



Prevalence of *Candida* Species from Cases of Vulvovaginitis in Women using Contraceptives in Four Selected States of North Central Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author LYA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AC and AB managed the analyses of the study. Authors AC and AB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Vulvovaginal candidiasis affects millions of women every year and in most developing countries such as Nigeria, vulvovaginal candidiasis is still received with little attention since it is considered to be a trivial disease.

Aim: The aim of this study is to determine the prevalence of *Candida* species from cases of vulvovaginitis in women using contraceptives in four selected states of North Central Nigeria.

Place and Duration of Study: The study was carried out in four selected states of North Central Nigeria and the Department of Microbiology, Federal University Lafia between the months of January to November 2018.

Study Design: A cross-sectional study design was utilized.

Material and Methods: One thousand six hundred samples of high vaginal swabs were collected in four States of North Central Nigeria. Four hundred samples were collected from women using contraceptive device in each state. Two high vaginal swabs were collected. Germ tube test, direct

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examination and lactophenol cotton blue examination was carried out. The high vaginal swabs was streaked on Sabouraud dextrose agar (SDA), and subsequently isolates from SDA were streaked on CHROM agar and incubated aerobically at 37°C.

Results: Five species of *Candida* were isolated from 710 women with vulvovaginal candidiasis. *Candida albicans* was the most frequent isolate which accounted for 43.23% of the species isolated. The prevalence of vulvovaginal candidiasis across the four selected states in the North Central zone is 44.37%. The prevalence of vulvovaginal candidiasis in relation to age in the study area shows that the lowest prevalence (32.67%) of candidiasis infection was recorded among women below 20 years. The distribution of contraceptive usage reveals that the use of injectables was the most predominant contraceptive method used in the study area. The cross tabulated results at 95% confidence interval showed a statistically strong significance at 0.001 level for demographic characteristics which include age, marital status, and educational status in the study area. A significant difference at 0.294, 0.351 was recorded for diabetes and HIV status respectively.

Conclusion: The high prevalence rate of vulvovaginal candidiasis in this study poses a great threat to women's reproductive health, hence the need for continuous epidemiological surveys among women in Nigeria.

Keywords: Prevalence, Vulvovaginitis, contraceptive.

1. INTRODUCTION

Within women of reproductive age, Vulvovaginal candidiasis (VVC) is considered the second most common cause of genital infection and it represents a problem of public health globally, yet its exact incidence has not been identified. Nearly 5–10 million females seek gynaecologic advice for vaginitis every year world-wide [1,2]. Women with vulvovaginal candidiasis triggered by hormonal changes such as puberty, pregnancy, menopause, use of hormonal contraceptive or hormone replacement therapy have a 25% lifetime risk [3].

Vulvovaginal candidiasis is caused by the overgrowth of *Candida* species in the vagina and is characterized by itching, erythema and curd-like vaginal discharge [4]. A number of fungal species belonging to the genus *Candida* can cause acute vulvovaginal infection (VVC) [5]. However, *Candida albicans* is by far the most prevalent etiological agent [6]. Other *Candida* species that cause vaginitis most often are *C. glabrata* and *C. tropicalis*. Ray et al., 2007 and Akah et al., 2010 [7,8] reported that over the years, there has been a consistent increase in the frequency of cases caused by *Candida glabrata*.

A number of factors such as age, pregnancy, uncontrolled diabetes mellitus, HIV/AIDS and long-term broad spectrum antibiotic treatment have been identified as predisposing factors to vulvovaginal candidiasis [9]. Other risk factors include the use of contraceptives, diaphragms, orogenital sex, douching [8,10] and diet with high

glucose content [11]. The changes in the composition and function of the vaginal microbiota may expose females to *Candida* infection [12].

Family planning aids in the protection of women from high risk pregnancies, unsafe abortions, reproductive tract infections and STIs including HIV/AIDS [13].

There are several types of contraceptive methods which are widely used by women and these include; Combination hormonal contraceptives, Progesterone preparations, intrauterine contraceptive devices, Barrier method (condoms) and sterilization which involves vasectomy in males and sterilization in females [14].

