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Identification and Antifungal Susceptibility Pattern of *Candida* Species Causing Oral Thrush and Vaginal Candidiasis

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ABSTRACT

Candidiasis: A disease, which though common yet often neglected, has caused more havoc than reported and with the increase in antimicrobial resistance, antifungal agents that are more effective are to be used. The aim of this work is to identify *Candida* species causing oral thrush and vaginal candidiasis in Awka, Nigeria and evaluate the effect of Fluconazole and Nystatin in-vitro against them. Using standard microbiological tests in identification, 80 samples (43.24%) were positive for *Candida* out of the 185 samples gotten from the study subjects. *Candida albicans* (55%), *C.tropicalis* (35%) and *C.glabrata* (10%) were isolated from the oral cavity while *Candida albicans* (35%), *C.krusei* (31.67%), *C.tropicalis* (15%), *C.dublinensis* (8.33%), *C.glabrata* (5.00%), and *C.parapsilosis* (5.00%) were isolated from the vagina. Fluconazole (50µl) and Nystatin (100 Units) were employed in the anticandidal sensitivity test using agar well diffusion method. More susceptibility to Nystatin than Fluconazole was recorded. From the oral cavity, *C.tropicalis* was the most susceptible while *C.glabrata* and *C.parapsilosis* were the most susceptible species from the HVS samples. This reveals the increase in isolation of non-albicans *Candida* (NAC) and their growing resistance to Fluconazole which is commonly used hence the need to employ Nystatin as a first line drug for the treatment of oral thrush and vaginal candidiasis.

KEYWORDS: *Oral thrush; Vaginal candidiasis; Fluconazole; Nystatin; Candida species*

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INTRODUCTION

"Candidiasis is caused by pathogenic yeasts, *Candida species*, which are opportunistic pathogens present in the normal microbiome" (Somanon *et al.,* 2020). *C. albicans* stands out as the most common causative species but non-albicans *Candida* (NAC) such as *C. lusitaniae, C. tropicalis, C. krusei,* etc. are beginning to appear as colonizers and pathogens that can cause superficial and systemic infections (Karin *et al.,* 2013).

One of the most prevalent fungal infections that affect the oral mucosa is oral candidiasis (Singh *et al.,* 2014) caused mainly by *Candida albicans* which though a normal flora of the mouth have been isolated from the mouths of about 60% of dentate patients over 60 years old (Arya and Rafiq, 2020) and implicated for the infection. It is mostly prevalent among people whose immune system has been compromised (Alhamzi and Al.Maqtari 2018) and more than 90% of immunocompromised persons, which are HIV patients, present with oral candidiasis. Oral candidiasis is mainly of three types, which are erythematous, pseudomembranous, and chronic hyperplastic candidiasis (Raesi *et al.,* 2019). White patches on the throat, tongue, and other areas in the oral cavity are distinguishing features of oral candidiasis (Raesi *et al.,* 2019) and amongst its symptoms are soreness and difficulty in swallowing. It has been reported that the co-adhesion of *C. albicans* to other bacteria in the oral cavity and the various synergistic relationships developed enables its persistence and intensifies its colonization in the host (Taissa *et al.,* 2020).

Vulvovaginal candidiasis (VVC) another type of candidiasis affects the lower genital tract of approximately 75% of women of childbearing age and has remained a common problem worldwide despite therapeutic advances (Ezeadila *et al.,* 2020; Sobel, 2007). Its symptoms include burning, genital

itching, and a white "cottage cheese-like" discharge. It is more common in people who are immunocompromised (e.g. with diabetes mellitus), during pregnancy and when using oral contraceptives (Arya and Rafiq, 2020).

Candida infections are treated with antifungal medications mainly from the azoles and polyenes families (Fang *et al*., 2020). The less side effects and oral availability of itraconazole and fluconazole, encourages their use in the treatment and chemoprophylaxis of systemic fungal infections (Livermore, 2004). Polyenes (Amphotericin B and Nystatin), due to their fungicidal nature stands as the drug of choice in treating superficial and mucosal candidiasis (Miranda-Cadena *et al.,* 2018) though Amphotericin B is rarely used due to its nephrotoxicity. However, the level of resistance to these drugs is increasing. Some *Candida* species such as *C. glabrata* and *C. krusei*, are naturally resistant to fluconazole and the majority of fluconazole-sensitive isolates of *C. dubliniensis* encodes multidrug transporters that mediate fluconazole resistance during clinical therapy (Karin *et al.,* 2013). There is a need to determine the efficacy of top used antifungal drugs to ascertain the most effective agent in certain geographical areas.

