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Prevalence and antimycotic susceptibility profile of *Candida* species in the oral cavities of HIV/AIDS patients and pregnant women in Nsukka, Nigeria

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ABSTRACT

Aims: The present study determined *Candida* species occurrence in the mouth of HIV/AIDS, pregnant women and healthy individuals, and *Candida* susceptibility to antimycotics.
Methodology and results: A cross-sectional study of the *Candida* species occurrence and their *in vitro* sensitivity to nystatin, clotrimazole, ketoconazole and fluconazole was studied. Oral swabs were taken from the dorsum of the tongue of 63 HIV/AIDS patients, 38 pregnant women and 53 apparently healthy humans served as control. All participants gave their consent. The samples were cultured for isolation of *Candida* species. The susceptibility of commonly used antimycotics against *Candida* species was determined using agar-well and disc-diffusion methods. The broth dilution method was adopted in the determination of the minimum inhibitory concentration of the drugs. The rate of *Candida* species colonization in HIV/AIDS patients was higher than that among pregnant women and apparently healthy humans. *Candida albicans* had the highest frequency. *Candida* species displayed higher sensitivity to nystatin and clotrimazole than ketoconazole and fluconazole. *Candida albicans* exhibiting low sensitivity to fluconazole were found in the mouth of AIDS patients, pregnant women and apparently healthy humans.
Conclusion, significance and impact of study: *Candida* species prevalence was highest among HIV patients. *Candida albicans* had the highest frequency among the study groups and had reduced susceptibility to fluconazole. The findings of the study underscore the need for regular surveillance and susceptibility testing of *Candida* isolates before prescription of antimycotics, especially in cases of chronic infection, as well as the development of safe and efficacious treatment alternatives since *Candida* isolates are becoming less susceptible to fluconazole.

Keywords: *Candida* colonization, oral infection, antifungal drugs, *Candida* susceptibility

INTRODUCTION

Candida species colonize the mucosal surfaces of the mouth, gastrointestinal and urogenital tracts as normal flora of humans and animals. They are also the most implicated opportunistic fungal pathogen in human infections (Hauman *et al.*, 1993; Firacative, 2020; Gerós-Mesquita, 2020; Romo and Kumamoto, 2020) and the most opportunistic oral fungal pathogen of clinical importance (Bandara *et al.*, 2019). The genus *Candida* is aerobic, thin-walled small yeast made up of 350 heterogeneous species, but a few implicated in opportunistic infections of humans or animal. Studies have shown the emergence of non-albicans and *Candida* species resistant to antimycotics as important agents of superficial and invasive fungal infections, especially among patients with weakened immunity (Abrantes *et al.*, 2014; Yapar, 2014; Pendleton *et al.*, 2017; Tsega and Mekonnen, 2019). Among HIV-seropositive individuals, a weakened immune system due to low CD4 counts as a result of HIV infection correlates with increased *Candida* carriage and oral candidiasis, the earliest oral manifestation and a most important indicator of HIV infection (Nugraha *et al.*, 2018). During pregnancy, the elevated levels of progesterone and estrogen as well as increased acidity in the mouth as a result of exposure to gastric acid due to persistent morning sickness, favour *Candida* growth (Annan and Nuamah, 2005; Africa and Turton, 2019; Tsega and Mekonnen, 2019). Progesterone decreases plasma bicarbonate level, which results in the reduced pH, a condition that supports the proliferation of *Candida* species (Naveen *et al.*, 2014; Africa and Turton, 2019). Dietary cravings common during pregnancy may likely lead to increased sugar consumption resulting in increased *Candida* colonization (Rio *et al.*, 2017; Talapko *et al.*, 2021).

Candida species presence in the mouth is a likely occurrence among 40-70% of healthy individuals

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(Zegarelli, 1993; Gerós-Mesquita, 2020). The asymptomatic existence of *Candida* species in the mouth increases the likelihood of developing clinical diseases, especially under predisposing conditions. Both pregnancy and HIV/AIDS are among the predisposing condition that favours the yeast colonization in the mouth. Infection of the mouth during pregnancy is worthy of consideration as early dental caries in children have been linked to mothers who have high caries (Africa and Turton, 2019; Tsega and Mekonnen, 2019). Oral infection also lowers the quality of life, causes pain, reduces the sense of taste and interferes with nutritional intake and administration of medication in serious cases (Osaigbovo *et al*., 2017).

The most frequently implicated yeast in oral infection has been *C. albicans*, but reports abound on the increasing incidence of oral candidiasis caused by non-albicans *Candida* species (Pfaller *et al*., 2005; Skrodeniene *et al*., 2006; Nweze and Ogbonnaya, 2011; Mulu *et al*., 2013). The result of the drastic rise in candidiasis is the increase in antimycotic usage which was accompanied with the outbreak of drug-resistant *Candida* strains (Vandeputte *et al*., 2012; Mulu *et al*., 2013; Singh and Chakraborty, 2017). Epidemiological data on the *Candida* species occurrence and level of drug resistance varies worldwide.

The treatment of candidiasis in Nigeria involves the use of common antimycotics such as nystatin, ketoconazole, clotrimazole and fluconazole. Despite the extensive antifungal drug use against fungal infections in Nigeria, insufficient information exists on the level of resistance of *Candida* isolates from the mouth to these antifungal drugs. There are also few reports on the *Candida* species occurrence in the mouth of HIV/AIDS patients and pregnant women in Nigeria and their drug resistance profile. The study determined the *Candida* species occurrence and antimycotic sensitivity of *Candida* species isolated from the mouth of AIDS patients, pregnant women and apparently healthy individuals in Nsukka, Enugu State, Nigeria.

MATERIALS AND METHODS

Participants' inclusion and exclusion criteria

Purposive sampling technique was used to determine the sample size. The participants of the cross-sectional study were volunteers made up of three groups, namely (a) HIV-seropositive individuals who were not taking any antifungal or antiretroviral drugs; (b) Pregnant women within the second trimester who were HIV-seronegative and not on any antifungal medication; (c) Apparently healthy individuals (HIV-seronegative, non-pregnant, non-diabetic, not on the antibiotic or antifungal treatment or steroid, non-contraceptive user). This last group served as control. Participants who were diabetic or currently on treatment, especially with antibiotics or antifungal drugs, were excluded. All participants who gave their consent and met the inclusion criteria participated in the study, while those who did not give consent voluntarily were excluded. The purpose of the study was explained to the volunteers as well as how samples will be collected. Any participant currently on antifungal medication was excluded, while participants who used such drugs 2-3 months ago were accepted in the study. The expectant mothers were visiting prenatal clinics while HIV/AIDS patients were visiting Bishop Shanahan Hospital or medical diagnostic laboratory for the first time in Nsukka, Enugu State, Nigeria.

Ethical approval

Approved for the study was gotten from the Faculty of Biological Sciences Ethical Committee, University of Nigeria, Nsukka with reference number unn/fbs/ec/1030. Verbal approval was also obtained from the management of the hospital/clinic after consideration of the proposed study.