Relatively few epidemiological data on the distribution of *Candida* spp. involved in VVC and contraceptive usage are available; hence, this study was carried out to determine the occurrence of *Candida* spp. causing vulvovaginitis amongst women using contraceptives in four selected states of North Central Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Nigeria has six geopolitical zones which were carved out based on similar cultures, ethnic groups, and common history. The North Central zone consists of seven states situated geographically in the middle belt region of the

country, spanning from the West, around the confluence of the River Niger and the River Benue. These states include Benue, Nasarawa, Niger, Plateau, Kogi, Kwara, and the Federal Capital Territory, Abuja. This research was conducted in four selected states of the North Central which include Nasarawa, Niger, Benue and the Federal Capital Territory, Abuja. These states were selected by simple random sampling.

2.2 Study Design

A hospital based, cross sectional study design was used to determine the prevalence of *Candida* spp causing vulvovaginitis amongst women and also to assess demographic characteristics in relation to contraceptive usage. A well structured questionnaire was used to obtain bio demographic data and analyze risk factors.

2.3 Study Population

This study involved only women (outpatients) between the age group of 18-45 years who use various contraceptive and visit the Gynaecology and Obstetrics unit of the hospitals.

2.4 Inclusion Criteria and Exclusion Criteria

All women using contraceptives were recruited for this study. Anyone with an underlining disease such as HIV, diabetes e.t.c. was noted and included in the study.

All women who were pregnant, currently menstruating and those who did not give their consent for sample collection were excluded from this study. Women below the ages of 18 and above 50 years were excluded from the study. Women above 50 years may be experiencing menopause and may have no need for contraceptives.

2.5 Sample Examination

2.5.1 Sample Collection

Four hundred samples of high vaginal swabs were collected from women using contraceptive device in each state. A total of one thousand six hundred samples were collected. These samples were collected on their clinic days with the assistance of a gynaecologist. Two high vaginal swabs, one after the other was collected. The high vaginal swab was collected by inserting a sterile vaginal speculum into the vagina; a sterile cotton wool swab was inserted into the posterior

vaginal fornix and rotated gently. The swab stick was withdrawn and replaced in its case and labelled appropriately with the patient's information. The swab sticks was preserved in normal saline and taken to the Microbiology Laboratory of Federal University Lafia for analysis. One of the swabs was used for direct smear examination. The second swab stick was pressed firmly against the inner wall of the tube above the fluid level to remove excess fluid from the swab. It was then streaked on Sabouraud dextrose agar (SDA) and incubated aerobically at 37°C.

2.6 Sample Processing

2.6.1 Direct examination

Specimens of vaginal swabs were examined by placing the specimen on a clean, dried, grease – free slide mounted with a drop of KOH 10%, and examined under both low (10×) and high (40×) power fields of the microscope for the presence of fungal elements. The details regarding the hyphae, spores, budding cells and pseudo-hyphae were noted [15].

2.6.2 Culture and isolation

The high vaginal swab was directly streaked onto Sabouraud Dextrose Agar medium (SDA) and incubated for 24-48 hours at 37°C to obtain pure isolates. The fungal growths were preserved in screw-capped tubes containing 5ml of Sabouraud dextrose agar slants; they were tightly wrapped with parafilm and stored at 4°C in refrigerator.

2.6.3 Lactophenol cotton blue examination

With the aid of a sterile wire loop, a colony of the *Candida* cell was picked from the petri dish and placed on clean, dried, grease free slide mixed with drop of lactophenol cotton blue, covered with cover-slip and examined under light microscope for the budding cells of *Candida*.

2.6.4 Germ tube test

The germ tube test is a presumptive test for the identification of *Candida albicans*. The procedure of Menza et al., 2013 [16] was used to conduct the test. A loopful of the yeast cells was inoculated into 0.5 mL of human serum (negative for HBV, HCV, and HIV infections) and incubated at 37°C for three hours. A drop of the incubated serum was placed on a microscope slide and covered with a cover slip and examined under

the microscope using the x10 and x40 objective lenses for the presence of germ tube. Germ tube is considered as a lateral tube without septum and has no constriction at initiating site [17].

2.6.5 Cultivation on selective medium (CHROM agar)

Purified single colonies from Saboraud dextrose agar (SDA) was inoculated on CHROM agar using an inoculating loop and incubated at 37°C for 48 hours. The method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species following differential exoenzyme activity [18]. *Candida* isolates were classified according to their colours on CHROM agar based on the manufacturer's protocol.

