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Prevalence and Speciation of Non-albican Vulvovaginal Candidiasis in Zaria

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Abstract
Candida species are versatile microorganisms which live normally in the skin, mouth, gastrointestinal tract, and genitourinary tract. In healthy people, *Candida* species usually live as benign commensals and produce no disease. However, they are the most common cause of fungal infections in immunosuppressed individuals, leading to a range of non-life threatening mucocutaneous diseases to threatening invasive systemic diseases. Among *Candida* spp, the more important pathogenic species are *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C.krusei.* The research set out to determine the prevalence of non albicans vulvovaginal candidiasis and pattern of distribution of the different Candida species in Zaria.A cross sectional study of female genital swabs collected from 400 women with features suggestive of vulvovaginal candidiasis attending Gynaecology clinics in four selected hospitals in Zaria. The swabs collected between a period of February 2012 to March 2013 were analysed by microscopy and culture in the Medical Microbiology laboratory, Ahmadu Bello University, Zaria. Data on demographic details were also obtained; using structured questionnaires. Of the 400 patient samples examined 163(40.8%) were culture positive for *Candida spp*. Of these 163 isolates, 84 were germ-tube negative giving a prevalence of 21% for non albicans candida (NAC). Of the 84 isolates of NAC, 51(60.7%) were *Candida parapsilosis*, 18 (21.4%) were *Candida tropicalis*, while 15 (17.9%) were *Candida glabrata*. Vulvovaginal candidiasis was found to be more prevalent among the age group 21-30 years in general. Vulvovaginal candidiasis is a prevalent infection among women. More than half of the infections are due to non-albicans Candida. NAC is becoming increasingly relevant in the aetiology of Vulvovaginal candidiasis and health providers need to be aware of this as the treatment options differ.
Keywords: Non albicans Candida, Vulvovaginal candidiasis, Vulvovaginitis

1. Introduction

Candida species are versatile microorganisms which live normally in the skin, mouth, gastrointestinal tract, and genitourinary tract. In healthy people, *Candida* species usually live as benign commensals and produce no disease. However, they are the most common cause of fungal infections in immunosuppressed individuals (Achkar and Fries, 2010), leading to a range of non life threatening mucocutaneous diseases to threatening invasive systemic diseases. Among *Candida* spp, the more important pathogenic species are *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C.krusei (*Pfaller and Diekema, 2004; Centre for Disease Control and Prevention, 2010; *Al*-Ahmadey and Mohammed, 2014)

It is estimated that at least 75% of healthy adult women will experience one episode of *Candida* vulvovaginitis during their reproductive lives and that 5% will have recurrent infectious episodes (Achkar and Fries, 2010). *Candida albicans* is responsible for infection in 80 to 90% of cases, although the incidence of vulvovaginal candidiasis (VVC) due to non- albicans Candida (NAC) *s*uch as *C. glabrata* has increased steadily over the past few decades (Marot –Leblond *et al.*, 2009). Risk factors for VVC include sexual activity, recent antibiotic use, steroid therapy pregnancy, and immunosuppression from such conditions as poorly controlled HIV infection or diabetes mellitus (Van Schalkwyk and Yudin,2015)

The signs and symptoms of uncomplicated VVC include a thick cheese–like discharge associated with intense vaginal and vulvar pruritus, pain, burning, erythema, and/or edema. There may be associated dyspareunia. Complicated VVC may be defined as recurrent vulvovaginitis (4 or more episodes in 1year period) associated with severe symptoms, the result of a non-albicans species, or present in a compromised host (Van Schalkwyk and Yudin, 2015).

Although published works in the Northern part of Nigeria in relation to genitourinary Candidiasis are scarce, Okungbowa *et al*. (2003) reported that *Candida glabrata* is more predominant among symptomatic individuals, following a survey of seven cities in the Southern part of Nigeria in contrast to the existing belief that *Candida albicans* is the most prevalent.

Some isolates of non *Candida albicans* are resistant to the azole group of antifungal drugs which are commonly used. *Candida krusei* has been found to be innately resistant to Fluconazole, *C.glabrata* has been reported to acquire resistance *in vitro* and *in vivo* and *C.dubliniensis* isolates have been observed to rapidly develop resistance to fluconazole (Arjuna *et al.,* 2005)

Due to the changing epidemiology of *Candida* and availability of newer antifungal drugs with different antifungal spectra, many physicians may no longer be able to make therapeutic decisions based on broad

identification of fungi as yeasts and molds but may need to study species levels so as to enhance proper treatment (Nadeem *et al.,* 2010). This research aims to determine the prevalence of non albicans vulvovaginal candidiasis and the pattern of distribution of associated different species in Zaria.