Sample collection

Oral swab was collected from the dorsum of the tongue of each participant using a sterile cotton swab (Evepon sterile swab stick^{R}) during the periods of March 2006 to February 2007. Information on the participant's age, pregnancy status, HIV status, oral health status and drug use were recorded during sample collection.

Culture of oral swab/identification of isolates

The sample was streaked on prepared sterile Sabouraud dextrose agar (SDA) plate and kept in an incubator at 37 °C for 48 h. White creamy colonies which grew on SDA after incubation were sub-cultured on SDA slant for identification and further study. *Candida* isolates were identified using standard microbiological procedures, which included germ tube test, sugar assimilation test, chlamydospore production and inoculation on chromogenic agar (Anaele and Okafor, 2020). Yeast colonies were further subcultured on two *Candida* chromogenic agar plates, namely Fluka *Candida* Ident agar and Oxoid Chromogenic *Candida* agar plates. Quality control organisms used were *C. albicans*, ATCC 90028 and *C. krusei*, ATCC 6258.

Antifungal susceptibility test

Four commonly used antifungal drugs, namely fluconazole, ketoconazole, clotrimazole and nystatin were purchased from a drug shop in Nsukka. Fluconazole (Diflucan and Lucon), ketoconazole (Fungral and Nizoral), clotrimazole (Canesten) and nystatin (Nystatin Pharmamed) were dissolved in 20% dimethyl sulfoxide (DMSO) to give a final stock concentration of 5000 µg/mL for fluconazole, ketoconazole and clotrimazole, and 5000 IU/mL for nystatin. All drugs were divided into 2 mL aliquots and stored at 4 °C in the refrigerator for use within 7 days. The susceptibility testing method was the agar-well diffusion method, according to Magaldi *et al.* (2004), which was similar to the disk diffusion method described in the document M44-A of Clinical and

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Laboratory Standards Institute (CLSI, 2004), formerly National Committee for Clinical and Laboratory Standards (NCCLS). Mueller-Hinton agar (25 mL per plate) supplemented with 2% glucose and 0.5 µg of methylene blue per mL (MHA-GMB), with a depth of 4.0 mm were used. The agar surface was inoculated using a sterile swab dipped in a yeast suspension adjusted to the turbidity of 0.5 McFarland standards. Instead of using drug-impregnated disks as described in the CLSI method, the drugs were added to the wells punched in the solidified agar using a 6 mm sterile cork borer. The bottom of each well was sealed with 50 µL of sterile molten MHA-GMB to ensure that the drugs do not diffuse under the agar layer when added. After solidification, each well was labelled with the drug name and the DMSO control. Twenty-five microliters of each drug were dispensed against the corresponding well and a pre-diffusion period of one hour was observed at 4 °C in the refrigerator. The plates were incubated at 37 °C for 18-24 h. A commercial fluconazole (25 µg) disk (Oxoid) and 20% DMSO served as positive and negative controls, respectively. Quality control organisms used were *C. albicans*, ATCC 90028 and *C. krusei*, ATCC 6258. The tests were carried out in duplicates on two separate occasions and the results were expressed as the duplicate means. The antifungal drug activity was measured in millimeters (mm), representing the diameter of the clear zone of growth inhibition. Inhibition zone diameter was interpreted either as susceptible or resistant. Mean IZD 15 mm was interpreted as susceptible while 14 mm was resistance.

Minimum inhibitory concentration (MIC) determination

Agar dilution technique was adopted for MIC determination. Drugs were initially dissolved in 20% DMSO and subsequent dilution in sterile Nutrient Broth reduced the concentration to 10,000-19.5 µg/mL and 10,000-19.5 IU/mL for fluconazole and nystatin, respectively. One milliliter of each drug concentration was added to 19 mL of already prepared sterile molten MH-GMB agar in test tube (cooled to about 45 °C) and gently mixed by inverting the tube two or three times. The drug-MH-GMB agar blend, poured and solidified in sterile Petri plates, served as the test plates while drug-free agar plate served as control. Each of these plates was divided into five sections in pie shape and labelled with corresponding *Candida* isolate to be tested. A loopful of standardized yeast suspension was spotted on top of the corresponding pie in the solidified agar, starting with the controls and working from the lowest drug concentration (10^{th} plate) to the highest drug concentration (1^{st} plate). The culture plates stayed undisturbed for 1 h to permit adsorption of the inoculum into the surface of the medium. Both the test plates and the control were kept in the incubator at 37 °C for two days and monitored for growth. Once evidence of yeast colony was seen in the control, the presence or absence of growth in the other ten test plates was recorded. This test was done in duplicates.

Statistical analysis

The data obtained were presented as means and percentages while differences observed between groups were subjected to Chi-square test to determine if the observed difference was significant or not, with probability set at 5% level of significance. The software used was statistical package for social sciences (SPSS) version 15.0

RESULTS

Participants' characteristics

A total of 154 volunteers made up of 63 HIV/AIDS patients, 38 pregnant women and 53 apparently healthy humans (AHH) participated in the study. Among the HIV-infected volunteers were 27 males and 36 females, while AHH volunteers were made up of 13 males and 40 females within the age range 12-67 years. Sixty-five volunteers had symptoms of oral infection (presence of white patch on the tongue): 2 (3.08%) pregnant women and 63 (96.92%) HIV-infected patients.

Prevalence of *Candida* species

Candida species was isolated from 61 (31.61%) volunteers who participated in the study. The *Candida* species isolation frequency from oral tracts of AIDS patients, pregnant women and AHH showed that the highest percentage of *Candida* positive samples 46 (73.02%) was among AIDS patients, followed by pregnant women 11 (28.95%), but was least in AHH 4 (7.50%) as displayed in Table 1. The difference observed in the occurrence of *Candida* among these three groups was significant ($p<0.05$). *Candida* species were also significantly ($p<0.05$) higher among subjects with oral infection 48 (73.85%) than those without symptoms 13 (14.61%).

Four *Candida* species which include *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* were isolated from AIDS patients, pregnant women with exception of *C. parapsilosis* and AHH with exception of *C. krusei* and *C. parapsilosis*. With chromogenic agar, mixed *Candida* cultures were observed in some *Candida* positive samples. *Candida albicans* and non-albicans *Candida* species occurrence in the dorsum of tongue indicated that *C. albicans* was highest among AIDS patients (75.00%), pregnant women (64.29%) and AHH (75.00%) (Table 1).

Candida albicans frequency in both male and female AIDS patients was highest, followed by *C. parapsilosis* and *C. tropicalis*. *Candida krusei* was found in the oral cavity of male AIDS patients but not among female AIDS patients (Table 1).

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Table 1: Distribution of *Candida* species isolated from oral cavities of AIDS patients, pregnant women and apparently healthy humans according to sex.

