ORIGINAL RESEARCH

Beyond Decay: Exploring the Age-associated Variations in *Streptococcus mutans* and *Lactobacillus* in Dental Caries

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Abstract

Background: *Streptococcus mutans* and *Lactobacilli* play an important role in the etiopathogenesis and progression of dental caries (DC). Their quantification and identification may be helpful for epidemiological and early intervention measures.

Objectives: We conducted the study to evaluate the colony counts of *S. mutans* and *Lactobacillus* with the location of DC and correlate their prevalence with the age of the patient.

Materials and methods: The study population comprised 60 patients with DC. They were divided into two groups according to age, and each group was further divided into three subgroups based on involvement of enamel, dentin, and pulp by DC. The swab samples were collected, and organisms were isolated using Mitis Salivarius Bacitracin (MSB) Agar and *Lactobacillus* MRS Agar. Manual counting of colonies on plates illuminated by transmitted light was done. Results were summarized and analyzed statistically.

Results: The caries prevalence was found to be higher in children, with females being more affected. In both groups, posterior teeth were more affected, and occlusal/incisal surface caries were more common. The mean colony count of *S. mutans* (61.3%) and *Lactobacillus* (63.4%) was significantly higher in group I compared to group II. In both groups, the mean colony counts of *S. mutans* were higher in enamel, followed by dentin and pulp. In contrast, in both groups, the mean colony counts of *Lactobacillus* were higher in pulp, followed by dentin and enamel.

Conclusion: Bacterial colony counts may help in taking specific measures against specific organisms and thereby prevent the development of new carious lesions.

Keywords: Colony count, Dental caries, *Lactobacillus*, *Streptococcus mutans*.

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Introduction

Dental caries (DC) is a chronic infection caused by normal oral microbial flora.[1] Imbalance between pathological factors leading to the demineralization of tooth tissue and protective factors causing remineralization leads to DC.[2] *Streptococcus mutans*, the major cariogenic organism,[3] along with *Lactobacilli*, is implicated as important contributory bacteria in tooth decay and progression of the disease.[4]

Quantification and identification of *S. mutans* and *Lactobacillus* may be helpful for epidemiological and early intervention measures. We conducted the study to evaluate the colony count of *S. mutans* and *Lactobacillus* with the location of DC and to correlate their prevalence with the age of the patient.

Materials and Methods

The study population comprised 60 patients divided into two groups. Group I comprised 30 patients aged between 5 and 15, and Group II comprised 30 patients aged 16 years and above. Further, the 30 patients were divided into three subgroups in both Group I and Group II, and the salivary samples were taken accordingly.

- Group I (a) and Group II (a) comprised patients affected with caries involving only the enamel.
- Group I (b) and Group II (b) comprised patients affected with caries extending to the dentin.
- Group I (c) and Group II (c) comprised patients affected with caries extending to the pulp.

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The informed consents were obtained after the patients and their parents were informed of the study and the related procedures. Approval from the Institutional Ethics Committee had been obtained prior to the study.

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The diagnosis of caries was done clinically by means of visual and tactile methods and by use of intraoral periapical radiographs. Patients having signs and symptoms of DC were included in the study, and those with evidence of periapical infections, periodontal infections, systemic diseases, and those undergoing fluoride treatment were excluded from the study.

Mitis Salivarius Bacitracin (MSB) Agar Base (HiMedia Laboratories, Mumbai) was used for *S. mutans*. Mitis salivarius agar is used for the isolation of streptococci (Fig. 1), especially *Streptococcus mitis*, *Streptococcus salivarius*, and *Enterococcus faecalis* from grossly contaminated specimens. It was modified by adding 0.2 units/mL bacitracin to make it a selective medium for *S. mutans*. Around 90.07 gm of agar powder was suspended in 1000 mL of distilled water. This mixture was heated until boiling to dissolve the medium completely. Autoclaving at 15 lbs pressure (121°C) for 15 minutes was done for sterilization. Then the solution was cooled to 50–55°C, and 1 mL of sterile 1% potassium tellurite solution was added. The mixture was mixed well and poured into sterile Petri plates for bacterial culture.