3. RESULTS

Five species of *Candida* were isolated from 710 women with vulvovaginal candidiasis. *Candida albicans* was the most frequent isolate which accounted for 43.23% of the species isolated. Out of the non-albicans *Candida* species 19.01% were *C. glabrata*, 15.77% were *C. tropicalis*, 8.87% and 13.09% was accounted for *C. parapsilosis* and *C. krusei* as shown in Table 1.

Table 1. Occurrence of *Candida* isolates from 710 patients with vulvovaginal candidiasis

Species	Number of isolates	% of the total isolates
<i>C. albicans</i>	307	43.23
<i>C. glabrata</i>	135	19.01
<i>C. tropicalis</i>	112	15.77
<i>C. parapsilosis</i>	63	8.87
<i>C. krusei</i>	93	13.09
Total Isolates	710	100

The prevalence of vulvovaginal candidiasis across the four selected states in the North Central zone is 44.37%. The highest prevalence of 53.00% was recorded in Nasarawa State followed by Niger state with a prevalence of 51.00%, Benue had 39.25% prevalence while the lowest prevalence of 35.00% was recorded in Abuja as shown in Table 2.

The prevalence of vulvovaginal candidiasis (culture positive) in relation to demographic characteristics and their respective P-values are shown in Table 3. The prevalence of vulvovaginal candidiasis in relation to age in the study area shows that the lowest prevalence

(32.67%) of candidiasis infection was recorded among women below 20 years while the highest prevalence of 52.03% was recorded among women who were within the age group of 21-30 years. Six hundred and thirteen were HIV negative and Seventeen of them were positive to vulvovaginal candidiasis. Twenty Six women did not know their status out of which five of them had VVC. The prevalence of VVC in relation to HIV status in the study Area is 4.60%. The prevalence of vulvovaginal candidiasis in relation to diabetes is 8.93%. One hundred and forty three women had diabetes out of which 44(30.76%) had VVC. Ninety nine (6.79%) women were positive to vulvovaginal candidiasis did not have diabetes.

The highest prevalence of VVC was recorded among the married women in the study area with a prevalence of 46.94% while the unmarried had a prevalence of 29.0%. The highest prevalence of vulvovaginal candidiasis in relation to educational status was recorded amongst women with tertiary education (54.58%). The lowest prevalence of 33.4% was recorded among women with secondary education.

Table 4 shows the distribution of contraceptive usage which reveals that the use of injectables was the most predominant contraceptive method used in Abuja, Nasarawa and Niger States while the least contraceptive method was the use of implants in the study area.

4. DISCUSSION

Several studies on the prevalence of *Candida* species have led to the general agreement that *C. albicans* is the most commonly isolated specie in patients with vulvovaginal candidiasis. This study also reveals that *C. albicans* had the highest occurrence of 43.23% and this may be due to the fact that *C. albicans* are capable of forming hyphae in the murine vagina, candidalysin expression and consequently activating the NLRP3 inflammasome, which contributes to elicit robust immunopathogenicity [19].

Our finding of *C. albicans* as the most major species is in concordance with several studies carried out earlier by Jimoh et al.,(2016), Nnadi and Singh, (2017), Mnge et al.,(2017), Sasikala and Udayasri, (2018), Rajeshwari, (2019) who reported higher rates of 48.5%, 49.1%,45.4% , 46.1% and 54% in the United states, Nigeria, South Africa and India [20,21,22,23,24].

Candida albicans has been reported to be the most prominent species isolated from clinical samples of patients diagnosed with VVC; however, there has been a notable shift in the etiology of candidiasis with non-*albicans Candida* (NAC) species gaining prominence in recent times. Although *C. albicans* was the most common species isolated in this present study, the frequency of non-*albicans Candida species* isolated was 56.74% and this may be attributed to immunosuppression or uncontrolled diabetes. In separate studies conducted by Haleim et al., (2015), Jhinuk et al., 2015, [25,26] the recovery rate of non-*albicans Candida species* was 57.5% and 62% in Iran and India. Deorukhkar and colleagues also reported

that NAC species accounted for over 60% of their isolates in a similar study conducted in India [27].

Of the non-*albicans candida species*, *C. glabrata* had the highest prevalence of 19.01%. This may be attributed to the ability of *C. glabrata* to adapt and survive in macrophages as an immune evasion strategy, thus avoiding the innate immune response to pathogens. This adaptation to intracellular survival is related to its ability to prevent toxic phagolysosome environments by modifying its phagosome, suppressing Reactive Oxygen Species (ROS) production and producing minimal proinflammatory response [28].

