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 Gynecology 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 C. 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-Ahmady 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) such 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 1 year 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 1-2 drops 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°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 ×40 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 41 years 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.

<table>
<thead>
<tr>
<th>Age</th>
<th>C. parapsilosis Frequency (%)</th>
<th>C. tropicalis Frequency (%)</th>
<th>C. glabrata Frequency (%)</th>
<th>Total Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>8(53.3)</td>
<td>4(26.7)</td>
<td>3(20)</td>
<td>15(17.9)</td>
</tr>
<tr>
<td>21-30</td>
<td>24(58.5)</td>
<td>8(19.5)</td>
<td>9(22)</td>
<td>41(48.8)</td>
</tr>
<tr>
<td>31-40</td>
<td>16(64)</td>
<td>6(24)</td>
<td>3(12)</td>
<td>25(29.7)</td>
</tr>
<tr>
<td>41-50</td>
<td>3(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>3(3.6)</td>
</tr>
<tr>
<td>51-60</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>84(100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>C. parapsilosis Frequency (%)</th>
<th>C. tropicalis Frequency (%)</th>
<th>C. glabrata Frequency (%)</th>
<th>Total Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>16(59.3)</td>
<td>7(25.9)</td>
<td>4(14.8)</td>
<td>27(32.1)</td>
</tr>
<tr>
<td>Married</td>
<td>35(62.5)</td>
<td>10(17.9)</td>
<td>11(19.6)</td>
<td>56 (66.7)</td>
</tr>
<tr>
<td>Divorced</td>
<td>0(0)</td>
<td>1(100)</td>
<td>0(0)</td>
<td>1(1.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education status</th>
<th>C. parapsilosis Frequency (%)</th>
<th>C. tropicalis Frequency (%)</th>
<th>C. glabrata Frequency (%)</th>
<th>Total Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>9(81.8)</td>
<td>2(18.2)</td>
<td>0(0)</td>
<td>11(13.1)</td>
</tr>
<tr>
<td>Secondary</td>
<td>13(59.1)</td>
<td>5(22.7)</td>
<td>4(18.2)</td>
<td>22(26.2)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>14(56)</td>
<td>7(28)</td>
<td>4(16)</td>
<td>25(29.8)</td>
</tr>
<tr>
<td>None</td>
<td>15(57.7)</td>
<td>4(15.4)</td>
<td>7(26.9)</td>
<td>26(30.9)</td>
</tr>
</tbody>
</table>

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 antifungal 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 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.

Acknowledgement
We thankfully acknowledge the members of staff of Department of Medical Microbiology Ahmadu Bello University Teaching Hospital, Zaria and all the women that participated in the study for their tireless cooperation.

Reference

teenagers (between the ages of 13-19) reporting at the Tamale Teaching Hospital (THH) with vaginal discharge problems. *International Journal of Research in Environmental Science and Technology*, 4: 47-51.


Zeng, J., Zong, L.L., Mao, T., Huang, YX & Xu ZM, (2011)“Distribution of *Candida albican* Genotype and *Candida* Species Is Associated with the Severity of Vulvovaginal Candidiasis,” *Journal of Southern Medical University*, 31(10) 1649-1653.