



**PREVALENCE AND DISTRIBUTION OF *CANDIDA* ISOLATES FROM HIGH VAGINAL SWAB SAMPLES OF SOME WOMEN IN NNEWI, ANAMBRA STATE, NIGERIA.**

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**ABSTRACT**

*Candida* species normally colonize the skin and mucosal surfaces of humans in an asymptomatic manner but can become a most significant cause of disabling and lethal infection in immuno-suppressed persons. This study was designed to determine the prevalence and distribution of *Candida* isolates from some women attending Life Specialist Hospital, Nnewi, Nigeria. Ninety nine (99) high vaginal swab (HVS) samples were collected from HIV-negative (76 pregnant and 23 non-pregnant/apparently healthy) women in Nnewi. Samples were cultured on appropriate media and identified using standard techniques. Statistical analysis was done using Chi-square test ( $p < 0.05$ ). A total of 33 of the 99 participants were colonized with *Candida* species giving a prevalence of 33.3% while a total of 34 *Candida* isolates were recovered. The prevalence of vaginal *Candida* isolates among the pregnant and non-pregnant women was 36.8% and 21.7% respectively with the age group 26-35 years (in pregnant women) having the highest prevalence. Meanwhile, pregnant women in their second trimester had the highest prevalence. *Candida tropicalis* was the most frequently isolated species (13; 38.3%) followed by *C. albicans* (11; 32.4%), *C. glabrata* (4; 11.8%), *C. krusei* (4; 11.8%), *C. dubliniensis* (1; 2.9%) and *C. kefyr* (1; 2.9%). The prevalence of non-*albicans* *Candida* (NAC) species was 67.6%. This study suggests that all pregnant women be subjected to routine laboratory tests to rule out the possibility of vaginal *Candida* infection and also that proper identification of yeasts to species level be done since more non-*albicans* species of *Candida* are now implicated in many infections.

**KEYWORDS:** *Candida* isolates, Prevalence, Distribution, Women, Awka, Nnewi.

**INTRODUCTION**

*Candida* species are eukaryotic opportunistic pathogens that reside on the mucosa of the gastrointestinal tract as well as the mouth, oesophagus and vagina.<sup>[1]</sup> Although this commensal organism normally colonizes mucosal surfaces in an asymptomatic manner, it can become one of the most significant causes of a disabling and lethal infection.<sup>[2]</sup> No other mycotic pathogen produces as diverse a spectrum of disease in humans as does *Candida*. Most infections involve the skin or mucous membranes. This occurs because *Candida* is a strict aerobe and finds such surfaces very suitable for growth.<sup>[3]</sup>

The incidence and prevalence of fungal infections have increased significantly over the years<sup>[4]</sup> and *Candida* species have been strongly implicated. *Candida* infections have become more frequent in recent times. They account, to a large extent, for increases in mortality and morbidity rates in patients with weakened immunity.<sup>[4]</sup> *Candida* species are the most common cause

of infections in AIDS patients.<sup>[5]</sup> *Candida* infections are ranked fourth among nosocomial bloodstream infections in the United States with mortality rates close to 50%<sup>[6]</sup> and are among the top ten most frequently isolated nosocomial bloodstream pathogens in Europe.<sup>[7]</sup> This can be attributed to the rise in risk population. Patients who receive immunosuppressive therapies are more likely to develop *Candida* infections<sup>[8]</sup> and these include cancer patients and individuals who undergo invasive procedures.

