

Original Research Article

Non-Albicans Candida among Symptomatic Vulvovaginal Candidiasis Women in Onitsha, Nigeria

Ejike Chinwe E¹; Agbakoba Nneka R²; Ezeanya Chinyere C³; Emele Felix E⁴; Oguejiofor Charlotte B⁵

¹Department of Medical Microbiology, Chukwuemeka Odimegwu Ojukwu University, Awka Campus, Nigeria.

²Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

³Department of Medical Microbiology, Edo University Iyamho, Edo State, Nigeria.

⁴Department of Medical Microbiology and Parasitology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria

⁵Department of Obstetrics and Gynecology, Nnamdi Azikiwe University Nnewi Campus, Nnewi, Nigeria.

Corresponding Author: Ezeanya Chinyere C

ABSTRACT

Background: Non-albicans Candida (NAC) species have emerged as important causes of Vulvovaginal candidiasis (VVC) among women of reproductive age. The aim of this study was to establish an association between NAC and symptomatic VVC among female patients in Onitsha, Nigeria.

Method: High vaginal specimens from a total of 876 women were evaluated mycologically by standard methods, involving microscopic examination and culture using sabouraud dextrose agar (SDA) and Brilliance *Candida* agar. Susceptibility of isolates to fluconazole and itraconazole were tested using agar dilution method.

Result: Of the 876 samples; yeasts were isolated in 484 (55.3%). The overall occurrence of Non-albicans Candida species was 45%: *Candida glabrata* (18.2%), *Candida krusei* (11.6%), *Candida tropicalis* (4.1%) and *Candida dubliniensis* (4.1%) and other non-albicans (7%). Among the subjective symptoms presented, vulval pruritus was the commonest (81%), followed by vaginal discharge (70%). High resistance to Itraconazole was observed among non-albicans increasingly involved in VVC. *C. tropicalis*, *C. dubliniensis*, *C. krusei* and *C. glabrata* showed 100%, 50%, 40% and 25% resistance to Itraconazole respectively. Susceptibility to Fluconazole was 100%.

Conclusion: The epidemiological study revealed that NAC is associated with symptomatic VVC. Vulvovaginal Candidiasis is of serious public health concern which affects millions of women annually. Non-albicans Candida has gradually been characterized as the cause of VVC thus posing an emerging threat.

Keywords: Symptomatic Vulvovaginal Candidiasis, Non-albicans Candida species, Fluconazole, Itraconazole

INTRODUCTION

Vulvovaginal candidiasis (VVC) occasionally denoted as ‘Candida vaginitis, thrush, or monilial infection’ is a fungal infection caused by uncharacteristic growth of *Candida* found in the mucosa of the female genital tracts. This ensues once there is a disruption of general condition or impairment of immune system. ^[1]

Vulvovaginal candidiasis is among the commonest infections of the genital tract of women within child bearing age. ^[2-4] Almost three quarter of all women undergoes at least one incidence of VVC throughout their lifespan and approximately half of them undergo several incidents. ^[5,6]

Emele *et al.* ^[2] and Nwadioha *et al.* ^[4] reported *Candida albicans* as the most significant pathogenic species. Other

pathogenic Non-albicans Candida (NAC) species that have been isolated from humans include *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida dubliniensis* and *Candida lusitanae*.^[7,8]

However, due to a variety of interferences including single dose treatment, low-dosage azole maintenance regimens, and the use of over-the-counter antimycotics, the proportion of NAC species appears to be increasing.^[9-11] Reports circulated in the most recent decade insinuate a NAC prevalence of 10% - 30% in patients with VVC.^[12-17] Differences in NAC prevalence figures may be reflective of variances among the respective patient groups sampled with respect to cultures, topographical location, and prescribing precedents.

In the early few years, there has been some evidence put forward querying the pathogenicity and overall implication of NAC species. These sources suggest that symptomatic patients positive for NAC species require no treatment as their symptoms may be a consequence of another diagnosis.^[18,19] One major hypothesis of such claim is that 27% of patients treated for NAC infection experienced persistence symptoms regardless of negative culture. However, a case-specific determination of NAC significance may be necessitated in women with other hypothetical diagnoses. It is important to mention that *Candida* infection can exist despite negative cultures. With the introduction of polymerase chain reaction, an additional study group of women with vulvovaginal symptoms, nonetheless negative cultures can be detected.

Other data also suggests that NAC infections demand major concern. Several authors have reported that NAC species seem to be associated with serious or recurrent cases of VVC. NAC infections have demonstrated superiority over *Candida albicans* in association with more severe symptoms. Antifungal resistance among NAC is a concern as the predominant NAC

species (*C. glabrata* as well as other strains including *C. krusei*) in published studies, reports non-reliably respond to azoles. Consequently, detection is needed to better direct therapy.

