Original Article

Isolation and Identification of Nonoral Pathogenic Bacteria in the Oral Cavity of Patients with Removable Dentures

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Abstract

Aims and Objectives: Dentures in the oral cavity may act as a reservoir of microorganisms, which may be related to systemic infections. The aim of this study was to investigate the nonoral pathogenic bacteria in the oral cavity of patients with removable dentures in Shiraz, Southern Iran.

Materials and Methods: 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.

Results: 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, *Raoultella ornithinolytica*, *Raoultella planticola*, *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 (P = 0.004).

Conclusions: Based on our 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.

Keywords: *Dental prosthesis, Gram-negative bacilli, nonoral pathogenic bacteria, oral cavity, saliva*

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Introduction

The normal microbial population included bacteria and fungi in the human oral cavity is very diverse.[1] Among all microbial strains, anaerobic Gram-negative bacteria and Streptococci (as Gram-positive bacteria) are the main types of human mouth's normal flora. Any alterations in this normal population affect the oral health because these microorganisms have pivotal roles in inhibiting of colonization of pathogenic microbiota.[1,2]

Complete or partial dentures are used by adults and elderly peoples in response to different factors that affect tooth extraction.[3,4] Dentures were shown to serve as a reservoir for oral bacteria and halitosis development that can be a concern for denture wearers.[5,6] Use of removable dental prosthesis induces some changes in the oral microbial population. In certain cases, this situation leads to dental prosthetic- or denture-associated stomatitis.[7]

Nonoral flora can cause diseases in patients who used contaminated denture or in technicians due to occupational hazards.[8] In two reports, colonization of denture materials with *Candida albicans* was identified as etiological cause of denture-associated stomatitis (sore mouth), which affects 24%–75% of denture wearers.[9-11]

Hence, new developments related to denture materials have been undertaken to decrease bacterial and yeast

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colonization to reduce denture stomatitis with appropriate denture hygiene.[12,13] Increased life span and dynamic development of prosthetic treatment resulted into an increase in the number of people using removable dentures for a long period that can be a potential pathogen factor for oral mucosa being in contact with this material. Prosthetic stomatopathy was reported in 20%–70% of patients with removable dentures.[14] Removable dentures have been proposed as a reservoir for pathogens to cause systemic infections.[15,16] Therefore, this study was conducted to determine the nonoral pathogenic bacteria in the oral cavity of patients with removable dentures.

Materials and Methods

Ethical statement

The protocol of this study was approved by the Local Ethical Committee of Shiraz University of Medical Sciences (8819-1), and all participants signed informed consent before entering the study. All subjects were informed about the study. The demographic details in relation to age, sex, oral hygiene practice, and medical history of each participant were recorded.

Reagents

Eosin methylene blue (EMB), thioglycollate broth, blood agar, barium chloride, sulfuric acid, crystal violate, safranin, lugol solution, acetone, ethanol, oxidase, and catalase reagents were purchased from Merck (Germany). API20E kit was obtained from Biomerieux (France). All other chemicals were analytic grade and of commercially available.

Participants

In a cross-sectional study from January 2014 to October 2015, 100 saliva samples were provided from 50 men and 50 women with removable dentures (case group). They were wearing their present dentures at least for 1 year and all were admitted to the Department of Prosthodontics, Dental School, Shiraz University of Medical Sciences, Shiraz, Iran. The sampling method was convenience sampling. The exclusion criteria were systemic conditions that could affect the bacterial flora and any use of antibiotics before the study. Among them, 90 were without any systemic disease, and seven suffered from hypertension. Their systemic diseases were under control and they used related drugs routinely. A control group consisted of 100 age- and sex-matched participants having their own dentition (wearing no dentures) and all were admitted to other departments of dental school for routine checkups, scaling, or filling.

Saliva sampling and microbial culture

The unstimulated whole saliva was collected between 10 am and 12 pm and at least 60 min after the last intake of drink or food. The subjects were instructed to spit unstimulated saliva into sterile Falcon tubes containing 3 ml thioglycollate broth. Each sample was centrifuged at 12,500 rpm for 10 min and the supernatant was discarded. 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 using a standard streak plate method. All culture plates were incubated at 37°C for 24 h, and the growth of bacteria was observed as pink- and white-colored colonies. The suspected colonies were subjected to Gram stain for identification of Gram-negative rod bacteria. Once identified, the colonies were further subjected to biochemical reactions by API20E Kit (Biomerieux).

Statistical analysis

All data were analyzed using SPSS software (SPSS Ltd., Hong Kong). The Chi-square test was used to correlate the positive and negative cases with dentures. Two-independent sample *t*-test was used to compare the groups regarding gender and age. Statistically, significant difference was considered when $P < 0.05$.

Results

The frequency of detected bacteria in relation to age in different groups is presented in Table 1. Except for *Enterobacter cloacae* isolate ($P = 0.03$), there was no significant difference between both groups for the presence of other bacteria. For Gram-negative rods, a significant difference was visible between case and control groups ($P = 0.004$). *E. cloacae* and Gram-negative bacilli (Enterobacteriaceae and nonfermenting) were significantly more visible in case group than the control group. There was no significant correlation between age and presence of bacteria in the oral cavity ($P = 0.07$).