The epidemiological landscape of *Candida* infection has evolved in the last two decades. Although *Candida albicans* remains the chief aetiological agent, infections caused by other species are increasing.<sup>[9]</sup> *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei* are among the main non-*Candida albicans* *Candida* (NCAC) species. They, along with *Candida albicans*, are responsible for more than 90% of invasive candidiasis cases.<sup>[9]</sup>

The incidence of relatively unknown species has increased too. Since it was first isolated from the oral cavities of HIV-positive patients in Ireland<sup>[10]</sup>, the global prevalence of *C. dubliniensis* has long been established. It has also been seen in specimens from other body sites such as vagina, gastrointestinal tract and skin.<sup>[11]</sup> *C. guilliermondii* and *C. rugosa* are growing in importance.<sup>[12]</sup> Increased use of broad-spectrum antibiotics, glucocorticoid therapy, increasing age, transplantation, increased use of intravenous catheters, total parenteral nutrition, and invasive procedures have contributed to this.<sup>[13]</sup>

The frequency and distribution of *Candida* species vary in different geographical regions.<sup>[14]</sup> *Candida albicans* has been found to predominate in Northern and Central Europe and the United States, while non-*albicans* species were more frequently isolated in South America, Southern Europe and Asia.<sup>[15]</sup> *Candida* species can adapt to varied anatomical sites and as a result, they cause a wide range of infections, from superficial to deep-seated infections.

Not very much is known about the prevalence and species distribution of *Candida* colonizing the vagina of women in Nigeria especially among women in the Southeastern region where the burden of HIV/AIDS is huge. The aim of this study therefore, was to determine the prevalence and species distribution of *Candida* isolates from women in Nnewi, Anambra State, Nigeria.

## MATERIALS AND METHOD

### Study Area

The study was conducted in one major city in Anambra State- Nnewi in the South-East geopolitical zone of Nigeria. Life Specialist Hospital (LSH), Nnewi, Anambra State, Nigeria was selected based on convenience.

### Study Design

A descriptive cross-sectional study was conducted from January to July, 2016 and a random sampling method was used.

### Ethical Consideration

Ethical clearance was obtained from the management board of the hospital.

### Sample Collection

A total of 99 high vaginal swab samples (from HIV-negative pregnant/non-pregnant women attending Life Specialist Hospital, Nnewi) were collected. Structured questionnaires were used to gather relevant information from the patients after a written consent was obtained from them. The samples were transported to the Department of Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Awka, for analysis.

## Culture of the High Vaginal Swab (HVS) Samples

The samples were inoculated onto Sabouraud dextrose agar plates supplemented with Chloramphenicol (50µg/ml) and incubated aerobically at room temperature for 24-48hours as described by.<sup>[16]</sup> After the period of incubation, representative discrete colonies on the plates were picked and sub-cultured on Sabouraud dextrose agar using streaking techniques and incubated to obtain pure cultures. The pure cultures were then stored on agar slants of Sabouraud dextrose agar in Bijou bottles and stored at 4°C for further use

## Characterization and Identification of the Yeast Isolates

The yeast isolates were identified on the basis of colony characteristics, microscopic morphology, physiological and biochemical characteristics such as growth on chromogenic *Candida* Agar, cornmeal agar, growth at elevated temperature (45°C) germ tube test, sugar fermentation test and also by comparing with photomicrographs in the colour Atlas of pathogenic fungi by.<sup>[17]</sup>

## Statistical Analysis

The Chi- Square Test was used to test the occurrence of *Candida* species and comparisons of proportions between groups. SPSS software version 21.0 was used for the analysis. The level of significance was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The present study was carried out to determine the prevalence and species distribution of *Candida* isolates from some women attending Life Specialist Hospital in Nnewi, Anambra State, Nigeria. A total of 34 isolates of *Candida* species were recovered from the high vaginal swab samples. The occurrence of the different *Candida* species in the high vaginal swab (HVS) samples is presented in table 1. However, one (1) sample yielded a combination of two species (*Candida albicans* and *Candida dubiniensis*). Thus, the number of HVS samples positive for *Candida* species was actually 33, giving *Candida* colonization prevalence of 33.3%.

It was observed in this study (Table 1) that *Candida tropicalis* was the most common species isolated from HVS of the women (13, 38.2%) followed by *C. albicans* (11, 32.4%). This finding does not agree with the reports by some other researchers<sup>[18,19,20,21]</sup> in which *Candida albicans* was the most prevalent *Candida* species. The work of<sup>[22]</sup> revealed that *Candida glabrata* was the most predominant *Candida* species from genitourinary tracts. These differences may be as a result of the fact that the present study combined urinary and high vaginal swab isolates. However,<sup>[23]</sup> reported that *C. tropicalis* had a higher incidence (26.4%) than *C. glabrata* (20.6%) and also speculated that widespread and inappropriate use of antimycotic treatments (self medication, topical use, long-term treatments and repeated candidal episodes)

could have given rise to the increasing detection of non-*albicans* species.

