Prevalence of *Candida* spp. and age-related disparities amongst women presenting with vaginitis at the Obstetrics and Gynaecology (O&G) Clinic in a Tertiary hospital in Port Harcourt, Nigeria

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Abstract

Background: Vaginitis, an infection of the lower genital tract in women, is known to be triggered by the overgrowth of the vagina's naturally occurring microorganisms.

Objective: This study looked at the prevalence of *Candida* spp. and age-related disparities amongst women presenting with vaginitis at the Obstetrics and Gynaecology (O&G) clinic in a tertiary hospital in Port Harcourt, Nigeria.

Methods: One hundred high vaginal swabs were collected from pregnant and non-pregnant women and examined microscopically and microbiologically.

Results: Age-group 20–29 years had the highest incidence of candidal vaginitis. There was a higher occurrence of yeast cells in pregnant than in non-pregnant participants while the non-pregnant women had a greater level of bacterial cells. Forty (40) of the samples contained yeasts of *Candida* species representing a 40% prevalence. Three species of Candida were identified with *C. albicans* dominating. Of the 40 samples positive for *Candida* spp., 30 (75.0%) were confirmed to be *C. albicans*. The remaining isolates were *C. tropicalis* (15.0%) and *C. parapsilosis* (10.0%).

Conclusion: The findings in this study would play a role in the future management of *Candida*-induced vaginitis.

Keywords: *Candida*, epidemiology, prevalence, vaginitis, vulvovaginal candidiasis.

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Introduction

Vaginitis is an infection of the lower genitals in women often caused by yeasts and other fungi. It is known to be triggered by the overgrowth of *Candida* in the vagina and is a common infection of the female genital tract[1]. It may also be caused by *Trichomonas vaginalis*, *Gardnerella vaginalis* and *Chlamydia trachomatis* or a combination of these various microbial groups. When caused by *Candida* spp., it is often referred to as vulvovaginal candidiasis (VVC) and is characterised by itching, curd-like vaginal discharge and erythema[2,3]. Studies show that almost 70% of females will suffer candidal vaginitis at least once in their lifetime while in approximately 50% of women, the infection will occur multiple times. This figure goes up to 75% in women of child-bearing age[1,3,4]. The factors that predispose women to VVC include hormonal fluctuation, such as occur during pregnancy and the menstrual cycle, diabetes, sustained antibiotic use and use of oral contraceptives[3,5]. It has been reported, however, that 5% – 10% of apparently healthy women experience persistent vaginal candidiasis devoid of defined predisposing factors[6].

Candida spp. are part of the natural vaginal flora in 20.0% – 50.0% of healthy women but may become pathogenic under certain conditions including the presence of invading pathogens or biochemical changes in the vaginal environment making them common aetiologic agents of vaginitis[1]. Invading pathogens alter the normal microflora of the vagina while biochemical changes in the vagina stimulate the rapidproliferation of the natural *Candida* population, enhance their attachment to the epithelial cells of the vagina and promote germination of daughter yeast cells so that normal asymptomatic colonisation becomes symptomatic *Candida* infection[1,7]. The evidence

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further suggests that the protracted antimicrobial use causes irritation in body tissues making them susceptible to penetration and adherence by *Candida* spp.[8].

Beyond the epidemiological import of documenting trends in pathogenic prevalence and noting the likely sources of infection, recording these changes has strong implications for treatment regimens and management. In Nigeria, there is a paucity of data on the distribution of *candidal vaginitis* and the species involved. There is only limited work on *candidal vaginitis* in South Southern Nigeria. This study sought to establish the different Candida species involved in vaginitis in women attending the Obstetrics and Gynaecology (O&G) clinic in a tertiary hospital in Port Harcourt, Nigeria.

Materials and methods

Study design

This was a cross-sectional study employing both biological and demographic data. The study population consisted of adult females attending the Obstetrics & Gynaecology clinic of the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria with symptoms of vaginitis.

The sample size of 100 was derived using the formula:

$$n = \frac{PQ}{(e/1.96)^2}$$

Where n = sample size; P* = working proportion = 6.89%; Q =100 _ P = 100 _ 6.89 = 93.11%; e = margin of sampling error tolerated (5%), at 95% degree of confidence.

*The working proportion (P) was determined by the prevalence of *Candida*-induced VVC based on other studies in the region.

Ethical considerations

All the participants provided informed consent for inclusion in the study and were assured that all the information provided would be used solely for the purposes of this study and treated confidentially. Ethical approval was given by the UPTH.

