Original Article

# Comparison and Analysis of Acidogenic Bacteria in Saliva of Rampant Caries, Single Tooth Caries, and Non-Carious Individual

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## Abstract

Dental caries is a highly prevalent infectious disease that causes tooth decay with the reported prevalence of 51.9% in 5-year-old children to as high as 85.0%. The study comprised total of 150 young children with deciduous dentition with no history of drug therapy 3 months before saliva sampling, systemic disease, and active orthodontic treatment reported in the Outpatient Department of Triveni Institute of Dental Sciences, Bilaspur. Caries was diagnosed using the Decayed, Missing due to caries, Filled Teeth and Decayed, Missing due to caries, and Filled tooth Surfaces (DMFS) scores. Sample of saliva was taken through stimulating salivary gland by chewing paraffin stimulated saliva and collected in a cotton swab and assessed for the quantitation of Streptococci, Lactobacilli, and Actinomyces in among three groups. These bacteria were cultured on Mitis salivarius HiVeg agar base, Actinomyces agar, and Rogosa SL HiVeg™ agar broth, respectively, for streptococcus, Actinomyces, and *Lactobacillus,* respectively. There was highly significant difference in mean Streptococci count between the three groups ($P = 0.001$). The Tukeys *post hoc* test showed highly significant difference ($P = 0.001$) between all the groups. Within the limitation of the study, it can be concluded that higher mean microbial count was observed in individuals with multiple caries, than individuals with single caries and was least in the control group; the Streptococcal and Lactobacilli count was found to be higher in the subjects with rampant caries as compared to subjects with single caries lesion while the Actinomyces count was significantly higher in caries free individual as compared to subjects with caries.

**Keywords:** Dental caries, Rampant caries, Saliva.

## Introduction

Dental caries is a highly prevalent infectious disease that causes tooth decay with the reported prevalence of 51.9% in 5-year-old children to as high as 85.0% in adults aged 65–74 years as per the nationwide survey conducted by Dental Council of India in 2004 which also pointed toward an increase in dental caries with age.[1] Children visiting to dental office has often present early childhood caries, which is a very common and consequential chronic disease affecting young children and has been defined as "the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger."[2]

Individual tooth surfaces have vastly different susceptibilities to caries, with the pit and fissure (occlusal) surfaces the most susceptible, and the smooth (labial and lingual) surfaces the least susceptible. The most frequent sites of attack are the occlusal surfaces of the first and second permanent molars. In addition, it was reported that neighboring approximal tooth surfaces differ in their caries susceptibility, implying that one surface may show obvious radiographic signs of caries, while the neighboring surface does not.[3,4]

The etiology of the condition is complex and involved multiple standpoints including molecular/biochemical, microbiological,

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behavioral, social, and environmental system. Recently, a unified conceptual model[5] was suggested that connects the environmental social risk factors of the mothers and their children. The mothers are at the center of the model, which usually comes from an unfavorable social environment, with a low socioeconomic, educational level, and health behavior. This model represents proximal and distal determinants of early childhood caries; proximal risk factors such as bacteria such as *Streptococcus mutans*, *Lactobacillus* spp., sugar in take, oral hygiene (visible dental plaque), saliva, and the use of preventive dental services have been identified as predictors of the disease and the distal risk factors such as social determinants of health and population health.[6,7]

In the view of aforementioned model and scarce literature, we estimated the total salivary count of *Streptococcus*, *Lactobacillus,* and Actinomyces in children with multiple carious lesions, isolated carious lesion and caries-free individuals.

## Materials and Methods

The study comprised total of 150 young children with deciduous dentition with no history of drug therapy 3 months before saliva sampling, systemic disease, and active orthodontic treatment reported in the Outpatient Department of Triveni Institute of Dental Sciences, Bilaspur. They were distributed in a three group comprising 50 children in each group: GROUP I-Individuals with multiple carious lesion; GROUP II-Individuals with single carious lesions; and GROUP III-Individuals who are caries free.

