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Research Article

A Comparative Study of Microorganisms Adhered to Different Surfaces of Complete Dentures

Arati Sharma[1]*, Bidhan Shrestha[2], Prakash K Parajuli[3], Pramita Suwal[4] and Raj K Singh[5]

[1]Assistant Professor, Department of Prosthodontics, College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

[2]Lecturer, Department of Prosthodontics, Kantipur Dental College and Research Center, Kathmandu, Nepal

[3]Associate Professor, Department of Prosthodontics, College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

[4]Additional Professor, Department of Prosthodontics, College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

[5]Professor, Department of Prosthodontics, College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

***Corresponding Author:** Arati Sharma, Assistant Professor, Department of Prosthodontics, College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Nepal.

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Abstract

Purpose: To study the types of aerobic microorganisms adhering to the polished and tissue surfaces of complete dentures after 24 hours and 1 week of wearing the dental prosthesis and to know whether there is any decrease or alteration of adherence of microorganisms on the polished and tissue surfaces of complete dentures.

Materials and Methods: A total of 35 patients visiting the department of Prosthodontics for complete dentures without any known systemic and oral diseases were included in the study. Dentures were fabricated according to the standard prosthodontic procedures accepted in the Department of Prosthodontics. Each patient was examined twice, once after 24hrs of denture insertion, then after one-week of denture in use wherein plaque samples were collected from the polished surface and the tissue surface. The samples were taken to the microbiology lab for microbiological investigations within half an hour of sample collection and different microorganisms were identified.

Results: *Streptococcus* spp. were the constant finding from all the samples. The second most commonly isolated microorganisms were *Staphylococcus* spp. An unexpected spectrum of opportunistic pathogens including aerobic Gram negative bacilli and *Candida albicans* were also found. The Gram-negative bacilli isolated were, *Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter anitratus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris*, and *Citrobacter freundii*. At 1-week of dentures in use, there was increase in the percentage of gram negative bacteria and decrease in the percentage of gram positive bacteria as compared to that at 24hrs from both the polished and tissue surfaces of the complete dentures. The increase was greater on the tissue surface.

Conclusion: Wide range of microorganisms are present in denture plaque and differences do occur between polished surface and tissue surface. A significant finding was the high isolation frequency of Staphylococcus spp. from the polished surfaces of the dentures which increased at 1 week but decreased from the tissue surfaces.

Keywords: *Denture Plaque; Microorganisms; Adherence; Complete Denture; Plaque*

Introduction

Synthetic acrylic resins are susceptible to microbial adhesion. The adherence of microorganisms is an essential and necessary first step in successful colonization of microorganisms. After adherence, aggregation and growth of microorganisms occur in the absence of appropriate denture hygiene, free salivary flow and mucosal cleansing by the tongue [1] thus forming the denture plaque

In the plaque, a variety of harmful products may be produced by both the yeasts and the bacteria, which may provoke mucosal inflammation and once the mucosa is inflamed its barrier function against microbial products is diminished [1]. Oral bacteria may be risk factors for a number of prevalent systemic diseases also [2,3].

Dentures offer a reservoir for microorganisms associated with these infections. Therefore, attention should be paid to the bacterial population in denture as a potential source of oral and systemic diseases. In addition to the significant gram-positive and fungal isolates, the gram-negative infections that become systemic are of particular concern because they possess lipopolysaccharides (endotoxin), which may initiate cascades of harmful cytokines such as tumor necrosis factor [4]. The already difficult chemotherapy of these microorganisms has been further complicated in recent years by the well-documented overall increase in antimicrobial resistance. Therefore, it is essential that clinicians be cognizant of the importance of appropriate prosthesis hygiene so that denture-related diseases can be avoided.

Thus, the purpose of this study is to compare the types of aerobic microorganisms adhering to the polished and tissue surfaces of complete dentures after 24 hours and 1 week of wearing the dental prosthesis and to know whether there is any decrease or alteration of adherence of microorganisms on the polished and tissue surfaces of complete dentures.