Patient eligibility and inclusion criteria

All participants had to have presented with symptoms of vaginitis. Women who voluntarily consented to participate were considered eligible and recruited for the study. All those who did not assent or did not have symptoms were not included.

Sample collection

A total of 100 high vaginal swabs (HVS) samples were collected from 60 symptomatic pregnant and 40 symptomatic non-pregnant females between the ages of 20 – 49 years attending the O & G clinic of the UPTH. Samples were collected from patients with at least one of the following symptoms: vaginal discharge, itching and/or burning sensation. The data were analysed anonymously.

Microscopy of HVS Smears

Microscopic examination of the high vagina swab smears was via wet preparation. A drop of 10.0% potassium hydroxide solution was added to a smear of HVS on a clean glass slide, covered with a coverslip and then examined under the microscope. The wet preparation was done to check for motile organisms like *Trichomonas vaginalis*, yeast cells, pus cells, epithelial cells and bacterial cells.

Isolation of bacteria

The presence of bacteria in the swab samples was examined via isolation on Nutrient Agar and Mannitol Salt Agar. The swab samples were suspended in sterile normal saline and inoculated onto agar plates following serial dilution. Incubation was at 37^{o}C for 24 hours. Biochemical testing was employed in the identification of the isolates. The tests carried out included sugar fermentation tests, oxidase test, H_2S production, citrate utilisation, motility, indole formation, urea hydrolysis, catalase test, coagulase test, lysine decarboxylase and lysine deaminase production, Methyl Red test, Voges Proskauer test and Gram's staining[9].

Characterisation of *Candida* species

The swabs were immediately streaked on Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (500mg/ml) and incubated at 37^{0}C for 48 hours, then examined for the cream coloured pasty colonies indicative of yeast colonies. Identification was done using direct microscopy, culture and biochemical methods and the prevalence determined. Cultures identified as yeast were tested for germ-tube and chlamydospore formation using human serum and corn meal agar respectively.

Germ tube test

A small portion of the yeast was inoculated into human serum in a test tube and incubated at 37°C for 3 hours. A drop of the serum-yeast suspension was then examined under the microscope using lactophenol blue stain[9].

Statistical analyses

Statistical Package for the Social Sciences (SPSS)® 21.0 (International Business Machines Corporation (IBM), NY, USA) was used to analyse the data. Statistical significance of data sets was determined at $p \leq 0.05$.

Results

Fig. 1 depicts the age distribution in the study. The results of the microscopic examination of the samples are shown in Fig. 2. Age group 20 – 24 showed the greatest numbers of yeast, epithelial and pus cells. *Trichomonas vaginalis* was only found in this group as well. Comparisons between the two groups showed that there was a higher occurrence of yeast cells in pregnant than in non-pregnant participants while the non-pregnant women had a greater number of bacterial cells (Fig. 3).

