

# Prevalence of *Candida* spp. among healthy denture and nondenture wearers with respect to hygiene and age

Bharathi Prakash, Malathi Shekar<sup>1</sup>, Biswajit Maiti<sup>2</sup>, Indrani Karunasagar<sup>2</sup>, Sreeshma Padiyath<sup>3</sup>

Department of Microbiology, University College, Hampankatta, Mangalore, <sup>1</sup>Department of Fishery Microbiology, Karnataka, Veterinary, Animal and Fisheries Sciences, University College of Fisheries, Mangalore, <sup>2</sup>Faculty of Biomedical Science, Nitte University Centre for Science Education and Research, (NUCSER), Nitte University, Deralakatte, Mangalore, Karnataka, <sup>3</sup>Department of Microbiology, Kurunji Venkatramana Gowda Medical College, Sullia, India

## Abstract

Dentures are inert and nonshading surfaces and therefore get easily colonized by *Candida* species. Subsequent biofilm produced by them lead to denture stomatitis and candidiasis. This study was aimed to understand the prevalence of *Candida* species among healthy denture and nondenture wearers with respect to their age and hygiene status. Swabs were collected from 50 complete dentures and 50 non-denture wearers and processed on Sabouraud's dextrose agar. Identification of *Candida* species was done by staining and a battery of biochemical tests. Data obtained was correlated with age & oral hygiene and statistical analysis was performed. *Candida* was isolated from both denture and nondenture wearers. Prevalence of different *Candida* species was significantly higher in denture wearers and found predominated by *C. albicans*, *C. tropicalis*, *C. dubliensis* and *C. glabrata*. Among nondenture wearers, *C. albicans* and *C. tropicalis* were isolated. Prevalence of *Candida* increased with increasing age among denture wearers. Men presented declining denture hygiene compared to women with increasing age. In comparison to nondenture wearers, multispecies of *Candida* colonized the dentures thus presenting higher risk of candidiasis especially with increasing age.

**Key Words:** Age, *Candida* spp., denture, gender, hygiene

## Address for correspondence:

Dr. Indrani Karunasagar, Nitte University Centre for Science Education and Research, Nitte University, Deralakatte, Mangalore - 575 018, Karnataka, India.  
E-mail: karuna8sagar@yahoo.com

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## INTRODUCTION

Human oral cavity is a reservoir of approximately 700 species of microorganisms including 20 species of *Candida*.<sup>[1,2]</sup> *Candida* is not considered harmful in healthy hosts but may cause opportunistic infections resulting in candidiasis.<sup>[3]</sup> Old age necessitates wearing artificial dentures which results in changes in the oral environment and consequently oral flora. Microbes from the oral environment colonize the denture

surface to form adherent biofilm which is dependent on the denture characteristics, species of *Candida* and oral hygiene practices.<sup>[4,5]</sup> Dentures made from synthetic polymers like polymethylmethacrylate are micro-porous in nature and, therefore, cause *Candida* to easily adhere and colonize. In addition, several host factors such as diet, immune competence, surface roughness, denture cleansers, cleaning methods, saliva with food particles, age, hormonal imbalance and other predisposing factors facilitate the adhesion and colonization on the dentures surface.<sup>[6]</sup> Plaque, stain, and calculus accumulate on the dentures in a manner similar to natural teeth.<sup>[7]</sup> The subsequent proliferation of oral microbes and the formation of plaque on the nonshading surface may initiate pathogenesis from the oral to the systemic front. A less disturbed and relatively stagnant posterior and the interior region of denture is found to be contaminated by a load of oral microbes.<sup>[8]</sup>

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Among *Candida* species that colonize the surface in the oral cavity, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida krusei* and *Candida kefyr* are reported to be associated with both clinical and nonclinical conditions.<sup>[4-8]</sup> *C. albicans* is frequently isolated from the normal oral cavity; however only a few are associated with Candidiasis.

Adherence, a virulence trait of *Candida* enables it to resist the flushing action of saliva, and the resultant colonization is, followed by infection. Literature shows normal denture flora is less studied than the flora of denture induced pathologies. Hence, this is an effort to contribute toward the denture microflora and to look at the prevalence of *Candida* species from healthy denture and nondenture wearers (NDWs). The results generated were correlated with age and oral hygiene of the subjects.