Sample source	No. sample	Subjects with oral infection, n (%)	*Candida* positive sample, n (%)	*Candida* species isolated, n (%)				Total
				C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. krusei*	
AIDS patients	63	63(100)	46(73.02)[a]	36(75.00)	3(6.25)	8(16.67)	1(2.08)	48
Male	27	27(100)	19(70.37)	15(71.43)	2(9.52)	3(14.29)	1(4.76)	21
Female	36	36(100)	27(75.00)	21(77.78)	1(3.70)	5(18.52)	0	27
Pregnant women	38	2(5.26)*	11(28.95)[b]	9(64.29)	2(14.29)	0	3(21.43)	14
AHH	53	0	4(7.50)[c]	3(75.00)	1(25.00)	0	0	4
Male	13	0	0	0	0	0	0	0
Female	40	0	4(10. 00)	3(75.00)	1(25.00)	0	0	4
Total	154	65	61(39.61)	48(72.72)	6(9.091)	8(12.12)	4(6.06)	66

χ^2 = 53.975; Degree of freedom (df) = 2; p<0.05; AHH = Apparently healthy humans; * = All *Candida* positive.

Table 2: Minimum inhibitory concentration values of nystatin and fluconazole against selected isolates.

Candida isolates	*Candida* species	Nystatin (IU/mL)	Fluconazole (µg/mL)
$H01^G$	*C. albicans*	7.81	R
$H08^W$	*C. parapsilosis*	3.91	15.63
$H17^G$	*C. albicans*	7.81	R
$H60^W$	*C. parapsilosis*	1.95	7.81
$R30^W$	*C. albicans*	7.81	R
$R35^G$	*C. albicans*	7.81	R
$R53^{Wa}$	*C. albicans*	1.95	15.63
$R58^B$	*C. tropicalis*	1.95	7.81
C. albicans	ATCC 90028	1.95	7.81
C. krusei	ATCC 6258	7.81	R

Key: R = Resistant even at 500 µg/mL; H01-H60 = Isolates from HIV patients; R30-R58 = Isolates from oral tract of pregnant women.

In vitro antimycotic susceptibility

The susceptibility to antimycotics indicated that *C. albicans* isolates were more sensitive to nystatin 47 (97.92%) and clotrimazole 43 (89.58%) than ketoconazole 19 (39.58%) and fluconazole 2 (4.17%). *C. albicans* was least sensitive to fluconazole when compared to *C. tropicalis* (50%), *C. parapsilosis* (87.50%) and *C. krusei* (75%) as specified in Figure 1.

Candida species susceptibility to the three study groups is shown in Figure 2. *Candida* isolates from AIDS patients showed higher sensitivity to nystatin 47 (97.92%) and clotrimazole 43 (89.58%) than ketoconazole 23 (47.92%) and fluconazole 9 (18.75%). Isolates from AIDS patients and pregnant women were more susceptible to nystatin and clotrimazole than isolates from AHH. A significant difference in *Candida* sensitivity to clotrimazole was observed among HIV patients, pregnant women and AHH (p=0.05).

Fluconazole MIC (with the lowest number of susceptible *Candida* isolates) was 7.80-15.63 µg/mL and nystatin MIC (with the highest number of susceptible *Candida* isolates) was 1.95-7.81 IU/mL to selected sensitive *Candida* isolates. Some of the fluconazole resistant isolates remained resistant even at 500 µg/mL fluconazole (Table 2).

DISCUSSION

Candida species occurrence among AIDS patients, pregnant women and apparently healthy humans (AHH) in the present study showed that *Candida* carriage in HIV/AIDS patients (73.02%) was significantly higher than in pregnant (28.95%) and apparently healthy humans (7.50%) (Table 1). The occurrence of more than one *Candida* species per clinical sample was observed and we identified four *Candida* species in the study namely: *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. Absence of *C. glabrata* in the present study was not a limitation of the chromogenic agar used as *C. glabrata* was identified from another sample source using the same media (Anaele and Okafor, 2020).

The observed differences in *Candida* carriage and symptoms of oral infection among AIDS patients may be related to the immune status. AIDS has been associated with T-cell dysfunction, various monocyte functional abnormalities, including defective chemotaxis and

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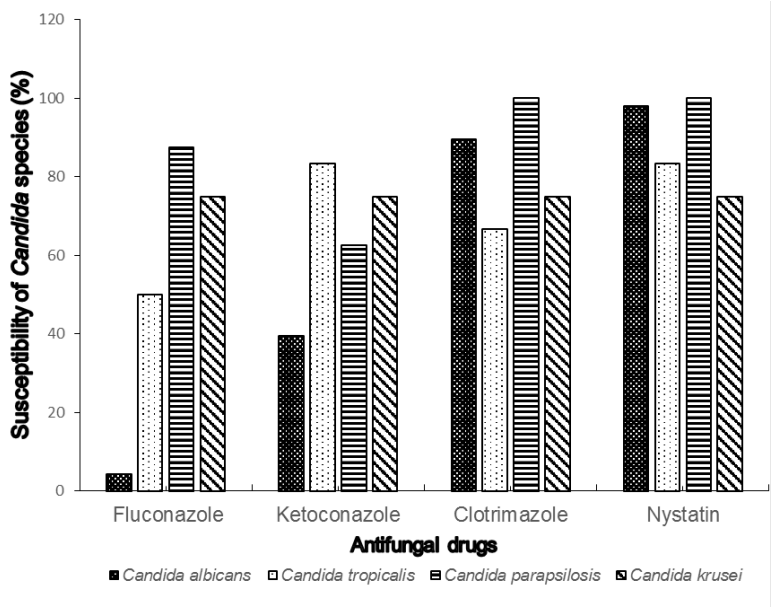

Figure 1: Susceptibility of different *Candida* species to antifungal drugs.

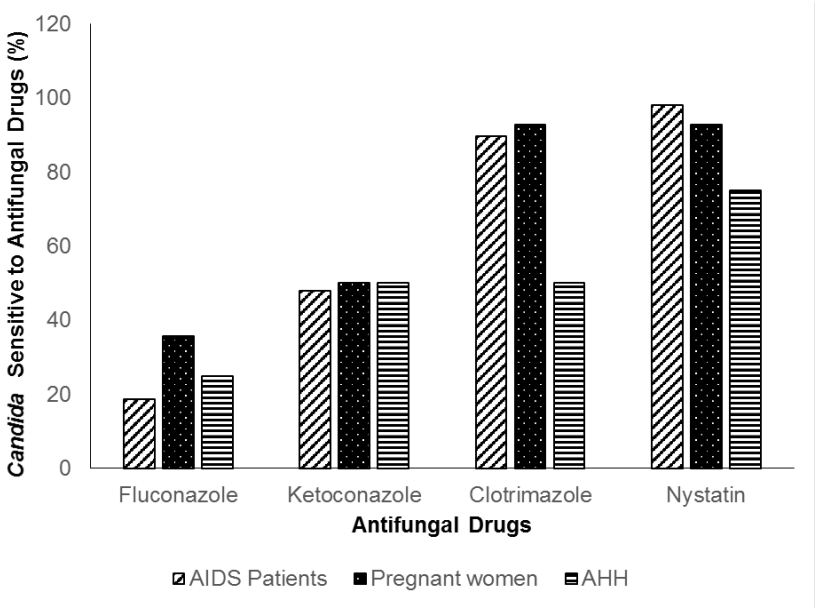

Figure 2: Sensitivity of *Candida* isolates obtained from oral cavity of AIDS patients, pregnant women and apparently healthy humans (AHH) to antifungal drugs.

