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Prevalence of oral *Candida* carriage in denture wearers

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Abstract

Objective To compare the prevalence and species of *Candida* in the oral cavity of denture wearers and non-denture wearers.

Materials and methods A total of 80 subjects were studied: 40 denture wearers and 40 non-denture wearers, matched by age and sex, comprised the experimental and control groups, respectively. Each subject was instructed to perform oral rinsing using a phosphate-buffered saline solution, which was expectorated and processed for the recovery of *Candida* on Sabouraud's dextrose agar. Isolates were speciated by culturing on chromogenic candida agar and noting species-specific colony characteristics.

Results The prevalence of *Candida* carriage was 85.00% in denture wearers and 77.50% in non-denture wearers. *C. albicans* was the most frequently isolated species, followed by *C. tropicalis, C. glabrata, C. parapsilosis* and *C. krusei.* The distribution of *Candida* species among each patient varied from one to four species. The differences in prevalence and distribution of *Candida* species in asymptomatic denture wearers compared to non-denture wearers were not statistically significant ($p > 0.05$).

Conclusion Asymptomatic denture wearers and non-denture wearers did not differ in *Candida* carriage.

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Key words: *Candida; carriage; denture; prevalence*

Introduction

Among the several hundred species of microorganisms in the oral cavity, yeasts, especially members of the genus *Candida,* are representative of the few fungi considered to be commensal oral flora. *Candida albicans* is the most common species isolated from the human oral cavity, while other species such as *C. glabrata, C. tropicalis,* and *C. dubliniensis,* are less frequently found.[1,2] The reported prevalence of *Candida* in normal healthy adults varies considerably among population groups, ranging from 6 to 55.4%,[3] with a median of 34.4%.[3] Interestingly, when broken down by age, the prevalence of the *Candida* in clinically healthy adults ranged from 3 to 48%, whereas prevalence is more consistent in symptom-free children, ranging from 45 to 65%.[3] Furthermore, *Candida* prevalence is related to consumption of fermentable carbohydrate[4] and salivary flow rate.[5] Isolation of *Candida* has been investigated for associations with dental caries risk, as well as denture wearing status.[6]

The most common oral yeast infection is caused by members of the genus *Candida.* Candidiasis is an opportunistic infection that results in pathological changes to mucosal surface of the oral cavity.[7-10] Patients with candidiasis may display various symptoms including burning, painful sensation, change of taste, and swallowing difficulty, but most often are asymptomatic.[9] The infection is usually cured with antifungal medications, but recurrences may be problematic in immunocompromised patients such as patients treated in intensive care units, cancer patients receiving radiation or chemotherapy, organ transplant patients and HIV-positive patients.[11]

Recently, some *Candida* species (spp.), including *C. tropicalis, C. glabrata, C. krusei,* and *C. parapsilosis,* have been recovered with increasing frequency from cases of candidiasis.[1,2,12] Each species differs in the production of putative virulence factors and sensitivity to antifungal agents. Greater emphasis has now been placed on identification of isolates to the species level. Differentiating the *Candida* spp. is helpful in choosing proper treatment regimen as some species may be resistant to certain groups of antifungal drugs.[13-17] Infection caused by non-albicans *Candida* spp., such as *C. tropicalis, C. glabrata,* and *C. krusei,* have been reported to be less responsive to the currently used fluconazole.[18,19] There are numerous case reports describing the colonization and infection of immunocompromised patients on long-term regimens of oral antifungal agents, from whom drug resistant *C. krusei* and *C. glabrata* have been recovered.[20-22] Host defenses have been reported to be less effective in patients infected by *C. glabrata* than *C. albicans.*[23] Therapeutically, itraconazole, a triazole antifungal with a broad spectrum of activity, has *in vitro* activity against many of the non-albicans *Candida* species, specifically *C. glabrata.*[14-15] Echinocandins, anidulafungin, caspofungin, micafungin and the newer triazoles, including posaconazole and voriconazole are antifungal drugs that also exhibit potent activity against *Candida* spp. However, echinocandins, appears to be less potent against some species, such as *C. parapsilosis* and *C. guilliermondii.*[16] *C. dubliniensis,* a species that is very similar to *C. albicans* has been reported to have reduced susceptibility to azole drugs.[17,24-26]