### Caries assessment

Caries was diagnosed using the decayed, missing due to caries, filled teeth (DMFT) and decayed, missing due to caries, and filled tooth surfaces (DMFS) scores. Teeth lost to trauma or exfoliation was not included in the final DMFT scores. Carious lesion was assessed as below:

Without drying the teeth, examinations were performed with dental mirrors and blunt sickle-shaped explorers under a dental chair light, according to the WHO recommendations.[8] The examiner applied standardized and routinely used WHO diagnostic criteria. First, caries teeth were identified, and a diagnosis of caries was made only when there was clear evidence of loss of tooth substance. White or brown spots in enamel, the surfaces of which remained intact and glossy, were not considered to be caries. Caries were recorded as present when a lesion in a pit or on a smooth tooth surface had a detectably softened floor, undermined enamel, or softened wall.

"Sticky" and discolored fissures were accepted as caries only if there was clear evidence of cavitation beginning below the fissure. On approximal surfaces, caries were recorded when the explorer had entered a lesion.

### Microbial measurement

Sample of saliva was taken through stimulating salivary gland by chewing paraffin (stimulated saliva) and collected in a cotton swab and assessed for the quantitation of Streptococci, Lactobacilli, and Actinomyces in among three groups. These bacteria were cultured on Mitis salivarius HiVeg agar base, Actinomyces agar, and Rogosa SL HiVeg™ agar broth, respectively for *Streptococcus*, Actinomyces, and *Lactobacillus,* respectively.

The plates were incubated in an anaerobic atmosphere at 37°C for 48 h and the quantitative microbial assessment was done by a colony counter. Statistical analysis for the comparison of total Streptococcal, *Lactobacillus,* and Actinomyces count in single and multiple caries lesions and caries free individuals will be carried out by one-way ANOVA and *post hoc* Tukey's HSD test. Pearson product-moment correlation was used to determine the relationship between DMFT and DMFT status of the children and their salivary Streptococci, Lactobacilli, and Actinomyces salivary count. $P = 0.05$ was considered significant at 95% confidence interval.

## Results

The participants were 78 males (52%) and 72 females (48%) with a mean age of 4.24 ± 2.15 years. The DMFT score was 5.32 ± 2.20, 1 ± 0, and 0 for Group I, II, and III, respectively.

There was highly significant difference in mean Streptococci count between the three groups ($P = 0.001$). The Tukeys *post hoc* test showed highly significant difference ($P = 0.001$) between all the groups (Table 1).

There was highly significant difference in mean *Lactobacillus* count between the three groups ($P = 0.001$). The Tukeys *post hoc* test showed no significant difference ($P = 0.119$) between group 2 and 3 (Table 2).

There was highly significant difference in mean Actinomyces count between the three groups ($P = 0.001$). The Tukeys *post hoc* test showed that there was highly significant difference between group 1 and group 2 ($p < 0.001$ ) and between group1 and group 3 ($p < 0.001$ ). There was significant difference between group 2 and group 3 ($p < 0.05$). Group 3 having the highest count, whereas group 1 having the least count (Table 3).

Overall, it was observed that group 1 was having maximum of *S. mutans* count and Lactobacilli count followed by group 2 and 3, respectively, whereas Actinomyces count is maximum in Group 3 (Figure 1).

Correlation of DMFS score with microbial count Streptococci count and the DMFT scores in the subjects with multiple caries showed a strong positive correlation ($r = 0.82$). Similarly, a strong positive correlation was observed for the Streptococci count and the DMFT/DMFT scores for subjects with isolated caries ($r = 0.85$). Lactobacilli counts and DMFT/DMFT scores in Groups I and II showed a moderate positive correlation ($r = 0.55$; $r = 45$) while Actinomyces count and DMFT score showed low correlation($r = 25$, $r = 20$) in either of the groups (Figures 2 and 3).

**Table 1: Intergroup comparison of streptococci count in saliva**

| Groups | Mean* | SD* | SE | Minimum | Maximum | F-value | *P*-value |
|---|---|---|---|---|---|---|---|
| Group 1 | 52.00 | 5.40 | 0.76399 | 43.00 | 60.00 | 222.637 | **<0.001**** |
| Group 2 | 41.44 | 5.82 | 0.82421 | 30.00 | 50.00 | | |
| Group 3 | 30.76 | 3.58 | 0.50674 | 21.00 | 36.00 | | |