Materials and Methods

A total of 35 patients visiting the department of prosthodontics, college of dental surgery (CODS), B.P. Koirala Institute of Health Sciences, Dharan, Nepal for conventional complete denture treatment agreed to participate in the study. Inclusion criteria were all the patients visiting the department of Prosthodontics for CD except those with any known systemic disease, infectious disease, unhealthy oral mucosa, any history of antibiotic therapy for the last 3- months, any adverse oral habits like smoking and patients not willing to give consent. CD were fabricated according to the standard prosthodontic procedures accepted in the department of Prosthodontics by the same person. Each patient was examined twice, once after 24hrs of CD insertion, then after one-week of CD in use. Ethical clearance was obtained from Institutional Ethical Review Board of BPKIHS, Dharan, Nepal.

Sample collection

CD was taken out from the patient's mouth and the layer of saliva over it was washed-off with sterile normal saline [4]. Sterile cotton swab [2,5] stick was used to take the plaque sample by rotating the stick 5 times antero-posteriorly and medio-laterally respectively [4]. Two plaque samples were collected from each patient. One plaque swab was taken from the tissue surface (TS) including the rugae area crossing the mid-line and another plaque swab was taken from the polished surface (PS) including the posterior border of the denture.

Transport of the sample

Each swab sample was vortexed with hand in 2 ml of normal saline in a sterile vial and taken to the microbiology lab for culture within half an hour of sample collection.

Culture and colony counting

Membrane Filter (Millipore; 0.45-micron meter, 047 mm) was placed in a sterile petridish and the diluted sample was spread over the membrane filter. It was left to dry for sometime and then transferred with a sterile forceps 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.

Sub cultures

Sub-cultures were performed repeatedly on Blood Agar and Mac Conkey Agar until pure bacterial isolates were obtained. Each representative colony was described according to its size, shape, margin, elevation, pigment, density and surface. Thus, differences in colony morphologies helped to some extent to identify microorganisms. Also, the type of haemolysis present or absent in the blood agar and presence or absence of Lactose fermentation in Mac Conkey agar helped to some extent to distinguish the microorganisms.

Preparation of Smear and Gram-staining

After describing the colony morphology, the microorganisms were further identified by their differences in cellular morphologies and staining properties. For this, smear was prepared and gram-staining was done. Microscope slides were cleaned and dried thoroughly. The surface of the slide over which the smear was to be spread was flamed. The slide was labelled and a drop of sterile normal saline was put on it. A portion of colony from the culture plate was picked up with a sterile straight wire and emulsified with the normal saline and spread evenly. The smear was air dried, and then heat fixed by passing the dried slide three times slowly over the flame and allowed to cool. The inoculating wire was flamed and reflamed each time before picking up a portion of a colony. All these procedures were performed in a disinfected area within 1-feet of a burning Bunsen burner.

The heat fixed smear was covered with crystal violet stain for one minute. Then, the stain was rapidly washed off with clean water. Remaining water on the slide was tipped off and the smear was covered with Lugol's Iodine for one minute. Iodine was washed off with clean water. Then, decolourisation was done rapidly with Acetone and the smear was washed immediately with clean water. The counter stain was done with 0.1% Carbol-Fuchsin for 30 seconds. The stain was washed off with clean water. The smear was air dried and examined under oil immersion using a compound microscope. Findings were recorded. Thus, the microorganisms were identified as either gram positive or gram negative and either coccus-shaped or rod-shaped.

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.

All the data collected were entered in Microsoft Excel and data analysis was done using SPSS version 10 (SPSS, Inc., Chicago, IL). McNemar's test was applied.

Results

Results obtained from the 35 complete dentures at 24hrs

The colony count obtained were ≤ 200 from all the samples taken from polished surfaces and 25 samples of tissue surface as shown. Sub-cultures obtained a total of 73 isolates from polished surfaces of the complete dentures; 70 isolates from tissue surfaces.

Among the 73 isolates, 53 were Gram positive cocci (GPC) and 20 were Gram negative bacilli (GNB). Among those 53 isolates of Gram positive cocci, 35 isolates (48%) were *Streptococcus* spp., 18 (24.66%) were *Staphylococcus* spp. and among the 20 Gram negative bacilli, 3 (4.10%) were *Klebsiella pneumoniae*, 4 (5.48%) were *Enterobacter cloacae*, 6 (8.22%) were *Acinetobacter anitratus*, 1 (1.37%) was *Pseudomonas aeruginosa*, 1 (1.37%) was *Proteus vulgaris*, 5 (6.85%) were *Citrobacter freundii*.

