

## Original Article

# The Comparison of Predominant Oral Micro-Flora in Subjects with and without Complete Denture Referred to Yazd Dentistry Department

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### Abstract

**Introduction:** Human oral cavity consist a mass of microorganisms, which may altered by application the complete removable denture in edentulous patients. The aim of present study was to compare the dominant oral micro-flora between edentulous denture users with dentate elderly. The aim of the present study was to compare the dominant oral micro-flora between edentulous denture users with dentate elderly.

**Materials and Method:** In the current analytical, cross-sectional study, 30 edentulous patients with complete removable denture and 30 dentate subjects, who had been admitted for non-prosthetic treatments, were randomly selected in Yazd dentistry department. Their oral cavity samples were obtained using sterile cotton-tip swabs for direct smear analysis and cultivation on the selective fungal and bacterial media. Isolated predominant bacteria and fungi were enumerated and identified by microbiological differential diagnosis tests. The data were analyzed with SPSS software with T test and the differences were considered statistically significant when  $p < 0.05$ .

**Results:** The non-aureus staphylococcus and alpha-hemolytic streptococci showed the highest positive culture among the isolated microorganisms in both groups whereas beta hemolytic streptococci showed the least percentage of isolated microorganisms in both groups. The higher density of non-aureus Staphylococci,  $\alpha$ -hemolytic Streptococci, Gram negative cocobacillus, non-pathogenic Neisseria, Candida and Corynebacterium were recovered from oral samples of denture users in comparison with dentate subjects ( $P = 0.0001$ ). A statistically significant correlation between the number of isolated microorganisms and the duration of denture utilization in denture users was also seen ( $P = 0.013$ ).

**Conclusion:** The results of the present study showed that complete denture can act as a predisposal factor in the overgrowth of several oral micro-flora particularly Candida, non-aureus Staphylococci,  $\alpha$ -hemolytic streptococci, gram negative cocobacillus, non-pathogenic Neisseria, and Corynebacterium, which emphasized the users denture hygiene.

**Key words:** Oral cavity, Micro-flora, Complete denture.

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## Introduction

Human oral cavity micro-flora plays an important role in maintaining a healthy oral mucosa in normal teethered and even edentulous denture users <sup>[1]</sup>. These microorganisms are highly diverse containing about 700 bacterial and fungal species of which more than half have not yet been isolated and differentiated <sup>[2]</sup>. An increasing population of edentulous elderly is being seen in the world <sup>[3]</sup> and in Iran reported as 52/5% in Hamadan, 32.8% in Torbat-e Heydarieh and 40.7% in Yazd <sup>[4]</sup>. There are several methods for replacement of normal teeth such as using complete removable denture, implants and over denture implants, the complete removable denture which was known as the most common type of treatment in edentulous patients <sup>[5, 6]</sup>. Since the completely edentulous patients are usually the elderly, the age-related changes in their salivary flow and oral micro-flora will affect the health of their oral tissues <sup>[7]</sup>. Denture stomatitis, formerly known as denture sore mouth is an inflammatory disorder of the mucosa following the use of complete dental prostheses reported in about 60% of denture wearers <sup>[8]</sup>. The attachment of the elderly's oral micro-flora on the denture fitting mucosal surface particularly on maxillary part resulted in microbial plaque, which may have caused denture stomatitis <sup>[9]</sup>.

Studies in the literature have shown that oral microorganisms such as periodontopathic bacteria can cause the development of systemic

diseases such as aspiration pneumonia <sup>[10, 11]</sup>. Hamalainen et al. <sup>[12]</sup> also reported that periodontal infections and complete dentures may act as reservoirs for potentially harmful pathogens.

In order to show the influence of denture-wearing and age on the oral microflora, Marsh et al. reported in a study that the proportions of staphylococci and mutans streptococci were raised in denture wearers. The isolation frequency of yeasts from plaque was also significantly higher in denture wearers <sup>[12]</sup>.

