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DENTURE WEARERS SHOW MORE DIVERSITY OF LACTOBACILLUS SPP. THAN KLEBSIELLA SPP. COMPARED TO NON-DENTURE WEARERS.

Microbiology

Bharathi Prakash* Asst. Prof. and Head, Department of Microbiology, University College, Mangalore University, Mangalore *Corresponding Author

Prof. Dr. Indrani Karunasagar Director (R&D), Nitte (Deemed to be University) , Centre for Science Education & Research, Deralakatte, Mangalore

Dr. Iddya Karunasagar Senior International Food Safety Consultant, Nitte (Deemed to be University) Deralkatte, Mangalore.

G D Khedkar Director, Paul Hebert's Centre for DNA Barcoding & Biodiversity Studies, Dr. BAM University, Auranagabad

ABSTRACT

Oral cavity is an ideal place for endogenous microbes to colonize as biofilm on the dentures causing oral infection. The study comprises denture wearers and non-denture wearers. The prevalence of Lactobacillus and Klebsiella Spp from denture flora of test and palatal flora of control groups was studied correlating the outcome with food, hygiene and age of the subjects. Samples were collected, cultured on to specific media and identified by standard methods. Data was statistically analyzed using student's "t" and "chi-square test". The result was correlated with the subject's denture/oral profile and microbial prevalence. In both the groups *Lactobacillus* spp. (78%) were the predominant and diverse bacterial isolates than *Klebsiella* Spp.(38%). Six *Lactobacillus* spp. were isolated from the test and four in control group. *K. pneumonia* and *K. oxytoca* were the only species in both the groups. Correlation was found between the higher prevalence of Lactobacillus than Klebsiella spp. and their lifestyle.

KEYWORDS

Denture flora, Lactobacillus, Klebsiella, Diet, Age.

INTRODUCTION

Dentures offer a reservoir for microorganisms. [1] The inert and micro porous nature of the denture attracts microbes easily to colonize on it. Various factors contribute for microbial adhesion on the dentures. It accumulates plaque, stain and calculus in a way similar to natural teeth which may initiate the pathogenesis at the oral and systemic front.[2] The surface roughness, type of denture material, denture cleansers, cleaning methods, denture cleaning frequencies and denture/oral hygiene correlating saliva, food particles age and host immunity are some of the factors that facilitates the adhesion of microbes on the dentures and oral cavity. The biofilm formation on denture and subsequent oral pathologies are due to the inert nature of denture material, Within 24 hours of exposure, microorganisms can substantially contaminate removable oral appliances.[3] Oral bacteria like Lactobacillus and Klebsiella have been implicated in bacterial endocarditis, aspiration pneumonia, gastrointestinal infection and chronic obstructive pulmonary disease, malodour. depressive symptoms especially in elderly people.[4,5] With this background the prevalence of Lactobacillus and Klebsiella species were studied in correlation with subject's lifestyle.

MATERIALS AND METHODS

The samples of the study were collected over a period of ten months from subjects visiting a dental institute from a town of Dakshina Kannada district, Karnataka. Informed consents were obtained from the subjects prior to the study. In the study design, the subjects aged between 31-80 yrs. and who were not on any immunosuppressive, antibiotics or antifungal medications were included. The subjects were divided into two groups; as 'test' and 'control' groups. The test group included 50 healthy denture wearers with twenty eight men and twenty two women (mean age 54.38±10.31). For denture wearers the inclusion criteria was wearing dentures for minimum of one year and who visited the Prosthodontics department of the institute. In the control group 50 healthy non denture wearers with thirty men and twenty women were included (mean age 51.92±9.2). For sample collection in test group, the palatal mucosa of the candidates was examined for no signs of denture induced stomatitis and oral lesions. Their living standards and socioeconomic status were not considered but as and when the subject was available, the samples were collected. A questionnaire was prepared to document the oral and denture profile of the subject in both the groups. It includes subject's daily habits of consuming food and beverages, denture cleaning frequencies, maintenance, smoking, chewing tobacco etc. Denture and oral, hygiene was visually examined by the prosthodontist and they were graded as good, average and poor.