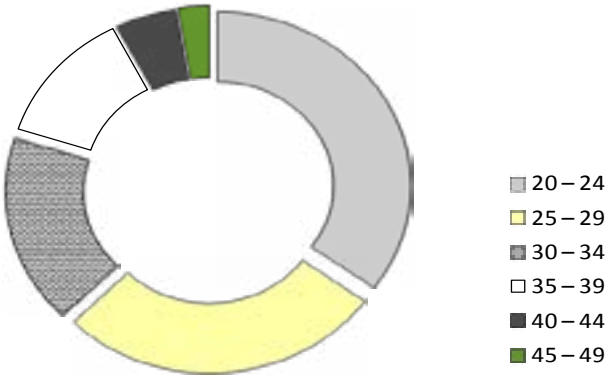

Figure 1: Age distribution of participants

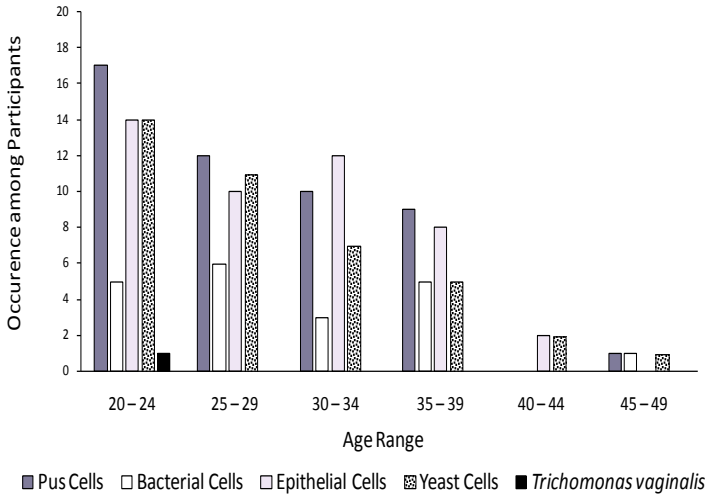

Figure 2: Microscopy of high vaginal swab samples

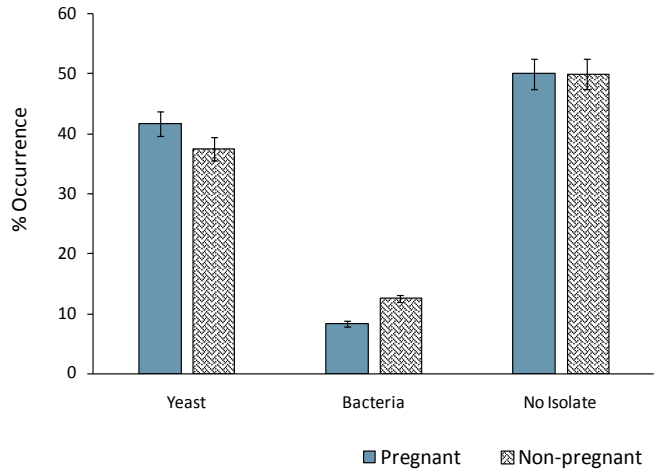

Figure 3: Observed microscopy amongst pregnant and non-pregnant participants

There was a 40.0% prevalence of *Candida*-induced vaginitis in this study with *Candida albicans* being the dominant aetiologic agent. The prevalence of non-*C. albicans* species was 10.0%. Of the one hundred samples collected, forty (40) showed growth of *Candida* spp., forty (40) showed no microbial growth while twenty (20) showed bacterial growth. Following the germ tube and sugar fermentation tests, it was observed that 30 (75.0%) of the 40 isolates were *Candida albicans* 6(15.0%) were *Candida tropicalis* and 4 (10.0%) were *Candida parapsilopsis*. The observed isolates are presented in Fig. 4 while the occurrence of the *Candida* isolates is illustrated in Fig. 5 among some bacterial isolates encountered which included *Staphylococcus aureus* and *Streptococcus sp.* (Fig. 4). The results also showed that individuals aged 20–29 years had the greatest number of *Candida* isolates but ages 40–49 had the highest frequency of isolation (Figs. 6 and 7).