Walter et al. reported that denture plaque was mainly caused by Gram-positive and Gram-negative coccal forms, however, *Candida* was only rarely observed. They also concluded that in the early stages of denture stomatitis development, a bacterial inflammation very similar to bacterial gingivitis was mainly seen due to lack of oral hygiene versus a fungal infection <sup>[9]</sup>. However, several other studies reported *Candida* colonization especially *C. albicans*, as the main etiologic agents responsible for the development of this opportunistic infection <sup>[14, 15]</sup>.

Studies revealed that microbial biofilm, which attached and colonized on denture hard surfaces, can be re-colonized in the mouth of denture users if a denture is worn <sup>[16, 17]</sup>. Researchers reported that a number of pathogenic microorganisms, which normally harbored in the

mouth of dentate patient, were also present in the oral cavity, when they were in the edentulous state [17-19]. Since many opportunistic microorganisms are capable of adhering and surviving on the denture acrylic resin surfaces, dental prostheses may act as a potential source of infection in elderly denture users. A wide diversity of oral and non-oral micro-flora such as *Staphylococcus* spp., *Streptococcus* spp., *Candida* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Enterococcus* spp., and *Actinomyces* spp. are associated with denture biofilm. The presence of such microbial contamination may result in several local and systemic infections [20] such as periodontal disease, caries, mucosal inflammation, urinary tract infections, pneumonia, abscess and even endocarditis [21, 22].

However, complete removable denture usually used for aesthetic concerns is also used in order to return the natural teeth functions in edentulous elderly. Maintaining good denture hygiene plays an essential role in the prevention of denture stomatitis.

The general purpose of the current study was to compare the oral microbial flora between edentulous patients with complete denture and dentate subjects.

## Materials and Methods

### Sample collection

In the present analytical cross-sectional study, 30 edentulous patients with complete denture

and 30 dentate patients, who had been admitted for non-prosthetic treatments in Yazd dentistry department, were randomly selected. In order to reach a minimum of 6 unit differences in the average microbial colony counts between groups, the significant level of 5%,  $\alpha=5\%$ ,  $\beta=2\%$ ,  $S=80\%$  and based on the following formula, 30 subjects were chosen for each group in present study.

$$n = \frac{(z_{\beta} + z_{\alpha})^2 2s^2}{(\bar{x}_1 - \bar{x}_2)^2}$$

Subjects with underlying diseases such as diabetes, malignancies, immunosuppressed and recent antibiotic users were excluded from the current study. Subjects of whose last eating and brushing less than two hours had elapsed were also excluded from the present study. All subjects were in the age range of  $65.8 \pm 12.5$  years and were also matched based on age and sex groups. Each patient completed a medical and dental history and signed an informed consent document before accepting oral sample collection.

Their demographic data were collected using a designed questionnaire and oral cavity samples were then collected using 2 sterile cotton-tip swabs rubbed on their oral mucosa [23].

### Laboratory diagnosis of oral micro-flora

Two sterile swab samples were used for oral sample collection. One sample was used for preparation of two direct smears, stained (Gram

stain) for determination of microscopic bacterial and fungal contamination. The second swab sample was inserted in the sterile container containing 1ml of sterile physiological saline (0.85% solution of Sodium chloride) as the transport media and sent to microbiology laboratory for microbiological analysis within 2 hours.

The sterile container containing collected swab sample was shaken and a 10 µl of transport media was cultured on Sabouraud dextrose agar (Oxoid, Uk) containing 50µg/l Chloramphenicol for isolation of *Candida* species. The plates were incubated for 48 hours at 30°C; isolated *Candida* colonies were enumerated and determined based on germ tube test (for detection of *Candida albicans* and non-*albicans* *Candida* species).

