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Charaterization of *Candida* species isolated from pregnant women with vaginal discharge attending anternatal clinic in parts of Edo State, Nigeria

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ABSTRACT

During pregnancy, women are more prone to vaginal infections due to immunological changes. These infections are mainly caused by different species of *Candida*, a reality that makes distinguishing *Candida* species vital since non-*albicans* strains often resist common antifungals, making accurate diagnosis key to better maternal outcomes. Therefore, this study aimed at isolating and characterizing *Candida* species from pregnant women with vaginal discharge. The sample size consisted of 220 subjects (151 pregnant and 69 non-pregnant women). Samples were collected following standards guidelines and analyzed for *Candida* by culturing on Sabouraud dextrose agar (SDA) and CHROMager™ *Candida* (CAC). The overall prevalence of vulvovaginal candidiasis (VVC) was 34.55%, and VVC infection was more common among women >30 years. VVC infection was more common in women in their second trimester (48.05%). There was a significant difference with gestational age ($p \leq 0.05$). *Candida albicans* was the most common cause of VVC in both pregnant (75.86%) and non-pregnant (77.78%) subjects. There was no significant variation ($p > 0.05$) in the species of *Candida* isolated from pregnant and non-pregnant women. Overall, *C. albicans* was the most common species isolated from pregnant women. This was followed by *Candida krusei* (10.34%), *Candida dublinensis*, *Candida. glabrata* (5.17% each) and *Candida parapsilosis* (1.72%); while mixed infection, both *C. dublinensis* and *C. parapsilosis* were found in 1.72%. Among the non-pregnant women, *C. albicans* was the most common species isolated, followed by *C. dublinensis* and *C. glabrata* (11.11% each). *C. krusei* and *C. parapsilosis* were not isolated from non-pregnant women. Routine screening of vulvovaginal candidiasis and other vaginal infections should be conducted during antenatal visits to enable early detection and treatment.

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1. INTRODUCTION

*Candida*s is is a fungal infection caused by yeast belonging to the genus *Candida* [1]. There are over 20 species of *Candida* that can cause infection in humans, the most common of which is *Candida albicans* [2], [3]. Although *Candida* is a normal flora in certain parts of the body, overgrowth of

these organisms can cause symptoms of candidiasis depending on the area of the body that is affected [4]. Invasive candidiasis occurs when *Candida* species enter the bloodstream and spread throughout the body [5]-[7]. Vulvovaginal candidiasis is *Candida* infection of the vagina, and pregnant women are particularly at high risk due to physiological changes in pregnancy characterized by weakening of the immune system to accommodate the growing foetus [8].

Vaginal discharge is the fluid secreted from the thin glands in the vagina and cervix. It may be either physiological or pathological [9], [10]. Causes of vaginal discharge include non-infective, non-sexually transmitted infections and sexually transmitted infections. Non-infective conditions are caused by physiological conditions such as the level of oestrogen and progesterone, menopause, cervical polyps, as well as genital tract malignancy [8], [9]. Abnormal vaginal discharge is most commonly caused by infection, with 70% of all cases associated with vulvovaginal candidiasis, bacterial vaginosis, or *Trichomonas vaginalis* [11], [12]. The most common infection in pregnancy in most studies is *C. albicans* infection [13]-[15]. This infection could result in significant negative maternal, foetal, and even neonatal outcomes [8], [16]. The higher incidence rate of vaginal infections in pregnant women than in non-pregnant women is attributed to the increase oestrogen content of vaginal mucosa [17], [18].

Pregnancy alters the immune system, leaving women more susceptible to infections. Among these, vaginal infections are especially frequent, with vulvovaginal candidiasis, which is primarily caused by *Candida albicans*, being a common concern. Despite its prevalence, data comparing *Candida* infections in pregnant versus non-pregnant women remain scarce in many parts of Edo State. This lack of information complicates efforts to establish effective screening and treatment protocols during prenatal care. From a clinical standpoint, identifying the exact *Candida* species is crucial because not all respond equally to antifungal medications. This can result in persistent infections and failed treatments. Therefore, accurate species identification directly influences how cases are managed, helps prevent complications, and ultimately supports better health outcomes for expectant mothers. In this work, we intend to provide updated microbiological data that is lacking about *Candida* species causing infections in pregnant and non-pregnant women in a part of the world that has limited facilities for basic healthcare. Hence, this study aims to determine the variation in the *Candida* species causing vulvovaginal candidiasis in pregnant and non-pregnant women attending primary health care centres in Ekpoma, Edo State, Nigeria, where the state-owned Ambrose Alli University is located.

