

Original Research Article

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## Species Identification and Antifungal Susceptibility Profile of *Candida* Isolates Obtained from Oral Lesions in Patients Attending Outpatient Department of Academic Dental Hospital

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### ABSTRACT

*Candida* species is the only fungal pathogen that causes variety of afflictions that ranges from superficial mucosal infections to life-threatening disseminated mycoses. Oral candidiasis is a common fungal infection caused by an overgrowth or infection due to *Candida* spp. *Candida albicans* is considered as the primary etiology of various clinical types of candidiasis including oral lesions. However, in recent years research studies have highlighted the greater recognition of non-*albicans Candida* (NAC) spp. The present study was conducted with an aim to study species distribution and antifungal susceptibility profile of *Candida* isolates obtained from oral lesions. HIV infection, diabetes, dentures and malignancy were main predisposing factors. *C. albicans* (79.8%) was the predominant isolate. NAC spp. were isolated from 21(20.2%) cases. Fluconazole resistance was observed in 9.6% of *C. albicans* whereas 23.8% of NAC spp demonstrated resistance to fluconazole. From this study, it can be concluded that, although an epidemiological shift towards non *albicans Candida* species is noted in recent years, *C. albicans* still remains the pervasive pathogen. Antifungal susceptibility testing of *Candida* isolates is extremely important for selection of most appropriate therapeutic agent.

#### Keywords

Antifungal resistance, *Candida albicans*, Oral candidiasis

#### Article Info

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### Introduction

The incidence of fungal infections has dramatically increased worldwide (Razzaghi-Abyaneh *et al.*, 2014). While HIV/AIDS has been an important predisposing factor for the rise, other conditions like malignancies, use of broad spectrum antibiotics, indwelling medical devices and diabetes have also contributed to the increase. Among various

fungal infections, candidiasis has greatest effect due to its frequency and the severity of complications associated with it (Lopez-Martinez 2010).

*Candida* species is the only fungal pathogen that causes variety of afflictions that ranges from superficial mucosal infections to life-threatening disseminated mycoses (Seneviratne *et al.*, 2008; Deorukhkar *et al.*,

2014). Fungi belonging to genus *Candida* are commensals and harmlessly colonize various niches of human body like the oral cavity, gastrointestinal tract, vagina and skin. Under certain circumstances, this “innocuous commensal” is transformed into a disease-causing “parasitic” form. This transition is dependent on both host’s predisposing factors and virulence of infecting strain (Deorukhkar *et al.*, 2014).

Oral candidiasis is a common fungal infection caused by an overgrowth or infection due to *Candida* spp (Akpan and Morgan, 2002). The incidence of oral candidiasis varies as per age and certain predisposing factors. Although, oral candidiasis is rarely fatal, it often leads to local discomfort, dysphagia and alteration in sensation of taste that result in poor nutrition, slow recovery from illness and prolonged hospital stay (Akpan and Morgan, 2002). In most of the cases *Candida albicans* is considered as the primary etiological agent for various clinical types of candidiasis including oral lesions. However, in recent years research studies have highlighted the emergence of non-albicans *Candida* (NAC) spp like *C. tropicalis*, *C. glabrata* and *C. krusei* (Raju and Rajappa, 2011) which have different drug susceptibilities. Species identification of the isolates has therefore, become necessary for initiation of species-directed therapy.

The present study was conducted with an aim to study species distribution and antifungal susceptibility profile of *Candida* isolates obtained from oral lesions.

### **Materials and Methods**

The present study is a part of PhD thesis in the Department of Microbiology, Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram in collaboration with Sinhagad Dental College and Hospital, Pune, Maharashtra, India. The protocol of study was

approved by Institutional Ethics Committee. The study included OPD patients presenting with oral lesions. Informed consent was obtained from all participants.

A total of 2 oral swabs were collected from these patients. Out of these, one swab was used for preparation of smear for Gram staining whereas, other swab was inoculated on Sabouraud dextrose agar (SDA) slope. The SDA slope was incubated at 37°C for 7 days and observed daily for growth of *Candida* spp.

*Candida* spp. produces curdy white, opaque, flat, smooth and pale colored colonies with sweet smell similar to that of ripe apple (Lynch 1994). The *Candida* isolates were identified upto species level as per standard mycological protocol which included germ tube test, sugar fermentation and assimilation tests and growth pattern on CHROM agar *Candida* (Koneman *et al.*, 1985).