Accumulation of denture plaque was observed to be higher in the posterior maxillary fitting surface regions than the anterior regions and in the interior rather than the surface of denture.[6] Hence the tissue contacting surface of upper denture surface was considered for isolation of microbes in this study. Using a sterile wet swab, the tissue contacting surface of the dentures were wiped thoroughly and processed for microbiological examination within one hour. Each swab was immersed in five ml of physiological saline and vortexed to disperse the adhering bacteria to the swab. A loopful of this suspension was plated onto the selective media such as Mac Conkeys agar (MCA) and Rogosa agar (RA) (Hi Media, Mumbai.). The MCA plates were incubated at 37°C for 24 hrs while the RA plates were incubated for 48 hrs at 37°C. The plates were assessed by semi quantitative standard method.[7] Representative colonies from MCA and RA plates were picked and subjected to a series of biochemical tests for identification of the bacteria. Standard procedures were applied for the microbial culture and identification. Data generated was analysed using 'student's *'t'* and "chi-square test".

RESULTS:

Denture flora from test group and palatal flora from control group showed few predominant and some less prevalent isolates with diversity on MCA and RA medium. The predominant bacterial isolates were Lactobacillus and Klebsiella spp in both the groups. Among Lactobacilli *L. casei, L. plantarum, L. fermentum, L. brevis, L. salivarius and L. acidophilus* were the isolated species in test group from RA. While control group showed Lactobacillus species same as test group except the absence of *L. brevis* and *L. acidophilus*. On MCA, *K. pneumonia* and *K. oxytoca* were the common species in both the groups. Apart from more prevalent Lactobacillus and Klebsiella species, *E.coli, Staphylococci, Enterococci,* and *Pseudomonas* species were the less prevalent isolates in both the groups while Serratia *spp* were found only in the test group. The study showed significant difference in the prevalence of denture and oral isolates with P value, $p < 0.001$, as shown in the table 1.

Table.1: Prevalence of Lactobacillus and Klebsiella spp. in test and control group

Most prevalent bacteria from test and control group			
Sl No.	**Microbial Isolates**	**Test group**	**Control group**
A. Prevalence of Lactobacillus spp.		**% in Test group =78 % (n=50)**	**% in Control group =36% (n=50)**
1.	*L. fermentum*	23%	3%
2.	*L. plantarum*	33%	36%
3.	*L. casei*	33%	56 %
4.	*L. salivarius*	11%	2%
5.	*L. brevis*	2%	Nil
6.	*L. acidophilus*	2%	Nil
B. Prevalence of Klebsiella spp		% in Test group=78%(n=50)	% in control group= 34% (n=50)
1.	*K.pneumonia*	82 %	94 %
2.	*K. oxytoca*	18 %	6 %
C. Less prevalent bacteria		**% In Test group (n=50)**	**% In control group (n=50)**
3.	Staphylococci Spp.	18%	10%
4.	Pseudomonas Spp.	5%	2%
5.	*E.coli*	2%	2%
6.	Serracia Spp.	2%	Nil

However, the prevalence and distribution of *Lactobacillus* species in both the groups also showed significant difference with x^2 test P values as X2=16.176, p< 0.001. But in case of *Klebsiella*, though the percentage of occurrence in both the groups was different, there were only two common species i.e. *K. pneumonia* and *K. oxytoca* with x^2 test and P values as, x^2=19.642, p< 0.001.

When other factors from subjects profile were correlated with the microbial findings, in test and control group, highest population in the study belonged to 51 to 60 yrs. of age. Percentage of 'average', 'denture and oral' hygiene subjects were more compared to 'good and poor' and they belonged to age group of 41 to 70 yrs. There was no subject in age group of 70 to 80 yrs. in control group. The numbers of 'poor' 'denture and oral hygiene' subjects were more in 61 to 70 yrs. age group and not found in the 31 to 40 yrs. age group. 'Good and Poor' hygiene subjects in both the groups were more or less same. In control group, the Lactobacillus and Klebsiella isolates were maximum in 41 to 60 yrs. while in test group those were highest in 41 to 70 yrs of age group. The details of age distribution, denture and oral hygiene status of test and control groups, gender and prevalence of Lactobacillus and Klebsiella spp. isolated from test and control group are given in the table 2 and 3.