2. METHOD

The laboratory investigation for this study was carried out at the Microbiology laboratory of the Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma. Edo State.

2.1. Study design

A community-based cross-sectional study design was used among pregnant and non-pregnant women who registered in Ujuolen and Upkenu Primary Health Care centers in Ekpoma, Esan-West Local Government area, Edo State, Nigeria.

2.2. Inclusion/exclusion criteria

The inclusion criteria for this study are: all consenting pregnant and non-pregnant women indicated for high vaginal swab culture, attending Primary Health Care Centers, Esan West, with no history of antifungal usage in the last two months. The excluded group in this study: non-consenting women; women not indicated for high vaginal swab culture; not attending PHCs; with a history of antifungal usage in the last two months.

2.3. Area of study/sample size

The primary health care centers where pregnant women go for antenatal care are located in Esan-West Local Government Area of Edo State, Nigeria. Ekpoma is the administrative headquarters of Esan-West Local Government Area and has a total population of 167,721 (NPC, 2006). The inhabitants are mainly farmers, artisans, government workers, and students of tertiary institutions, of which Ambrose Alli University is one of them. The number of subjects used in this study was guided by an upper limit to give 95% confidence at an expected prevalence of about 37% from a previous study [19].

$$Sample\ size = \frac{Z^2Pq}{d^2}$$

Where Z = confidence level (95%) = 1.96.

P = expected prevalence = 37% = 0.37.
q = 1.0 – p =1 1-0.37 = 0.63.
d = margin of error = 0.065.
Sample size = $\frac{1.96^2 \times 0.37 \times 0.63}{0.065^2}$.
Sample size = 211.95~212 samples.

2.4. Data collection instruments/procedure

The study comprised 220 subjects: 151 pregnant women who registered in the Primary Health Care Centre for antenatal care and 69 non-pregnant women who also visited the health facility. The socio-demographic information used for the study was collected from the records of the subjects in the health facilities. The specimens were collected from each subject by means of a sterile swab stick by the attending physician. They were properly labelled with an identification number and sent immediately to the laboratory for analysis.

2.5. Isolation, identification, and characterization of *Candida*

The sample on the swab stick was used to make a primary inoculum on the surface of prepared Sabouraud dextrose agar (SDA), streaked with the aid of a wire loop, and incubated at 37 °C for 48 hours. After incubation, the plates were observed for fungal growth, and the isolates were identified as *Candida* using the Gram staining technique. The Gram stain revealed gram-positive yeast-like budding cells. The SDA isolates were inoculated in *Candida* selective medium, using CHROM^{TM} *Candida* (CAC), and incubated at 37 °C for 72 hours to ensure detection of mixed cultures. On SDA, *C. albicans* showed a white coloured, smooth, and yeast-like appearance; *Candida parapsilosis colonies were white to creamy, shiny, and smooth; Candida dubliniensis was cream to white; Candida glabrata colonies were smooth and white-coloured*; while *Candida krusei* showed rough colonies. On CAC, *C. albicans* was green; *C. glabrata* was pink-purple; *C. parasiplosis* was white to pale pink, and *C. krusei* appeared as pink colonies. The CAC method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species after differential exoenzyme activity.

2.6. Statistical analysis

Statistical analysis was done using the Chi-square test and the student t-test to determine level of significance. A p-value of less than or equal to 0.05 ($p \leq 0.05$) was considered to statistically significant.

3. RESULTS AND DISCUSSION

Table 1 shows the socio-demographic characteristics of the study population. A total of 220 participants were included in the study. The majority were between the ages of 21–30 years (68.6%), while 13.7% were aged 0 – 20 years and 18.2% were older than 30 years. With respect to occupation, merchants (32.7%) and students (30.5%) constituted the largest groups, followed by tailors (10.5%), housewives (10.0%), and hairstylists (9.1%). Smaller proportions were teachers (2.7%), food vendors (2.3%), farmers (0.9%), and others, such as nurses, secretaries, and soldiers (1.4%). Regarding pregnancy status, 68.6% were pregnant, distributed across first (6.8%), second (35.0%), and third (26.8%) trimesters, while 31.4% were non-pregnant.