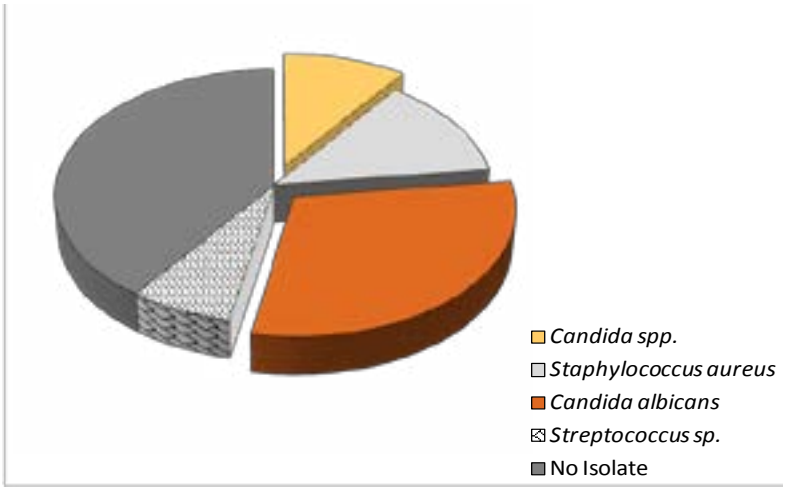

Figure 4: Distribution of observed isolates

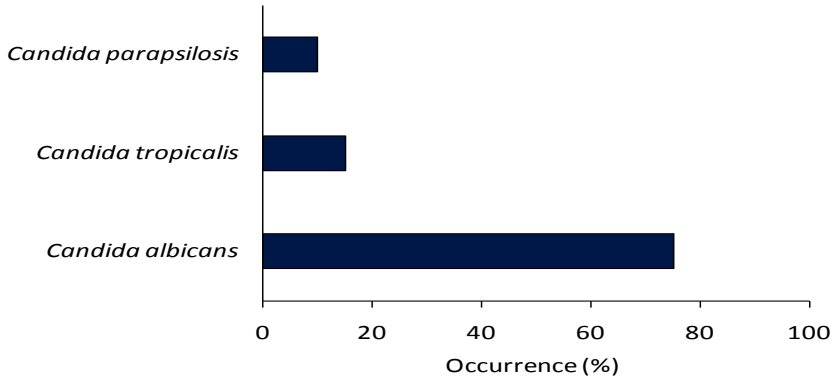

Figure 5: Occurrence of *candida* species isolated

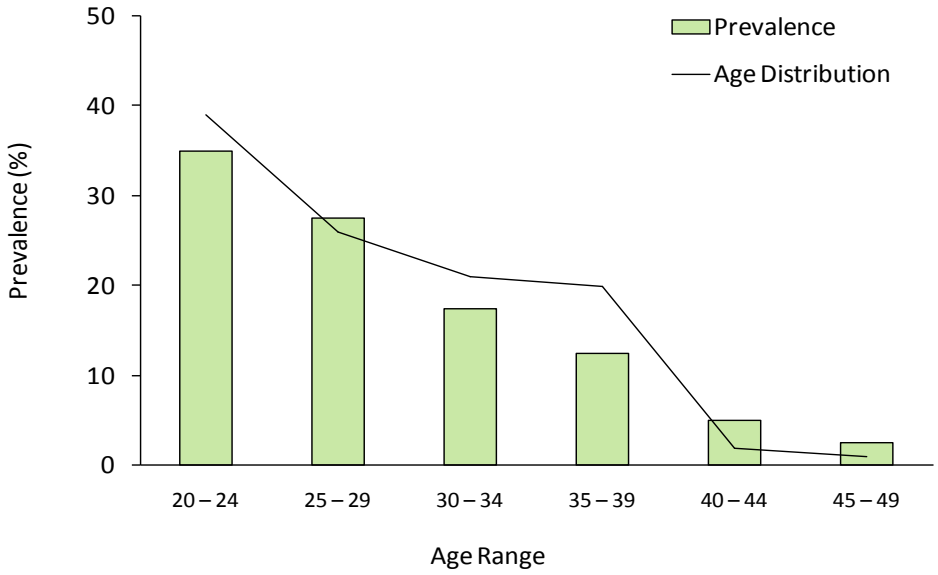

Figure 6: Prevalence of *candida*-induced vaginitis according to age showing age distribution

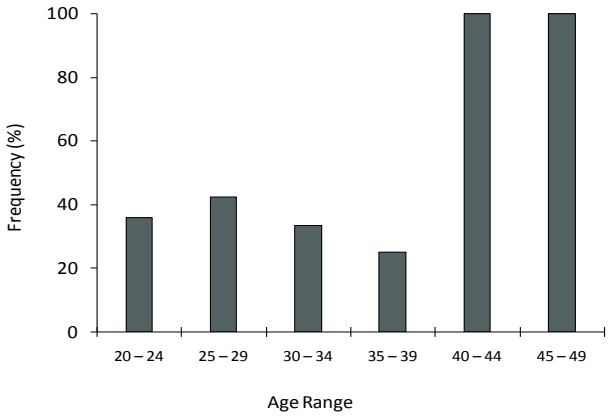

Figure 7: Frequency of isolation of *candida* spp. with respect to age

Discussion

The results showed that women in age group 20–29 years had more *Candida*-induced vaginitis and were also the dominant group in the clinic since they had the greatest number of positive *Candida* isolates. This study further showed that the age group 20 – 29 years had the greatest prevalence of *Candida* spp. Some studies have shown that the prevalence of *Candida* increased significantly in pregnant women below 35 years but decreased in pregnant women above 35 years[3,7]. Another similar study also reported the prevalence of *C. albicans* and non-*C. albicans* to be 6.5% and 7.5% respectively out of 200 individuals predominantly aged 20 to 30 years[1]. Findings from Alo et al.[10] were different as they observed a higher prevalence in the 36 to 40 age-group while the age-group 20 – 25 years had the lowest prevalence. The differences in the prevalence of candidal vaginitis have been linked to high sexual activity, use of contraceptives, poor hygiene and antibiotics/ drug abuse as is common in the younger, more sexually active age-groups[11,12].