Table.2: Age distribution, oral hygiene, gender and prevalence of Lactobacillus and Klebsiella among healthy denture wearers (test group).

	Lactobacillus (36)							**Klebsiella (37)**						
Age group	**Denture hygiene**						**Total**	**Denture hygiene**						**Total**
	Good		**Average**		**Poor**			**Good**		**Average**		**Poor**		
	M	**F**	**M**	**F**	**M**	**F**		**M**	**F**	**M**	**F**	**M**	**F**	
31 to 40	-	-	1	3	-	-	4	-		1	2	-	1	4
41 to 50	1	1	2	2	1	1	8	2	1	2	3	1	1	10
51 to 60	2	3	4	3	1	-	13	1	3	2	5	-	-	11
61 to 70	-	1	3	1	3	1	9		2	4	1	5		12
71 to 80			-	-	2		2	-	-	-		-	-	-
Total	3	5	10	9	7	2	36	3	6	9	11	6	2	37
	8		19		9			9		20		8		37
	Male -20, Female -16, Total -36							**Male -19, Female -18, Total -37**						

Table.3: Age distribution, oral hygiene, gender and prevalence of Lactobacillus and Klebsiella among healthy non denture wearers (Control group).

	Lactobacillus (17)							**Klebsiella (16)**						
Age group	**Oral hygiene**							**Oral hygiene**						**Total**
	Good		**Average**		**Poor**			**Good**		**Average**		**Poor**		
	M	**F**	**M**	**F**	**M**	**F**	**Total**	**M**	**F**	**M**	**F**	**M**	**F**	
31 to 40	-	1	2	-	-	-	3	-	-	-	1	-	-	1
41 to 50	-	2	2	1	-	1	6	1	1	2	2	-	1	7
51 to 60	1	-	1	2	1	1	6	-	-	1	3	1	1	6
61 to 70	-	-	-	-	2	-	2	1	-	1	-	-	-	2
71 to 80	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total	1	3	5	3	3	2		2	1	4	6	1	2	-
	4		8		5		17	3		10		3		16
	Male -9, Female -8, Total -17							Male -07, Female -09, Total -16						

The difference found in the distribution of Lactobacillus and Klebsiella spp. in both the groups among male and female was statistically insignificant. Out of the total subjects in both the groups, 25% were vegetarians and 75% were non vegetarians. Among denture wearers 71% were cleaning dentures with water after every meal.19% of the denture wearers cleaning dentures only once and out of them majority (18%) were from 61 to 70 yrs of age group. Remaining subjects were cleaning dentures at least twice a day.

DISCUSSION

Dentures are made up of polymers primarily polymethyl methacrylate. Insertion of the denture itself brings changes in the physiology and the normal flora of palate. Tissue contacting surface of the denture being less disturbed and relatively stagnant, enhances easy colonization of microbes especially acidogenic bacteria and *Candida*.[8, 9] The rate of adhesion of *Candida* spp. isolated from denture plaque to exfoliated buccal epithelial cells has been shown to be very low, indicating that dentures supports microbial colonization than mucosal epithelia. Similar mechanism might be true for bacteria in the oral cavity hence the denture flora is more than oral flora.[10] Thus denture wearers are at higher risk of developing oral infections.

Many studies state that in addition to Gram positive cocci and Gram negative rods, *Lactobacilli* and yeast are commonly isolated from the

dentures.[11] Our study also reports the similar result with predominant Lactobacillus and Klebsiella species. Lactobacilli are the part of human normal oral flora. In edentulous people Lactobacilli do not have significant role in pathogenesis. Whatever may be the sampling method or the identification technique, among oral Lactobacilli, most species belong to *Lactobacillus casei* group.[12, 13] Supporting this statement, our study shows highest prevalence of *L. casei* in both test and control group while *L. plantarum* was found in equal prevalence among test group.