Table 2. Prevalence of Vulvovaginal Candidiasis (VVC) in four selected states of North Central Nigeria

States	Number examined	Number positive	Prevalence (%)
Nasarawa	400	211	53.00
Abuja	400	140	35.00
Niger	400	202	51.00
Benue	400	157	39.25
Total	1600	710	44.37

Table 3. Prevalence of vulvovaginal candidiasis (culture positive) in relation to demographic characteristics

Variables	Number Examined	Number Positive	P-Value
Age			
≤20	303(18.93%)	99(32.67%)	0.0001
21-30	492 (30.75)	256(52.03%)	
31-40	600(37.50%)	254(42.33%)	
41-50	205(12.81%)	101(49.26%)	
Total	1600(100%)	710(44.37%)	
HIV Status			
Positive	74(4.62%)	50(67.56%)	0.351
Negative	613(38.31%)	17(2.77%)	
Unknown	26(1.62%)	7(26.92%)	
Total	1600(100%)	74(4.62%)	
Diabetes			
Positive	143 (8.93%)	44 (30.76%)	0.294
Negative	1457 (91%)	99 (6.79%)	
Total	1600 (100%)	143 (8.93)	
Marital Status			
Married	1376(86%)	646(49.94%)	0.0001
Unmarried	224(14%)	64(28.57%)	
Total	1600(100%)	710(44.37%)	
Educational Status			
Informal education	142(8.87%)	69(12.47%)	0.0001
Primary	435(27.18%)	195(13.6%)	
Secondary	532(33.25%)	178(13.6%)	
Tertiary	491(30.68%)	268(13.4%)	
Total	1600(100%)	710(44.37%)	

Table 4. Distribution of contraceptive usage in the study Area

Contraceptive type	Nasarawa State	Abuja	Niger State	Benue State	Total
IUCD	84	82	75	158	399
Injectables	119	145	134	67	465
Implants	100	43	66	100	309
Pills	97	130	125	75	427
Total	400	400	400	400	1600

The studies of Trama et al., 2005, Hasanvand et al., 2017 and Gharaghani et al., 2018 [29,30,31] demonstrated that *C. glabrata* was the predominant yeast among the non-albicans *Candida* species with recovery rates of 14.3%, 20%, 7% in the United States of America and Iran.

From this study, the prevalence of vulvovaginal candidiasis among contraceptive users in the study area is 44.37%. This rate is much lower than 84.5% reported in Kano, Nigeria by Ugwa in 2015 [32]. However, the prevalence rate from this study is higher than the prevalence rates of 14%, 30% and 17% reported in similar studies conducted by Emeribe et al., 2015; Olufunmilola et al., 2016 and Aniebue et al., 2018 [33,34,35] among non pregnant women in Abuja, Ibadan, Oyo State and Enugu State respectively. This variation in prevalence may be due to differences in the population studied and geographical area.

The highest prevalence of vulvovaginal candidiasis (53.00%) was recorded in Nasarawa State while the lowest prevalence of 35.00% was recorded in Abuja as shown in Table 2. These prevalence rates may be due to the influx of patients into these health facilities during this study. Furthermore, this study was carried out in secondary and tertiary health facilities which excluded women who may report to primary health-care facilities which are mostly involved in treating VVC. Information regarding the prevalence of vulvovaginal candidiasis among contraceptive users in Nigeria is unknown.

The highest prevalence of 52.03% of candidiasis infection was recorded among women who were within the age group of 21-30 years. This may be due to hormonal influences, predominant nutritional types or a possible higher number of sexual partners as reported by Gonçalves et al., 2016 [36]. The high prevalence within this age group is in concordance with the findings of Nurat et al., 2015 Najwan et al., 2017; Moradi et al., 2018 [37,38,39] who reported the highest prevalence rates of 33.8%, 48.9% and 36.8%

within this age group. However, our findings contradicts the report of Olugbenga et al., 2014 and Maikenti et al., 2016 [40,41] who recorded the highest prevalence rate of 36% and 26% within the age group of 31-35 years and 16-20 years respectively.

The lowest prevalence (32.67%) of candidiasis infection was recorded among women below 20 years. This may be due to high vaginal immunity against *Candida* species and the onset of reproductive hormones production among teenagers as reported by Goncalves et al. [36].