Table 1: Presence of nonoral pathogens in saliva of removable denture wearer and nondenture wearing control groups

Presence of bacteria	Denture wearer	Control group without denture	*P*
Escherichia coli	1	0	0.999
Enterobacter cloacae	8	1	0.035
Klebsiella pneumoniae	2	3	0.999
Nonfermenting Gram-negative bacilli	4	1	0.212
Raoultella ornithinolytica	2	0	0.497
Raoultella planticola	1	1	0.999
Kluyvera spp.	3	0	0.246
Enterobacter aerogenes	0	1	0.999
Gram-negative bacilli (*Enterobacteriaceae* and nonfermenting Gram-negative bacilli)	21	7	0.004

The Gram-negative rods were prevalent more in men than women but with no significant correlation between gender and presence of the nonoral pathogens ($P = 0.08$).

Discussion

In the present study, the difference for microbial population in saliva samples between participants with removable dentures and the control group was compared revealing a variety of pathogens in removable dentures including *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. *E. cloacae* and Gram-negative bacilli (Enterobacteriaceae and nonfermenting) were significantly more in case group than the control group. Potentially pathogenic bacteria, including Gram-negative bacilli of *Acinetobacter*, *Pseudomonas*, *Moraxella Micrococcus*, and *Alcaligenes* species were reported as sources of contamination in commercial dental laboratories.[17,18]

The number of studies on denture plaque is lower than those performed on dental plaque with controversies on similarity of microbial flora. It was shown that Bifidobacteria were seen in denture plaque at the same level to those of carious lesions and *Bifidobacterium dentium* cannot be sustained in an edentulous mouth.[19] The presence of *Treponema denticola* and *Fusobacterium nucleatum* in edentulous area and the oral hygiene status of the mucosal or denture surfaces were demonstrated to affect the colonization by the bacteria.[20]

A typical biofilm with morphology of columnar microcolonies surrounded by maxillary epithelial cells was previously reported during imaging.[21] Changes in the mouth condition in response to a denture can lead to lack of saliva accessibility and tongue-related mechanical cleaning.[22,23] In addition, dentures were shown to harbor a mixed species of bacterial biofilm.[24,25]

Dentures can play significant roles in harboring of pathogens that cause inhalation pneumonia.[26] Some unusual microorganisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *E. cloacae*, and *Pseudomonas aeruginosa* were shown to be isolated from dentures.[27]

An increase in pneumonia was reported in individuals working in dental laboratories and exposed to lathe aerosol.[17] Manipulation of *Mycoplasma pneumoniae* contaminated prostheses caused infection in 10 subjects working in dental prosthetic laboratories,[28] while all of these studies denote to the public importance of removable dentures.

The microbial strains which detected in implants and adjacent teeth at 10 years after implantation were periodontitis-related strains in 6.2%–78.4% of the implants. A significantly higher count was noted for *Tannerella forsythia*, *Parvimonas micra*, *F. nucleatum*/*necrophorum*, and *Campylobacter rectus* in implants than the other teeth. In comparison to whom with removable maxillary prosthesis, it was demonstrated that *C. albicans* was the most frequently isolated yeast species in both groups.[29]

K. pneumoniae was found as the dominant bacterial species in cases wearing removable maxillary prosthesis with and without denture stomatitis lesions.[30] It was shown that the normal microbial population of oral cavity changed with increase in age due to immune system alteration and further colonization with nonoral bacterial species such as Staphylococci and Enterobacteriaceae.[31,32]

In our study, *E. coli* was isolated just from one patient, and other Gram-negative rods were visible in remained denture wearing patients. Unusual oral cavity microorganisms have been previously isolated from dentures and were reported by other researchers.[33-37] Umeda *et al*. reported *Helicobacter pylori* in the oral cavity of a patient with periodontal pockets that existed even after extirpation of the bacterium from the stomach.[38]

The tongue surface was also evaluated by some researchers. It was shown that 43% of the individuals had the microorganisms on the tongue dorsum, which was more prevalent in the age range of 40–50 years and who had not consumed cigarette. However, they could not find any correlations between detected species and the presence of dentures, indicating that tongue can act as an initial reservoir of the microorganisms. In the present study, different nonoral pathogens were noted in 21 cases and 7 controls. It seems that finding the source of these species in other parts of the body and indication of those site as a reservoir for denture infection must be addressed in future studies.[11,35,39]

In the present study, there was no statistically significant difference for age and gender between patients and the control group. Similar results have been reported before by Agwu *et al*. in HIV-positive patients.[3] Our findings are in line with the above-mentioned study and indicated that wearing of removable dentures can be considered as a risk factor for colonization of different species of microbiota and with public health importance.

Conclusions

Based on the findings in the present study that nonoral pathogenic bacteria are detected from the saliva of the denture wearers, considering the possibility that the oral cavity may act as a potential origin of pathogenic species

that may cause infection in other body sites, general and oral health measures in patients with removable dentures should be adopted to decrease the risk of cross-infection.

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Conflicts of interest

There are no conflicts of interest.

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