The higher prevalence of *Lactobacilli* spp. observed among older denture wearing subjects can be attributed to their age and their denture hygiene. Oral Lactobacilli creates acidogenic environment by acid formation thereby nurturing acid loving microbes in the oral cavity. Studies indicate a strong correlation between the salivary Lactobacilli count and pathologies like dry mouth and depressive symptoms.[14] Therefore our data showing higher prevalence of Lactobacillus spp. among elderly people in test group may lead to similar pathologies. Though *Lactobacillus* is not pathogenic it may act as a "catalyst" for adhesion and biofilm formation for other microbes.

A strong correlation was found between microorganisms of dentures and that of pharyngeal mucosa.[15] Respiratory pathogens are known to preferentially colonize teeth or dentures, rather than soft tissue.[16] In contrast to several studies stating that in the denture plaque, Gram-negative rods are relatively few compared to Gram positive cocci and short rods, we could isolate *Klebsiella* species from 78% of the denture wearers**.** High prevalence of *Klebsiella* in our study shows that the dentures can act as a reservoir for these opportunistic pathogens which may cause mucosal and respiratory infections like aspiration pneumonia.

Klebsiella species are not generally considered as members of oral microbiota. Thus it's occurrence in such high number is of significance as a potential pathogen. Previous studies states that isolates of *Klebsiella* and *Enterobacter* emit foul odours *in vitro* resembling bad breath with concomitant production of volatile sulphides and cadaverine whereas both compounds are related to bad breath in denture wearers. *K. oxytoca* found on the tongue scrapings and denture was also found to be associated with oral malodour.[17] Our study shows much lesser numbers of *K. oxytoca* in both test and control group compared to K. *pneumonia.* The denture wearers also reported the similar denture and oral foul odour, which may be due to the microbial biofilm involving Klebsiella spp. Various studies state that not any one but multiple factors, mainly bacterial species including anaerobes are responsible for the oral malodour.[1819]

The route of entry of oral and denture microbes might be from external sources like food and drink than from endogenous flora. The highest prevalence of Lactobacillus in both the groups might be from dairy products. As per the subjects profile, milk, curd and buttermilk were the part of their daily diet which might have contributed to their denture and oral flora with multispecies of Lactobacillus. Subjects profile in the age group of 50 to 70 yrs, showed daily intake of rice with curd /buttermilk as it conditions gut due to probiotics. This correlates with the fact that highest numbers of Lactobacillus spp. found in the age group of 51 to 70 yrs. which might have contributed to their oral flora. Similarly the source of Klebsiella might be water, food and other source as dentures were kept in a container with tap water at night. The effect of consuming vegetarian and / or non-vegetarian diets, tea, coffee and other drinks like homemade decoctions (kashayas) were found insignificant on these microbial isolates and their prevalence in this study.

To get rid of the denture biofilm, its associated infections and denture malodour, good denture hugiene is necessary. Geriatric denture wearer's especially dependent ones find it difficult to clean the dentures on a regular basis.[20] Poor denture hygiene was observed highest among age group of 51 - 70 yrs. compared to other age groups. Inadequate and irregular cleaning of denture /oral cavity makes the hygiene even poorer and this declines with age.

This study was focused mainly on the isolation of Lactobacillus and Klebsiella Spp., present in the oral and denture flora by conventional techniques. But molecular identification techniques might help to explore wider range of non-cultivable flora in both the groups.

CONCLUSION:

Our study concludes that denture wearers although free from diseases are asymptomatic carriers of abundant microflora thereby exposing themselves to the greater risk of infections. Regular cleaning of dentures having any type of diet for both the genders with growing age is necessary to keep good oral and overall health. Innovation of biofilm resistant material for denture fabrication is needed to minimize potential oral pathologies in elderly people

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