MATERIALS AND METHODS

Study Area

The study was carried out in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra state, Nigeria, after been approved by the appropriate ethical committee. Sample size of 185 clinical specimens from the oral cavity and high vaginal swabs (HVS) samples were used. Oral swab specimens were taken from patients (male and female) who presented with clinical symptoms of oral thrush aged 18-45 years while HVS were taken from female patients who presented with clinical symptoms of vaginal candidiasis aged 18 – 45 years with sterile swab sticks. Both were separately processed.

Identification of Isolates

All samples were cultured on SDA supplemented with 0.05 $mgml^{-1}$ of chloramphenicol, and then were incubated at 37ºC for 24-48 hrs. Yeast growths were identified by characteristic colonial morphology of *Candida* on SDA. Pure cultures were then subjected to Gram stain, germ tube test, culture on CHROMagar *Candida* medium and urease test for confirmative species identification. A positive germ tube test is confirmatory for *Candida albicans* provided the set guidelines be followed.

Antifungal Susceptibility Testing

The antifungal agents used were fluconazole (50µg/ml) and Nystatin (100 units) and the test was done according to the agar well diffusion method described by Perez *et al.,* (1990). 50µg/ml of Fluconazole was prepared from dilution of the powder and commercially prepared 100 units Nystatin was used. To standardize the inoculum, 24hrs old pure culture grown on SDA was dissolved in normal saline and adjusted to 0.5 McFarland standard as described by Cheesbrough, (2010). This suspension was then cultured on Muller Hinton agar. The resulting inhibition zone diameters (IZDs) after 24 hours were measured and recorded, as susceptible (S), Susceptible Dose Dependent (SDD), or resistant (R) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2009).

RESULTS

Isolation and identification of *Candida* species

A total number of 80 samples (60 HVS and 20 oral cavity specimens) were positive for *Candida* spp. The prevalence rate of candidal colonization of the vagina and oral cavity was 60% and 23.5% respectively. Using standard conventional methods in identification and characterization of the *Candida* isolates, results as shown in Table 1 revealed that *Candida albicans* was prominent in both anatomical sites. The NAC were also well isolated.

Table 1: Distribution of *Candida* Species

Oral Cavity		
Candida species	**Frequency**	**Percentage (%)**
Candida albicans	11	55
Candida tropicalis	7	35
Candida glabrata	2	10
Total	**20**	**100**
HVS		
Candida albicans	21	35
Candida krusei	19	31.67
Candida tropicalis	9	15
Candida dublinensis	5	8.33
Candida glabrata	3	5
Candida parapsilosis	3	5
Total	**60**	**100**

On Sabouraud dextrose agar (SDA) medium, the *Candida* isolates had circular form, raised elevation, entire margin/border, and creamy smooth surfaces. They were Gram positive and their morphology under the microscopic can be seen in Fig 1 and Fig. 2. Some showed blastoconidia with multilateral budding and some pseudohyphae. Other identification results are shown in Table 2 and Fig. 3 and 4.

Table 2: Distinctive Growth Features of *Candida* species

Chromagar Candida	Germ Tube Test	Urease Test	Candida Species Identified
Leaf Green	+	_	Candida albicans
Blue	_	_	Candida tropicalis
Dark Green	_	_	Candida dublinensis
Pink	_	+	Candida krusei
Purple	_	_	Candida glabrata
Off-white/Creamy	_	_	Candida parapsilosis