Table 1. Socio-demographic characteristics of the study population

Characteristics	Variable		Frequency (n = 220)	Percentage (%)
Age (years)	0 – 20		29	13.68
	21 – 30		151	68.64
	> 30		40	18.18
Occupation	Merchant		72	32.73
	Student		67	30.45
	Tailor		23	10.45
	Housewife		22	10.00
	Hairstylist		20	9.09
	Teacher		6	2.73
	Food vendor		5	2.27
	Farmer		2	0.91
	Others (nurse, secretary, & soldier)		3	1.36
Pregnancy status	Pregnant	1st	15	6.82
		2nd	77	35.00
		3rd	59	26.82
	Non-pregnant		69	31.36

Table 2 shows the distribution of *Candida* (species and intensity of growth) according to pregnancy status. There were no significant variations (p>0.05) in the species of *Candida* isolated from pregnant and non-pregnant women. *C. albicans* was the most common species isolated from pregnant women and non-pregnant women, 75.86% and 77.78% respectively. In pregnant women, this was followed by *C. krusei* (10.34%), *C. dubliniensis* and *C. glabrata* (5.17% each), and *C. parapsilosis* was found in (1.72%), while mixed infection, both *C. dubliniensis* and *C. parapsilosis* were found in 1.72%. Among the non-pregnant *C. albicans* was the most common species isolated, followed by *C. dubliniensis* and *C. glabrata* (11.11% each). *C. krusei* and *C. parapsilosis* were not isolated from non-pregnant women. Figures 1-4 show the appearance of various species of *Candida* on CHROMagar *Candida*. There was also no significant variation (p>0.05) in the intensity of *Candida* growth according to pregnancy status, but the non-pregnant women had heavier growth (22.22%) compared to their pregnant counterparts (18.97%).

Table 2. Distribution of *Candida* species and intensity of growth among pregnant and non-pregnant women

Variable	Category	Pregnant (n = 58) (%)	Non-pregnant (n = 18) (%)	X^2	p-value
Species	*C. albicans*	44 (75.86)	14 (77.78)	3.962	0.555
	C. dubliniensis	3 (5.17)	2 (11.11)		
	C. glabrata	3 (5.17)	2 (11.11)		
	C. krusei	6 (10.34)	0 (0.00)		
	C. parapsilosis	1 (1.72)	0 (0.00)		
	Mixed	1 (1.72)	0 (0.00)		
Intensity of growth	Heavy	11 (18.97)	4 (22.22)	0.092	0.762
	Moderate	47 (81.03)	14 (77.78)		

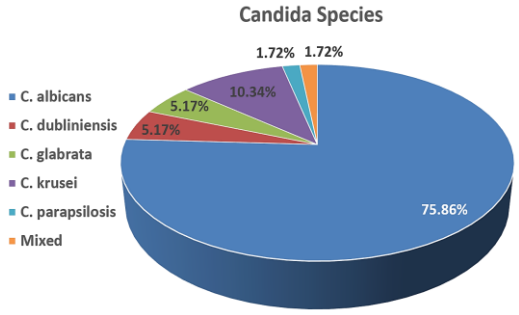

Figure 1. Proportion of *Candida* species isolated from pregnant women attending antenatal Clinics in Juoelen and Ukpenu Primary Health Care Centres, Esan West Local Government Area, Edo State, Nigeria

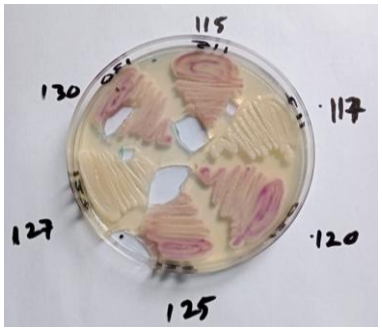

Figure 2. CHROMagar *Candida* plate showing *C. krusei* (pink- 115, 120, 125, and 130) and *C. glabrata* (white – 117 and 127)

Figure 3. CHROMagar *Candida* plate showing *C. parapsilosis* (faint pink-21), *C. albicans* (light green- 24, 29, 33, and 34); *C. parapsilosis* and *C. dublinensis*

Figure 4. CHROMagar *Candida* plate showing *C. dublinensis* (deep green, 11 and 14); *C. glabrata* (white - 9); *C. albicans* (light green- 6, 8, and 19)

4. DISCUSSION

Vaginal infections in pregnancy are associated with considerable discomfort and adverse pregnancy outcomes, including pre-term delivery, low birth weight, increased infant mortality, and predisposition to HIV/AIDS [8], [17]. Several microorganisms cause vaginal infections, and the most common vaginal infection is vulvovaginal candidiasis [20].