One-way ANOVA Test; *P* value < 0.05 considered significant at 95% CI

**Table 2: Intergroup comparison of *Lactobacillus* count in saliva**

| Groups | Mean* | SD* | SE | Minimum | Maximum | F-value | *P*-value |
|---|---|---|---|---|---|---|---|
| Group 1 | 36.4000 | 5.66407 | 0.80102 | 23.00 | 45.00 | 44.252 | <0.001** |
| Group 2 | 27.8400 | 7.59796 | 1.07451 | 14.00 | 38.00 | | |
| Group 3 | 25.4000 | 4.82764 | 0.68273 | 12.00 | 35.00 | | |

One-way ANOVA test; *P* value < 0.05 considered significant at 95% CI

**Table 3: Intergroup comparison of Actinomyces count in saliva**

| Groups | Mean* | SD* | SE | Minimum | Maximum | F-value | *P*-value |
|---|---|---|---|---|---|---|---|
| Group 1 | 11.9000 | 6.96712 | 0.98530 | 0.00 | 24.00 | 22.513 | <0.001** |
| Group 2 | 17.0200 | 5.87450 | 0.83078 | 0.00 | 27.00 | | |
| Group 3 | 20.0600 | 5.50106 | 0.77797 | 10.00 | 32.00 | | |

One-way ANOVA test; *P* value < 0.05 considered significant at 95% CI

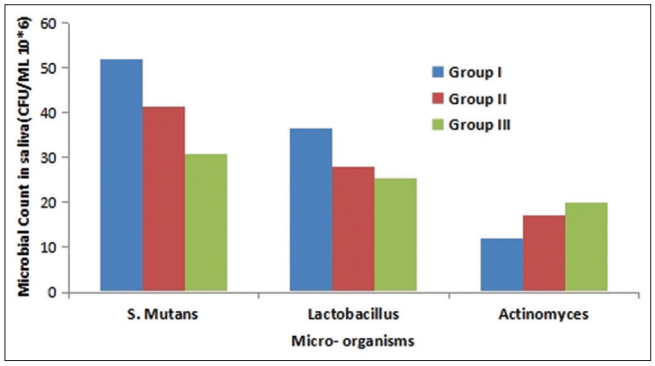


**Figure 1:** Comparative assessment of microbial count in saliva

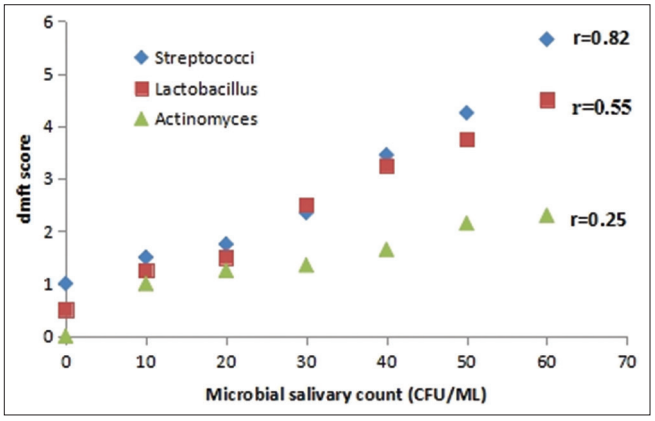


**Figure 2:** Corelation between salivary microbial count and DMFT score in Group 1

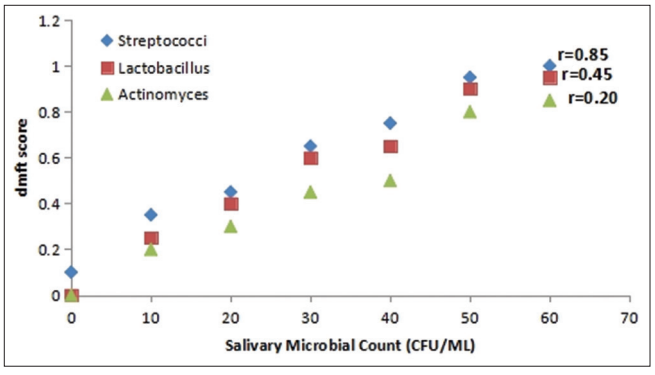


**Figure 3:** Corelation between salivary microbial count and DMFT score in Group 2

## Discussion

This study focused on the microbial aspect of the early childhood caries and caries involving single tooth process. Microbiota, diet, and host (teeth) all three are indispensable for caries development and considered as caries risk predictors. *Lactobacillus* and MS are considered as primary caries risk predictors and this stems from the fact that caries conducive conditions are associated with increased levels of these organisms in saliva. Actinomyces are members of the indigenous oral microbiota which have been implicated in root caries and are also thought to play a role in childhood caries initiation and progression.[7] Therefore, the purpose of the present study was to quantify the Streptococci, Lactobacilli, and Actinomyces counts in saliva of subjects with varying number of teeth affected by dental caries.

