International Journal of Dental Sciences and Research, 2018, Vol. 6, No. 3, 74-77
Available online at http://pubs.sciepub.com/ijdsr/6/3/4

DOI:10.12691/ijdsr-6-3-4

Salivary Microflora of Complete Denture Wearing Patients

Sharma A[1,*], Chaudhari B[2], Shrestha B[3], Singh R. K.[1]

[1]Department of Prosthodontics and Crown and Bridge, B.P. Koirala Institute of Health Sciences, Dharan, Nepal
[2]Department of ENT, NAMS, Bir Hospital, Kathmandu, Nepal
[3]Department of Prosthodontics, Kantipur Dental College and Hospital, Kathmandu, Nepal
*Corresponding author: aratisharma122@yahoo.com, arati.sharma@bpkihs.edu

Abstract **Objectives** of this study were to see the types of aerobic microorganisms present in the saliva of Complete Denture wearing patients and to see any change in isolation frequency and types of microorganisms at 1-week of dentures in use. **Materials and Methods:** Ethical clearance from the institutional ethical committee and written consent from the patients were taken. All the patients were assessed by taking a detailed history and clinical examination which were recorded in the pre-designed proforma. Standard procedures accepted in the Department of Prosthodontics and in the Department of Microbiology were performed. Each patient were examined twice, once at 24hrs of denture insertion and then at 1-week of dentures in use. A micropippet was used to collect the resting saliva from the floor of the mouth and was diluted in 1ml of normal saline in a sterile vial and was immediately taken to the microbiology lab for culture within half an hour of sample collection. **Result:** Microorganisms identified at 24hrs of Complete Denture insertion were *Streptococcus spp.* (n=35), *Staphylococcus spp.* (n=15), *Klebsiella pneumonia* (n=5), *Acinetobacter anitratus* (n=5), *Enterobacter cloacae* (n=4), *Citrobacter freundii* (n=4), *Pseudomonas aeruginosa* (n=1) and *Proteus vulgaris* (n=1). Two new microorganisms were isolated at 1-week, *Escherichia coli* (n=1) and *Candida* albicans (n=2). Frequency of isolation of the gram negative bacteria increased at 1-week except for *Proteus vulgaris*. **Conclusion:** Use of Complete Denture favours colonization of some microorganisms which appear in saliva of patients.

Keywords: *aerobic microorganisms, denture microflora, denture-plaque, oral microflora, salivary microflora*

Cite This Article: Sharma A, Chaudhari B, Shrestha B, and Singh R. K., "Salivary Microflora of Complete Denture Wearing Patients." *International Journal of Dental Sciences and Research*, vol. 6, no. 3 (2018): 74-77. doi: 10.12691/ijdsr-6-3-4.

1. Introduction

Oral microflora changes quantitatively and qualitatively with age, presence or absence of diseases, presence or absence of teeth and other surfaces to which they can get attached [1,2]. The resident microflora plays an active role in the maintenance of the healthy state by contributing to the host defenses and preventing colonization by exogenous microorganisms. Disease can be a consequence of disruption of this resident microflora [3]. Deeper understanding of the relation between the host and its resident microflora may lead to new strategies for the prevention of disease via the active maintenance of a health-associated microflora.

Placement of a prosthesis in the oral cavity results in profound alterations of the oral environment as the prosthesis becomes colonized with oral microorganisms and cuts off the underlying mucosa from the mechanical cleansing effect of the tongue and the free flow of saliva which may lead to a substantial shift in bacterial composition and can even lead to oral and systemic infections [4,5].

So, the rationale of this study was to see the types of aerobic microorganisms found in the unstimulated saliva of apparently healthy patients at 24hrs of CD (Complete Denture) insertion and to see any change in frequency of isolation and type of microorganisms at 1-week of dentures in use.

2. Materials and Methods

This study was conducted in the Department of Prosthodontics and Crown and Bridge and Department of Microbiology, BPKIHS, Dharan, Nepal after obtaining ethical clearance from the institutional ethical committee. Duration of the study was one year. A total of 35 patients were included. Exclusion criteria were patients with any known systemic disease, salivary gland disorder, infectious disease, unhealthy oral-mucosa, any history of antibiotic therapy for the last 3-months, any adverse oral habit, patients not willing to give consent and old denture wearer.

