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Original Research

Assessment of Presence of Different Bacterial Species in Denture Wearer

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ABSTRACT:
Background: Human oral cavity is a reservoir of approximately 700 species of microorganisms. 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 and oral hygiene practices. **Aim of the study:** To assess presence of different bacterial species in denture wearer. **Materials and methods:** The study was conducted in the Department of Prosthodontics of the Dental institution. For the study, 50 patients were selected, 25 males and 25 females, who were wearing removable dentures for more than a year and were admitted to the Department of Prosthodontics of the dental institute. 50 individuals with normal dentition and not wearing any dentures were selected as a control group. For the collection of saliva, it was made sure that unstimulated saliva is collected after at least one hour of the last intake of drink or food. The subjects were directed to spit unstimulated saliva into sterile Flacon tubes containing 3 ml thioglycolate broth. **Results:** We observed that non-oral pathogens were more commonly seen in denture wearers. Gram negative bacilli were seen in 12 denture wearers and 3 non-denture wearers. Similarly, E. coli and E. cloacae are more commonly seen in denture wearers. **Conclusion**: From the results of the present study, this can be concluded that patients wearing denture have non-oral pathogenic bacteria in the saliva, which can be a primary cause for increased cases of infections. Thus, improved measures for maintaining oral health should be followed by patients who wear dentures.
Key words: Denture, bacterial species, gram negative bacteria.

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INTRODUCTION:

Human oral cavity is a reservoir of approximately 700 species of microorganisms.[1,2] 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 and oral hygiene practices.[3] Dentures made from synthetic polymers like polymethyl methacrylate are micro-porous in nature and, therefore, cause microorganisms 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.[4] Plaque, stain, and calculus accumulate on the dentures in a manner similar to natural teeth.[5,6]The subsequent proliferation of oral microbes and the formation of plaque on the non-shading surface may initiate pathogenesis from the oral to the systemic front. Hence, the present study was planned to assess presence of different bacterial species in denture wearer.

MATERIALS AND METHODS:

The study was conducted in the Department of Prosthodontics. The ethical clearance for study protocol was obtained from ethical committee of the institution. A

written informed consent was obtained from the participants before entering the study after they were briefed about the protocol of the study. For the study, 50 patients were selected, 25 males and 25 females, who were wearing removable dentures for more than a year and were admitted to the Department of Prosthodontics of the dental institute. Patients who had systemic conditions which could affect the bacterial flora and who had taken antibiotics in last 2 months were excluded from the study. 50 individuals with normal dentition and not wearing any dentures were selected as a control group.

For the collection of saliva, it was made sure that unstimulated saliva is collected after at least one hour of the last intake of drink or food. The subjects were directed to spit unstimulated saliva into sterile Flacon tubes containing 3 ml thioglycolate broth. Then, the saliva sample was centrifuged at 12,500 rpm for 10 minutes and the supernatant was discarded. Then, the precipitate was suspended in 1 ml of phosphate buffered saline to obtain a concentrated sample suspension. One loop full of concentrated suspension was inoculated onto EMB and MacConkey agar culture media. The culture medias were then incubated at 37 C for 24 hours and the growth of bacteria was observed. Suspected colonies were subjected to gram staining to identify gram negative rod bacteria.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

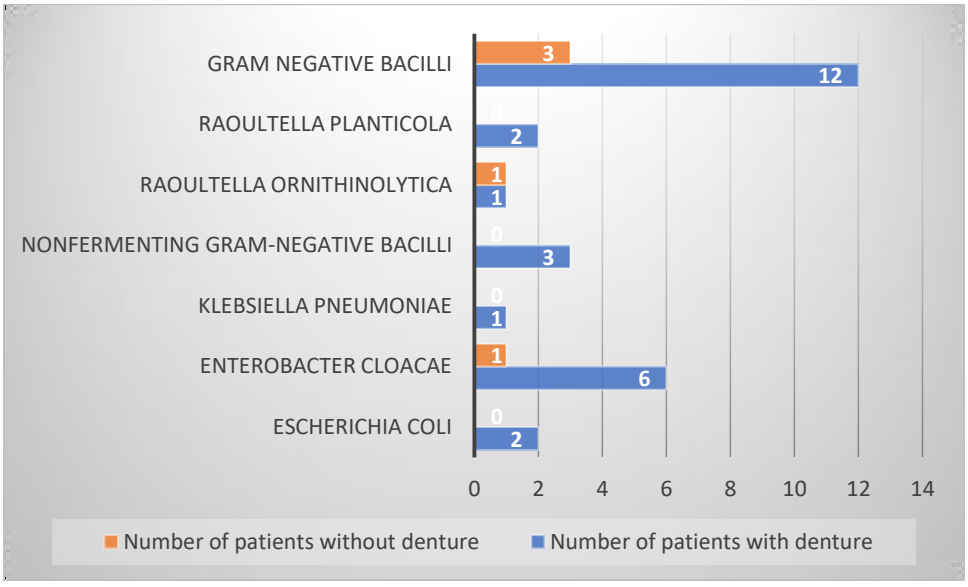
RESULTS:

