

Identification of Yeast Associated with Oral Candidiasis among Patients Attending Image Diagnostic Centre Port Harcourt, Rivers State, Nigeria

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Abstract:- Oral candidiasis is a fungal infection that occurs in the mucous membranes of the mouth. It is caused by yeast called *Candida*. This study was carried out to isolate and identify *Candida* species from the buccal mucosa and the dorsal and ventral surfaces of the tongue and teeth of patients attending Image Diagnostic Centre, Port Harcourt from May-July 2017. A total of 50 specimens were collected from patients attending the hospital. The specimens were studied by culture on Sabouraud dextrose agar plates. Identification was by Lactophenol staining technique and Biochemical tests using Periodic-acid Schiff stain. Antifungal susceptibility test using disc diffusion method was also done. The antifungal used for susceptibility test was Nystatin, Tinidazole and Fluconazole. Data were subjected to Analysis of variance (ANOVA). Out of 50 specimens, 28 patients were infected while 22 patients were non-infected. The highest number of patients infected was within the age interval of ≤ 20 years 10(20%) while the lowest was within the age interval of 21-23 years 3(6%). The total number of infected male and female patients was 13(46.4%) and 15(53.6%) respectively. The organisms isolated were *Candida albicans* 11(39.3%), *Candida krusei* 9(32.1%) and *Candida glabrata* 8(28.6%). *C. albicans* was susceptible to Nystatin with 11(39.3%) and resistant to Fluconazole and Tinidazole. *C. krusei* was susceptible to Fluconazole and Nystatin with 6(21.4%) and 3(10.7%) respectively and *C. glabrata* was susceptible to Nystatin and Tinidazole with 5(17.9%) and 3(10.7%) respectively. From the statistical analysis, P- value was >0.05 (.054, .022 and .008) hence, we conclude there is no significant difference in the antifungal susceptibility. P-value also showed >0.05 (.245 and .295) hence we conclude there is no significant different in the age distribution of *Candida* species isolated from oral swab while P-value >0.05 (.465 and .553) was obtained hence we conclude there is no significant different in the sex distribution of *Candida* species isolated from oral swab. Nystatin was found to be more effective in treatment of fungal infection caused by *Candida* species. Personal oral hygiene should be improved in order to reduce the risk of fungal infection which could lead to death if not properly managed.

Keywords:- Yeast, Oral candidiasis, Antifungal susceptibility test.

I. INTRODUCTION

Oral candidiasis also known as oral thrush, oropharyngeal candidiasis (James *et al.*, 2006) is a yeast infection that occurs in the mouth; most commonly encountered in general dental practice. However, it is not contagious and is usually successfully treated with antifungal medication. Oral thrush rarely causes complications but may cause opportunistic infections under various conditions. The condition can worsen with only weakened immune systems. Oral candidal infection usually involves compromised host immunity. The predisposing factors for oral candidosis may be local; involving poor oral hygiene, denture wearing or systemic such as leukemia, diabetes, malignancy, HIV, nutritional deficiency or medical treatments (Krishnan, 2012; Kundu *et al.*, 2012; Ahn *et al.*, 2014; Darteville *et al.*, 2018).

It is caused by *Candida* species, a normal flora in the oral cavity. However, when the fungus begins to grow uncontrollably, an infection can develop in the mouth. There different species of *Candida* implicated in oral candidiasis, but *Candida albicans* is the most frequently isolated species in humans (Delgado *et al.*, 2009; Hise *et al.*, 2009; Junqueira *et al.*, 2012; Li *et al.*, 2013). There are also significant increase in the prevalence of infections caused by species of *Candida* other than *C. albicans* such as *C. krusei*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* (Sant'Ana *et al.*, 2002; Li *et al.*, 2013; Kaur *et al.*, 2016). In humans, oral candidiasis is the most common form of candidiasis (Anil and Shubhangi, 2010). It is the most common fungal infection of the mouth (Bouquot *et al.*, 2002) and also represents the most common opportunistic oral infection in humans (Lalla *et al.*, 2013) with lesions occurring only when the environment favours pathogenic behaviour.

Oropharyngeal candidiasis is a very common oral sign in individuals with HIV and cancer care (Yamada and Alpers, 2009; Epstein *et al.*, 2012). The progression of oral candidiasis is often faster and more severe in patients with AIDS due to immunodeficiency and the emergency of antifungal resistance among *Candida* species isolates.