phagocytosis (Maródi *et al.*, 1991; Fidel, 2006). Since monocytes have known fungicidal activity, their dysfunction coupled with the T-lymphocyte defects and deficit may contribute to the predisposition to candidiasis in AIDS patients (Lehrer 1975; Maródi *et al.*, 1991). It is very important to monitor the epidemiology and ecology of these species in healthy subjects since *Candida* species causes opportunistic infections.

The disparity observed in the frequency of oral infection and *Candida* prevalence between pregnant and non-pregnant (AHH) women may be an indication of the biochemical and hormonal changes observed during pregnancy which might favour *Candida* growth. In this study, 28.95% of pregnant women were *Candida* colonized, with 5.26% having symptoms of oral infection, while 10% of healthy non-pregnant women (AHH) were colonized by *Candida* species but none showing

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symptoms of oral thrush. In Portugal, Rio *et al*. (2017) observed that oral yeast prevalence was higher among expectant mothers than in non-pregnant women but their observed difference was not statistically significant. In the USA, Xiao *et al*. (2019) reported that pregnancy status did not significantly influence C*andida* carriage in their study, but the overall immune status during pregnancy likely played a role. It is, therefore, necessary to give antenatal mothers timely oral care since current reports allude that the placenta may harbour a unique microbiome that is of high similarity to the oral cavity and may influence pregnancy outcome (Aagaard *et al*., 2014; Maki *et al.*, 2017). Additional evidence was provided by Gomez-Arango *et al*. (2017) who reported that both oral and gut microbiome of expectant mothers may impart to the placental microbiome.

Candida albicans was the predominant species in AIDS patients, pregnant women and AHH from the study (Table 1). Although *C. albicans* is a well-known opportunistic pathogen (de Melo *et al*., 2019; Hirayama *et al*., 2020), immune defense of the host must have played major role in determining pathogenicity since AHH were colonized by *C. albicans* but none had oral thrush. *C. albicans* is known to be a benign colonizer of mucosal surfaces and causes opportunistic infection when there are specific defects or changes in host immune defense (Vartivarian and Smith, 1993; Gerós-Mesquita *et al.*, 2020). Sánchez-Vargas *et al*. (2005) reported a high occurrence of *C. albicans* among HIV/AIDS patients and non-HIV adults. Other studies carried out in Nigeria, Cameroon, Indonesia and Saudi Arabia also revealed a high prevalence of *C. albicans* among HIV patients and apparently healthy adults (Darwazeh *et al*., 2002; Skrodeniene *et al*., 2006; Nugraha *et al*., 2018; Ambe *et al*., 2020).

We observed that our result was consistent with reports from Indonesia, Germany and South Africa where *Candida* carriage in HIV/AIDS patients were 77.2%, 73.8% and 81.3%, respectively (Schmidt-Westhausen *et al*., 1991; Patel *et al*., 2006; Nugraha *et al*., 2018). However, a lower *Candida* carriage was reported from Thailand (66.6%), India (65.3%), Italy (61.9%) and Hong Kong (54.8%) (Teanpaisan and Nittayanata, 1998; Tsang and Samaranayeke, 2000; Campisi *et al.*, 2002; Gugnani *et al.*, 2003). The trend in C*andida* carriage among HIV patients has not changed (Vidya *et al*., 2016). A number of factors could have influenced the high C*andida* carriage. Diet has been implicated as a major factor that could contribute to high yeast carriage among undernourished HIV-infected children in Nigeria and a low prevalence among comparable well-fed children resident in the United States (Jabra-Rizk *et al*., 2001). Kadir *et al*. (2005) also reported that in Turkey, age and diet affected yeast carriage of the population. The subjects of the present study were Africans and their diet was rich in carbohydrate which enhances the proliferation of *Candida* and attachment to cells of membranous tissue (Pizzo *et al*., 2000; Talapko *et al.*, 2021). Moreover, within Africa, the rate of carbohydrate intake varies, exposing the reason for disparity observed in reports on yeast carriage in Africa. In Kalahari, their diet is rich in animal protein but low in carbohydrates since the people are known to be nomads, while in Guateng, their carbohydrate intake is usually high since the population is urbanized (MacKeown and Faber, 2004). As such, the carrier rate in Kalahari was 30.4%, while that of semi-urban area of Guateng was 58% among patients with unknown HIV status. In this present study, the population consumes more of their traditional foods (Bambara nut or 'okpa', local beans, corn and other local delicacies), which have been found to be also rich in protein in addition to carbohydrate (Ene-Obong and Obizoba, 1995). Thus, the difference observed in carrier rate among HIV patients in Nsukka, Nigeria (73.02%) and Guateng, South Africa (81.3%) may be due to differences in diet.

The *Candida* carriage rate among apparently healthy humans (AHH) in this study was low. The low incidence was also observed among HIV-negative subjects from other parts of the world: 10.8% in Thailand, 13.8% in Germany and 29.3% in Italy (Schmidt-Westhausen *et al*., 1991; Teanpaisan and Nittayananta, 1998; Campisi *et al.*, 2002) but South African carrier rates were different (63-68%) (Hauman *et al*., 1993; Patel *et al.*, 2006). Milan *et al*. (2001) observed a high frequency of yeast occurrence among household members living with AIDS patient, which indicates the possibility and ease of strain colonization and transfer. This could explain the high carrier rate among South African HIV-negative subjects with a population of more than 5.0 million living with AIDS and low carrier rate among Nigerian HIV-negative subjects with a population of 3.6 million HIV-infected (UNAIDS, 2004).

The current antimycotic susceptibility study indicated optimal susceptibility of the isolates to nystatin and clotrimazole but minimal to ketoconazole and fluconazole. It was also observed that *Candida* isolates from healthy individuals were less sensitive to nystatin and clotrimazole in comparison to isolates from AIDS patients and pregnant women. This could be attributed to community overuse or abuse of clotrimazole and nystatin in the treatment of other fungal infections such as vaginalis and cutaneous infections (Ocan *et al*., 2015; Anaele and Okafor, 2020). Elevated use of clotrimazole and nystatin in a community could reduce the susceptibility of *Candida* isolated from healthy individuals to such drugs. Fluconazole and ketoconazole use are often associated with doctors' prescription of those drugs for systemic infections. Pregnant women are known to often use only drugs prescribed by medical doctors. Resistance to azole drugs gradually develops during antifungal therapy (Müller *et al*., 1999; Morschhäuser, 2002).