2. Material and Methods

Study Area:

The study was conducted in Zaria, Nigeria. Zaria is located on a plateau at a height of 2,200 feet above sea level. It is situated in the centre of Northern Nigeria (Mortimore, 1970).The inhabitants of Zaria are predominantly Hausa and Fulani Muslims. Culturally and religiously polygamy is widely practiced with mostly purdah housewives who are economically and socially dependent on their husbands and relatives. Predominant ethnic groups are hausa and Fulani with a mixture of other ethnic groups and people from other neighbouring countries like Niger and Chad. Local indigenes are mainly farmers, herdsmen, traders, artisans, tailors, drivers and few civil servants.

Study Population

The target population was women with history suggestive of vulvovaginal candidiasis and who are clinically diagnosed.

Inclusion criteria: All symptomatic(vaginal discharge, severe itching) Out-Patients attending Gynaecology clinic and symptomatic in-patients who were not on antifungal therapy.

Exclusion criteria: All asymptomatic patients who present at the Gynaecology clinic for other clinical problems apart from vulvovaginitis. All who declined to participate, pregnant women and also symptomatic patients who were on antifungal therapy were excluded.

Sample Size and Collection

This was a hospital based cross sectional study conducted from February 2012 to March 2013 at four selected hospitals in Zaria. Women with a history of vaginal discharge, severe itching and who were clinically diagnosed as having vulvovaginal candidiasis were recruited consecutively from Gynaecology clinics in the four selected hospitals in Zaria. Ethical approval for the study was obtained from the ethical committee of Kaduna State Ministry of Health, while informed written consent was obtained from every patient before sample collection.

Sample Processing

A pair of high vaginal swab (HVS) was collected from every patient aseptically with the help of speculum and posterior vaginal wall retractor by a nurse or doctor. The samples were transported to the laboratory within one hour of collection for analysis and when any delay was encountered the samples were refrigerated at 4°C to 8°C. One of the paired swabs was used for wet mount preparation using a standard method described by Forbes *et al,* (2007) and Cheesbrough, (2009).The swab was emulsified in 1-2drops of saline solution on a slide, and then covered with cover slip. Yeast cells were identified as large oval cells at ×40 magnification. The other swab was used for culture and streaked on Sabouraud Dextrose Agar (SDA) and incubated at 37^{0}C for 48.hours. Candida species were identified by their usual spherical oval cell shape with terminal, sub-terminal or multipolar budding or sometimes hyphae under the x40 magnification. Colonies suggestive of *Candida species* on SDA agar were further identified and characterized using germ tube test and colour production on CHROMagar Candida (Oxoid). Data were entered and analyzed using Statistical Package for Social Sciences for windows version 20.0. Data on the sociodemographic characteristics was obtained from participants using self administered questionnaire. Result was presented in chart and descriptive statistics was used.

3. Results

Of 400 patients samples examined, 163(40.8%) were culture positive for *Candida spp*. Of the 163 positive isolates, 84 were germ-tube negative giving a prevalence of 21% for non albicans candida (NAC). Of the 84 isolates of NAC, 51(60.7%) were *Candida parapsilosis*, 18(21.4%) were *Candida tropicalis* while 15(17.9%) were *Candida glabrata.* Using the CHROMagar Candida, beige and smooth colonies were identified as *Candida parapsilosis,* blue, discrete and smooth colonies as *Candida tropicalis* and cream, smooth and shining colonies as *Candida glabrata*. The women stratified according to age showed the age group 21-30 years had the highest prevalence of non-albicans vulvovaginal candidiasis; 41(48.8%), while those in age group 41years and above had the least prevalence NAC; 3(3.6%). A higher prevalence of 56(66.7%) was observed among married women when compared to single women; 27(32.1%). However, the lowest prevalence was recorded among those who are divorced. Prevalence in those without formal education was 26(30.9%), followed by those with tertiary education with a prevalence of 25(29.8%).Patients with secondary education had a prevalence of 22(26.2%), while primary education had the least 11(13.1%). *C. parapsilosis* was the most prevalent non-albicans Candida (60.7%) among the study participants (Fig.1).