The identification of *Candida* spp. is essential since the pathogenicity profile and sensitivity to a particular antifungal agent vary between different species (Costa *et al.*, 2009; Negri *et al.*, 2009). With the increasing use of antifungal agents in the treatment of oral candidiasis in hospitals, other health sectors and indiscriminate use by individuals, these seem to have contributed to the development and spread of antifungal-resistant strains and thus constituting a major public health issue (Darteville *et al.*, 2018). Due to the differences that has been observed in the behaviour of *Candida* spp. and the increasing number of resistant strains to current antifungal therapies, there is need for continuous antifungal susceptibility testing to monitor the resistance of these organisms (Marcos-Arias *et al.*, 2012; Li *et al.*, 2013; Idelevich *et al.*, 2014). Therefore, this study was carried out to identify and determine the antifungal susceptibility pattern of *Candida* species from the buccal mucosa and the dorsal and ventral surfaces of the tongue and teeth of patients attending Image Diagnostic Centre, Port Harcourt.

II. MATERIALS AND METHODS

➤ *Specimen Collection and isolation*

A total of fifty (50) oral swab specimens were collected randomly from the patients. The label on each of the sterile swab stick contained their data such as name, sex, age and location. The specimen was collected by gently rubbing a sterile cotton swab stick over the buccal mucosa and the dorsal and ventral surfaces of the tongue and teeth. The swab was used to inoculate a plate of Sabouraud dextrose agar and was streaked out using wire loop. The plates were sealed tightly and incubated at room temperature of 25°C for 3 days.

➤ *Characterization of isolates*

The oral isolates were identified based on their colonial morphology; Lactophenol stain and Periodic acid–Schiff stain (Carson *et al.*, 2009).

➤ *Antifungal Susceptibility Test*

Disk diffusion method described by Magaldi *et al.* (2004) was used. The antibiotics used were Nystatin, fluconazole and tinidazole. The antibiotics solution was prepared by dissolving 25mg of Fluconazole, 250mg of Nystatin and 250mg of Tinidazole in 100ml of sterile water respectively. The disks prepared and labelled N, F and T were dipped into nystatin, fluconazole and tinidazole solutions respectively using sterile forceps for 10 minutes. The disks were dried in hot air oven. The surface of Sabouraud dextrose agar was streaked using swab stick containing the inoculum. The disks were placed on the inoculated plates using sterile forceps. The plates were incubated for 48hrs at 37°C to determine the zone of inhibition and recorded.

➤ *Data Analysis*

The data were analyzed using Analysis of variance (ANOVA) to determine the significant difference at 95% confidence limit in the occurrence of fungi in the oral cavity.

III. RESULTS

In this study, 50 patients were examined; of which 56% of the patients were infected with *Candida* species. The highest number of patients infected was within the age interval of ≤ 20 years 10(20%) while the lowest was within the age interval of 21-23 years 3(6%) as shown in Table 1.

Age Interval	% Infected	% Non- Infected	Total
≤20	10(20)	7(14)	17(34)
21-23	3(6)	4(8)	7(14)
24-26	6(12)	2(4)	8(16)
27-29	5(10)	3(6)	8(16)
29-32	4(8)	6(12)	10(20)
Total	28(56)	22(44)	50(100)

Table 1:- Distribution of *Candida* species isolated from buccal mucosa and the dorsal and ventral surfaces of the tongue and teeth of patients attending Image Diagnostic Centre, Port Harcourt based on age.

Key
%- Percentage

Table 2 shows the percentage distribution of *Candida* species isolated from buccal mucosa and dorsal and ventral surfaces of the tongue and teeth of the patients by sex. The total number of infected male and female patients was 13(46.4%) and 15(53.6%) respectively. The females were more infected than the males.

Sex	% Infected	% Non-Infected	Total
Female	15(30)	10(20)	25(50)
Male	13(26)	12(24)	25(50)
Total	28(56)	22(44)	50(100)

Table 2:- Percentage distribution of *Candida* species isolated from buccal mucosa and the dorsal and ventral surfaces of the tongue and teeth of the patients attending Image Diagnostic Centre, Port Harcourt by sex.

Key
%- Percentage

Table 3 shows the percentage occurrence of *Candida* species by culture among patients attending Image Diagnostic Centre, Port Harcourt. A total of 28 *Candida* species were isolated. The species recovered were *Candida albicans* 11(39.3%), *Candida krusei* 9(32.1%) and *Candida glabrata* 8(28.6%).