In the present study, 50 denture wearer patients and 50 controls underwent testing of saliva. Table 1 shows Pathogens present in oral cavity of denture wearers and non-denture wearers. We observed that non-oral pathogens were more commonly seen in denture wearers. Gram negative bacilli were seen in 12 denture wearers and 3 non-denture wearers. Similarly, E. coli and E. cloacae are more commonly seen in denture wearers. On comparing, the results are statistically non-significant for all the pathogens except for Gram negative bacilli.

Table 1: Pathogens present in oral cavity of denture wearers and non-denture wearers

Bacteria	Number of patients with denture	Number of patients without denture	p-value
Escherichia coli	2	0	0.32
Enterobacter cloacae	6	1	0.65
Klebsiella pneumoniae	1	0	0.25
Nonfermenting Gram-negative bacilli	3	0	0.44
Raoultellaornithinolytica	1	1	0.98
Raoultellaplanticola	2	0	0.69
Gram negative bacilli	12	3	0.002

Figure 1: Pathogens present in oral cavity of denture wearers and non-denture wearers



DISCUSSION:

In the present study, we observed that non-oral pathogens are more common in patients who wear removable dentures. This could be due to failure to clean the denture and oral cavity properly and lodgment of food in the denture. The results were statistically non-significant except for Gram negative bacilli. On comparing the results with previous studies from the literature, it was found that the results are consistent. O'Donnell LE et al compared the microbiomes of denture wearers, and to understand the implications of these towards inter-kingdom and host-pathogen interactions within the oral cavity. Swab samples were obtained from 123 participants wearing either a complete or partial denture; the bacterial composition of each sample was determined using bar-coded illuminaMiSeq sequencing of the bacterial hypervariable V4 region of 16S rDNA. Sequencing data processing was undertaken using QIIME, clustered in Operational Taxonomic Units (OTUs) and assigned to taxonomy. The dentures were sonicated to remove the microbial flora residing on the prosthesis, sonicate was then cultured using diagnostic colorex Candida media. Samples of unstimulated saliva were obtained and antimicrobial peptides (AMP) levels were measured by ELISA. They have shown that dental and denture plaques are significantly distinct both in composition and diversity and that the oral microbiome composition of a denture wearer is variable and is influenced by the location within the mouth. Dentures and mucosa were predominantly made up of Bacilli and Actinobacteria. Moreover, the presence of natural teeth has a significant impact on the overall microbial composition, when compared to the fully edentulous. Furthermore, increasing levels of Candida spp. positively correlate with Lactobacillus spp. AMPs were quantified, though showed no specific correlations. This was the first study to provide a detailed understanding of the oral microbiome of denture wearers and has provided evidence that DS development is more complex than simply a candidal infection. Both fungal and bacterial kingdoms clearly play a role in defining the progression of DS, though we were unable to show a defined role for AMPs. Daniluk T et al determined the fungi occurrence rate in the oral cavity of denture wearer patients in comparison to those without dentures. The examinations were conducted in patients treated in two clinical departments of the University Hospital. Demographic data and those connected with basic diseases were collected and the evaluation concerning dentition and oral hygiene was performed. Samples for mycological examinations from the tongue dorsa, palatal mucosa, and mucosal surfaces of dentures were collected from patients with dentures while tongue and palate swabs were taken from those without dentures. For culture and identify of fungi standard methods were used. Dental and mycological examinations were performed in 95 patients, out of which 57 (60.0%) used complete or partial dentures and 38 (40.0%) had their own dentition (without dentures). Oral cavity revealed only growth of Candida albicans species, more frequently in patients with dentures (38/57; 66.7%) than in those without dentures (11/ 38; 28.9%). C. albicans statistically significantly more frequently was isolated in denture wearer patients with diabetes mellitus and without diabetes comparing to such groups of patients but without dentures. Among 32 patients with diabetes mellitus, 14 (43.8%) revealed C. albicans; this rate was comparable with 9/23 (39.1%) patients without diabetes. A similar analysis, conducted in 25 surgical patients with abdominal cancer and 15--without--cancers, did not show statistically significant differences in the incidence rate of C. albicans; it also concerned denture wearers (14/16; 87.5%) and non-wearing dentures (5/9; 55.6%) with cancer. In 37 (64.9%) wearer patients denture stomatitis was observed, associated mainly with C. albicans infections (29/37; 78.4%). Mycological findings from the study do not indicate that diabetes mellitus or advanced cancer has a significant effect on oral colonisation by Candida albicans or other species of Candida genus. [7, 8]