## MATERIALS AND METHODS

The study was carried out on 100 subjects aged between 35 and 80 years. It included a group comprising 50 denture wearers (DW) and 50 NDWs. The DW group comprised 28 men and 22 women with a mean age of  $54.38 \pm 10.31$ , who had been using removable complete dentures for a minimum of 1-year. The NDW comprised 31 men and 20 women with mean age  $51.92 \pm 9.2$ . The age, denture hygiene and denture wearing status of subjects are given in listed in Table 1. The DW attended the dental clinics of the Prosthodontic Department of K.V.G. Dental College; Sullia were considered for the study. Prosthodontists examined the patients for any oral infections. DWs having any Candidial infection were rejected from the study. NDW were healthy individuals with no systemic disease and no clinical signs of oral infection including Candidiasis. All the individuals under study, who received or were currently on antibiotics, antifungal, steroids or immunosuppressive drugs in the past 6 months, were excluded from the study. A questionnaire was prepared to document the subject's profile. Denture and oral hygiene were graded as good, average and poor based on the prosthodontist's report. Informed consent was obtained from all subjects of this study.

The most of the denture plaque occurs on the denture-base of the upper denture<sup>[8]</sup>, and hence the tissue contact surface of the upper denture was considered for isolation of *Candida* in our study. For NDW sample collection was from their palatal mucosa and in DW the samples were collected from the tissue bearing area of the upper denture by scraping it with a sterile swab. The swabs were processed for microbiological examination by immersing it in 5 ml of physiological saline. This was vortexed to disperse the adhering bacteria. A loopful of the suspension was plated on Sabouraud's

dextrose (SD) agar (Hi Media, Mumbai, India) containing gentamycin (2 mg/dL) and chloramphenicol (5 mg/dL) and incubated for 48 h at 37°C. Typical colonies suggestive of *Candida* spp. were picked up and stained by Gram's and the lactophenol blue to observe their morphology and subjected to a battery of biochemical test. Identification of *Candida* was done by studying surface growth in SD broth, carbohydrate assimilation test, carbohydrate fermentation test, serum germ tube test and studying chlamydo spores formation on Corn meal agar. Results obtained analyzed using Student's *t*-test and Chi-square test.

## RESULTS

*Candida* species isolated and identified from both DW and NDW are listed in Table 2. There was a significant difference in the numbers of *Candida* species isolated from DW and NDW [Table 3]. *Candida* spp. was isolated from DW among all age groups, while only 52% of the NDW showed the presence of *Candida* species. Among NDW, although a higher proportion (60%) of *Candida* was isolated from subjects between the age group 66-75, there existed no significant difference between age and prevalence of *Candida* species.

Denture wearers harbored a mixed species of *Candida* which was predominantly *C. albicans* (58%), followed by *C. tropicalis* (28%), *Candida dubliensis* (12%) and *C. glabrata* (2%) wherein all the age groups showed more than 2 different *Candida* species. In contrast, among NDW, *C. albicans* was observed to be the dominant (96.2%) species. Except for one individual (in the age group 36-45), who was positive for *C. tropicalis*, rest all other age groups were negative for both *C. tropicalis* and *C. dubliensis* [Table 3].

**Table 1: Age, hygiene and denture wearing status among men and women**

Age group in years	Men						Women						Total
	DW			NDW			DW			NDW			
	G	A	P	G	A	P	G	A	P	G	A	P	
36-45	2	2	0	3	3	0	2	5	1	3	5	1	27
46-55	4	4	1	3	4	2	2	3	0	0	3	1	27
56-65	1	4	4	3	6	3	3	3	0	4	1	0	32
66-75	0	2	4	1	0	2	1	1	1	0	1	1	14
Total	7	12	9	10	13	7	8	12	2	7	10	3	100

DW: Denture wearers, NDW: Non denture wearers; hygienic status indicated as G: Good, A: Average and P: Poor

**Table 2: Distribution of *Candida* isolates in denture and nondenture wearers**

Age groups in years	DW		NDW	
	Total tested	% Prevalence	Total tested	% Prevalence
36-45	12	12 (100)	15	8 (53.3)
46-55	14	14 (100)	13	7 (53.9)
56-65	15	15 (100)	17	8 (47.1)
66-75	9	9 (100)	5	3 (60.0)