All the patients were assessed by taking a detailed history and clinical examination which were recorded in the pre-designed proforma. Dentures were fabricated

according to the standard prosthodontic procedures accepted in the Department and sample collection, transport, culture and identification of microorganisms were performed according to the standard methods followed in the Department of microbiology. Each patient was examined twice, once at 24hrs of denture insertion and then at 1-week of denture in use.

2.1. Sample Collection

Patient was asked to take out the denture from the mouth. Sterile surgical gloves and mouth masks were worne. A micropippet was used to collect the resting saliva from the floor of the mouth and was diluted in 1ml of normal saline in a sterile vial and was immediately taken to the microbiology lab for culture within half an hour of sample collection.

2.2. Culture and Colony Counting

Membrane filter (Millipore 0.45 micron meter, 047mm) was placed in a sterile petridish and the diluted sample was spread over the membrane filter. It was left to dry for some time and then transferred with a sterile forcep on the Blood Agar. All these procedures were performed in a disinfected area within-1-feet of a burning Bunsen burner. After incubation of 12hrs, colony counting was done under the colony counter.

2.3. Sub-Cultures

Sub-cultures were performed repeatedly on Blood Agar and Mac Conkey Agar until pure bacterial isolates were obtained.

2.4. Gram-staining

Smear was prepared, gram-staining was done and was examined under oil immersion using compound microscope. Up-to this stage microorganisms were identified as either gram positive, gram-negative or Candida.

2.5. Biochemical Tests

To aid in the more definitive identification of the microorganisms, a series of biochemical tests were performed like catalase test, coagulase test, triple sugar iron test, sulphide indole motility test, urease test and citrate test.

3. Result

A total of 35 complete Denture patients ranging in age group from 54 years to 72 years (mean 65.40 yrs; SD±4.265) who visited the Clinic of Prosthodontics, CODS, BPKIHS, Dharan, Nepal from March 2012 to May 2013 were included in the study. Among them 17 were males and 18 were females. Colony counts, Gram-staining and Identified microorganisms from their saliva at 24hrs and at 1-week of dentures in use are shown in tables below [Table 1-Table 3].

Table 1. Colony Counts obtained from the saliva of 35 CD patients (35 samples)

Characteristic		Number of Samples (%)	
		At 24hrs	At 1-Week
Colony Counts	≤ 200	14 (40%)	1 (2.86%)
	≥ 300	21 (60%)	34 (97.14%)

Table 2. Result of Gram-Staining

Characteristic		Number of Isolates (%)	
		At 24hrs	At 1-Week
Gram Staining	GPC	50 (71.43%)	49 (50%)
	GNB	20 (28.57%)	47 (48%)
	Candida	0 (0%)	2 (2.04%)
Total No. of Isolates		70 (100%)	98 (100%)

Table 3. Microorganisms identified from the saliva samples

Characteristic		Number of Isolates (%)	
		At 24hrs	At 1-Week
Microorganisms Identified	*Streptococcus spp.*	35 (50%)	35 (35.81%)
	Staphylococcus spp.	15 (21.43%)	14 (14.28%)
	Klebsiella pneumoniae	5 (7.14%)	11 (11.22%)
	Enterobacter cloacae	4 (5.71%)	8 (8.16%)
	Acinetobacter anitratus	5 (7.14%)	10 (10.20%)
	Citrobacter freundii	4 (5.71%)	10 (10.20%)
	Escherichia-coli	0 (0%)	1 (1.02%)
	Pseudomonas aeruginosa	1 (1.42%)	6 (6.12%)
	Proteus vulgaris	1 (1.42%)	1 (1.02%)
	Candida albicans	0 (0%)	2 (2.04%)
Total No. of Isolates		70 (100%)	98 (100%)

Increase in the frequency of higher colony counts (≥300) and increased frequency of isolation of Gram Negative Bacilli (GNB) were seen at 1-week of dentures in use. Two new microorganisms which were not been isolated at 24hrs of denture insertion but isolated at 1 week of dentures in use were *Candida albicans* and *Escherichia-coli.* Increase in isolation frequency at 1-week of dentures in use was seen with *Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter anitratus and Citrobacter freundii.* Whereas, no increase in isolation frequency was seen with *Proteus vulgaris.* Low frequency of isolation as compaired to others was seen with *Proteus vulgaris, Escherichia-coli* and *Candida albicans.*