The antifungal susceptibility testing was done by disc diffusion method and interpreted according to Clinical Laboratory Standards Institute’s M44-A guidelines (CLSI, 2004). Isolates were tested for antifungal drugs like amphotericin B, fluconazole, ketoconazole and itraconazole. Antifungal discs were procured from Himedia Laboratories Pvt. Ltd Mumbai. Demographic and clinical features of patients were recorded and analyzed.

### **Results and Discussion**

During the study period, a total of 460 patients with oral lesions attended the OPD of dental hospital. Out of these, 364 (79.1%) were males and 96 (20.9%) were female patients. The mean age of patients was 41 years (range: 20-75 years).

A total of 322 (70%) patients were tobacco chewers, cigarette smoking was reported by 9 (1.9%) patients. HIV infection, diabetes,

dentures and malignancy were main predisposing factors. A total of 62 patients with oral lesions were positive for HIV infection. Oral carcinoma was the commonest malignancy seen. Poor oral hygiene was seen in 36 (7.8%) cases.

Leukoplakia, pseudomembranous thrush and angular cheilitis were common oral lesions in patients. In HIV infected individuals, pseudomembranous thrush was the common clinical type of oral lesions.

*Candida* spp. were isolated from 104 (22.6%) patients. The species wise distribution of *Candida* isolates is shown in figure 1. *C. albicans* was the predominant isolate 83 out of 104(79.8%). NAC spp. were isolated from 21(20.2%) cases. They were *C glabrata* 7, *C tropicalis* 7, *C krusei* 3 and *C guilliermondii* 4.

The antifungal susceptibility profile of *Candida* isolates is shown in Table 2. A total of 13 (12.5%) isolates were resistant to fluconazole. Fluconazole resistance was observed in 9.6% of *C. albicans* whereas 23.8% of NAC spp demonstrated resistance to fluconazole. However, there was no statistical difference observed between fluconazole resistance between NAC spp. and *C. albicans* (Fischer's exact test,  $P = 0.13$ ). In the present study, a total of 3 *Candida* isolates were SDD (Susceptible dose dependent) to fluconazole.

Itraconazole resistance in *C. albicans* and NAC spp were 4.8% and 4.7% respectively. Ketoconazole resistance was observed only in *C. albicans* isolates. Amphotericin B resistance was noted in 7 (8.4%) *C. albicans* isolates. Among NAC spp. amphotericin B resistance was observed in only *C. tropicalis* isolates

Oral candidiasis is one of the most common clinical presentations of *Candida* reported by clinicians of different specialties worldwide

(Razzaghi-Abyaneh *et al.*, 2014). Oral candidiasis presents in many forms. In the present study leukoplakia, pseudomembranous thrush and angular cheilitis were common oral lesions seen. Leukoplakia due to *Candida* spp. is characterized with white localized patches with irregular borders that are difficult to remove (Lopez-Martinez 2010). Pseudomembranous candidiasis is characterized by white membranes on the oral mucosa and tongue (Samaranayake and Nair, 1995). It is made up of necrotic material and desquamated epithelia invaded by yeast cells and hyphae (Samaranayake and Nair, 1995). In the current study, pseudomembranous thrush was most commonly seen in HIV infected patient. Similar observation was reported by Samaranayake and Nair (Samaranayake and Nair, 1995).

A variety of local and systemic factors are implicated for oral candidiasis. These include mechanical factors like ill-fitting dentures, short term factors like antibiotic therapy and factors related to immune and underlying disease status of the host. In the present study 13.5% of patients with oral lesions were HIV infected. Oral lesions are reported in about 64% of HIV/AIDS patients in India (Deorukhkar *et al.*, 2012). Pseudomembranous candidiasis is the most common oral lesion reported in HIV infected individual (Coleman *et al.*, 1997). It occurs in 17-43% cases with HIV infection and in more than 90% of AIDS patients (Deorukhkar *et al.*, 2012).

In this study diabetes, dentures and malignancy were other risk factors associated with oral candidiasis. In diabetes, the presence of glucose enhances growth of *Candida* in saliva and its adherence to buccal epithelial cells (Akpan and Morgan 2002). Presences of dentures create a micro environment for *Candida* growth. Oral candidiasis occurs in as many as 65% of geriatric population wearing dentures (Akpan and Morgan 2002).