Daniluk T et al conducted another study to determine bacterial composition in the oral cavity of patients with removable dentures and with own dentition (without dentures). Bacteriological investigations were performed in 55 patients from the department of internal medicine (32 diabetic patients) and 40 patients treated in surgical department (25 patients with malignancy). Palate mucosa and tongue dorsa swabs were collected from two groups of patients, and additionally swabs from mucosal part of denture surfaces in prosthetic patients. Cultures in oxygenic and microaerophilic (5% CO2) conditions were conducted on solid non-selective and selective media as well as media enriched with 5% sheep blood. Standard procedures of bacterial culture and identification were applied. Among 95 of examined patients, 57 (60.0%) with removable dentures and 38 (40.0%) had their own dentition. As far as prosthetic patients were concerned, the rate of bacterial isolations from palate, tongue dorsa and denture plaque swabs were generally comparable; in number and species compositions. Statistically significant differences were observed in the bacterial composition of denture plaques, palate and tongue dorsa in patients with and without abdominal cancers. Patients without cancer did not reveal staphylococci and enteric bacteria in the samples from a various sites of their oral cavities. These bacteria were most common in cancer patients. Similar (in number and species) composition of bacteria occurred in palate and tongue swabs in patients without dentures. The incidence rate of aerobic bacteria in denture plaques and palatal mucosa of patients with (37/57; 64.9%) and without (20/57; 35.1%) denture associated stomatitis were comparable (except for Neisseria spp.). Generally, there were no statistically significant differences in species composition of bacteria isolated from the hard palate and tongue dorsa in patients with and without removable dentures. Staphylococcus spp. and Gram-

negative enteric bacilli were isolated more often from denture plaque, palate and tongue dorsa of cancer patients than from patients without cancer. Staphylococcus spp. was isolated more frequently from denture plaques of diabetic patients compared with non-diabetic patients. No significant differences observed in isolation frequencies (%) of aerobic bacteria in denture plaques and palatal mucosa of patients with and without denture associated stomatitis. Derafshi R et al investigated the nonoral pathogenic bacteria in the oral cavity of patients with removable dentures in Shiraz, Southern Iran. The bacterial flora of saliva samples from 50 men and 50 women with removable dentures and 100 age- and sex-matched controls with normal dentate were compared using culture, Gram staining, and API20E Kit methods. All data were analyzed using SPSS software. Except for Enterobacter cloacae isolate ($P = 0.03$), there was no significant difference between both groups for the presence of Escherichia coli, Klebsiella pneumoniae, nonfermenting Gram-negative bacilli, Raoultellaornithinolytica, Raoultellaplanticola, Kluyvera spp., and Enterobacter aerogenes. No significant correlation was noticed between age and presence of bacteria in the oral cavity. The Gram-negative rod bacteria were more in males, but the difference was not significant. When a total of Gram-negative rods were considered, there was a significant difference between case and control groups. Based on their findings that nonoral pathogenic bacteria are detected from the saliva of the denture wearers, general and oral health measures in patients with removable dentures should be adopted to decrease the risk of cross infection.[9, 10]

CONCLUSION:

From the results of the present study, this can be concluded that patients wearing denture have non-oral pathogenic bacteria in the saliva, which can be a primary cause for increased cases of infections. Thus, improved measures for maintaining oral health should be followed by patients who wear dentures.

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