Conventional laboratory methods for identifying yeasts to the species level rely on criteria such as colony and microscopic morphology, growth characteristics, carbon source fermentation, as well as appearance on differential media.[27,28] Isolates of *C. albicans* are typically identified by their ability to form germ tubes or chlamydospores under the appropriate conditions.[29] New methods for the rapid isolation and identification of clinically important *Candida* spp. with differential and selective media have been developed and widely accepted.[28,30] These media are usually composed of a Sabouraud's dextrose agar base with chromogenic substrates that can detect specific enzymatic activity in target organisms. These enzymes cleave a colorless substrate, releasing chromogenic molecules within the

colonies that allow them to be clearly seen and differentiated.[31] A broad-spectrum antibacterial agent, usually chloramphenicol, is added to the agar to inhibit bacterial growth. *C. albicans, C. dubliniensis, C. tropicalis, C. kefyr, C. glabrata, C. krusei, C. parapsilosis, C. lusitaniae, C. guilliermondii, C. stellatoidea, C. pseudotropicalis* and *C. famata* are species typically isolated from clinical specimens.[32,33]

The wearing of dentures has been associated with overgrowth of oral *Candida,* leading to denture stomatitis.[10] Studies to identify *Candida* spp. in patients with denture stomatitis have yielded conflicting results. Some studies claimed that a single species was responsible for the infections,[34] whereas others isolated multiple species of *Candida.*[35] The present study was performed to compare the prevalence and species identities of *Candida* recovered from the oral cavities of removable denture wearers and non-denture wearers.

Materials and methods

Subject selection

A total of 80 subjects participated in the study. Forty removable partial denture wearers comprised the experimental group and 40 age and gender matched non-denture wearers comprised the control group. All subjects were patients who attended the dental clinics of the Department of Oral Medicine and Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University during the peroid of June 2008 to February 2009. Inclusion criteria for subject selection were healthy individuals with no systemic disease, and no clinical sign of *Candida* infection. Individuals who smoked, received or were currently taking antibiotics, antifungals, steroids or immunosuppressive drugs in the past 6 months were excluded from this study. All subjects were informed and signed the consent forms approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University prior to their participation.

Collection and identification of samples

Salivary samples were collected using the oral rinse technique.[36] Briefly, each subject was requested to rinse the mouth for 60 seconds with 10 milliliters of sterile phosphate-buffered saline (PBS; 0.01 M phosphate-buffered saline solution, pH 7.2) and expectorate the rinse into a 15 milliliter sterile container.[37] Subjects who wore removable dentures were asked to remove the appliances prior to the collection of samples. The samples were immediately transported on ice to the microbiology laboratory. Each oral rinse was centrifuged at 3500 rpm for 10 minutes. The supernatant was discarded. The pellet was resuspended in 1 milliliter of sterile PBS. One hundred microliters of the concentrated oral rinse was inoculated onto Sabouraud's dextrose agar (BBL, USA) and incubated at 37° C for 48 hours. The remaining samples were stored at -80° C. If *Candida* colonies appeared on the Sabouraud's dextrose agar, then chromogenic candida agar (Oxoid, Basingstoke, England) was inoculated using 100 microliters of the oral rinse supernatant and incubated for 48 hours for colony study.[30] *Candida* spp. were identified by the color of the colonies using the color reference guide supplied by the manufacturer (Table 1).[30] When color identification was equivocal, fermentation assay of glucose, sucrose, maltose, lactose and galactose was performed. The *Candida* spp. were also identified by the ability to produce chlamydospores on glutinous rice agar.[29,38]

Statistical Analysis

Data were statistically analyzed using the SPSS program version 15. The difference in distribution of the *Candida* species between groups was based on comparison of frequency distributions by a chi-square test. A p value < 0.05 was considered to be significant.