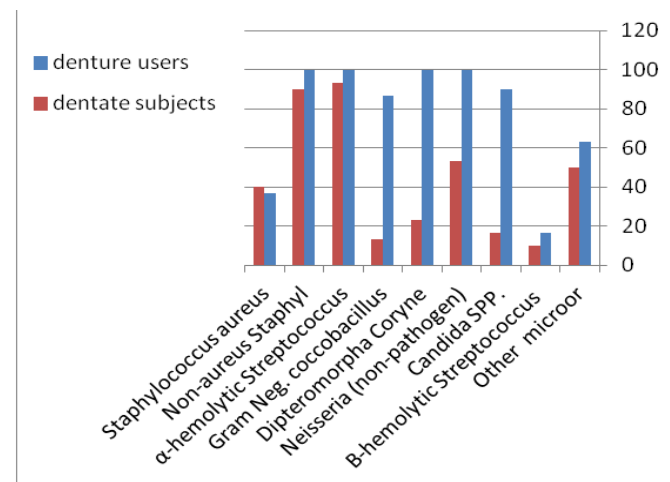
In order to analyze bacterial contamination, two 10 µl of shaken transport media were also cultured on Blood agar (Merck, Germany) and MacConkey agar (Oxoid, Uk) plates, incubated at 37°C for 24 hours. The isolated bacterial

colonies were also enumerated and identified using specific media, and specific identification tests such as Oxidase, Catalase, Coagulase, DNase and Novobiocin sensitivity testing. The data were analyzed with SPSS software using T test and the significant level was set at  $P = 0.05$ .

## Results

Non-aureus staphylococcus and alpha-hemolytic streptococci showed the highest positive culture among the isolated microorganisms in both denture user [100%] and dentate subjects (90-93.3%), but beta hemolytic streptococci showed the minimum percent of isolated microorganism (Figure 1) in oral sample culture of both groups (16.7% in denture users and 10% in normal dentate subjects).

The positive culture with *Dipteromorpha* *Corynebacteria*, non-pathogen *Neisseria* and *Candida* species was mostly recovered from edentulous denture user's samples rather than the dentate subjects.



**Figure 1.** Frequency (percent) of predominant micro-flora isolated from oral culture in case and control groups

In relation to microorganisms' density (CFU, colony forming units) recovered from oral cavity samples, no statistically significant difference was seen between B-hemolytic Streptococcus ( $p=0.388$ ) and Staphylococcus aureus ( $p= 0.087$ )

in both groups, whilst a higher density of all the other isolated organisms was seen in edentulous patients with denture compared with dentate subjects (Table 1).

**Table 1.** Comparison the average (SD) of predominant micro-flora (CFU/ml) isolated from oral culture in case and control groups

Isolated micro-flora	Case (with denture)	Control (normal teeth)	P value
	Average (SD)	Average (SD)	
Staphylococcus aureus	2271 (530.7)	357 (192.8)	0.087
Non-aureus Staphylococcus	34766.6 (12931.2)	2673.4 (968.8)	0.0001
$\alpha$ -hemolytic Streptococcus	22866.7 (2580.9)	2533.3 (1485.2)	0.0001
Gram Neg. coccobacillus	4335.6 (825.2)	59.5 (184.8)	0.0001
Dipteromorpha Corynebacteria	11383.3 (6736)	273.3 (93.6)	0.0001
Neiseria (non-pathogen)	25433.3 (2137.5)	1330.3 (882.3)	0.0001
Candida SPP.	586 (190)	50 (16.7)	0.001
B-hemolytic Streptococcus	10 (3.2)	4 (1)	0.388
Other microorganisms	1840.2 (522.7)	983.3 (120.1)	0.682

T test

A statistically significant correlation was observed between the number of isolated Microorganisms and the duration of using the denture in edentulous group ( $P=0.013$ ). A higher density of isolated Candida species was recovered from male oral samples than from female ones ( $P=0.045$ ). However, no statistically

significant difference in the case of isolated bacterial micro-flora was observed.