Owing to the significance of NAC species in medical practice, we aimed to establish the current prevalence of NAC among symptomatic vulvovaginal candidiasis patients and the species-specific distribution in such infection.

METHODOLOGY

Patient's Population

The participants recruited were women of child bearing age who visited a secondary care provider Hospital in Onitsha, Nigeria with symptoms suggestive of VVC (such as vaginal discharge, pruritus vulva, genital burning, dysuria and dysmenorrhea) and had given their informed consent.

Clinical Samples Used and Method of Collection

The Sample used for the investigation was high vaginal swab. This was collected (by the clinician) from the posterior fornix of the vagina (high vagina), using cotton - wool tipped swab sticks; two swab samples were collected from each patient. Some of the vaginal discharge in the speculum was also transferred to a sterile container.

Samples Processing:

In the laboratory, the samples were processed without delay as follows:

Determination of pH:

The vaginal swab in the sterile containers was used to determine pH of the vagina. The vaginal discharge was transferred to a strip of pH. The pH strip was observed for immediate colour change. The new colour of the strip was then compared with a standardized colour reference chart (provided by the manufacturers), in order to estimate the actual pH. The pH values were recorded.

Microscopic Examination of Wet Mount (KOH)

A drop of KOH solution was placed on a clean slide. One of the two vaginal

swabs was rolled in the drop of KOH to produce light suspension of the vaginal exudate. It was covered with cover slip. The slide was placed in a microscope and examined using 10x and 40x objective with the condenser iris closed sufficiently to give good contrast.

Cultural Techniques Used

The second swab was inoculated onto Sabouraud dextrose agar (SDA), as well as Brilliance Candida agar; both were incubated at 37°C and examined for growth daily for 4 days.

The yeast isolates on SDA were sub-cultured onto brilliance Candida agar and incubated for 48hrs at 35-37°C.

Identification of Isolates

Presumptive identification: The presumptive identification of the isolates was based on colonial morphology and colour reaction on the chromogenic medium, in line with the manufacturer's guidelines (Oxoid, UK).

Statistical Analysis

The statistical method used was Chi square test, one way analysis of variance (ANOVA).

RESULTS

Of the eight hundred and seventy-six (876) women who enrolled in this study, four hundred and eighty-four (484) or 55.4% were positive for Candida species. Amongst the 484 women, *Candida albicans* accounted for 264 (54.6%) of cases. *Candida glabrata* 88 (18.2%). *Candida krusei* 56 (11.6%), *Candida dubliniensis* 20 (4.1%), *Candida tropicalis* 24 (4.9%). Other non albicans species 32 (6.6%) (Figure 1).

Cultural isolation of Non-albicans *Candida* species from patients presenting different symptoms associated with VVC is seen in Figure 2. Among the symptoms of VVC, vulval pruritus occurred most frequently (81%) and associated with *C. glabrata*, followed by vaginal discharge (70%) which was associated with *C. krusei*. The frequency of NAC cases among the patients with and without multiple

symptoms was seen to be high in *C.dubliniensis*.

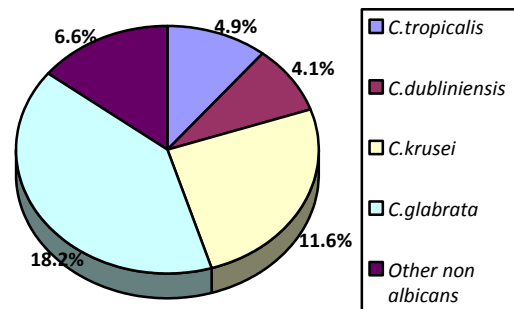


Figure 1: Non albican Candida species isolated among Vulvovaginal Candidiasis patients

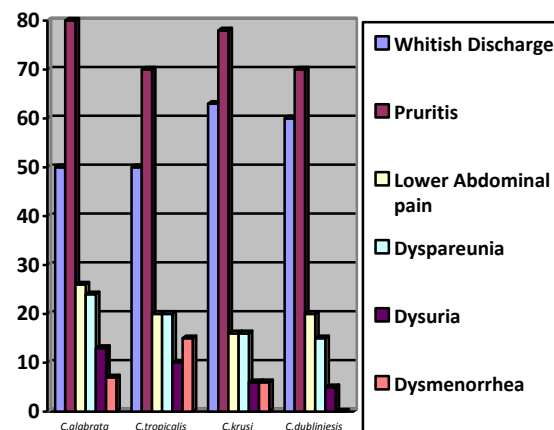


Figure 2: Symptoms Presented By Patients With Vulvovaginal Candidiasis ($X^2 = 83.50$; $p < 0.05$)