4. Discussion

Aerobic gram positive cocci (GPC) were isolated from higher numbers of the samples at 24hrs of CD insertion as compared to GNB. This finding is in agreement with the results of other authors [6,7]. *Streptococcus spp.* were a constant finding from all the samples both at 24hrs and at 1-week of CD in use. The second most commonly isolated microorganisms were *Staphylococcus spp.* The reason

may be that they are frequently found in the human respiratory tract and on the skin and a large proportion of their carriage is through the anterior nares of the nasal passages and they are not always pathogenic. *S. epidermidis* has the ability to adhere to biomaterials surface and develop as biofilm, which constitutes an important virulence factor and the most important pathogenic mechanism of staphylococcal infection [7]. These staphylococci have emerged in the last years as the most frequently isolated pathogen in nosocomial sepsis, associated with implanted medical devices, namely, prosthetic heart valves and joints, central venous catheters, urinary catheters, contact lenses, and hip prostheses [8]. *Staphylococcus aureus* is a major pathogen of increasing importance due to the rise in antibiotic resistance.

An unexpected spectrum of opportunistic pathogens including aerobic GNB and *Candida albicans* were also found. Isolation of aerobic GNB was in contrast to the finding of Ohman S C et al. [9] who found the bacteria of Enterobacteriacae familiy in a few cases only in their study. But, it was in agreement with the findings of other studies [2,3,6,10,11].

In our study the GNB isolated were, Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter anitratus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, and Citrobacter freundii. These are the microorganisms of the family Enterobacteriacea. They are opportunistic respiratory pathogens.

The frequency of isolation of *Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter anitratus and Citrobacter freundii.* increased at one week indicating that the presence of denture favoured colonization of those microorganisms. Whereas, very low frequency of isolation of *Proteus vulgaris* and *Escherichia coli* indicated that presence of dentures did not favour their colonization. The yeast isolated was *Candida albicans.* It was not isolated from any of the samples at 24hrs. At 1 week, it was isolated from 2 samples. This finding is in agreement with the fact that *Candida albicans* disappears from the oral cavity with the loss of all the teeth and reappears after rehabilitation with CD [12].

The low isolation frequency of *Candida albicans* as compared to other microorganisms from the saliva of CD wearers may also be due to the fact that the interaction between different micro-organisms in the biofilm may have an impact on colonization and subsequent infections. Several researchers have studied interactions among *Candida* and bacteria in an attempt to determine how oral bacteria may modulate *Candida* adherence and colonization. The influence of *Streptococcus salivarius* has been reported to decrease *Candida* adherence [13]. *Staph aureus* does not coagglutinate with yeast cells whereas Escherichia *coli*, a fimbriate strain produces a mannose-sensitive agglutination of C. *albicans* [14]. The study by El-Azizi M A *et al.* showed that, with the exception of the glycocalyx producer *P. aeruginosa*, the preformed biofilms of bacteria significantly reduced adhesion and biofilm growth of *C. albicans* [13].

Further work is required on the cell walls of the microorganisms to determine the specific adhesins and how these complex molecules relate to the salivary pellicle that forms the surfaces at different denture base materials. According to the ecological plaque hypothesis, it may be the proportions of pathogens, present that cause the change from health to disease, rather than the presence or absence of particular species but, it is also important to know which microorganisms are present in the oral cavity for the diagnosis and rational treatment of systemic as well as oral infections.

The medical community has placed little importance on daily CD care in denture wearers for the prevention of systemic infections [2]. Therefore, while diagnosing oral or systemic infections in an elderly CD wearing patient, physician must pay attention to CD plaque microorganisms so that life threatening infections can be prevented. Knowledge of the types of microorganisms in CD wearers can also be used to evaluate the efficacy of different denture cleansers in preventing colonization of the CD by those pathogenic microorganisms [15,16,17,18].