### Discussion

Since the number of edentulous and denture users is increasing, investigating their oral micro-flora is becoming increasingly important. The aim of the current study was to compare the

frequency and density of certain microorganisms that are able to survive in the oral cavity of edentulous wearing dentures and normal subjects with natural teeth. Several previous studies <sup>[23-27]</sup> reported a decrease in and even disappearance of many bacteria such as *Streptococcus mutans*, a troublesome anaerobes usually found in human oral cavity in edentulous people. Many bacteria and also *Candida* which need a hard surface for attachment and colonization were also isolated from the mouth of denture wearers with poor denture <sup>[22, 24]</sup>.

Controversial results concerning the dominant and common inhabitant recovered before and after using insertion of denture in complete denture wearer have been observed <sup>[3]</sup>. For instance, *Strep.mutans* was reported as a common gram positive bacterium isolated from mucosa and saliva of denture wearing edentulous subjects <sup>[19, 27]</sup>. However, this bacterium was not isolated from both case and control groups in the present study supported by Marsh and Kononem's study <sup>[13, 19]</sup>. Gram Neg. coccobacillus, non-pathogen *Neisseria* species, *Dipteromorpha Corynebacteria* and *Candida* SPP. were determined as the prevailing bacteria in the oral samples of denture wearers in the present study. However, Culter et al. <sup>[29]</sup> reported bacteroides, lactobacilli, actinomyces, streptococci and neisseriae as common isolates after insertion of denture. The only interpretation for these differences is that the oral samples were cultured after at least one year from

insertion of denture, while the oral samples in Culter's study were analyzed 1 and 4 weeks after insertion of denture. The distribution and colonization of *Staphylococcus aureus* did not indicate any differences in the current study as this organism was equally found in oral samples of both case and control subjects in the current study supported by Culter <sup>[29]</sup> and Kononem's <sup>[19]</sup> study.

Different species of *Candida* especially *C. albicans* as the most common etiologic agent of denture stomatitis were isolated from 90% of denture wearers in comparison with 16.7 % of normal subjects in the present study supported by many studies in the literature <sup>[30, 32]</sup>. However, in a few studies such as those of Latteef <sup>[33]</sup> and Culter <sup>[29]</sup> this fungus was only in a small number of samples collected from oral samples of patients with denture. This may be the result of differences in sample collection since sterile swabs were used in the current study as Plaque was collected in Culter's study and the whole saliva samples were collected in Latteef's study. Denture stomatitis is known as the most common oral disorder and is reported in about 67% of complete denture wearers resulting from the attachment and colonization of *Candida* on the hard surfaces of denture <sup>[30, 33]</sup>.

Among Gram negative bacteria, commensal *neicerria* species (*Sicca*, *Flava*, and *Mucosa*) were isolated from 100% of the samples from denture wearers compared with 53.3% of the normal subjects which represented a statistically

significant higher frequency and density in the case group. This result was in line with Al-Aswad's findings which indicate a greater percentage of Neisseria (39%) in mucosa of denture wearers<sup>[35]</sup>. However, it contrasted with the study of Latteef's who reported that there was no difference after one week of denture insertion in denture users<sup>[33]</sup>. A wide variation in the prevalence of oral microbial flora in edentulous patients was revealed, which was related to site and method of sample collection, selective medium and enrichment cultivation and interpretations. This variation was also affected by the duration of complete denture usage. Our case group (denture users) was used at least more than one year and even patients with more than 5 years also were also present in the current study. The simple sterile cotton-tip swabs rubbed in different sites of the subjects' mouth was also used in the present study for sample collection and evaluation of oral microbial flora as used by several studies in the literature<sup>[36-39]</sup>.

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## Conclusion

The results of the present study confirmed that the insertion of a denture in patients with no previous denture experience was associated with significant changes in the composition of their oral micro-flora. These changes may persist and could result in plaque formation with considerable pathogenic microorganisms in edentulous denture users. A more extensive longitudinal study on the flora of patients with denture is required to show the formation of plaque rich in Streptococcus, coccobaciluls or Candida which are specifically associated with the risk of denture stomatitis.

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