Among the 70 isolates obtained from the tissue surfaces 50 isolates were GPC and 20 isolates were GNB. 35 isolates (50%) were *Streptococcus* spp., 15 isolates (21.43%) were *Staphylococcus* spp., 8 isolates (11.43%) were *Klebsiella pneumoniae*, 2 isolates (2.85%) were *Enterobacter cloacae*, 4 isolates (5.71%) were *Acinetobacter anitratus*, 1 isolate (1.42%) was *Pseudomonas aeruginosa*, 2 isolates (2.85%) were *Escherichia coli*, 1 isolate (1.42%) was *Proteus vulgaris*, 2 (2.85%) isolates were *Citrobacter freundii*. Isolation of microorganisms between polished surface and tissue surface was not statistically significant ($p > 0.05$) at 24 hrs.

Micro-organisms isolated	Number of Samples		*p value**
	Polished surface	Tissue surface	
Streptococcus spp.	35	35	1.00
Staphylococcus spp.	18	15	0.37
Klebsiella pneumoniae	3	8	0.06
Acinetobacter anitratus	6	4	0.50
Enterobacter cloacae	4	2	0.50
Pseudomonas aeruginosa	1	1	1.00
Escherichia coli	0	2	
Proteus vulgaris	1	1	1.00
Citrobacter freundii	5	2	0.37
Candida albicans	0	0	

Table 1: *Microorganisms Identified at 24 hours from polished and tissue surface of complete denture.*

** McNemar's test*

Results obtained from the 35 complete dentures at 1 week

The colony counts obtained from the 35 complete dentures were ≤ 200 counts from 28-samples of the polished surfaces and ≥ 300 counts from 7 samples of the polished surfaces; from the tissue surfaces only one sample showed colony count ≤ 200 and remaining 34-samples showed colony count ≥ 300.

Sub-cultures obtained a total of 109 isolates from the polished surfaces, 96 isolates from the tissue surfaces and 98 isolates from the saliva samples also. Among the 109 isolates of the polished surfaces, 60 isolates were GPC, 47 were GNB and 2(1.83%) were *Candida albicans*. 35(32.11%) isolates were *Streptococcus* spp. and 25(23%) were *Staphylococcus* spp. Among the gram-negative bacilli 11(10.10%) were *Klebsiella pneumoniae*, 7(6.43%) were *Enterobacter cloacae*, 6(5.50%) were *Acinetobacter anitratus*, 7(6.43%) were *Pseudomonas aeruginosa*, 3(2.76%) were *Escherichia coli*, 3(2.76%) were *Proteus vulgaris* and 10 (9.17%) were *Citrobacter freundii*.

Among the 96 isolates of the tissue surfaces, 47 isolates were GPC, 47 isolates were GNB and 2 (2.08%) were *Candida albicans*. 35 (36.46%) isolates were *Streptococcus* spp., 12 (12.50%) isolates were *Staphylococcus* spp., 9(9.38%) isolates were *Klebsiella pneumoniae*, 10 (10.41%) isolates were *Enterobacter cloacae*, 6 (6.25%) isolates were *Acinetobacter anitratus*, 7 (7.30%) were *Pseudomonas aeruginosa*, 3(3.13%) were *Escherichia coli*, 2 (2.08%) were *Proteus vulgaris*, 10 (10.41%) were *Citrobacter freundii*. *Staphylococcus* species were isolated significantly higher in number ($p = 0.000$) from polished surface compared to tissue surface. However, no any significant differences were found in other isolated microorganisms.

Micro-organisms isolated	Number of Samples		p value*
	Polished surface	Tissue surface	
Streptococcus spp.	35	35	1.00
Staphylococcus spp.	25	12	0.001
Klebsiella pneumoniae	11	9	1.00
Acinetobacter anitratus	6	6	1.00
Enterobacter cloacae	7	10	1.00
Pseudomonas aeruginosa	7	7	0.625
Escherichia coli	3	3	1.000
Proteus vulgaris	3	2	1.000
Citrobacter freundii	10	10	1.00
Candida albicans	2	2	

Table 2: *Microorganisms Identified at 1 week of CD insertion from polished and tissue surface of complete denture.*

** McNemar's test*

Discussion

Aerobic gram positive cocci (GPC) were isolated from significantly higher numbers of the samples at 24hrs of CD insertion as compared to the gram-negative bacilli (GNB). This finding is in agreement with the results of other authors [6].