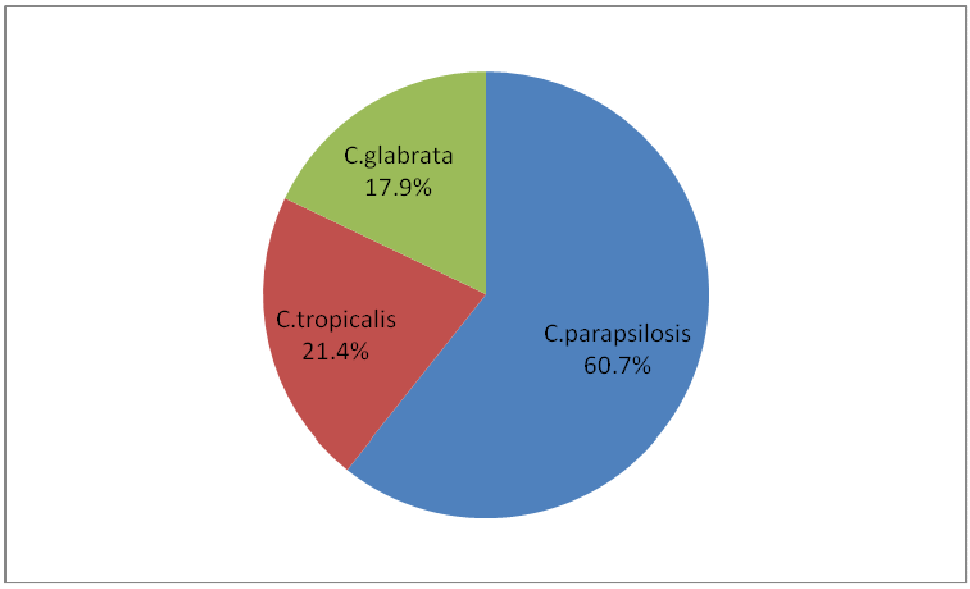

Figure 1: Proportion of different species of *non albicans Candida* isolated

Table 1: Distribution of *non albicans Candida* species by sociodemographic variables of study participants.

Age	*C. parapsilosis* Frequency (%)	*C.tropicalis* Frequency (%)	*C.glabrata* Frequency (%)	Total Frequency (%)
<20	8(53.3)	4(26.7)	3(20)	15(17.9)
21-30	24(58.5)	8(19.5)	9(22)	41(48.8)
31-40	16(64)	6(24)	3(12)	25(29.7)
41-50	3(100)	0(0)	0(0)	3(3.6)
51-60	0(0)	0(0)	0(0)	0(0)
>60	0(0)	0(0)	0(0)	0(0)
				84(100)
Marital status				
Single	16(59.3)	7(25.9)	4(14.8)	27(32.1)
Married	35(62.5)	10(17.9)	11(19.6)	56 (66.7)
Divorced	0	1(100)	0	1(1.2)
				84(100)
Education status				
Primary	9(81.8)	2(18.2)	0(0)	11(13.1)
Secondary	13(59.1)	5(22.7)	4(18.2)	22(26.2)
Tertiary	14(56)	7(28)	4(16)	25(29.8)
None	15(57.7)	4(15.4)	7(26.9)	26(30.9)
				84(100)

4. Discussion

The overall prevalence of Candida vulvovaginitis of 40.8% observed in this study demonstrates that vulvovaginal candidiasis is a prevalent condition in women in Zaria. This prevalence was however lower than that of 55% obtained by Whong *et al,* (2005) among women in Zaria. The disparity could be due to the fact that they used a smaller sample size and their study was limited to one hospital compared to this study where a larger sample size and four different hospitals were visited giving a broader coverage. Enweani *et al*. (2001) also reported a prevalence of 51.5% among patients using contraceptives and those not using contraceptives (40.6%) in Edo State, Nigeria. The value obtained among the non contraceptive users was similar to the finding in this study while that of the contraceptive users was higher. Contraceptive use especially of the estrogen containing form is a known risk factor for vulvovaginal candidiasis due to its effect on the vaginal epithelium which promotes growth and adherence of Candida to vaginal epithelium.