In this study, stimulated whole saliva was used to assess the microbial aspect of caries. Questions concerning reliability of saliva over plaque or oral swab samples can be raised. However, reports in the literature indicate that detection of *Streptococcus* and Lactobacilli in stimulated saliva was in

excellent agreement with either pooled plaque or oral swab samples. Most culture based diagnostic tests are designed to detect bacteria in saliva, likely due to convenience as well as because saliva reflects the overall bacterial levels in the oral cavity. Furthermore, the chewing of paraffin wax also liberates bacteria from the tooth surfaces into the saliva.[9] Results by author have shown that number of *Streptococcus* and Lactobacilli in dental plaque samples does not explain the variation in caries better than the number in stimulated whole saliva Caufield *et al*. have shown that detection of *Streptococcus* in unstimulated saliva samples was however discordant with that in either plaque or oral swab samples.[10] These findings collectively justify the use of stimulated saliva for the quantitative assessment of *Streptococcus*, Lactobacilli, and Actinomyces.

In the present study, a mean DMFT score of 7.32 was found, being much higher than the findings of the studies on similar population in Srinagar, Jammu, and Kashmir (1.80).[11] Narmada (3.50), Gujrat,[12] and 3.74 in Udupi Taluk, Karnataka,[13] the differences may relate obey to living conditions, which mark differences in the levels of caries by socioeconomic status and social disadvantages and the existence of barriers to access oral health services. Counts of *S. mutans* in this study show conformity with the previous reports. We did reported statistically significant differences between children with caries active and those who were free of caries with respect to colony forming units of *S. mutans* or *Lactobacillus* spp. These findings were in agreement with a previous report by Singh *et al*. (2015) in their investigation of 80 children in India found a 40 children with active caries, had a 77.5% presence of *S. mutans*.[14] These results led us to consider that *S. mutans* was the microorganism with the most abundant cariogenic activity in the oral cavity, and its presence in high numbers is generally associated with caries, which is the reason why the American Academy of Pediatric Dentistry has recommended its reduction; however, it should be considered that there is evidence of other species of bacteria that may be responsible for the initiation and development of caries (AAPD).[15] In a previous study, a positive association between ECC and plaque *S. mutans* levels, than that in saliva, theoretically dental plaque is more appropriate for estimating *S. mutans* infection in individuals, because tooth surfaces are the natural habitat of this organism.[7]

Regarding *Lactobacillus* spp., the findings of the present research also correspond with those found in similar studies, in which the presence of *Lactobacillus* spp. is strongly related with the presence of caries in children. In this study, both caries-free children and children with ECC had similar *Lactobacillus* spp. counts.[16,17]

The third microorganism studied was Actinomyces and the mean Actinomyces counts in caries free individuals were higher as compared to single caries and rampant caries. This result was in accordance with the previous studies who stated that Actinomyces were less numerous in plaque and saliva from carious lesions as compared to caries free subjects.[18,19] The result could be attributed to the reason that *S. mutans* prevents the establishment of Actinomyces due to the fact that *S. mutans* could utilize salivary proteins as source of nutrition allowing it to grow rapidly whereas in case of Actinomyces they grow slowly because they cannot utilize it, reflecting the influence of microbial interaction on growth rate. Results were in accordance with the findings of the previous studies who also had seen the microbial interaction on growth rate and increase in number of Actinomyces in caries free subjects.[20,21]

The present study had limitations. The convenience sample selected from institution may not represent the overall conditions in Bilaspur district. The results were applicable only to other populations with similar characteristics. For a future study, it would be advisable to have a matched control group to compare the results.

## Conclusions

Within the limitation of the study, it can be concluded that higher mean microbial count was observed in individuals with multiple caries, than individuals with single caries and was least in the control group; the Streptococcal and Lactobacilli count was found to be higher in the subjects with rampant caries as compared to subjects with single caries lesion while the Actinomyces count was significantly higher in caries free individual as compared to subjects with caries.

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