Reduced *Candida* susceptibility to antifungal drugs has been reported elsewhere. In a report by Vazquez *et al*. (1993) on 60 *C. albicans* recovered from fluconazole refractory oropharyngeal candidiasis patients, resistance to fluconazole was 78%, while cross-resistance was recorded for itraconazole (7%), ketoconazole (11%) and clotrimazole (41%). Salobrena *et al*. (1999) reported that none of the *Candida* isolates was resistant to nystatin

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while resistance to fluconazole (7.9%) and ketoconazole (2.9%) was observed in their study. Tseng *et al*. (2005) also reported increased *Candida* resistance to fluconazole (25.7%). Azole drugs such as fluconazole are fungistatic while polyenes such as nystatin are fungicidal drugs. The fungistatic activity of azole drugs may be responsible for the reported decrease in *Candida* drug susceptibility. Diverse resistance mechanisms have been observed in *Candida* which could explain the reduced susceptibility to azoles. Resistance to fluconazole has been reported following the presence of point mutations in *ERG11*, which involves the replacement of amino acid (Whaley *et al*., 2017). Other mechanisms include increased expression of *ERG11* and drug efflux pumps, *ERG3* gene inactivation, aneuploidy and other chromosomal abnormality including loss of alternative forms of the same gene known as loss of heterozygosity (LOH) have been described in *Candida* resistance to azole (Berkow and Lockhart, 2017; Whaley *et al*., 2017). It has been reported that various processes frequently combine over time to result in fluconazole resistance (Morschhäuser, 2002).

The emergence of fluconazole resistance is a problem in public health. The reports by Johnson *et al*. (1995), Tumbarello *et al*. (1997) and Magaldi *et al*. (2001) indicated an increased occurrence of resistant strains among patients on long-term therapy in 1995 (40%),1997 (43%) and 2000 (45%). In another study, Patel *et al*. (2006) reported that 55.6% of patients on nystatin and 18.9% on amphotericin B required additional treatment. In Nigeria, fluconazole susceptibility studies by Enwuru *et al*. (2008) indicated that 78.4% of oropharyngeal *Candida* isolates from HIV-patients were sensitive (MIC 8 µg/mL), 12.1% were susceptible dose-dependent (MIC 16-32 µg/mL) and 9.5% were resistant (MIC 64 µg/mL). Nweze and Ogbonnaya (2011) also reported resistance among oral *Candida* isolates to fluconazole (11.7%), voriconazole (1.7%), flucytosine (8.3%) and itraconazole (7.5%) but none was resistant to amphotericin B among HIV patients screened. Osaigbovo *et al*. (2017) reported reduced susceptibility to fluconazole in 24% of 75 *Candida* from their study. A recent report indicated that 24.6% of *Candida* isolated from the mouth were not sensitive to fluconazole (Ambe *et al*., 2020).

Antifungal susceptibility of *Candida* species varies in different regions and settings. Some reports indicated *C. albicans* reduced susceptibility to fluconazole higher than 20%. Mohamadi and Motaghi (2014) reported 33.3% *C albicans* resistance. Abaci and Haliki-Uztan (2011) also reported a reduced susceptibility of *C. albicans* to fluconazole (44%) and amphotericin (61%) in Turkey. These reports show the need for regular surveillance of antifungal susceptibility testing. This may likely result from the practice of self-medication or inappropriate drug usage, which is high in the study area (Ocan *et al*., 2015; Anaele and Okafor, 2020).

It has been postulated that extensive azole or fluconazole usage promotes resistant strains selection, changing colonization to a resistant subset (Mathema *et al*., 2001). The reduced susceptibility to azoles observed in the study calls for regular antifungal susceptibility surveillance, especially for patients at risk of oral candidiasis as well as the development of new antifungal substances since fluconazole, the safest antifungal agent available, is becoming less effective.

Another finding of serious concern was that *C. albicans* showed reduced susceptibility to fluconazole, a trend that has been observed for more than two decades. Skrodeniene *et al*. (2006) reported that the frequency of fluconazole resistance was higher in *C. albicans* strains than non-albicans *Candida* species. A report from India indicated *C. albicans* loss of sensitivity to azoles and other antimycotics (Sirnivasan and Kenneth, 2006; Singh and Chakraborty, 2017). Another drug-resistant research on bloodstream infection in Taiwan indicated that *C. albicans* (47.3%) and *C. glabrata* (70%) from patients were resistant (Hsueh *et al*., 2002). In Johannesburg, South Africa, a low frequency of resistance was observed by Owotade *et al*. (2016) among *C. albicans* isolates from the mouth of immunocompromised patients.

The most striking observation from the current study was that these *Candida* isolates that were not sensitive to fluconazole were found among AIDS patients, pregnant and apparently healthy humans. Lattif *et al*. (2004) suggested factors contributing to fluconazole resistance which include the extent of patient's immunosuppression, the magnitude of fluconazole usage, contribution of other chemotherapeutic drugs and intrinsic resistance. However, the present result revealed that health status (AIDS) or hormonal changes (pregnancy) did not affect the *Candida* species sensitivity to fluconazole. Also, prior exposure to fluconazole did not influence susceptibility as these pregnant women and apparently healthy human used in this study indicated that they had never used fluconazole.

However, there is a possibility of transmitting azole-resistant strains from HIV-infected oropharyngeal candidiasis patients to asymptomatic family members which portrays an under-appreciated risk for families living with HIV-infected members (Müller *et al*., 1999). Mode of transmission of drug-resistant strains between family members may be through the exchange of contaminated fomites such as food, utensils and toys. Acquisition of azole-resistant strains by asymptomatic patients could have future clinical implications, especially in high-risk populations resulting in oropharyngeal candidiasis refractory to azole. In another study, Lupetti *et al*. (2002) reported horizontal transfer of *C. parapsilosis* from nurses to the newborns in the neonatal intensive care unit. Another distinct possibility is the spread of colonizers with reduced drug sensitivity by sexual transmission (Mathema *et al*., 2001). Also, of serious concern is the dominance of *Candida* strains with minimal or no susceptibility to antimycotics, causing infection, especially if the individual becomes immunosuppressed at a later time. Fluconazole resistance is a problem in candidiasis treatment since fluconazole, a known drug for prophylaxis and treatment of fungal infections especially among HIV/AIDS patients in Africa, has lost its efficacy. The situation calls for further research in Nigeria, where *in*

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vitro resistance will be matched with *in vivo* response, especially among a human population with HIV/AIDS. There is a pertinent need for alternative antifungal drugs with a good safety profile or modification of the activity of fluconazole through the use of adjuvants.

