

Detection and prevalence of *Candida* among pregnant women in Ibadan, Nigeria

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Abstract: This study aimed at detecting and determining the prevalence of *Candida* among pregnant women at Adeoyo maternity hospital in Ibadan, Southwestern Nigeria. One hundred antenatal clinic attendees at Adeoyo Maternity Hospital, Ibadan, were recruited for this study. Samples of high vaginal swab (HVS) were collected, transported, stored and processed using standard laboratory procedures. Additional information was obtained using a proforma specially designed for this purpose. The results showed that a total of 26 *Candida species* were isolated and identified as *Candida albicans* [17(65.4%)] and other *Candida species* [9(34.6%)]. However, vaginal colonization by *Candida* was not age-dependent ($P>0.05$). The rate of *Candida* infection was found to be 26.0% ($n=26$) among the pregnant women; no isolate was recovered from those greater than 30 years of age, while the peak age of infection was 20-29 years of age 34.9% ($n=22$). Those less than 20 years of age accounted for 23.5% ($n=4$) of the entire colonization and/or infections. Twenty-six pregnant women (26.0%) without symptoms or signs of infection harboured *Candida albicans* or other yeast species in the genital tract. They were given treatment with clotrimazole. The implications of the presence of *Candida* in the genital tract are discussed. The vaginal colonization by *Candida* among the 100 tested pregnant women was 26.0% emphasizing the importance of routine screening of pregnant women thereby assisting in prevention of invasive neonatal candidal infections. The incidence of asymptomatic vaginal candidiasis was found to be high among these pregnant women in Ibadan; proper and well coordinated sex education should be organized for adolescents and pregnant women in order to prevent candida infections. Therefore, a thorough medical examination and culture of HVS is highly recommended for pregnant women to ensure detection of vulvovaginal infection caused by *Candida species* among these immunosuppressed persons. It is concluded that clotrimazole is an effective antimycotic agent which can be used for vulvovaginal candidiasis during pregnancy without causing side effects.

Key words: Antimycotic agent • *Candida* • Colonization • Clotrimazole • HVS • Pregnant women • vulvovaginal candidiasis • Nigeria

INTRODUCTION

The adverse effects of infectious diseases in many developing countries, in particular, in sub-Saharan Africa is considerable and, within those countries, economically

disadvantaged persons are most likely to contract communicable diseases and least likely to access appropriate treatment [1]. *Candida* is the fourth most common cause of nosocomial bloodstream infection in the United States [2]. Three quarters of women experience

vaginitis in their lifetime and 30% of vaginitis is caused by *Candida*. Vulvovaginal candidiasis is a common clinical finding among women especially the sexually active group, even though there has been a sustained increase in both the variety and potency of antifungal drugs over the past three decades; the disease apparently appears not to have sufficiently yielded to these breakthroughs. Three quarters of all women experience at least one episode of vulvovaginal candidiasis in their lifetime and about one half of these women experience a recurrence [3].

Candidiasis is the most common opportunistic fungal infection. Disease manifestation of candidal infection can vary with type of host immunodeficiency. Lymphocytes and cell-mediated immunity are important in the prevention of mucosal candidiasis. Therefore, patients with T-cell deficiency, such as human immunodeficiency virus (HIV), have a propensity to develop recurrent and/or persistent mucocutaneous candidiasis [3].

Candidal vaginitis is the most common form of mucosal candidiasis. Vulvovaginal candidiasis is usually secondary to overgrowth of normal flora *Candida* species in the vagina [3]. Vaginitis accounts for 10 million office visits per year. Invasive candidiasis is the most common invasive fungal infection in the United States and other countries of the world, Nigeria inclusive. However, there is an increasing shift toward infections caused by non-*albicans* *Candida* species with 40-60% of the species currently being reported as non-*albicans* species [3].