Results

The denture wearer and non-denture wearer groups each consisted of 26 males and 14 females with mean ages of 57.43 ± 10.82 years (range 33 - 79 years) and 56.65 ± 11.24 years (range 32 - 83 years), respectively. The prevalence of oral *Candida* was 85.00% in denture wearers and 77.50% (p = 0.568) in non-denture wearers (Table 2). Carriage of either a single species or multiple species was comparable in both groups with 64.71% of denture wearers and 64.52% of non-denture wearers harboring only a single species (Table 3). *C. albicans* was the most frequently isolated species between both groups at 73.53% and 54.84% in denture wearers and non-denture wearers, respectively. The differences in prevalence and distribution of *C. albicans, C. tropicalis, C. glabrata* and *C. parapsilosis* did not differ statistically between denture wearers and non-denture wearers (Table 4). In subjects who hosted more than one species of *Candida,* no significant difference between denture wearers and non-denture wearers were found in the total numbers of species isolated (Table 5). As shown in Table 6, denture wearers who harbored one species of oral *Candida* most often carried *C. albicans,* followed by *C. tropicalis, C. parapsilosis* and *C. glabrata.* The order of frequency differed for non-denture wearers. *C. albicans* was still the most common species isolated, but it was followed by *C. parapsilosis, C. glabrata, C. tropicalis,* and *C. krusei.* Nevertheless, there were no overall statistical differences in prevalence and distribution of the *Candida* species between the two groups.

Table 1 Color of *Candida* spp. on chromogenic agar (Oxoid)[30]

***Candida* species**	**Color on chromogenic agar**
C. albicans, C. dubliniensis	Green
C. tropicalis	Blue
C. glabrata, C. kefyr, C. lusitaniae, *C. parapsilosis*‡	Beige-yellow, brown
C. krusei	Dry, fuzzy brown-pink

‡*C. glabrata, C. kefyr, C. lusitaniae,* and *C. parapsilosis* appear as a variety of beige, yellow, brown. Sugar fermentation assays needed.

Table 2 Prevalence of oral *Candida* between denture wearers and non-denture wearers

	Prevalence of *Candida*		***p*-value**
	Number	**%**	
Denture wearer (N = 40)	34	85.00	0.568
Non-denture wearer (N = 40)	31	77.50	

Table 3 Prevalence of oral *Candida* hosted one or mixed species between denture wearers and non-denture wearers

	N	Single *Candida* spp. (%)		Mixed species (%)
		Albicans	Non-albicans	
Denture wearer	34	13 (38.24)	9 (26.47)	12 (35.29)
Non-denture wearer	31	11 (35.48)	9 (29.03)	11 (35.48)
p-value		0.520	0.522	0.589

N = Number of subjects from whom *Candida* was recovered

Table 4 Comparison of numbers of denture wearers and non-denture wearers according to species of *Candida*

	N	Prevalence of *Candida* species (%)				
		C. albicans	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*
Denture wearer	34	25 (73.53)	10 (29.41)	8 (23.53)	4 (11.76)	4 (11.76)
Non-denture wearer	31	17 (54.84)	8 (25.81)	5 (16.13)	9 (29.03)	4 (12.90)
p-value		0.129	0.788	0.543	0.121	1.000

N = Number of subjects from whom *Candida* was recovered

Table 5 The concurrent distribution of *Candida* species between denture wearers and non-denture wearers

	N	Number of *Candida* species found concurrently (%)				
		1	2	3	4	*p*-value
Denture wearer	34	22 (64.71)	8 (23.53)	3 (8.82)	1 (2.94)	0.673
Non-denture wearer	31	20 (64.52)	9 (29.03)	2 (6.45)	0 (0.00)	

N = Number of subjects from whom *Candida* was recovered

Table 6 Comparison of numbers of denture wearers and non-denture wearers when hosting one species of *Candida* according to species of *Candida*