This present study observed that non-*albicans* *Candida* species from HVS of pregnant and non-pregnant HIV-negative women are on the increase in recent times with a prevalence of 67.6% and agrees with the finding of<sup>[24]</sup> that non-*albicans* *Candida* species (with a prevalence of 63.3%) have emerged as an important cause of infection and that their isolation from clinical specimens can no longer be ignored as non-pathogenic isolates.

**Table 1: Occurrence of *Candida* species in the high vaginal swab samples.**

Species	Occurrence (%)
<i>Candida tropicalis</i>	13(38.2)
<i>Candida albicans</i>	11(32.4)
<i>Candida glabrata</i>	4(11.8)
<i>Candida krusei</i>	4(11.8)
<i>Candida dubliniensis</i>	1(2.9)
<i>Candida kefyr</i>	1(2.9)
<b>Total</b>	<b>34(100)</b>

**Table 2: Prevalence of Vaginal *Candida* isolates in Pregnant Women According to Age**

Age group	<i>Candida</i> positive	<i>Candida</i> negative	Percentage of <i>Candida</i> positive Women (%)
16 – 25	9	12	32.1
26 – 35	12	31	42.9
36 – 45	7	5	25
46 – 55	0	0	0
56 - 65	0	0	0
<b>Total</b>	<b>28</b>	<b>48</b>	<b>36.8</b>

Table 3 shows the prevalence of vaginal *Candida* colonization among the pregnant and non-pregnant women. It is observed from this table that the prevalence of vaginal *Candida* colonization was higher among pregnant women (36.8%) than non-pregnant women (21.7%). However, the difference was not statistically significant ( $p > 0.05$ ). This finding agrees with previous works in South Libya<sup>[28]</sup> and Saudi Arabia<sup>[26]</sup> which reported a higher prevalence in pregnant women (43.8% and 24.24%) than in non-pregnant women (37.8% and 17.45%) respectively. Pregnancy and vaginal *Candida* colonization have a relationship that indicates increase in hormonal influences and alteration in vaginal pH.<sup>[29]</sup>

The trimester-specific prevalence of vaginal *Candida* colonization is presented in table 4. The highest prevalence of vaginal *Candida* colonization was observed in the second trimester of pregnancy (43.2%)

The prevalence of vaginal *Candida* isolates among the pregnant women in relation to age is shown in table 2. It was observed in this study that among the pregnant women, age group 26 - 35 years had the highest prevalence of vaginal *Candida* isolates (42.9%) followed by those aged 16 – 25 years (32.1%). Age group 36 – 45 years had a prevalence of 25% (Table 2).<sup>[25]</sup> had a similar result.<sup>[23]</sup> from India had a highest prevalence of 49.58% among age group 26-35 years but had age group 18-25 years as the second predominant age group (35.53%) which is similar to the result of this study. Meanwhile,<sup>[26]</sup> from Saudi Arabia reported that age group 26-30 years had the highest frequency of *Candida* species followed by age group 31-35 years. Oestrogen hormone increases in concentration during pregnancy and creates conducive environment for the growth of *Candida* species,<sup>[27]</sup>

followed by third trimester (35.3%). First trimester had zero (0%) prevalence. This result concurs with reports given by<sup>[20]</sup> Kanagal and<sup>[19]</sup> that second trimester (54% and 61%) respectively had the highest prevalence of vaginal *Candida* colonization followed by third trimester (30% and 21.4%) respectively. The first trimester (16% and 16.7% respectively) had the lowest vaginal *Candida* prevalence. However, the result of this study contradicts that of<sup>[25]</sup> who observed that third trimester had the highest prevalence of vaginal *Candida* colonization (68.09%), followed by second trimester (21.28%) and first trimester (10.63%). Conditions such as pH and temperature encourage *Candida* colonization and are enhanced as age of pregnancy increases.<sup>[30]</sup> Again, many of the women in third trimester of pregnancy must have received treatment for vaginal symptoms in the past even as their pregnancy got to term.