The epidemiological data of this report, although generated about 14 years ago, can be useful to historical documentation and comparison. The present study does not strictly lose relevance based on age since it forms a background to subsequent developments and helps stakeholders trace the origin of clinical issues. Such defined epidemiological history is usually relevant in studies such as the determination of clonal relationships among infectious agents within a given locality or region. For instance, reference has been made to the studies of Nweze and Ogbonnaya (2011) and Osaigbovo *et al*. (2017). A collaborative study using their isolates and those described in this report will be a pointer to the possible origin and degree of persistence of the isolates in the study population. It may also raise research questions on the vehicle of transmission. In addition, a major part of the report is on the *Candida* drug sensitivity profile. This is a continuing global public health challenge that is as relevant today as it was twenty years ago.

CONCLUSION

The study has shown *Candida* species higher predominance in oral cavities of HIV-infected patients than pregnant women and healthy humans. The dominant species was *C. albicans* in the mouths of the three groups. Gender did not influence *Candida* species prevalence in the mouth. *Candida* isolates from the oral cavity were less susceptible to fluconazole but highly susceptible to nystatin. *Candida* isolates that displayed minimal sensitivity to fluconazole were found among AIDS patients, pregnant and apparently healthy humans. Based on the aforementioned, there is a need for regular surveillance of the prevalence and susceptibility of *Candida* isolates to antifungals. There is also a need to boost or modify the activity of fluconazole or develop new antifungals with a good safety profile since *Candida* isolates are becoming less susceptible to fluconazole.

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AUTHORS' CONTRIBUTION STATEMENT

ENA designed the study, conducted the experiment, analyzed the data, managed literature searches and wrote the first version of the manuscript. JIO supervised the study contributed to the design, data interpretation and review of the manuscript. The final manuscript was read and approved by both authors.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethical Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, with reference number unn/fbs/ec/1030. Verbal approval was also obtained from the management of the hospital/clinic after consideration of the proposed study. The study objectives and protocols were explained to the participants/volunteers. All participants gave their consent after reading the introductory letter for the study.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J. and Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine* **6(237), 237ra65.**

Abaci, O. and Haliki-Uztan, A. (2011). Investigation of the susceptibility of *Candida* species isolated from denture wearers to different antifungal antibiotics. *African Journal of Microbiology Research* **5 (12), 1398-1403.**

Abrantes P.M.D.S, McArthur C.P. and Africa C.W.J. (2014). Multi-drug resist ant (MDR) oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon. *Diagnostic Microbiology and Infectious Diseases* **79 (2), 222**

Africa, C. W. and Turton, M. (2019). Oral health status and treatment needs of pregnant women attending antenatal clinics in KwaZulu-Natal, South Africa. *International Journal of Dentistry* **2019, Article ID 5475973.**

Ambe, N. F., Longdoh, N. A., Tebid, P., Bobga, T. P., Nkfusai, C. N., Ngwa, S. B., Nsai, F.S. and Cumber, S. N. (2020). The prevalence, risk factors and antifungal sensitivity pattern of oral candidiasis in HIV/AIDS patients in Kumba District Hospital, South West Region, Cameroon. *The Pan African Medical Journal* **36, 23.**

Anaele, E. N. and Okafor, J. I. (2020). The influence of age, health care and hygienic habits on *Candida* species prevalence in the human oral cavity and genitourinary tract. *International Journal of Tropical Disease and Health* **41(11), 1-13.**

Annan, B. D. R. T. and Nuamah, K. (2005). Oral pathologies seen in pregnant and non-pregnant women. *Ghana Medical Journal* **39(1), 24-27.**

Bandara, H. M. H. N., Panduwawala, C. P. and Samaranayake, L. P. (2019). Biodiversity of the

human oral mycobiome in health and disease. *Oral Diseases* **25(2), 363-371.**

Berkow, E. L. and Lockhart, S. R. (2017). Fluconazole resistance in *Candida* species: A current perspective. *Infection and Drug Resistance* **10, 237-245.**

Campisi, G., Pizzo, G., Millici, M. E., Mancuso, S. and Margiotta, V. (2002). *Candida* carriage in the oral cavity of human immunodeficiency virus-infected subjects. *Oral Medicine* **93(3), 281-286.**

CLSI, Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (2004). Method for antifungal disk diffusion susceptibility testing of yeasts: Approved guideline. Document M44-A. Clinical and Laboratory Standards Institute, Wayne, PA.

Darwazeh, A. M. G., Al-Dosari, A. and Al-Bagieh, N. H. (2002). Oral *Candida* and nasal *Aspergillus* flora in a group of Saudi healthy dentate subjects. *International Dental Journal* **52(4), 273-277.**

de Melo, A. P. V., Zuza-Alves, D. L., da Silva-Rocha, W. P., de Souza, L. F. C., Francisco, E. C., de Azevedo Melo, A. S. and Chaves, G. M. (2019). Virulence factors of *Candida* spp. obtained from blood cultures of patients with candidemia attended at tertiary hospitals in Northeast Brazil. *Journal de Mycologie Medicale* **29(2), 132-139.**

Ene-Obong, H. N. and Obizoba, I. C. (1995). Protein quality of some Nigerian traditional diets based on the African yam bean (*Sphenostylis stenocarpa*) and pigeon pea (*Cajanus cajan*). *Plant Foods for Human Nutrition* **48(4), 297-309.**

Enwuru, C. A., Ogunledun, A., Idike, N., Enwuru, N. V., Ogbonna, F., Aniedobe, A. and Adeiga, A. (2008). Fluconazole resistant opportunistic oro-pharyngeal candida and non-candida yeast-like isolates from HIV infected patients attending ARV clinic in Lagos, Nigeria. *African Health Sciences* **8(3), 142-148.**

Fidel, P. L. (2006). *Candida*-host interactions in HIV diseases: Relationships in oropharyngeal candidiasis. *Advances in Dental Research* **19, 80-84.**

Firacative, C. (2020). Invasive fungal disease in humans: Are we aware of the real impact? *Memórias do Instituto Oswaldo Cruz* **115, e200430.**

Gerós-Mesquita, Â., Carvalho-Pereira, J., Franco-Duarte, R., Alves, A., Gerós, H., Pais, C. and Sampaio, P. (2020). Oral *Candida albicans* colonization in healthy individuals: Prevalence, genotypic diversity, stability along time and transmissibility. *Journal of Oral Microbiology* **12(1), 1820292.**

Gomez-Arango, L. F., Barrett, H. L., McIntyre, H. D., Callaway, L. K., Morrison, M. and Nitert, M. D. (2017). Contributions of the maternal oral and gut microbiome to placental microbial colonization in overweight and obese pregnant women. *Scientific Reports* **7(1), 2860.**

Gugnani, H. C., Becker, K., Fegeler, W., Basu, S., Chattopadhya, D. Baveja, U., Satyanarayana, S., Kalghatgi, T. and Murlidhar, A. (2003). Oropharyngeal carriage of *Candida* species in HIV-infected patients in India. *Mycoses* **46, 299-306.**