DW: Denture wearers, NDW: Non denture wearers

**Table 3: Distribution of different *Candida* spp. among denture and nondenture wearers**

Age groups in years	Subjects	% occurrence of <i>Candida</i> species			
		<i>C. albicans</i>	<i>C. dubliensis</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>
36-45	DW (n=12)	M-2, F-5 7 (58.3)	-	M-1, 1 (8.3)	M-1, F-3 4 (33.3)
	NDW (n=8)	M-4, F-3 7 (87.5)	-	-	F-1 1 (12.5)
46-55	DW (n=15)	M-5, F-3 8 (57.1)	F-1 1 (7.1)	-	M-4, F-2 6 (42.9)
	NDW (n=7)	M-5, F-2 7 (100.0)	-	-	-
56-65	DW (n=14)	M-6, F-2 8 (57.14%)	M-2, F-2 4 (26.7)	-	M-1, F-1 2 (13.3)
	NDW (n=8)	M-5, F-3 8 (100.0)	-	-	-
66-75	DW (n=9)	M-4, F-2 6 (66.7)	M-1 1 (11.1)	-	M-1, F-1 2 (22.2)
	NDW (n=3)	M-2, F-1 3 (100.0)	-	-	-

DW: Denture wearers, NDW: Non denture wearers, M: Male, F: Female, (-): Nil

Prevalence of *Candida* spp. was higher in males than females. Among male DWs prevalence of *C. albicans* increased with age with, highest seen in the age group 66–75 (66.7%) and lowest between 36 and 45 (58.3%), while in female DWs, it was highest in the age group 36–45 (41.7%) and lowest in subjects of the group 66–75 (22.2%). Oral hygiene had an influence on the prevalence of *Candida* spp., in both the groups. Males had poor oral hygiene than females.

## DISCUSSION

Insertions of the denture bring about changes in the physiology and normal flora of the palate. Tissue contact surface of the denture is less disturbed and enhances the easy colonization of microbes, especially acidogenic bacteria and *Candida*.<sup>[9]</sup> Studies on *Candida* prevalence and colonization with respect to gender and age among DWs with stomatitis have been reported, however, the prevalence and colonization by *Candida* in healthy denture and NDWs is less studied. This study gives an insight about carriers' status of the *Candida* species. There are studies which demonstrated that denture insertion induces plaque formation favoring the increased population of potentially pathogenic bacteria and *Candida* spp.<sup>[10,11]</sup> The higher prevalence of different *Candida* species in DWs in contrast to NDW, confirms the reports on denture insertion inducing plaque formation.

*Candida* spp. readily forms a biofilm on denture acrylic surfaces and, therefore, are isolated more frequently from denture plaque than from dental plaque.<sup>[12]</sup> In this study, there was a significant difference ( $P < 0.001$ ) in the prevalence of *Candida* spp. between DW and NDW.

Our findings on the prevalence and species diversity of *Candida* from denture samples are similar to the study of Davenport<sup>[13]</sup> who demonstrated higher level of *Candida* spp. on the denture surface compared to the palatal mucosa.

*Candida* colonization increases with increasing age irrespective of the denture wearing status.<sup>[14]</sup> Denture insertion accelerates the colonization and biofilm formation. In our study, this trend was seen only in male DWs. The higher prevalence among male

DWs in both the groups differ from the study of Arenforf and Walker<sup>[2]</sup> where females were the frequent carriers of *Candida* species rather than males. Aging causes a progressive increase in *Candida* counts in the oral cavity<sup>[15]</sup> with higher counts being observed among elderly DWs than NDWs.<sup>[6]</sup>

The predominant *Candida* spp. among DWs was *C. albicans* followed by *C. tropicalis* and *C. dubliensis*. This is different from an earlier report<sup>[16]</sup> wherein *C. glabrata* was the dominant species. *Candida* was isolated more in males than females in both DW and NDW. Morphological similarities pose a problem in the identification of some species such as *C. albicans* and *C. dubliensis*.<sup>[17]</sup> The isolation of *C. dubliensis* from denture stomatitis and HIV patients has been reported.<sup>[18]</sup> In contrast to this, we isolated six *C. dubliensis* from healthy DWs with no signs of candidiasis. *C. dubliensis* were confirmed by the abundant chlamydoconidia formed on corn meal agar and positive germ tube test. To get rid of the denture plaque associated infections and denture malodor, adequate cleaning of the dentures and vigilance is needed.

Thus, it can be concluded that DWs though apparently free from diseases they are asymptomatic carriers of multispecies *Candida*. This may expose themselves to the risk of candidiasis with predisposing factors like old age and decreasing immunity. Compared to NDWs, diverse and increased prevalence of *Candida* species is seen among DWs, who are prone to get candidiasis and denture stomatitis. Therefore adequate and regular cleaning of the denture is a prerequisite for better oral and systemic health.

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