**Table 3: Prevalence of Vaginal *Candida* Colonization among Pregnant and Non-pregnant Women.**

Status	Number Sampled (%)	<i>Candida</i> positive	<i>Candida</i> negative	Percentage of <i>Candida</i> positive women
Pregnant	76(76.8)	28	48	36.8
Non-pregnant	23(23.2)	5	18	21.7
<b>Total</b>	<b>99(100)</b>	<b>33(33.3%)</b>	<b>66(66.7%)</b>	

**Table 4: Trimester-Specific Prevalence of Vaginal *Candida* Colonization.**

Trimester	Number sampled (%)	<i>Candida</i> positive	<i>Candida</i> negative	Percentage of <i>Candida</i> positive women
First (0 - 3 months)	5(5.1%)	0	5	0
Second (4 – 6 months)	37(37.4)	16	21	43.2
Third (7 – 9 months)	34(34.3)	12	22	35.3
Non-pregnant	23(23.2)	5	18	21.7
<b>Total</b>	<b>99(100)</b>	<b>33(33.3%)</b>	<b>66(66.7%)</b>	

Of the 99 women examined, 62 (62.6%) were symptomatic while 37 (37.4%) were asymptomatic. In this study, symptomatic women had a higher prevalence of vaginal *Candida* colonization (41.9%) than asymptomatic women (18.9%) as shown in table 5. The difference was observed to be statistically significant. This agrees with reports by<sup>[25]</sup> and<sup>[31]</sup> where symptomatic women had 32.9% and 26.7% respectively while asymptomatic women had 26.9% and 21.7%

respectively. Although symptomatic women had a higher prevalence, asymptomatic women had a prevalence that is worthy of note.<sup>[31]</sup> suggested that unnoticed asymptomatic *Candida* infections could lead to other severe complications. The finding of this study suggests that all pregnant women whether symptomatic or asymptomatic should be subjected to routine laboratory tests to rule out the possibility of vaginal *Candida* infection.

**Table 5: Prevalence of Vaginal *Candida* Colonization in Symptomatic and Asymptomatic Women.**

Clinical manifestation	Number sampled (%)	<i>Candida</i> positive	<i>Candida</i> negative	Percentage of <i>Candida</i> positive women
Symptomatic	62(62.6)	26	36	41.9
Asymptomatic	37(37.4)	7	30	18.9
<b>Total</b>	<b>99(100)</b>	<b>33(33.3%)</b>	<b>66(66.7%)</b>	

## CONCLUSION

In this study, it was observed that the prevalence of vaginal *Candida* colonization was higher in pregnant women (36.8%) than non-pregnant women (21.7%) though, the difference was statistically not significant. The highest vaginal *Candida* prevalence was observed among pregnant women in age group 26-35 years. Women in second trimester of pregnancy had the highest prevalence (43.2%) of vaginal *Candida*.

*Candida tropicalis* was the most frequently isolated species (13; 38.3%) followed by *C. albicans* (11; 32.4%). Also, the prevalence of non-*albicans* *Candida* (NAC) species was 67.6%. The symptomatic women had a higher prevalence (41.9%) of *Candida* than their asymptomatic counterparts (18.9%) and this difference was observed to be significant statistically.

Since unnoticed asymptomatic *Candida* infections could lead to other severe complications as suggested by some researchers, this study suggests that all pregnant women whether symptomatic or asymptomatic should be subjected to routine laboratory tests to rule out the possibility of vaginal *Candida* infection. Also, this study recommends proper identification of yeasts from clinical specimens to species level especially in recent times when the epidemiology of *Candida* infections is changing rapidly and more non-*albicans* species of *Candida* are implicated in many infections

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