Hauman, C. H. J., Thompson, I. O. C., Theunissen, F. and Wolfaardt, P. (1993). Oral carriage of *candida* in healthy and HIV-seropositive persons. *Oral Surgery, Oral Medicine, Oral Pathology* **76(5), 570-572.**

Hirayama, T., Miyazaki, T., Ito, Y., Wakayama, M., Shibuya, K., Yamashita, K., Takazono, T., Saijo, T., Shimamura, S., Yamamoto, K., Imamura, Y., Izumikawa, K., Yanagihara, K., Kohno, S. and Mukae, H. (2020). Virulence assessment of six major pathogenic *Candida* species in the mouse model of invasive candidiasis caused by fungal translocation. *Scientific Reports* **10(1), 3814.**

Hseuh, P. R., Chen, M. L., Sun, C. C., Chen, W. H., Pan, H. J., Yang, L. S., Chang, S. C., Ho, S. W., Lee, C. Y., Hsieh, W. C. and Luh, K. T. (2002). Antimicrobial drug resistance in pathogens causing nosocomial infections at a university hospital in Taiwan, 1981-1999. *Emerging Infectious Diseases* **8, 63-68.**

Jabra-Rizk, M. A., Falkler, W. A., Enwonwu, C. O., Onwujekwe, D. I., Merz, W. G. and Meiller, T. F. (2001). Prevalence of yeast among children in Nigeria and the United States. *Oral Microbiology and Immunology* **16(6), 383-385.**

Johnson, E. M., Warnock, D. W., Luker, J., Porter, S. R. and Scully, C. (1995). Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidiasis. *Journal of Antimicrobial Chemotherapy* **35, 103-114.**

Kadir, T., Uygun, B. and Akyüz, S. (2005). Prevalence of *Candida* species in Turkish children: Relationship between dietary intake and carriage. *Archives of Oral Biology* **50, 33-37.**

Lattif, A. A., Banerjee, U., Prasad, R., Biswas, A., Wig, N., Sharma, N., Haque, A., Gupta, N., Baquer, N. Z. and Mukhopadhyay, G. (2004). Susceptibility pattern and molecular type of species-specific *Candida* in oropharyngeal lesions of Indian human immunodeficiency virus-positive patients. *Journal of Clinical Microbiology* **42(3), 1260-1262.**

Lehrer, R. I. (1975). The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms. *Journal of Clinical Investigation* **55(2), 338-346.**

Lupetti, A., Tavanti A., Davini, P., Ghelardi, E., Corsini, V., Merusi, I., Boldrini, A., Campa, M. and Senesi, S. (2002). Horizontal transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. *Journal of Clinical Microbiology* **40(7), 2363-2369.**

MacKeown, J. M. and Faber, W. M. (2004). Frequency of food items consumed by young rural and urban African children -- Essential knowledge to provide dietary advice in caries prevention. *International Dental Journal* **54(5), 284-290.**

Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M. T. Olaizola, C. and Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases* **8(1), 39-45.**

Magaldi, S., Mata, S., Hartung, C., Verde, G., Deibis, L., Roldán, Y. and Marcano, C. (2001). *In vitro* susceptibility of 137 *Candida* sp. isolates from HIV positive patients to several antifungal drugs. *Mycopathologia* **149, 63-68.**

Maki, Y., Fujisaki, M., Sato, Y. and Sameshima, H. (2017). *Candida* chorioamnionitis leads to preterm birth and adverse fetal-neonatal outcome. *Infectious Diseases in Obstetrics and Gynecology* **2017, Article ID 9060138.**

Maródi, L., Korchak, H. M. and Johnson, R. B. (1991). Mechanisms of host defense against *Candida* species. I. Phagocytosis by monocytes and monocyte-derived macrophages. *Journal of Immunology* **146, 2783-2789.**

Mathema, B., Cross, E., Dun, E., Park, S., Bedell, J., Slade, B., Williams, M., Riley, L., Chaturvedi, V. and Perlin, D. S. (2001). Prevalence of vaginal colonization by drug-resistant *Candida* species in college-age women with previous exposure to over-the-counter azole antifungal. *Clinical Infectious Diseases* **33, e23-e27.**

Milan, E. P., Kallás, E.G., de O. Costa, P. R., Da Matta, D. A. and Lopes Colombo, A. (2001). Oral colonization by *Candida* spp. among AIDS household contacts. *Mycoses* **44(7-8), 273-277.**

Mohamadi, J. and Motaghi, M. (2014). Anti-fungal resistance in *Candida* isolated from oral and diaper rash candidiasis in neonates. *Bioinformation* **10 (11), 667-670.**

Morschhäuser, J. (2002). The genetic basis of fluconazole resistance development in *Candida albicans*. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1587(2-3), 240-248.**

Müller, F. M., Kasai, M., Francesconi, A., Brillante, B., Roden, M., Peter, J., Chanock, S. J. and Walsh, T. J. (1999). Transmission of azole-resistant isogenic strain of *Candida albicans* among human immunodeficiency virus-infected family members with oropharyngeal candidiasis. *Journal of Clinical Microbiology* **37(10), 3405-3408.**

Mulu, A., Kassu, A., Anagaw, B., Moges, B., Gelaw, A., Alemayehu, M., Belyhun, Y., Bladglegne, F., Hurissa, Z., Moges, F. and Isogai, E. (2013). Frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients in northwest Ethiopia. *BMC Infectious Diseases* **13, 82.**

Naveen, S., Asha, M. L., Shubha, G., Bajoria, A. A. and Jose, A. A. (2014). Salivary flow rate, pH and buffering capacity in pregnant and non-pregnant women - A comparative study. *JMED Research* **2014, Article ID 506946.**

Nugraha, A. P., Ernawati, D. S., Parmadiati, A. E., Soebadi, B., Triyono, E. A., Prasetyo, R. A., Utami, S. B. and Sosiawan, A. (2018). Prevalence of *Candida* species in oral candidiasis and correlation with CD4+ count in HIV/AIDS patients at Surabaya, Indonesia. *Journal of International Dental and Medical Research* **11(1), 81-85.**

Nweze, E. I. and Ogbonnaya, U. L. (2011). Oral *Candida* isolates among HIV-infected subjects in Nigeria. *Journal of Microbiology, Immunology and Infection* **44(3), 172-177.**

Ocan, M., Obuku, E. A., Bwanga, F., Akena, D., Richard, S., Ogwal-Okeng, J. and Obua, C. (2015). House-hold antimicrobial self-medication: A systematic review and meta-analysis of the burden, risk factors and outcomes in developing countries. *BMC Public Health* **15, 742.**

Osaigbovo, I. I., Lofor, P. V. and Oladele, R. O. (2017). Fluconazole resistance among oral *Candida* isolates from people living with HIV/AIDS in a Nigerian tertiary hospital. *Journal of Fungi* **3(4), 69.**