Key: + = Positive; - = Negative

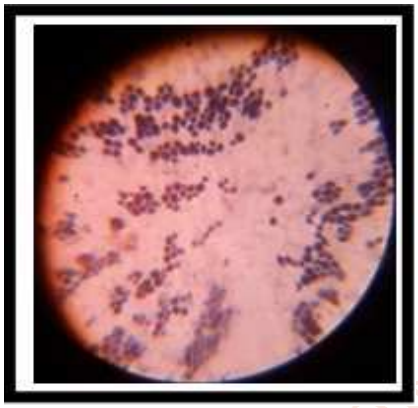

Fig. 1: Round to ovoid shape of Gram stained *Candida tropicalis*

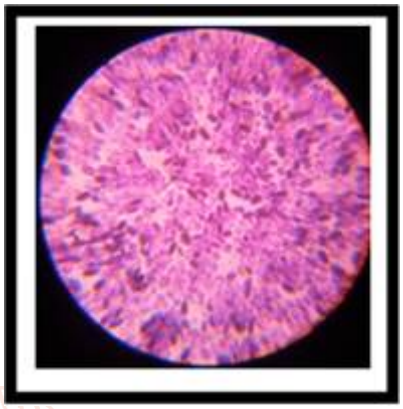

Fig. 2: Ellipsoidal shape of Gram stained *Candida albicans*

Fig 3: *Candida glabrata* (glossy purple) and *Candida krusei* (pink) on Chromagar

Fig. 4: *Candida tropicalis* on Chromagar

Antifungal Susceptibility Testing

The *Candida* isolates were more susceptible to Nystatin than Fluconazole in both samples. From the oral cavity, *Candida tropicalis* was the most susceptible to Nystatin (57.14%) as seen in Table 3. *Candida glabrata* and *Candida parapsilosis* were the most susceptible *Candida* species from HVS to Nystatin with 66.67% each (Table 4). High level of resistance of up to 100% by *Candida glabrata* and 94.74% by *Candida krusei* was encountered by Fluconazole.

Table 3: Invitro Susceptibility Profile of Selected *Candida* Isolates from the oral cavity to Fluconazole and Nystatin

DRUGS Fluconazole	Candida albicans	Candida tropicalis	Candida glabrata	Total (%)
S (%)	3(27.27)	2(28.57)	0(0)	5(25)
SDD (%)	2(18.18)	1(14.29)	0(0)	3(15)
R (%)	6(54.55)	4(57.14)	2(100)	12(60)
Nystatin				
S (%)	5(45.45)	4(57.14)	1(50)	10(50)
SDD (%)	4(36.36)	1(14.29)	1(50)	6(30)
R (%)	2(18.18)	2(28.57)	0(0)	4(20)

KEY: S-Susceptible, SDD-Susceptible Dose Dependent, R-Resistant

Table 4: Invitro Susceptibility Profile of Selected *Candida* Isolates from the Vagina to Fluconazole and Nystatin

DRUGS Fluconazole	Candida albicans	Candida krusei	Candida tropicalis	Candida dublinensis	Candida glabrata	Candida parapsilosis	Total (%)
S (%)	3(14.29)	0(0)	2(22.22)	0(0)	2(66.67)	3(100)	10(16.67)
SDD (%)	4(19.05)	1(5.26)	2(22.22)	2(40)	1(33.33)	0(0)	10(16.67)
R (%)	14(66.67)	18(94.74)	5(55.56)	3(60)	0(0)	0(0)	40(66.67)
Nystatin							
S (%)	10(47.62)	10(52.63)	4(44.44)	2(40)	2(66.67)	2(66.67)	30(50%)
SDD (%)	5(23.81)	4(21.05)	2(22.22)	1(20)	1(33.33)	1(33.33)	14(23.33)
R (%)	6(28.57)	5(26.32)	3(33.33)	2(40)	0(0)	0(0)	16(26.67)

KEY: S-Susceptible, SDD-Susceptible Dose Dependent, R-Resistant

DISCUSSION

More than 90% of immunocompromised persons, which are HIV patients, present with oral candidiasis (Raesi *et al.,* 2019) and also prevalent in this group is vaginal candidiasis which is currently ranked second in the most common infections of the vagina, next to bacterial vaginosis (Marcia *et al.,* 2010). In this study, the prevalence of oral candidiasis was 23.5%, which is in harmony with the findings of Ellen *et al.,* (2021) who reported a rate of 20.7%. However, Ngwa *et al.,* (2020) and Jasim *et al.,* (2016) had higher prevalence rates. While the prevalence rate of vaginal *Candida* colonization in this study was 60%, which is in similar range with the results of Umar *et al.,* (2017) and Elfeky *et al*. (2015).