Lactobacillus MRS Agar (HiMedia Laboratories, Mumbai) was used for the cultivation of all *Lactobacillus* species (Fig. 2). Around 67.15 gm of agar powder was suspended in 1000 mL of distilled water. The mixture was heated until boiling to dissolve the medium completely. Sterilization was done by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Finally, the contents were mixed well and poured into sterile Petri plates for bacterial culture.

Salivary samples, along with caries debris, were collected under sterile conditions from patients having signs and symptoms of DC using sterile cotton swabs (Fig. 3). The swabs were inserted at carious sites, kept for 1–2 minutes, taken out, and placed in a sterile vial containing 1 mL saline for transportation. A loopful (10 µL) of the saliva along with caries debris was streaked on different isolation media. The inoculated plates were incubated at their respective temperatures, that is, *S. mutans* for 48 hours at 37°C and *Lactobacillus* for 24 hours at 37°C. After bacterial cultivation, the bacterial count of *S. mutans* and *Lactobacillus* was done in colony forming units (CFUs) and recorded as CFU/mL × 10^3 for each sample. Manual counting of colonies on plates illuminated by transmitted light was done.

The results were tabulated and subjected to appropriate statistical analysis. Data were summarized as Mean ± standard error of the mean (SE). Groups were compared by independent Student's *t*-test. Groups were also compared by two-way analysis of variance (ANOVA), and the significance of mean difference within and between the groups was determined by Tukey's *post hoc* test after ascertaining normality by the Shapiro–Wilk test and homogeneity of variances by Levene's test. Categorical groups were compared by Chi-squared (χ^2) test. A two-tailed ($\alpha = 0.05$) *p*-value < 0.05 was considered statistically significant.

Fig. 1: Mitis salivarius agar and colonies of *S. mutans* on agar plate

Fig. 2: *Lactobacillus* MRS Agar and colonies of *Lactobacillus* on agar plate

Results

The demographic characteristics of the study population are summarized in Table 1.

Bacterial Colony Count

The bacterial colony counts of *S. mutans*, *Lactobacillus*, and total (*S. mutans* + *Lactobacillus*) according to groups (group I and group II) are summarized in Table 2.

The bacterial colony counts of *S. mutans*, *Lactobacillus*, and total (*S. mutans* + *Lactobacillus*) according to groups (group I and group II) and tooth involved (posterior and anterior) are summarized in Table 3. DC incidence was found to be higher in posterior teeth in both group I and group II. The mean colony counts of *S. mutans*

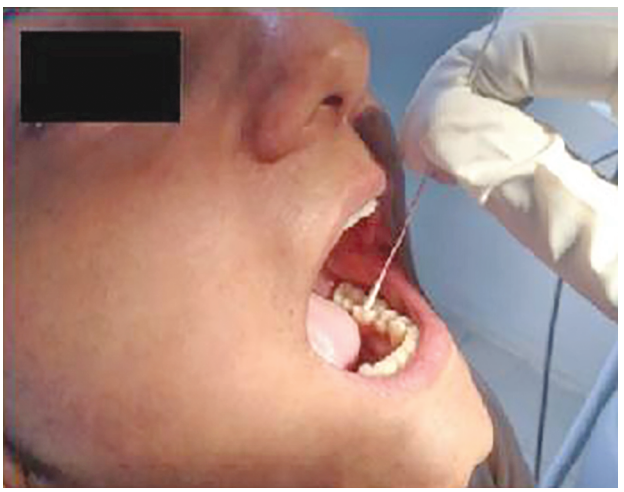

Fig. 3: Collection of saliva along with caries debris through sterilized aluminum swab stick

were higher in posterior than anterior teeth and higher in group I than group II, while those of *Lactobacillus* were higher in anterior than posterior teeth and higher in group I than group II.

The bacterial colony counts of *S. mutans*, *Lactobacillus*, and total (*S. mutans* + *Lactobacillus*) according to groups (group I and group II) and tooth surface (occlusal/incisal and smooth surface) are summarized in Table 4.