The prevalence of vulvovaginal candidiasis in relation to HIV status in the study area is 4.60%. This low prevalence could probably be due to the unwillingness of the women to reveal their HIV status due to the fear of stigmatization. Moreover, this particular data relied on the information provided by the patients in the questionnaires and HIV screening was not carried out. The patient's strict adherence to their antiretroviral regimen could also contribute to the low prevalence. In a similar study carried out by Ndukwu et al., (2016), 13% prevalence was reported among HIV positive patients in two tertiary Hospitals in Rivers State [42]. Similar studies by Wenwen et al., (2018) and Padmajakshi et al., (2018) revealed HIV prevalence rates of 18.3% and 22.6% among women with vaginitis in Sichuan, China and Karimnagar, India [43,44].

Previous studies by Deorukhkar et al., 2014 [27] has shown that Diabetes mellitus (DM) results in both increased rate of vaginal *Candida* colonization and infection with *Candida*. This current study established a relationship between VVC and diabetes mellitus with a total prevalence of 8.93% in the study area. The occurrence of VVC among women with diabetes may be attributed to poorly controlled diabetes mellitus which causes increased glycogen levels and other metabolic alterations, which lower vaginal pH resulting in *Candida* colonization at a rate higher than that of vaginal dysbiosis [45].

This prevalence of 8.93% is lower than the report of Udeani et al. (2018) [46] who recorded a prevalence of 30% among diabetic patients in Enugu, State. A prevalence rate of 18.8% was reported by Adebisi et al., (2015) and Gunther et al., (2014), in separate studies conducted in Ibadan, Oyo State, Nigeria and Paraná, Brazil [47,48].

The study revealed that marital status affects the prevalence of VVC. This study has shown a higher prevalence of VVC among the married (46.94%) compared to the unmarried (29.0%). This may be attributed to increased sexual activities among married women who may be more prone to develop vaginal yeast infection though the VVC is not a sexually transmitted disease. This is because the infection occurs in non-sexually active women and women can be vaginal colonized by *Candida* [36]. This finding agrees with Okunbowa et al., (2003), Ugwa et al., (2015) Fayemiwo et al.,(2018) who reported higher prevalence rate of 66.2%, 34.5% and 69.6% [49,32,50] among married women in separate studies conducted in Edo, Kano and Oyo state, Nigeria.

The highest prevalence of vulvovaginal candidiasis in relation to educational status was recorded amongst women with tertiary education (54.58%). This may be attributed to the fact that women who have acquired tertiary education may have obtained massive information related to health-care from their studies or through the Internet. This knowledge may predispose these women to embark on self medication which may have severe consequences such as reducing the host's immunity and becoming susceptible to VVC infection.

The lowest prevalence of 33.4% was recorded among women with secondary education. This is in conformity with the findings of Fayemiwo et al., (2018) who reported the highest prevalence among women with secondary education [50]. In contrast with this findings, Adane and Yeshiwork (2018) reported highest incidence among illiterate women [51].

The distribution of contraceptive usage in the study area indicates that injectables are the most common contraceptive methods used. This is in concordance with the 2017/2018 annual progress report of the FP2020 Core indicator summary which indicates that only 22.3% of Nigerian women use injectables, 17.0% use oral contraceptive pills, 7.1% use IUCD while 2.7%

use implants [52]. Najwan et al., 2017 [53] reported highest incidence rate (66.7%) among the oral contraceptive group in a similar study. The trend of contraceptive usage in this study is in concordance with the Nigerian Multiple Indicator Cluster Survey 2016/2017 report which also indicated that in the North Central States of Nigeria, 6.0% of women use injectables, 2.3% use oral contraceptive pills, 0.8% use IUCD while 2.0% use implants [54].

5. CONCLUSION

Vulvovaginal candidiasis poses a great threat to women's reproductive health and should not be considered as a trivial infection especially among women using contraceptives. The high prevalence rate of vulvovaginal candidiasis in this study highlights the importance of conducting continuous epidemiological surveys among contraceptive users in the country.

CONSENT AND ETHICAL APPROVAL

Ethical approval was sought from the Ministry of health of the concerned States and the ethical committees of the hospitals where the research was conducted. The samples were obtained with the informed consent of the women. The information obtained was kept confidential and the results were used for the purpose of which it was intended for.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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