The socio-demographic characteristics of the study show that most of the subjects were within 21–30 years of age (68.64%), merchants (32.73%), and pregnant (68.64%). Most of the pregnant women were in their second trimester, followed by the third (26.82%) and first trimester (6.82%). The high occurrence of second-trimester pregnant women in this study could be a result of the timing of the study or because most pregnant women in Edo State (63.0%) initiate antenatal care during the second trimester, as reported by [21].

In this study, the prevalence of vulvovaginal candidiasis (35.55%) supports the notion that at least one in every three women with vaginitis is infected with vaginal candidiasis [22]. Furthermore, the prevalence reported here is slightly lower than the 40% reported by [23], but far lower than the 84.5% reported by [24]. Among reproductive-aged women, the prevalence of vulvovaginal candidiasis was 34.55%, which aligns with 36% reported by [13] and [14], but is higher than the 10% reported in Abakaliki, South-Eastern Nigeria, by [23]. Variations in women's health hygiene across different study areas may account for these discrepancies.

Pregnant women in this study had a significantly higher prevalence of vulvovaginal candidiasis ($p \leq 0.05$) compared to their non-pregnant counterparts. This agrees with findings from Denmark [25], which also reported a higher prevalence in pregnant women. However, a study in Zaria, Nigeria, by [26] found a higher incidence of vulvovaginal candidiasis in non-pregnant women. The higher prevalence in pregnancy is likely due to increased reproductive hormones that elevate glycogen levels in vaginal tissue, providing a carbon source for *Candida* organisms [23].

There was no significant variation ($p > 0.05$) in the prevalence of vulvovaginal candidiasis with age in pregnancy in this study. However, [26] reported a significant variation, with the infection being more common in women above 30 years (44.44%). The prevalence of vulvovaginal candidiasis was highest in the second trimester (48.05%), similar to findings by [27] in Edo State, which also reported higher prevalence during this period. This may be due to depleting immunity during pregnancy [17], as well as issues around antenatal care. As noted by [21], since most pregnant women in Edo State begin antenatal care during the second trimester, vaginal infections may not have been properly managed before that time.

The present study also showed no significant variation in the prevalence of vulvovaginal candidiasis with occupation. *C. albicans* was the most prevalent *Candida* species causing infection in both pregnant and non-pregnant women, with 75.86% and 77.78% respectively. The predominance of *C. albicans* in this study agrees with the findings of [23] in Oyo State. However, studies from Abuja by [28] found a higher prevalence of *C. glabrata*. Similarly, studies in Southern Ethiopia among pregnant women reported *C. albicans* as predominant (62.30%), followed by *C. glabrata* (15.3%), *C. krusei* (14.1%), and *C. tropicalis* (5.9%) [29].

Although the distribution in this study differs, *C. albicans* remained the dominant cause of vulvovaginal candidiasis (75.86%) among pregnant women, followed by *C. krusei* (10.34%), *C. dublinensis* (5.17%), *C. glabrata* (5.17%), and *C. parapsilosis* (1.72%). As noted by [30], while *C. albicans* is the primary pathogen responsible for vulvovaginal candidiasis in both pregnant and non-pregnant women, and an epidemiological shift toward non-albicans *Candida* (NAC) species is increasingly being observed globally. Other *Candida* species isolated from pregnant women in this study include *C. krusei*, *C. dublinensis*, *C. glabrata*, and *C. parapsilosis*.

5. CONCLUSION

The present study has identified the presence of non-albicans *Candida* in causing VVC in pregnant and non-pregnant women. Therefore, routine screening of vulvovaginal candidiasis and other vaginal infections should be conducted during antenatal visits to enable early detection and treatment. Furthermore, public awareness and health education programs should be encouraged to educate particularly reproductive-aged women on the importance of personal hygiene, proper clothing choices, and differences between normal and abnormal vaginal discharge.

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The authors declare that no funding was received for this study.

AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

The authors state no conflict of interest.

ETHICAL APPROVAL

Ethical permission for this study was requested from and provided by the Ethics and Research Committee of Ambrose Alli University, Ekpoma. Consent for sample collection was obtained from the management of the Primary Health Centres (PHC), Esan West LGA. Mutual consent for the collection of samples was requested and obtained verbally from each of the subjects after educating them on the purpose of the research.

DATA AVAILABILITY

The data that support the findings of this study are available from the first author upon proper request. However, the data are not publicly available due to ethical restrictions.

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