Prevalence of non albicans vulvovaginal candidiasis was found to be 21% in this study, which signifies that non *albicans Candida* are becoming more prevalent in contrast to previous findings. A prevalence of 31% was documented by Nwadioha *et al.* (2013) in Kano which is a higher finding than in this research. The disparity observed may be due to the larger sample size used in their work. The values obtained from this recent study in Zaria and from Kano show emergence of non albicans candidiasis and other studies have also documented

similar observations (Arzeni, 1997; Mohanty, 2007). Conditions that could lead to immunosuppression such as AIDS, malignancy, use of corticosteroid and Diabetes mellitus may be associated with this emergence of non albican candidiasis but further studies to ascertain this are required.

As has been documented earlier on by Kumari *et al*., (2013) in India, more frequent isolation of non-albicans species from vulvovaginitis patients might be due to widespread and inappropriate use of antimycotic treatment in the form of self-medication, long-term maintenance treatments and repeated treatments for candidiasis episodes, as well as use of a single dose oral and topical azoles. *C.albicans* eradication by these means results in the selection of species such as *C. glabrata* that are resistant to commonly used agents. Although, this research did not explore these factors, it is an avenue for further research considering the prevalence of HIV in our environment.

In our study *C.parapsilosis* was the most prevalent NAC which was contrary to the finding of Whong *et al*,(2005) in Zaria where *C.krusei* was the most prevalent NAC. The disparity could be due to changes in the dynamics of the population over the past ten years and also fewer numbers of patients recruited for their study. Okungbowa *et al.,* (2003) reported *C.glabrata* as the most prevalent of NAC following survey of seven cities in the southern part of Nigeria. The difference observed in our study could be due to differences in geographical location and cultural practices of the population. Elsewhere in India, Deorukhtar *et al*., (2014) also reported a higher prevalent of *C.glabrata* in contrast to our findings. *C.parapsilosis* is an important agent causing candidaemia (Goncalves *et al*.2010,) although it has also been documented as a cause of other mucocutaneous candidiasis, The observed high prevalence in this work calls for further research in relation to genital candidiasis.

Age distribution of Candidiasis showed a higher prevalence of vulvovaginal candidiasis among the age group 21- 30years**.** This probably could be due to ovarian activity as well as sexual activity which peak in women of 20-30 years of age. During this period, the ovary produces adequate amount of estrogen, which favours *Candida* growth by maintaining the acidic pH in the vagina and enhancing the yeast adherence to vaginal epithelial cells (Akortha *et al*., 2009; Adetunde *et al*., 2011). This could also explain why there was lower prevalence at age 41years and above. The age distribution of vulvovaginal candidiasis observed in this work had a similar pattern with the findings of Okungbowa *et al.* (2003) in research carried out in southern part of Nigeria which showed highest incidence at age 26-30years. Nwadioha *et al.* (2010) also observed higher prevalence between age 21-30years in a research conducted in Jos. Similar observation was also made in Kano by Nwadioha *et al*. (2013) in Northern Nigeria.

Prevalence in relation to marital status showed a higher prevalence of vulvovaginal candidiasis among married women compared to the single and the divorced subjects. Although, not much information is available with regards to prevalence of vulvovaginal candidiasis and marital status, however, Okungbowa *et al.* (2006) also observed a higher prevalence among married women similar to observation in this work. The reason for this observation could be due higher regular sexual activity among the married as well as the probability of psychological stress in marriage which could predispose to hormonal changes suited to the growth of *Candida*.

The prevalence with regards to educational status showed the highest prevalence among the patients who had no formal education, which is followed by the patients who had tertiary education. These are the two extremes, the uneducated and the highly educated. Uneducated individuals are more likely to visit quacks for treatment and also may not be so particular with hygiene while the highly educated ones are more likely to do self medication thereby predisposing them more to Candida vulvovaginitis.

5. Conclusion

This current study has demonstrated that VVC is a prevalent infection among women and more than half of the infections are due to non-albicans candida (NAC). The study also identified *Candida parapsilosis* as the most prevalent non albicans candida causing vulvovaginal candidiasis. It is therefore important that there should be increased awareness among healthcare providers on the rising prevalence of non albicans candida as treatment options differ. The finding in this study further emphasizes the need for complete identification of *Candida* species in our routine laboratory investigations to ensure early diagnosis of *non albican Candida* infection. Due to the reduced susceptibility of non albicans Candida to azoles, prior identification of these species before treatment is therefore essential for informed and proper treatment.

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