Vulvovaginal candidiasis is more serious and more dangerous during pregnancy because the body changes due to conditions in the body (such as hormonal changes) and this can lead to the infection becoming chronic, or recurring. Previous use of antibiotics or the birth control pill can increase the chances of getting candidiasis during pregnancy (or at any other time) and hormone changes increase the risk of getting it while pregnant. Untreated *Candida* infection can lead to very serious health and medical problems and is worsened while pregnant. Bacteria such as *Lactobacillus acidophilus* balance *Candida* and prevent yeast overgrowth and pathogenic infection. Conditions that disrupt the balance of normal vaginal flora include antibiotic use, oral contraceptives, contraceptive devices, high estrogen levels and immunocompromised states such as diabetes mellitus and HIV [3]. Another risk factor for vulvovaginal candidiasis may be intrauterine contraceptive devices [4].

Symptoms classically are described as pruritus, vaginal irritation and dysuria. Thick, curd-like discharge is often present, but scant discharge may also characterize infection. Vaginal edema and erythema are present on examination. Epidemiologically, vaginal *Candida* infections are important as they may increase viral shedding in HIV-infected women [3]. Pregnancy brings yeast infections, often multiple ones per pregnancy. *Candida* and pregnancy often go hand in hand. Pregnancy is a very beautiful and natural thing but it often weakens the immune system of the mother making it easier for them to come down with other illnesses or disease. Some symptoms of *Candida* infection in pregnancy include; painful urination, pain during sex, enlarged or swollen vulva, white discharge from the vagina and itching or discomfort in the vaginal area.

The *Candida* fungus is both normal flora and an invasive pathogen. The range of infection with *Candida* species varies from a benign local mucosal membrane infection to disseminated disease. Severe disease is typically associated with an immunocompromised state including those vulnerable to iatrogenic pathogens in the intensive care unit or those with predisposing immunologic conditions such as malignancy, organ dysfunction, or immunosuppressive therapy [3]. *C. albicans* is the most common pathogenic species identified. Other species that are commonly found include *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* [3]. Non-*albicans* *Candida* accounted for 70% of candidemia in a Northern Indian pediatric intensive care unit. Other *Candida* species that have emerged are *C. parapsilosis* and *C. dubliniensis* [5]. *C. glabrata* and *C. krusei* have been identified as the leading causes of candidemia in patients with malignancy of hematologic origin [6]; *C. parapsilosis* has been identified as the leading cause of candidemia secondary to medical instrumentation such as central venous catheters, prosthetic devices and nosocomial spread [7]. *C. dubliniensis* has been identified in an immunocompromised patient with multifocal osteomyelitis in Germany [8] and in a patient with meningitis in Australia [9].

Mammary candidiasis, a condition that can affect breastfeeding women has also been reported [10]. Neonatal invasive candidiasis occurs with an incidence inversely proportional to birth weight. *Candida* colonization is found in approximately 30% of infants weighing less than 1500 grams at birth weight. Sources of invasive infection in one study included blood (70%), urine (15%), cerebrospinal fluid (10%) and peritoneal

fluid (5%). *C. albicans* and *C. parapsilosis* are the most common species found in neonates [3]. Neonates can also develop candidemia even after cesarean delivery due to premature rupture of amniotic membranes [11].

The diagnostic standard is the culture of anal and genital specimens obtained at 35 to 37 weeks of gestation or at delivery when at least one risk factor associated with neonatal infection is present. In order to detect pathogens in vaginal specimens, efficient standard culture and a rapid screening method is required to identify carriage of pathogens in pregnant women at the time of delivery [12].

To determine whether routine testing/screening for vaginal pathogens is necessary in our setting, there is need to determine the vaginal carriage rate of *Candida* amongst Nigerian women. The aim of this study therefore was to determine vaginal carriage rate of *Candida* among pregnant women in Ibadan, Southwestern, Nigeria.