Owotade, F. J., Gulube, Z., Ramla, S. and Patel, M. (2016). Antifungal susceptibility of *Candida albicans* isolated from the oral cavities of patients with HIV infection and cancer. *South African Dental Journal* **71(1), 8-11.**

Patel, M., Shackleton, J. T. and Coogan, M. M. (2006). Effect of antifungal treatment on the prevalence of yeasts in HIV-infected subjects. *Journal of Medical Microbiology* **55(9), 1279-1284.**

Pendleton, K. M., Huffnagle, G. B. and Dickson, R. P. (2017). The significance of *Candida* in the human respiratory tract: Our evolving understanding. *Pathogen and Diseases* **75(3), ftx029.**

Pfaller, M. A., Diekema, D. J., Rinaldi, M. G., Barnes, R., Hu, B., Veselov, A. V., Tiraboschi, N., Nagy, E., Gibbs, D. L. and the Global Antifungal Surveillance Group. (2005). Results from the ARTEMIS DISK global antifungal surveillance study: A 6.5-year analysis of susceptibilities of *Candida* and other yeast species to fluconazole and voriconazole by standardized disk diffusion testing. *Journal of Clinical Microbiology* **43(12), 5848-5859.**

Pizzo, G., Giuliana, G., Milici, M. E. and Giangreco, R. (2000). Effect of dietary carbohydrates on the *in vitro* epithelial adhesion of *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. *The New Microbiologica* **23(1), 63-71.**

Rio, R., Simões-Silva, L., Garro, S., Silva, M., Azevedo, Á. and Sampaio-Maia, B. (2017). Oral yeast colonization throughout pregnancy. *Medicina Oral Patologia Oral y Cirugia Bucal* **22(2), e144-e148.**

Romo, J. A. and Kumamoto, C. A. (2020). On commensalism of *Candida*. *Journal of Fungi* **6(1), 16.**

Sánchez-Vargas, L. O., Ortiz-López, N. G., Villar, M., Moragues, M. D., Aguirre, J. M., Cashat-Cruz, M., Lopez-Ribot, J. L., Gaitán-Cepeda, L. A. and Quindós, G. (2005). Oral *Candida* isolates colonizing or infecting human immunodeficiency virus-infected and healthy persons in Mexico. *Journal of Clinical Microbiology* **43(8), 4159-4162.**

Schmidt-Westhausen, A., Schiller, R. A., Pohle, P. D. and Reichart, P. A. (1991). Oral *Candida* and

Enterobacteriaceae in HIV-1 infection: Correlation with clinical candidiasis and antimycotic therapy. *Journal of Oral Pathology and Medicine* **20(10), 469-472.**

Singh, M. and Chakraborty, A. (2017). Antifungal drug resistance among *Candida albicans* and non-*albicans Candida* species isolates from a tertiary care centre at Allahabad. *Journal of Antimicrobial Agents* **3, 150.**

Sirnivasan, L. and Kenneth, J. (2006). Antibiotic susceptibility of *Candida* isolates in a tertiary care hospital in Southern India. Indian *Journal of Medical Microbiology* **24, 80-81.**

Skrodeniene, E., Dambrauskiene, A. and Vitkauskiene, A. (2006). Susceptibility of yeasts to antifungal agents in Kaunas University of Medicine Hospital. *Medicina (Kaunas)* **42(4), 294-299.**

Talapko, J., Juzbašic, M., Matijevic, T., Pustijanac, E., Bekić, S., Kotris, I. and Škrlec, I. (2021). *Candida albicans* - The virulence factors and clinical manifestations of infection. *Journal of Fungi* **7(2), 79.**

Teanpaisan, R. and Nittayananta, W. (1998). Prevalence of *Candida* species in AIDS patients and HIV-free subjects in Thailand. *Journal of Oral Pathology and Medicine* **27, 4-7.**

Tsang, C. S. and Samaranayake, L. P. (2000). Oral yeasts and coliform in HIV-infected individuals in Hong Kong. *Mycoses* **43, 303-308.**

Tsega, A. and Mekonnen, F. (2019). Prevalence, risk factors and antifungal susceptibility pattern of *Candida* species among pregnant women at Debre Markos Referral Hospital, Northwest Ethiopia. *BMC Pregnancy and Childbirth* **19(1), 527.**

Tseng, Y. H., Lee, W. T. and Kuo, T. C. (2005). *In vitro* susceptibility of fluconazole and amphotericin B against *Candida* isolates from women with vaginal candidiasis in Taiwan. *Journal of Food and Drug Analysis* **13(1), 12-16.**

Tumbarello, M., Taconelli, E., Caldarola, G., Morace, G., Cauda, R. and Ortona, L. (1997). Fluconazole resistant oral candidiasis in HIV- infected patient. *Oral Diseases* **3(Suppl 1), S110-S112.**

UNAIDS, Joint United Nations Programme on HIV/AIDS. (2004). Report on the global HIV/AIDS epidemic. UNAIDS, Geneva.

Vandeputte, P., Ferrari, S. and Coste, A. T. (2012). Antifungal resistance and new strategies to control fungal infections. *International Journal of Microbiology* **2012, Article ID 713687.**

Vartivarian, S. and Smith, C. B. (1993). Pathogenesis, host resistance and predisposing factors. *In*: Candidiasis: Pathogenesis, Diagnosis, and Treatment. Bodey, G. P. (ed.). Raven Press, New York. **pp. 59-84.**

Vazquez, J. A., Sanchez, V., Dmuchowski, C., Dembry, L. M., Sobel, J. D. and Zervos, M. J. (1993). Nosocomial acquisition of *Candida albicans*: An epidemiologic study. *Journal of Infectious Diseases* **168, 195-201.**

Vidya, K. M., Rao, U. K., Nittayananta, W., Liu, H. and Owotade, F. J. (2016). Oral mycoses and other opportunistic infections in HIV: Therapy and emerging problems – A workshop report. *Oral Diseases* **22(Suppl 1), 158-165.**

Whaley, S. G., Berkow, E. L., Rybak, J. M., Nishimoto, A. T., Barker, K. S. and Rogers, P. D. (2017). Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. *Frontiers in Microbiology* **7, 2173.**

Xiao, J., Fogarty, C., Wu, T. T., Alkhers, N., Zeng, Y., Thomas, M., Youssef, M., Wang L. Cowen, L. Abdelsalam, H. and Nikitkova, A. (2019). Oral health and *Candida* carriage in socioeconomically disadvantaged US pregnant women. *BMC Pregnancy and Childbirth* **19(1), 480.**

Yapar, N. (2014). Epidemiology and risk factors for invasive candidiasis. *Therapeutics and Clinical Risk Management* **10, 95-105.**

Zegarelli, D. J. (1993). Fungal infections of the oral cavity. *Otolaryngologic Clinics of North America* **26(6), 1069-1089.**

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