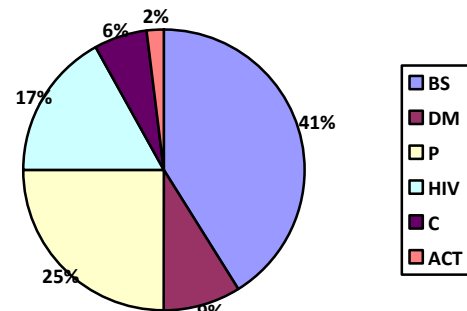


Figure 3: Presence of predisposing factors among VVC Patients

NB: BS: Broad Spectrum Antibiotics DM: Diabetes Mellitus P: Pregnancy C: Contraceptives ACT: Anti-cancer Chemotherapy

Out of 389 women that were associated with one or more predisposing factors to VVC, *Candida* was recovered from the vagina of 343 (88%), while 46 (12%) did not yield any yeast from the vagina. Among the factors that can predispose factors; use of broad spectrum

antibiotics was the most commonly encountered among the patients (41%) followed by pregnancy (25%), as can be seen in Figure 3.

The antifungal susceptibility testing in this study revealed that most NAC species were resistant to Itraconazole and this was most among *C. tropicalis*.

DISCUSSION

Though, *Candida albicans* is known to be the commonest cause of vulvovaginal Candidiasis as confirmed by Emele *et al* (2004) and Nwadioha *et al* (2010) from Nigeria. This study has clearly documented an increased prevalence (45%) of non-albicans Candida among VVC cases which is relatively higher than reports within the past decades in Nigeria. The high prevalence of Non -albican Candida noted in this study is comparable to studies from other parts of the world. Studies from Turkey by Guzel *et al* reported a 50% NAC prevalence among 474 women with acute and recurrent VVC in 2011. Furthermore, study from North India by Kumari *et al* [20] in 2013 reported prevalence of 67.6% among 232 patients of candida vulvovaginitis cases of child-bearing age in a tertiary care center. Another study in 2013 by Mintz and Martens [21] reported a 50% prevalence of NAC among 103 women with recurrent vulvovaginal symptomatology. This higher NAC prevalence probably reflects an increasing trend.

Following the speciation of NAC species in this study, *Candida glabrata* was most isolated NAC (18.2%) species. For most studies, *C. glabrata* is the most isolated NAC species responsible for VVC which is similar to report from this study. This can be compared with studies from different parts of the world where higher prevalence was reported except for studies by Shang *et al* [22] in China with *C. glabrata* having prevalence of 7.9% amongst the NAC species. Ahmed and Khan, [23] Guzel *et al* (2011), Mintz and Martens (2013), Sachin *et al*. [24] and Hedayati *et al* [25] all reported *C. glabrata* having a high prevalence of

36.7%, 35.0%, 28.6%, 28.1%, and 21.9% respectively compared to our study.

Non-Albicans Candida species are more likely to present more severe symptoms than *C. albicans* according to studies by Zeng *et al* in 2011. Although, it was observed in this study that *C. tropicalis* and *C. krusei* was higher (75%) each in women manifesting only one symptom whereas *C. glabrata* was the least isolated (17.1%). In addition, patients with multiple symptoms had the least isolated NAC species as *C. tropicalis* and *C. krusei* (25%) each and the highest as *C. dubliniensis* (40%). Thus, the statistical analysis of the result shows that number of symptom displayed by vulvovaginal candidiasis patients is dependent on the type of species infecting the patient ($X^2 = 83.50$; $P < 0.05$). As reported by Ahmed and Khan (2009) from Aligarh, India: vulval pruritus with and without vaginal discharge and vaginal erythema was the commonest symptoms among 215 women with vaginal candidiasis. Furthermore, in 2015 Hedrayati *et al* reported erythema concomitant with itching (40.9%) as the highest symptoms among 234 VVC patients from India. However, Girgoriou *et al* (2006) reports: NAC caused more frequent vaginal soreness and dyspareunia than *C. albicans*.

Vaginal Candidiasis was noted to be essentially a major health challenge of child bearing age in this study thus this could be as a result of sexual activity and consequently child bearing. Guzel *et al* in 2011 reported that having a history of child birth increased the risk of both acute and recurrent VVC ($P < 0.01$).

Some of the NAC species responded poorly to azole agents especially to Itraconazole which could contribute to the increased prevalence of NAC species among VVC patients. Non-albicans Candida species such as *C. glabrata* respond poorly to azole agents, most especially Fluconazole in VVC patients. [26] A possible explanation for the significant increase in infections caused by non-albicans Candida is probably related to indiscriminate use of antifungal

drugs such as azoles which are readily available over the counter and therefore easily abused; this would tend to favor the selection of the non-albicans species that may have been carried asymptotically in the vagina, since these are generally more resistant to azole than the albicans. Use of broad spectrum antibiotics was noted mostly with vulvovaginal Candidiasis patients, followed by pregnancy. Use of broad spectrum antibiotics could destroy the normal protective vaginal flora and promote colonization with Candida.

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