Candida species	No of Occurrence	% Occurrence
<i>Candida albicans</i>	11	39.3
<i>Candida krusei</i>	9	32.1
<i>Candida glabrata</i>	8	28.6
Total	28	100

Table 3:- Percentage occurrence of *Candida* species by culture among patients attending Image Diagnostic Centre, Port Harcourt

Key
%- Percentage

Table 4 shows the percentage antibiotic susceptibility pattern of *Candida* species isolated from buccal mucosa and dorsal and ventral surfaces of the tongue and teeth of the

patients. *C. albicans* was susceptible to Nystatin with 11(57.9%) and resistant to Fluconazole and Tinidazole. *C. krusei* was susceptible to Fluconazole and Nystatin with 6(31.6%) and 3(15.8%) respectively and *C. glabrata* was susceptible to Nystatin and Tinidazole with 5(26.3%) and 3(15.8%) respectively.

Organism	Tinidazole		Fluconazole		Nystatin	
	NS%	NR%	NS%	NR%	NS%	NR%
<i>Candida albicans</i>	0(0)	0(0)	0(0)	0(0)	11(57.9)	0(0)
<i>Candida krusei</i>	0(0)	0(0)	6(31.6)	0(0)	3(15.8)	0(0)
<i>Candida glabrata</i>	3(15.8)	0(0)	0(0)	0(0)	5(26.3)	0(0)
Total	3(15.8)	0(0)	6(31.6)	0(0)	19(100)	0(0)

Table 4:- Percentage Antibiotic susceptibility Patterns of *Candida* species isolated from patients attending Image Diagnostic Centre, Port Harcourt.

Key
%- Percentage
NS- Number Susceptible
NR- Number Resistant



Candida albicans



Candida glabrata

Fig 1:- Culture plates showing *Candida* colonies



Candida krusei

IV. DISCUSSION

Candidiasis is the most common oral fungal infection diagnosed in humans. This may result due to immune system dysfunction or as a result of local or systemic medical treatment (Muzyka, 2005; Krishnan, 2012). *Candida* species are one of the significant clinical pathogens implicated in most fungal infections; of which *Candida albicans* is the most significant pathogenic species associated with oral thrush but other species such as *Candida tropicalis*, *Candida krusei*; also known to be pathogenic have been implicated.

This present study revealed the presence *Candida albicans*, *C. krusei*, and *C. glabrata* to cause oral candidiasis which agrees with the work by done Marcos-Arias *et al.*, (2012) and Sanitá *et al.*, (2013). The prevalent of the *Candida* species was observed to be (56%); however, *Candida albicans* was found to be most occurring and predominant species which concur with other studies (Nejad *et al.*, 2011; Mbakwem-Aniebo *et al.*, 2012 and Kaur *et al.*, 2016).

Although, oral candidiasis can affect patients at any age but common among infants, the elderly and those with weak immune systems (Kuhn *et al.*, 2004; Mishra *et al.*, 2007) but in this study, it was mostly seen with people of ≤ 20 years of age. This could be as a result of poor oral hygiene as the rate of oral infections caused by *Candida* species is mainly attributed to individual's personal hygiene.

The susceptibility pattern of the isolates showed all the *Candida* species 19(100%) were susceptible to Nystatin. *C. albicans* was susceptible to Nystatin, *C. krusei* was susceptible to Fluconazole and Nystatin while *C. glabrata* was susceptible to Nystatin and Tinidazole. However, Nystatin was found to be more effective in treatment of fungal infection caused by the *Candida* species (Kundu *et al.*, 2012; Ahn *et al.*, 2014).

V. CONCLUSION

The presence of these pathogenic organisms is an indication of a potential health risk and should be treated as such. Therefore, it is very vital to Practice good oral hygiene to reduce the risk of oral candidiasis. Oral hygiene with the use of chlorhexidine mouth wash can help prevent oral candidiasis especially with people that have a weakened or poor immune function. From this study, the rate of resistance of *Candida* species to antifungal agents shows the prevalence of resistance is increasing, and as such appropriate antifungal therapy should be employed for the treatment of these infections. There is also need for continuous surveillance of antifungal susceptibility pattern of *Candida* species to evaluate and monitor the current trend of antifungal resistance; and to prevent fungal emergence resistance strains thus, providing a guide in the therapeutic choice and the clinical treatment.

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