There are a wide variety of reports on the prevalence of *candidal vaginitis*. The 40.0% prevalence in this study is much lower than that recorded by a study in Kenya that obtained a 90.38% prevalence of *candidal vaginitis* in pregnant women attending antenatal clinic. In some studies, the prevalence of *Candida albicans*-induced vaginitis was placed at 70.0 – 80.0%[13,14]. Other studies, however, recorded lower prevalences of 14.0 – 21.0% in both pregnant and non-pregnant women[1,15–17]. A study in Nnewi, Nigeria, obtained a comparable prevalence of 30.0%, similar to the present study[18] and in Ethiopia, a prevalence of 41.1% among 210 women[12]. Several factors could be responsible for the wide disparity in prevalence figures. The environment is one such factor and it has been found to play a role in the occurrence and dissemination of *Candida spp*. within hospital environments. One study found that the most commonly isolated non-*C. albicans* species in the hospital environment, including the coats of staff, were *C. glabrata* (37.62%), *C. parapsilosis* (25.74%) and *C. tropicalis* (16.86%)[19]. This agrees with the higher occurrence of *C. parapsilosis* over *C. tropicalis* in this study.

The findings in this study suggest that *Candida spp*. were the most significant causative organism of vaginitis while other possible causative species constituted less than 20.0% of the condition. *Trichomonas vaginalis* was isolated from only one (1) swab sample. This result agrees with the work of Richter et al.[4] who found other causative agents to be less than 1.69% of isolates and *Trichomonas vaginalis* occurred in only one (1) out of 593 isolates. Pregnant women showed a higher incidence of *Candida* infection. The marginal difference between the numbers of yeast cells observed in pregnant women compared to the number in non-pregnant women is somewhat unexpected as pregnant women are generally known to be more prone to yeast infections mainly because of their immunocompromised state and the changes in the pH of their vaginas.

The dominance of *Candida albicans* is well established. The occurrence of *Candida albicans* in 71.0% of VVC cases has been reported[4]. In India, *C. albicans* dominated with a recorded prevalence as high as 80.0% – 85.0% while in Ethiopia, *C. albicans* had a prevalence of 58.6% over other *Candida* species (41.4%)[12,20]. *Candida albicans*, *Candida tropicalis* and *Candida parasilopsis* are commonly associated

with VVC with *C. parapsilosis* having a higher prevalence than *C. tropicalis* as was found in the current study[21–23]; other studies also identified *C. krusei*, *C. lusitaniae* and *C. glabrata* in addition to the observed species[3,4,12]. *C. parapsilosis* has been highlighted as a predominant cause of vulvovaginal candidiasis behind *C. albicans*[24]. In contrast to the findings of this study, Grillot[25] opined that non-*C. albicans* VVC infections are most often due to Candida glabrata in 5.0–10.0% of cases and *Candida tropicalis* in less than 5% of cases. Dan et al.[9] found that non-*C. albicans* species accounted for 44.5% in asymptomatic women, 19.4% in women with recurring *vaginitis* and 21% in women with chronic symptoms. These figures are similar to the figures obtained in this study where non-*C. albicans* species made up 25.0% of isolates and 10.0% of all individuals tested. Mishra et al.[26] also validated *C. albicans* as the most common agent of UTIs and reproductory tract infection. Similar trends are recorded in the epidemiology of *candidal vaginitis* in studies in southern Nigeria[11,27]. The current findings, however, contradict an earlier report from Benin City, Nigeria that *C. glabrata* was the most commonly encountered Candida species in symptomatic VVC pregnant women[28].

A rise in the implication of non-*C. albicans* groups, especially *C. glabrata*, in vaginitis has been noted[21,29–32]. This shift is probably due to the increased misuse of over the counter drugs and antibiotics. Non-*C. albicans* infection is reported to be commonly linked to chronic but asymptomatic or mildly symptomatic vaginitis[21,30,33]. *Candida albicans*–linked infection, on the other hand, tends to be more symptomatic[9]. This might explain the low numbers of non-*C. albicans* isolates found in this study since the investigation was carried out on women with symptomatic vaginitis.

Conclusion

Vulvovaginitis is considered a complex infection as it involves many naturally occurring microorganisms in the vagina. In this study, there was a higher occurrence of yeast cells in pregnant than in non-pregnant participants while the non-pregnant women had a greater level of bacterial cells. Forty (40) of the samples contained yeasts of *Candida* species representing a 40% prevalence of vulvovaginal candidiasis. The highest prevalence was found amongst women in the age group 20 - 29 years. *Candida albicans* was the most prevalent causative agent. Other non-*C. albicans* species implicated were *C. parapsilosis* and *C. tropicalis*. These findings should help in the effective management of Candida-induced vaginitis. Continued surveillance would greatly support the management of this largely opportunistic infection. Further research aimed at determining possible risk factors, preventive therapy and treatment regimens is encouraged.

Conflict of interest

None declared.

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