Dental caries was found to be more on the occlusal/incisal surface in both group I and group II. The mean colony counts of *S. mutans* were higher on the smooth surface than on the occlusal/incisal surface and higher in group I than group II. In contrast, the mean colony counts of *Lactobacillus* were higher on the smooth surface than on the occlusal/incisal surface in group I. However, in group II, the mean colony counts of *Lactobacillus* were higher on the occlusal/incisal surface than on the smooth surface.

The bacterial colony counts of *S. mutans*, *Lactobacillus*, and total (*S. mutans* + *Lactobacillus*) according to groups (group I and group II) and tooth site (enamel, dentin, and pulp) are summarized in Table 5.

For each group, the comparison (*p*-value) of the mean difference in bacterial colony count between the sites is shown in Table 6.

Table 1: Demographic characteristics of two groups

Demographic characteristics	*Group I (n = 30) (%)*	*Group II (n = 30) (%)*
Age (years)		
Mean ± standard error of the mean (SE)	10.10 ± 0.45	30.30 ± 1.78
Sex		
Male	13 (43%)	11 (36%)
Female	17 (57%)	19 (64%)
Teeth involved		
Posterior (%)	18 (60%)	20 (67%)
Anterior (%)	12 (40%)	10 (33%)
Surface		
Occlusal/incisal	18 (60%)	16 (53%)
Smooth surface	12 (40%)	14 (47%)

Discussion

The oral cavity harbors one of the most complex microbiomes in the body, and oral bacteria are important contributors to the occurrence and progression of DC. DC is a prevalent chronic disease.[5] In the oral cavity, there is a biofilm (dental plaque) that comprises >800 species of microorganisms living in a complex community.

Table 2: Bacterial colony count (mean ± SE) of two groups

Bacterial colony count (CFU)	*Group I (n = 30)*	*Group II (n = 30)*	*t-value*	*p-value*
S. mutans	32180 ± 7309	12453 ± 4920	2.24	0.029
Lactobacillus	22517 ± 6062	8240 ± 3458	2.05	0.045
Total	54697 ± 6731	20693 ± 5498	3.91	<0.001

Table 3: Bacterial colony counts according to groups and tooth involved

		Group I		*Group II*		
Bacterial colony count (CFU)	*Tooth involved*	*N*	*Mean ± SE*	*N*	*Mean ± SE*	*p-value*
S. mutans	Posterior	18	32856 ± 9771	20	17375 ± 7167	0.999
	Anterior	12	31167 ± 11431	10	2610 ± 986	0.685
	p-value	–	0.513	–	0.223	–
Lactobacillus	Posterior	18	20433 ± 8012	20	5330 ± 2011	0.956
	Anterior	12	25642 ± 9589	10	14060 ± 9636	0.842
	p-value	–	0.331	–	0.755	–
Total	Posterior	18	53289 ± 9538	20	22705 ± 6879	0.993
	Anterior	12	56808 ± 9329	10	16670 ± 9473	0.968
	p-value	–	0.039	–	0.040	

Table 4: Bacterial colony counts according to groups and tooth surface

		Group I		*Group II*		
Bacterial colony count (CFU)	*Tooth surface*	*N*	*Mean ± SE*	*N*	*Mean ± SE*	*p-value*
S. mutans	Occlusal/incisal	18	31394 ± 9194	16	10381 ± 6066	0.999
	Smooth surface	12	33358 ± 12489	14	14821 ± 8148	0.985
	p-value	–	0.302	–	0.530	–
Lactobacillus	Occlusal/incisal	18	19533 ± 6839	16	11637± 6386	0.883
	Smooth surface	12	26992 ± 11435	14	4357 ± 1096	0.885
	p-value	–	0.834	–	0.162	–
Total	Occlusal/incisal	18	50928 ± 8273	16	22019 ± 7984	0.880
	Smooth surface	12	60350 ± 11625	14	19179 ± 7755	0.996
	p-value	–	0.076	–	0.017	