	N	*Candida* species (%)				
		C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. krusei*
Denture wearer	22	13 (59.10)	6 (27.27)	2 (9.10)	1 (4.55)	0 (0.00)
Non-denture wearer	20	11 (55.00)	1 (5.00)	5 (25.00)	2 (10.00)	1 (5.00)
p-value		0.788	0.053	0.167	0.493	0.288

N = Number of subjects hosting 1 *Candida* species

Discussion

Chromogenic agar is a useful medium for differentiating *Candida* spp. from samples with multiple species. It particularly enhances the ability to discriminate between *C. albicans* and other yeast species. When color-based differentiation of yeast colonies was ambiguous, however, sugar fermentation properties were tested. Formation of chlamydospores was also performed since they are produced only by the two closely related species, *C. albicans* and *C. dubliniensis.*[38] *C. dubliniensis* is a recently described *Candida* spp. that exhibits a high degree of similarity to *C. albicans* both phenotypically and in its sugar fermentation pattern.[39] Each of these species, forms green colonies on chromogenic agar.[40] The green colonies in this study were presumptively identified to be *C. albicans.* This assumption is due to the fact that *C. albicans* is the most commonly found fungal infection of the oral cavity. In expansion to this conclusion, *C. dubliniensis* is widely reported to be recovered from HIV-positive patients.[41-47] Even so, the presumptive *C. albicans* identification might possibly contain *C. dubliniensis* due to the limitation of the method used. PCR identification,[41] as well as assimilation of glycerol, D-xylose, methyl-a-D-glucoside and D-trehalose [API 20C AUX system (BioMerieux)],[48] that can specifically distinguish between these two species was not performed in this study.

In our current study, the prevalence of *Candida* in the denture wearing and non-denture wearing groups did not differ statistically. Furthermore, similar percentages of non-denture wearers and denture wearers harbored a single *Candida* species, most frequently *C. albicans. C. albicans* was also the most common species recovered from all subjects, whether they harbored one or more *Candida* species, which agrees with previous studies.[49-51] A recent study by Vanden Abbeele, *et al.*[52] reported that *C. glabrata* was the second most prevalent species in healthy denture wearers, whereas *C. tropicalis* was found to be the second most prevalent species in our study. However, our study found no statistical difference in carriage of any *Candida* spp. between the two groups. It has been reported that denture wearers, as well as the elderly, have a higher prevalence and density of oral *Candida* colonization.[53,54] However, eating habits, including frequency and types of food consumed, may favor oral *Candida* colonization within the elderly.[33] Furthermore, salivary flow tends to decrease with age.[55] This could explain the comparably high prevalences in both groups in our study.

Identification of *Candida* spp. has been found to be increasingly important for determining the appropriate course of treatment. *C. glabrata* is often found in significant numbers, with the highest frequency in denture wearers, among those with denture-induced stomatitis.[8,10] Campos, *et al.* reported that *C. albicans* was a dominant species in patients with denture stomatitis, whereas healthy denture wearers were more likely to harbor a diversity of yeast species. In a study of denture wearers without stomatitis, *C. glabrata* was isolated in 48% and *C. albicans* in 84% of subjects, with both species found in 41%.[56] As noted above, our results concurred with respect to the dominance of *C. albicans,* but *C. tropicalis* joined *C. glabrata* as the next most commonly isolated species. A study by Coco, *et al.* suggested that mixed *C. albicans* and *C. glabrata* biofilms could aggravate the clinical condition. However, it is not clear yet whether species co-existence plays an integral or antagonistic role in pathogenesis or virulence.[57] Furthermore, the co-existence of mixed species could complicate treatment modalities. Whether the co-existence of species is limited to certain combinations of species, and whether the co-existence is mutually beneficial, have yet to be determined.