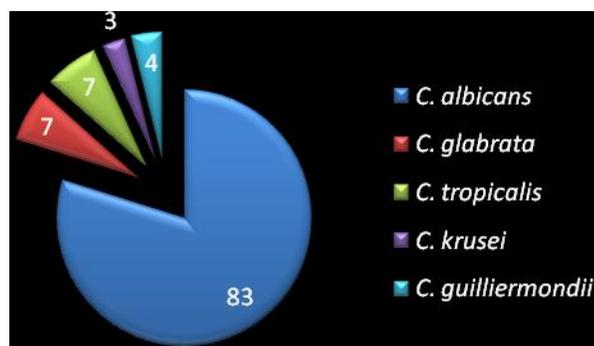
**Table.1** The lesion wise and species wise distribution of Candida isolate

Type of lesion	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>C. guilliermondii</i>
Pseudomembranous candidiasis (n=29)	26	1	1	1	-
Angular cheilitis (n=7)	6	1	-	-	-
Denture stomatitis (n=6)	4	1	1	-	-
Erythematous candidiasis (n=3)	2	-	-	-	1
Leucoplakia (n=12)	11	1	-	-	-
Tobacco pouch (n=7)	5	1	-	-	1
Oral cancer (n=15)	14	-	-	-	1
Oral submucous fibrosis (n=3)	2	1			
Multiple lesion (n=22)	13	1	5	2	1
<b>Total (n=104)</b>	<b>83</b>	<b>7</b>	<b>7</b>	<b>3</b>	<b>4</b>

**Table.2** Antifungal susceptibility profile of Candida isolates

<i>Candida</i> spp	Fluconazole			Itraconazole			Ketoconazole			Amphotericin B		
	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R
<i>C. albicans</i> n=83	74	1	8	79	-	4	74	2	7	74	2	7
<i>C. glabrata</i> n=7	5	2	-	7	-	-	7	-	-	7	-	-
<i>C. tropicalis</i> n=7	5	-	2	5	1	1	6	1	-	5	1	1
<i>C. krusei</i> n=3	3	-	-	3	-	-	3	-	-	3	-	-
<i>C. guilliermondii</i> n=4	1	-	3	4	-	-	3	1	-	3	1	-
<b>Total n=104</b>	88 (84.6 %)	3 (2.9%)	13 (12.5%)	98 (94.2 %)	1 (0.9 %)	5 (4.8 %)	93 (89.4%)	4 (3.8 %)	7 (6.7 %)	92 (88.4%)	4 (3.8 %)	8 (7.7 %)

**Fig.1** The species wise distribution of *Candida* isolates



The relationship between Candidial leukoplakia and malignancy is well recognized. Oral neoplasias can be further complicated by *Candida* infection (Samaranayake and Nair 1995).

In this study, *C. albicans* was the predominant isolate from oral lesions. Our observation was similar to that of Shafi *et al.*, (Shafi *et al.*, 2015) and Mane *et al.*, (Mane *et al.*, 2010). However, in the study of Deorukhkar *et al.*, (Deorukhkar *et al.*, 2012) NAC spp. were predominant isolates. Species variation may be due to various reasons including host factors like diet, oral hygiene and long treatment with fluconazole and use of commercially available kit system for identification of *Candida* spp. In the present study disc diffusion method was used for screening of antifungal resistance in *Candida* Spp. As compared to CLSI broth microdilution method, disc diffusion technique is comparatively less cumbersome and less time consuming (Deorukhkar *et al.*, 2012) and can be easily incorporated in laboratory for routine basis. As compared to amphotericin B and other azoles, *Candida* isolates demonstrated high resistance to fluconazole. Resistance to fluconazole is of concern because it is used as first line drug for

prophylaxis and treatment of candidiasis (Dismukes, 2000). It has good bioavailability, high water solubility and long half-life. Additionally, it is easy to administer and is comparatively less toxic (Deorukhkar and Saini, 2014).

Fluconazole resistance in the present study, was observed in 9.6% of *C. albicans* whereas 23.8% of NAC spp demonstrated resistance to fluconazole. NAC spp. are either intrinsically resistant to fluconazole or may acquire resistance during course of therapy.

From this study, it can be concluded that oral candidiasis is one of the most common affliction of the oral cavity and though an epidemiological shift towards non albicans *Candida* species is noted in recent years, *C. albicans* still remains the pervasive pathogen. Antifungal susceptibility testing of *Candida* isolates should be carried out for selection of most appropriate therapeutic agent.

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