MATERIALS AND METHODS

Study Area: The study was carried out in the municipal area of Ibadan, which is made up of five local government areas. Ibadan city lies 3°5' E and 7°23' N. The city is characterized by low level of environmental sanitation, poor housing and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

Study Population: A total of one hundred pregnant women of different ages and socioeconomic status attending antenatal clinic at Adeoyo maternity hospital, Ibadan, were enrolled in this study. The study was conducted over a period of six months starting from March to August, 2000 by recruiting consecutive consenting women residing at Adeoyo Maternity Hospital, Ibadan, Oyo State, Southwestern Nigeria until a total of 100 participants was attained. Other relevant information of all participants was obtained using a proforma specially designed for this purpose. The study was approved by the ethical review committee of the hospital.

Specimen Collection: Samples of High Vaginal Swabs were collected under aseptic condition using a speculum with the help of a gynaecologist. The specimens were transported in a commercially available collection and transport system for fungi, BBL Culture Swab Plus (Becton Dickinson, Heidelberg, Germany) to medical microbiology and parasitology laboratory for analysis.

Wet Preparation, Culture Isolation and Identification:

A sample of the exudate was transferred to a microscope slide. A drop of sterile physiological saline was added and mix. It was covered with a cover glass and examined under the microscope at $\times 10$ and $\times 40$. Gram stain was also performed on smears of the exudate made on another slide. The High vaginal swabs collected were inoculated onto Saborud dextrose agar, Blood agar, Chocolate agar, MacConkey agar plates and incubated aerobically at 37°C for 48hrs while Chocolate agar at and incubated at 35°C -37°C also for 48 hours inside candle extinction jar (or CO₂ incubator). *Candida* species were checked for on the chocolate agar plates. Germ tube test were performed on yeast isolates to confirm *Candida albicans*. A colony of the yeast was cultured into the 0.5ml of human serum in a sterile test tube and incubated at 37°C for 3 hrs. A loopfull was then placed on slide cover with cover glass and observed under the microscope at $\times 10$ and $\times 40$. Grams were also performed on organisms observed on plate after incubation. Biochemical characterizations were performed on the observed organism. Sensitivity test was done using agar disc diffusion method on the isolates where necessary. The isolates were identified to species level by conventional biochemical tests as described by Cheesbrough [13]. The results were analyzed using the χ^2 -test, with the level of significance set at $p < 0.05$.

RESULTS

There were 100 pregnant women used for this study. Of the 100 pregnant women, 26.0% ($n = 26$) showed positivity for *candida* colonization and/or infection (Table 1). Table 1 shows the frequency of occurrence and the distribution of *candida* isolates recovered from the pregnant women in Ibadan, Southwestern Nigeria. A total of 25 *candida* isolates were obtained in this study (Table 1), of which 17 (65.4%) were predominantly *Candida albicans* and 9(34.6%) was other *Candida* species.

Table 2 shows the distribution of *candida* colonization and/or infection of vagina in relation to age of the pregnant women in Ibadan, Nigeria. The rate of colonization and/or infection was found to be 26.0% ($n=26$); no isolate was recovered from those greater than 30 years of age, while the peak age of infection was found among 20-29 years of age 34.9% ($n=22$). Those less than 20 years of age accounted for 23.5% ($n=4$) of the entire colonization and/or infections. The vaginal colonization and/or infection by *candida* were age dependent ($P < 0.05$).

Table 1: Frequency and distribution of Candida isolates

Isolate	No. (%)
<i>Candida albicans</i>	17 (65.4)
<i>Candida</i> species	09 (34.6)
Total	26 (100.0)

Table 2: Distribution of candida colonization and/or infection of vagina in relation to age of the pregnant women in Ibadan, Nigeria

Age Group (years)	No. Tested	No. Positive for Candida (%)
Less than 20	17	04 (23.5)
20-29	63	22 (34.9)
30 and above	20	00 (00.0)
Total	100	26(26.0)

DISCUSSION

This study has shown that 26 *Candida* isolates were found colonizing and/or infecting the genital tract of pregnant women at Adeoyo maternity hospital in Ibadan, Southwestern Nigeria. *Candida albicans* and other *Candida* species had been isolated from several clinical specimens from different part of Nigeria [14, 15] and different parts of the world. According to Hedayati and Shafiei [3], of vulvovaginal candidiasis cases in the United States, 70-90% are caused by *Candida albicans*, while the remainder of infections is caused by other *Candida* species. The differences could be due to geographic, ethnic and socioeconomic factors, as well as differences in sampling and culturing techniques. Variations may also reflect differences in sexual practice and environmental factors such as hygiene and nutrition [16, 17].