Yeasts are demonstrable in 78 to 100% of patients with denture-induced stomatitis.[58] There was a 10-fold increase in the yeast counts in dental plaque obtained

from denture induced stomatitis patients when compared with healthy controls.[9] Improper denture care can promote growth of these commensal fungi.[10] Individuals who harbor *Candida* as an oral commensal may be at a higher risk of *Candida* infection than non-carriers.[59] However, a non-carrier with poor oral hygiene may contract the infection exogenously, while a *Candida* carrier with good oral hygiene may never show signs of infection.

This pilot study presented the data as the percent recovery for different *Candida* species. It affirms results from earlier studies, but also finds differences in the recovery of species other than *C. albicans.* The next logical step would be analytical studies with larger subject cohorts to determine, if preferential co-existence of particular *Candida* species can be linked to increased risk of denture stomatitis or its severity.

Conclusion

In the present study, we demonstrated that *C. albicans* was the most common species associated with oral carriage in both healthy denture wearers and non-denture wearers. The prevalence and distribution of *C. albicans,* as well as other oral candida spp., did not differ statistically between denture and non-denture wearers.

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ความชุกของเชื้อรา*แคนดิดา*ในช่องปากของผู้ใส่ฟันเทียม

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บทคัดย่อ

วัตถุประสงค์ เพื่อเปรียบเทียบความชุกและชนิดของเชื้อรา*แคนดิดา*ในช่องปากของผู้ใส่ฟันเทียมและผู้ที่ไม่ใส่ฟันเทียม

วัสดุและวิธีการ ผู้ที่เข้าร่วมศึกษาทั้งหมด 80 ราย ประกอบด้วยกลุ่มทดลอง ได้แก่ ผู้ที่ใส่ฟันเทียม และกลุ่มควบคุม ได้แก่ ผู้ที่ไม่ใส่ฟันเทียม กลุ่มละ 40 ราย ซึ่งมีเพศและอายุใกล้เคียงกัน อาสาสมัครผู้เข้าร่วมวิจัยทุกรายจะได้รับการเก็บตัวอย่างน้ำลายโดยการกลั้วปากด้วยสารละลายฟอสเฟตบัฟเฟอร์ซาลีน และตัวอย่างถูกนำมาเพาะเลี้ยงบนอาหารเลี้ยงเชื้อแซบูโรเด็กซ์โตรส อะการ์ และโครโมเจนิกแคนดิดา อะการ์ เพื่อศึกษาและแยกเชื้อตามคำแนะนำของผู้ผลิต

ผลการศึกษา ความชุกของเชื้อรา*แคนดิดา*ร้อยละ 85.00 ในผู้ที่ใส่ฟันเทียม และร้อยละ 77.50 ในผู้ที่ไม่ใส่ฟันเทียม โดยพบสายพันธุ์แคนดิดาอัลบิแคนส์บ่อยที่สุด ตามด้วยสายพันธุ์แคนดิดาทรอปิคัลลิส แคนดิดากลา-บราตา แคนดิดาพาราปซิโลซิส และแคนดิดาครูซีไอ พบการกระจายตัวของสายพันธุ์ของเชื้อรา*แคนดิดา*ในผู้ป่วยแต่ละรายได้ตั้งแต่ 1 ถึง 4 สายพันธุ์ แต่อย่างไรก็ตาม ความชุกและการกระจายตัวของสายพันธุ์ของเชื้อรา*แคนดิดา*ในผู้เป็นพาหะของเชื้อรา*แคนดิดา*ระหว่างผู้ที่ใส่ฟันเทียมและผู้ที่ไม่ใส่ฟันเทียมไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$)

สรุป ผลการศึกษาความชุกและชนิดของเชื้อรา*แคนดิดา*ในช่องปากของผู้ใส่ฟันเทียมและผู้ที่ไม่ใส่ฟันเทียมไม่มีความแตกต่างกัน

(ว ทันต จุฬาฯ 2552;32:101-12)

คำสำคัญ: *ความชุก; แคนดิดา; พาหะ; ฟันเทียม*