5. Conclusion

Use of complete dentures favoured colonization of some aerobic microorganisms which appeared in saliva of the patients like Streptococcus spp., Staphylococcus spp., Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter anitratus, Pseudomonas aeruginosa, Citrobacter freundii, and Candida albicans. Frequencies of isolation of Proteus vulgaris and Escherichia-coli were very low.

Limitation

Anaerobic culture was not done thus, missing many anaerobic microorganisms which may have been present in the saliva.

References

[1] Jorgensen E B. Ecology of Candida-associated Denture Stomatitis. *Microb Ecol Health Dis.* 2000; 12: 170-85.

[2] Sumi Y, Kagami H, Ohtsuka Y, et al. High correlation between the bacterial species in denture plaque and pharyngeal microflora. *Gerodontology.* 2003; 20: 84-87.

[3] Coulthwaite L VJ. Potential pathogenic aspects of denture plaque. *Br J Biomed Sci.* 2007; 64: 180-9.

[4] S.D. Harding, M. Wilson, C. Dickison, J. Howlett JH. The Cultivable Microflora of Denture Plaque from Patients with Denture-induced stomatitis. *Microb Ecol Health Dis.* 1991; 4: 149-57.

[5] Koopmans AS, Kippuw N de GJ. Bacterial Involment in Denture-induced stomatitis. *J Dent Res*. 67: 1246-50.

[6] Fatma Alzahraa M. Gomaa ZHH. Isolation and Identification of Microorganisms Associated With Removable Denture: Prevalence of Non Oral Pathogens. *Egypt Acad J Biol Sci. 2010; 2(2) 75-82*.

[7] P M. Presence of microorganisms on the fitting denture complete surface: study in vivo. *J Oral Rehabil.* 2000; 27: 708-13.

[8] Sousa C, Teixeira P, Oliveira R. Influence of Surface Properties on the Adhesion of Staphylococcus epidermidis to Acrylic and Silicone. *Int J Biomater.* 2009; 2009: 1-9.

[9] Ohman SC, Osterberg T, Dahlen G, et al. The prevalence of Staphylococcus aureus, Enterobacteriaceae species, and Candida species and their relation to oral mucosal lesions in a group of 79-year-olds in Goteborg. *Acta OdontolScand.*1995;53:49–54.

[10] Goldberg S, Cardash H, Browning H 3rd, Sahly H RM. Isolation of Enterobacteriaceae from the mouth and potential association with malodor. *J Dent Res.* 1997; 76: 1770-5.

[11] Conti S, dos Santos SS, Koga-Ito CY JA. Enterobacteriaceae and Pseudomonadaceae on the dorsum of the Human Tongue. *J Appl Oral Sci.* 2009; 17: 375-80.

[12] Budtz-Jørgensen E. Ecology of Candida-associated Denture Stomatitis. *Microb Ecol Health Dis.* 2000; 12: 170-185.

[13] Pereira-Cenci T, Del Bel Cury AA, Crielaard W TCJ. Development of Candida-associated denture Stomatitis: new insight. *J Appl Oral Sci. 2008; 16(2) 86-94*.

[14] Bagg J, Silverwood RW. Coagglutination reactions between Candida albicans and oral bacteria. *J Med Microbiol.* 1986; 22: 165-169.

[15] Paranhos Hde F, Panzeri H, Lara EH, Candido RC II. Capacity of Denture Plaque/Biofilm removal and Antimicrobial action of a new denture paste. *Braz Dent J.* 2000; 11: 97-104.

[16] Gornitsky M, ParadisI I, Landaverde G, et al. A clinical and microbiological evaluation of denture cleansers for geriatric patients in long-term care institutions. *J Can Dent Assoc.* 2002; 68: 39-45.

[17] da Silva FC, Kimpara ET, Mancini MN, Balducci I, Jorge AO K-IC. Effectiveness of six different disinfectants on removing Five microbial species and effects on the topographic characteristics of Acrylic resin. Journal of Prosthodontics. *J Prosthodont.* 2008; 17: 627-33.

[18] Dovigo LN, Pavarina AC, Ribeiro DG, et al. Microwave disinfection of complete dentures contaminated in vitro with selected bacteria. *J Prosthodont.* 2009; 18: 611-617.