification and frequency of occurrence of *Candida* isolate identified in this research is
156 shown in Table 5. These are *Candida albicans*, *Candida krusei*, *Candida pseudotropicalis* and
157 *Candida tropicalis*. *Candida albicans* had the highest frequency occurrence, closely followed by

158 *Candida krusei*. The rest two isolate *Candida pseudotropicalis* and *Candida tropicalis* had the
159 least frequency of occurrence in both hospitals.

160 Table 7 shows the Biochemical and Morphological Characteristic of *Candida* species isolated
161 from the research work. *Candida albicans* was able to utilize Glucose, Maltose, Lactose,
162 Galactose, Fructose, and Sucrose.

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

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

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

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

172 Table1. Number of HVS positive cases of *Candida* for pregnant women, General Hospital,
173 Minna Niger State.

Age group (years)	Numbers Of Samples Collected	Numbers Of Positives Samples	Numbers Of Negative Samples
11-20	2	1	1
21-30	10	8	2
31-40	7	4	3
41-50	1	0	1
Total	20	13	7

Comment [sn9]: Better to group 10-19\20-29 etc

175 Table2. Number of HVS positive cases of *Candida* in pregnant women attending IBB Specialist
 176 Hospital, Minna, Niger State.

AGE GROUP (YEARS) SAMPLE	NUMBER OF SAMPLE COLLECTED	NUMBER OF POSTIVE SAMPLE	NUMBER OF NEGATIVE
11 - 20	2	0	2
21 - 30	10	7	3
31 - 40	7	3	4
41 - 50	1	0	1
TOTAL	20	10	10

177

178

179 Table 3. HVS positive cases of candida for non-pregnant women attending General Hospital,
 180 Minna, Niger state.

AGEGROUP (YEARS) SAMPLES	NUMBERSOF SAMPLES COLLECTED	NUMBERSOF POSTIVE SAMPLES	NUMBERSOF NEGATIVE SAMPLES
11-20	2	0	2
21-30	10	3	7
31-40	7	2	5
41-50	1	0	1
TOTAL	20	5	15

Comment [sn10]: Avoid capitals letters in column head

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183

184 Table 4. HVS positive cases of *Candida* for non-pregnant women attending IBB Specialist

185

Hospital Minna, Niger state.

AGE GROUP (YEARS)	NUMBERS OF SAMPLES COLLECTED	OF NUMBERS OF POSTIVE SAMPLES	OF NUMBERS OF NEGATIVE SAMPLES
11-20	2	0	2
21-30	10	2	8
31-40	7	1	6
41-50	1	0	1
TOTAL	20	3	17

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187

188 Table 5. Frequency of occurrence of *Candida* species percentage in pregnant and non-pregnant

189

women attending General Hospital Minna, Niger- State.

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<i>Candida</i> species	Number of isolate in pregnant women	Numbers of isolate in non-pregnant women	Percentage in pregnant women	Percentage in non-pregnant women
<i>C. albicans</i>	9	2	45	10
<i>C. krusei</i>	2	1	10	5
<i>C. tropicalis</i>	1	1	5	5
<i>C. pseudotropicalis</i>	1	1	5	5
Total	13	5		

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192

193 Table 6. Frequency of occurrence of *Candida* species percentage in pregnant and non-pregnant
 194 women attending IBB Hospital Minna Niger State

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<i>Candida</i> species	Number of isolate pregnant women	of Numbers in isolate in non-pregnant women	Percentage in pregnant women	Percentage in non-pregnant women
<i>C. albicans</i>	5	2	25	10
<i>C. krusei</i>	3	1	15	5
<i>C. tropicalis</i>	1	0	5	0
<i>C. pseudotropicalis</i>	1	0	5	0
Total	10	5		

196

197

198 Table 7. Biochemical and Morphological Characteristics of the *Candida* isolate (Sugar
 199 Fermentation).

<i>Candida</i> Isolates	Morphological Characteristics	Fermentation Sugars
<i>C. albicans</i>	Chlamyospore: round oval in row, oval budding yeast that forms smooth, creamy, and numerous colonies, Pseudomycelium: numerous	Glucose + Maltose + Lactose + Galactose+ Fructose + Sucrose +

<i>C. krusei</i>	No chlamyospore. Budding cells with occasional pseudohyphae. Whitish growth, flat, dry and often with wrinkled small colonies	Elongated	Glucose - Maltose - Lactose - Galactose - Fructose - Sucrose -
<i>C. tropicalis</i>	No chlamyospore. Flat growth with smooth margin	Flat growth	Glucose + Maltose + Lactose - Galactose + Fructose + Sucrose -
<i>C. pseudotropicalis</i>	No chlamyospore. Creamy, round, smooth-walled colonies	Moist and smooth-walled	Glucose + Maltose - Galactose + Lactose +

200

201

202 DISCUSSION

203 The result showed that 45% and 25% of the pregnant women considered in the study in General
 204 Hospital and IBB Specialist Hospital respectively had *candida albicans*, while amongst the non-
 205 pregnant sample space, the infection rate was 10% for both study locations. This represents a
 206 high prevalence of candida infections in pregnant women relative to non-pregnant women.

207 The statistics indicating a higher incidence of infection amongst the study subjects in General
 208 Hospital compared to IBB Specialist Hospital may be attributed to the higher hygiene awareness
 209 among users of the Specialist Hospital; this may be premised on the differentials in the financial
 210 disposition of the two classes of subjects.

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

214 *Candida albicans* poses a threat to newborns, and neonatal thrushes are traced to contact with the
215 mother vagina during birth in infected pregnant women (Talaro, 1996). There also seems to be a
216 trend for recur during pregnancy as a result of the increased level of estrogens and cortoid, the
217 vaginal defence mechanism against such opportunistic infection of *Candida*. This distribution
218 studies showed that are the vast majority of the sufferers in pregnant and non-pregnant women
219 lie between the ages of 21 to 30 years and 31 to 40years. Pregnancy is uncommon in females
220 younger than 15 years or older than 40 years. Most pregnancy occurs between 20 and 35years of
221 age.

222 The *Candida* species isolated were *C. albicans*, *C. tropicalis*, *C. krusei* and *C pseudotropicalis*.
223 One or more of these were isolated from each positive case of the infection, meaning that were
224 may be multiple etiology. However, these organisms have been reported in cases of mycosis
225 (Haude *et al*, 1980, Talaro and Talaro 1996). Haude *et. al.* 1980) reported that *C.*
226 *pseudotropicalis* causes oral or genital thrush but rarely meningitis or encephalitis.

227

228 CONCLUSION

229 The study has revealed different *candida* species, in which *C. albicans* has the most frequency in
230 the positive cases in pregnant and non-pregnant women, and others are *C. krusei*, *C .tropicalis*,
231 *C. pseudotropicalis*. This suggests etiology, and also how these species of *Candida* ferment types
232 of sugar, and the shapes when emulsified in mammalian serum, and under microscopic
233 examination.

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Comment [sn11]: Make references if candidiasis causes neonatal infection?? Especially in asymptomatic women

Comment [sn12]: State the limitation of the study
How does this impact on health
Any treatment given if positive- ethical issues
Recommendations as a result of this study

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Comment [sn13]: References should follow the guidelines set by the journal

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