In the present study, *C. albicans* (65.4%, n=17) and other *Candida* species (34.6%, n=9) were recovered from the HVS. The overall carrier rates of 65.4% observed for *C. albicans* among other *Candida* isolates is comparatively higher than the report of Hedayati and Shafiei [3] who documented a 29.7% *Candida albicans* in their study. Hedayati and Shafiei [3] also documented other *Candida* species to be *Candida tropicalis* (48.4%) *C. guilliermondii* (14.1%), *C. krusei* (6.3%) and *C. glabrata* (1.6%). Welsh et al. (2010) identified *Candida* species as the most common fungal cause of nail disease in their study.

Haram and Digranes [18] reported an open trial of local clotrimazole therapy in 56 pregnant women with vulvovaginal candidiasis. In their study, Haram and Digranes [18] reported that six patients (10.7%) had slight complaints and 10 (17.9%) without symptoms or signs of infection harboured *Candida albicans* or other

yeast species in the genital tract. Pankajalakshmi and Taralakshmi [19] from Chennai, in their review, emphasized the emergence of candidiasis, cryptococcosis, aspergillosis, penicillosis and other pheohyphomycosis in patients infected with HIV. Lakshmi [20] and Shailaja *et al.* [21] reported on the isolations of *A. fumigatus*, *A. niger* and *Candida* species. *C. albicans* is reported as the most common 65.4% pathogens in this study. Although, been less common than bacterial infections, serious fungal infections occur in the immunocompromised patient both as new infection and as reactivation of latent disease [21].

In one recent study *Candida* accounted for 45% of invasive fungal infections in renal allograft patients [22]. It was also implicated as the cause of Fournier's gangrene in an immunocompromised patient in another study by Loulergue *et al.* [23]. Baradkar *et al.* [24] documented other *Candida* species among patients with bronchopneumonia originating from endobronchial inoculation or more commonly a hematogenously seeded, nodular diffuse infiltrate. Yildirim *et al.* [25] reported *Candida* in a patient without any history of underlying malignancy. Other species of *Candida* has been identified in a patient with meningitis in Australia [9] and in an immunocompromised patient with multifocal osteomyelitis in Germany [8]. Mendes *et al.* [26] documented that *Candida* species typically forms multiple microabscesses and small macroabscesses scattered throughout the brain. McGee *et al.* [27] also documented *Candida* species among immunocompromised patients with vaginitis and secondary to hematogenous spread. This hematogenous spread can also lead to acute renal infarction secondary to the infiltration of the renal parenchyma and occlusion of the hilar vessels.

In conclusion, this study demonstrated the common candida profile of colonized and/or infected vagina in this environment. We have demonstrated a pattern of pregnant women with vaginal infection similar in scale and serotype distribution to reports from the industrialized world but with a significantly worse outcome. It is concluded that clotrimazole is an effective antimycotic agent which can be used for vulvovaginal candidiasis during pregnancy without causing side effects [18]. Treatment should be given to culture positive women in order to prevent subsequent infection of the neonate and secondary infection to the mother. The need for appropriate health education to reduce candidal infection is very imperative and desirous. Important information to support future preventive strategies

includes estimate of rates of disease, timing of disease initial manifestations; and for vaccine development, description of serotype distribution in different populations. However, the incidence of asymptomatic vulvovaginal candidiasis was found to be high in these pregnant women in Ibadan; therefore, proper and well coordinated sex education should be organized for adolescents and pregnant women in order to prevent candidal infections.

ACKNOWLEDGMENTS

We thank the management and staff of Adeoyo Maternity Hospital, Ibadan, for permitting this study and the management and staff of Department of Medical Microbiology and Parasitology for permitting the use of the laboratory for bacteriological analysis.

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