Table 5: Bacterial colony counts according to groups and tooth site

		Group I		*Group II*		
Bacterial colony count (CFU)	*Tooth site*	*N*	*Mean ± SE*	*N*	*Mean ± SE*	*p-value*
S. mutans	Enamel	10	79500 ± 9788	10	32500 ± 12829	<0.001
	Dentin	10	15600 ± 6002	10	4100 ± 1345	0.857
	Pulp	10	1440 ± 521	10	760 ± 88	1.000
Lactobacillus	Enamel	10	1140 ± 442	10	2000 ± 996	1.000
	Dentin	10	3410 ± 1234	10	4020 ± 1382	1.000
	Pulp	10	63000 ± 8950	10	18700 ± 9707	<0.001
Total	Enamel	10	80640 ± 9654	10	34500 ± 12528	0.006
	Dentin	10	19010 ± 6118	10	8120 ± 1778	0.951
	Pulp	10	64440 ± 8973	10	19460 ± 9656	0.008

Table 6: For each group, comparison (*p*-value) of mean difference in bacterial colony count between the sites by Tukey's test

	S. mutans		*Lactobacillus*		*Total number of colonies*	
Comparisons	*Group I*	*Group II*	*Group I*	*Group II*	*Group I*	*Group II*
Enamel vs dentin	<0.001	0.065	1.000	1.000	<0.001	0.293
Enamel vs pulp	<0.001	0.028	<0.001	0.272	0.783	0.831
Dentin vs pulp	0.715	0.999	<0.001	0.413	0.008	0.942

It changes over time, and the microorganism population can shift between a healthy and pathological environment when factors such as sugar are enhanced.[6] The first microorganisms to colonize are termed pioneer species, and collectively they make up the pioneer microbial community. In the mouth, the predominant pioneer organisms are streptococci, in particular *S. mutans*, *S. salivarius*, *S. mitis*, and *S. oralis*.[7]

S. mutans is able to metabolize glucose, fructose, sucrose, lactose, galactose, mannose, cellobiose, glucosides, trehalose, maltose, and a previously unrecognized group of sugar-alcohols. In the presence of extracellular glucose and sucrose, *S. mutans* synthesizes intracellular glycogen-like polysaccharides (IPSs). *S. mutans* also produces mutacins (bacteriocins), which are considered an important factor in the colonization and establishment of *S. mutans* in the dental biofilm.[8] There is also a strong association between *Lactobacillus* spp. and caries.[9] *Lactobacilli* are isolated from deep caries lesions but rarely just before the development of DC and in early tooth decay. They are believed to be pioneering microorganisms in caries progression, especially in dentin. The level of *Lactobacillus* in saliva may be indirectly related to the progression of caries.[10] Studies have shown that *Lactobacilli* are a dominant part of the flora inhabiting deep cavities, and their number correlates with the presence of carbohydrates.[8]

The prevalence of caries was found to be higher in children, as reported in other studies.[11] The prevalence of caries was found to be higher in females in both groups (group I, 57%, and group II, 64%), as also reported in other studies.[12–14] However, a few studies reported that there was no statistically significant difference in caries prevalence between the two sexes.[15–17]

While additional species may play a role in DC development, a considerable amount of research has established *S. mutans* as a primary cariogenic pathogen.[18] It is noted that *S. mutans* and *S. sobrinus*, two species of the mutans streptococci, are the most significant in human caries, and studies of the microbial ecology of caries have been directed principally at these species.[9] *Lactobacilli* generally constitute a low proportion of the plaque microbiota. It has been suggested that *S. mutans* are the principal cariogenic pathogens, with *Lactobacilli* aiding in caries progression.[19] In our study, the mean colony count of *S. mutans* was significantly higher (61.3%) in group I compared to group II. Similarly, the mean colony count of *Lactobacillus* was significantly higher (63.4%) in group I compared to group II.

In both groups, posterior teeth were found to be more affected with caries than anterior teeth in our study. Comparing the two groups, group II (67%) had a higher prevalence than group I (60%). Similar findings were reported in other studies as well.[12,20,21] This could be due to the greater number of supplemental grooves present in posterior teeth, which act as sites for food accumulation and attraction of bacteria. Additionally, posterior teeth have a larger surface area for bacterial adhesion and multiplication, such as *S. mutans* and *Lactobacillus*. Correlating with the microorganisms, *S. mutans* were higher in posterior than anterior teeth. In contrast, the mean colony counts of *Lactobacillus* were higher in anterior than posterior teeth.