Candida albicans was the most frequently isolated species from both anatomical sites; closely followed by *C.tropicalis* (35%) in the oral cavity and *C.krusei* from HVS. This aligned with the work of Nejad *et al*., (2011), Wemedo and Duke (2020) and Shnawa *et al*. (2018). The predominance of *Candida albicans* in oral and vaginal candidiasis could be a result of the numerous virulence factors, which the species possess, amongst which are adhesins, the ability to form biofilms, hydrolytic enzymes and its dimorphic nature (Ngwa *et al*., 2020). In addition, is its higher degree of adherence to epithelial cells in the vagina than other species (Ezeadila *et al.,* 2020). On the other hand, isolation of NAC are more frequent than before as seen in this study and that of other researchers like Shrivastav *et al.* (2015), Amutaigwe *et al.,* (2017), etc. which infers that in the near future, VVC might be caused by mainly NAC and this calls for urgent attention. The core reasons for this drift might be frequent use of over the counter drugs, self-medication, and use of single dose antifungal medication (Emeribe *et al.,* 2015). The indiscriminate usage of these antifungal agents clears off *Candida albicans*, which appears to be more sensitive than other species thereby selecting and causing a rise in the trend of isolating NAC species (Ezeadila *et al.,* 2020).

Antifungal susceptibility testing in this study revealed that Nystatin was the more active drug against the *Candida* species isolated from the oral cavity and HVS. Though some species were sensitive to Fluconazole, Nystatin had a higher susceptibility rate of 50% each in both sites against the 25% and 16.67% (from the oral cavity and HVS respectively) recorded for Fluconazole. *Candida parapsilosis,* which was the most susceptible (100%) species to Fluconazole, also showed zero resistance to Nystatin likewise *Candida glabrata* thereby correlating with the findings of Kiguli *et al.,* (2015) and Gandhi *et al.* (2015). *Candida glabrata* (100%) and *C.krusei* (94.74%) were the most resistant species from the oral cavity and vagina respectively to Fluconazole in agreement with other studies (Miranda *et al.,* (2018), Ezeadila *et al.,* (2020) and Kiguli *et al.,* (2015)). While *C.tropicalis* (28.57%) and *C.dublinensis* (40%) were the most resistant species from the oral cavity and vagina respectively to Nystatin. The low percentage of resistance to Nystatin could be because of the fungicidal action exhibited by them when they bind to the pathogens, which are irreversible (Rati *et al*., 2015). More than half of the isolates in this study were resistant to Fluconazole especially the NAC, *Candida glabrata, Candida krusei and Candida dublinensis,* which showed little or no susceptibility to it. The results of Miranda *et al.,* (2018), Costa *et al.,* (2006) and Ngwa *et al.,* (2020) revealed similar pattern as all *Candida* isolates worked on were susceptible to Nystatin and resistance by both *Candida albicans* and NAC to Fluconazole was observed. Khan *et al.,* (2018) and Mohamadi *et al.,* (2015) also recorded high resistance of 62% and 76% respectively to Fluconazole by *Candida* species from HVS samples. This high level of resistance mainly by NAC (some intrinsically resistant) to Fluconazole is of utmost concern to health practitioners because Fluconazole is the most used antifungal agent for treatment of candidiasis, especially oral candidiasis, in developing countries (Mushi et al., 2016). Though the key causative agent of oral candidiasis has been

recognized as *Candida albicans*, the narrative is changing and NAC are increasingly been isolated from the oral cavity and showing resistance, which is because of the decrease in susceptibility to existing antifungal drugs (Miranda *et al*., 2018). According to Li *et al*., (2007), "*C. glabrata* was not seen as an important oral pathogen, but due to its opportunistic nature, and resistance to azoles, it is becoming increasingly involved in oral candidiasis, especially in HIV and cancer patients".

Conclusion

From this study, it can be concluded that *Candida albicans* remains the most isolated aetiologic agent of oral thrush and vaginal candidiasis though unlike before more non-albicans *Candida* are now been isolated. The increase in resistance by these species to Fluconazole, a top choice for prophylaxis and treatment of candidiasis, especially by the NAC is alarming therefore; it is recommended that Nystatin be prescribed in its stead. In addition, over the counter drugs used without proper diagnosis and the doctor's prescription should be discouraged and adequate antifungal testing should be carried out before treatment. This curtails resistance and retains the effectiveness of the few antifungal agents we have.

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