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. Some species of *Staphylococcus* have unique surface features which are responsible for the distinct adherence behaviour. (P) Some are known producers of the surface polysaccharides intercellular adhesin (PIA), which is identified as one of the main responsible factors for biofilm formation [7].

Staphylococcus spp. were more frequently isolated from the PS of CD as compared to the TS at 24hrs and at 1- week. The reason for this may be that the polished surface is more accessible to the pharyngeal microorganisms as compared to the tissue surface. Also, the swab samples from the PS of the CD were taken from the posterior border whereas tissue surface swabs were taken from the rugae area. Pellicles of different composition (different proportions of salivary proteins etc.) are present on the TS and PS of CD which attracts microorganisms differently.

The frequency of isolation of *Staphylococcus* spp. increased at 1-week from the PS but slightly decreased on the TS of the CD. Again, the reason may be easy accessibility to the surface, difference in the environment as the TS forms a closed protected environment whereas PS plaque may be considered as an open growth system.

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.(P) 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 [7]. *Staphylococcus aureus* is a major pathogen of

increasing importance due to the rise in antibiotic resistance and our study has shown that CD plaque can be a constant source of Staphylococcal infection and should be paid attention.

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*. [8] who found the bacteria of *Enterobacteriacae* familiy in a few cases only in their study. But, it was in agreement with the findings of Alzahraa F., *et al*. [6] Coulthwaite L and Verran J, [3] Goldberg S., *et al*. [9] Sumi Y., *et al*. [2] Conti S., *et al*. [10].

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 pneumoniae* was higher from the TS as compared to the PS at 24hrs. The reason may be that the environment under the CD favoured the adherence of *Klebsiella pneumoniae* more than the PS. It increased significantly at one week from the PS and not significantly from the TS. The reason may be that the initial colonizers on the PS modified the environment in 1-week thereby providing conditions for the colonization by *Klebsiella pneumoniae*.

Initial adherence of *Enterobacter cloacae* is very low to either PS or TS. Frequency of isolation of *Enterobacter cloacae* increased significantly at 1-week from the TS but from the PS the increase was not significant.

The initial adherence of *Pseudomonas aeruginosa* to CD surfaces is very low to either the PS or TS. The frequency of isolation increased significantly (at 10%) ($P = 0.070$) from both the PS and TS ($P = 0.070$) at 1- week.

The initial adherence of *Escherichia coli* to the PS did not occur. From the TS the increase in the frequency of isolation was not significant ($P = 1.000$) at 1-week.

The very low frequency of isolation of Proteus vulgaris at 24hrs shows that the initial adherence of it to CD surfaces cannot occur. It increased but not significantly from either PS ($P = 0.500$) or TS($P = 1.000$) at 1-week.

The frequency of isolation of *Citrobacter freundii* increased but not significantly from the PS ($P = 0.180$) and increased significantly from the TS ($P = 0.039$) at 1-week.

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 from the PS and 2 samples from the TS ($P = 1.000$). 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 [1].

The low isolation frequency of *Candida albicans* as compared to other microorganisms from the CD plaques 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 [11].

Staph. aureus does not coagglutinate with yeast cells whereas *Escherichia coli*, a fimbriate strain produces a mannose-sensitive agglutination of *C. albicans* [12].

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* [11].

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 diseases.

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 adhering to different surfaces of CD can also be used to evaluate the efficacy of different denture cleansers in preventing colonization of the CD by these pathogenic microorganisms [13-17].

Conclusion

A wide range of microorganisms both normal oral flora and extra-oral pathogens may be present in CD plaque on its different surfaces. Opportunistic pathogens including aerobic GNB and *Candida albicans* are also present in the CD plaque. In this study the GNB isolated were *Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter anitratus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris,* and *Citrobacter freundii*. A significant finding was the high isolation frequency of *Staphylococcus* spp. from the PS of the CD which increased at 1 week but decreased from the TS.

Though the frequency of isolation of GNB was low at 24hrs, it increased significantly at 1-week from both the PS and TS. Thus, CD can offer a reservoir for microorganisms associated with both oral and systemic infections in the absence of proper denture hygiene and attention should be paid to the bacterial population in CD as a potential source of both oral and systemic diseases.

Conflict of Interest

There is no any conflict of interest.

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