Dental caries was found to be more common on the occlusal/incisal surface in both group I and group II in our study. This finding has also been reported in other studies.[22–24] This could be due to the greater number and deeper developmental grooves present on the occlusal surface, allowing for more bacterial colonization. Additionally, pooling of saliva in these grooves and food accumulation may lead to an environment more prone to bacterial contamination and multiplication, such as *S. mutans* and *Lactobacillus*.

Comparing the microorganisms, the mean colony counts of *S. mutans* were higher on the smooth surface than on the occlusal/incisal surface and higher in group I than group II. In contrast, the mean colony counts of *Lactobacillus* were higher on the smooth surface than on the occlusal/incisal surface in group I. However, in group II, the mean colony counts of *Lactobacillus* were higher on the occlusal/incisal surface than on the smooth surface. *S. mutans* has a central role in the etiology of DC because they can adhere to the enamel salivary pellicle and other plaque bacteria. Mutans

streptococci and *Lactobacilli* are strong acid producers and, hence, create an acidic environment that increases the risk for cavities.[25]

In both groups, the mean colony counts of *S. mutans* were higher in enamel, followed by dentin, with pulp having the lowest counts. Additionally, in all three sites, the counts were higher in group I than group II. Comparing the mean colony counts of *S. mutans* within the groups (between tooth sites), the Tukey test revealed significantly lower colony counts in both dentin and pulp compared to enamel in both group I ($p < 0.001$) and group II ($p < 0.05$). Further, comparing the mean colony counts of *S. mutans* between the groups (group I vs group II), the Tukey's test revealed significantly lower colony counts at enamel in group II compared to group I ($p < 0.001$). However, the counts did not differ between the groups at dentin and pulp ($p > 0.05$), indicating that they were statistically the same.

In contrast, in both groups, the mean colony counts of *Lactobacillus* were higher in pulp, followed by dentin, with enamel having the lowest counts. Additionally, in all three sites, the counts were higher in group I than group II. Comparing the mean colony counts of *Lactobacillus* within the groups (between tooth sites), the Tukey's test revealed significantly higher colony counts in pulp compared to both enamel and dentin in group I ($p < 0.001$). In group II, however, the counts did not differ among the sites, indicating they were statistically the same. Further, comparing the mean colony counts of *Lactobacillus* between the groups (group I vs group II), the Tukey's test revealed significantly lower colony counts in group II compared to group I at pulp ($p < 0.001$). The counts were not different between the groups at both enamel and dentin ($p > 0.05$), indicating they were statistically the same.

The *S. mutans* group is more closely associated with DC in enamel, being primarily responsible for the initial phase of the lesion.[26] *S. mutans* from individuals with active caries were found to release significantly more calcium from hydroxyapatite than strains isolated from caries-free individuals.[27] Oral streptococci may be associated with the development of "low pH-carious dentin."[28] *Lactobacilli* are reported to be the most commonly isolated microorganisms in samples of carious dentin.[29] *Lactobacillus* is correlated with the progression of the caries process because it has a low capacity for adherence to the tooth surface.[26] Bacterial invasion of dentinal tubules commonly occurs when dentin is exposed following a breach in the integrity of the overlying enamel or cementum. While several hundred bacterial species are known to inhabit the oral cavity, a relatively small and select group of bacteria is involved in the invasion of dentinal tubules and subsequent infection of the root canal space. Streptococci are among the most commonly identified bacteria that invade dentin. Recent evidence suggests that streptococci may recognize components present within dentinal tubules, such as collagen type I, which stimulate bacterial adhesion and intratubular growth. Specific interactions of other oral bacteria with invading streptococci may then facilitate the invasion of dentin by select bacterial groupings.[30]

The limitations of our study include sample size and counting errors during the colony counting procedure. Better results could be achieved with a larger sample size and by using more precise colony counting methods, such as automated colony counters, to reduce human error.

Conclusion

Caries can be reduced by increasing the acid resistance of teeth and controlling carbohydrate consumption in the diet. By manipulating adhesion interactions, it may be possible to develop new methods to block adhesive reactions, impeding the development of biofilm-related oral diseases such as DC. Bacterial colony counts may help in taking specific measures against specific organisms and thereby prevent the development of new carious lesions.

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