

## Colony Forming Unit Levels of Salivary *Lactobacilli* and *Streptococcus mutans* In Orthodontic Patients

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*The purpose of this study was to compare the levels of Lactobacilli (LB) and Streptococcus mutans (SM) colony forming units (CFU) in the saliva of subjects before and after orthodontic appliance placement. This was a controlled, prospective two-group, two-measurement, clinical trial performed on 64 study patients, 12-15 years old. Subjects in the experimental group were sampled for LB and SM in stimulated saliva collected on the same day but prior to band and bracket placement. The subjects in the control group were sampled on their first screening appointment two months prior to band and bracket placement. The second samples of LB and SM were taken from the experimental and control groups after two months. Saliva was transferred to a selective agar carrier and incubated for 48 hours at 37°C / 99 °F. LB and SM colonies forming units were compared with standard densities. The experimental group wearing orthodontic appliances had significantly higher mean LB CFU counts than the control group at the 2 month follow-up (3.25 vs. 2.57, p = 0.0036). The two groups, however, did not show any difference in mean SM CFU counts at the 2 month follow-up (3.0 vs. 3.1, p = 0.66).*

*The results of this study showed that a higher number of CFUs of LB were associated with the group wearing orthodontic appliances after two months and may play a role in the increased levels of plaque seen in many orthodontic patients.*

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### INTRODUCTION

Orthodontic treatment increases the number of retention areas resulting in an accumulation of bacterial plaque.<sup>1</sup> Plaque removal is a problem, especially in individuals carrying orthodontic brackets

and ligatures. Many orthodontic patients have difficulty in mechanically removing plaque around brackets and underneath ill-fitting bands.<sup>2</sup> In addition, it has been suggested that in general, metal brackets used for orthodontics increase bacterial adhesion.<sup>3</sup> Specifically, the binding of *S. mutans* to metal brackets has been found to be higher than other materials.<sup>4</sup> Mutans streptococci, particularly *Streptococcus mutans*, and other so-called low-pH non-mutans streptococci are considered virulent members of the dental plaque microflora. It has been reported that *Lactobacilli* and *Streptococci* species create a low pH (below 5.5) oral environment due to the bacteria's byproducts. These bacteria have an acid tolerance that enables them to reproduce at the low pH found in dental plaque.<sup>5</sup>

Different caries predicting parameters have been studied and bacteria are considered to be one of the main factors in pathogenesis of dental caries. Therefore, determinations of both the *S. mutans* and the *Lactobacilli* counts have an important value for determining caries risk.<sup>6,8</sup> Evaluation of bacterial counts in patients is beginning to be an important aspect in caries risk assessment in modern dental practices. Recently, a new simple chair side microbial test has been developed to determine the *S. mutans* and *Lactobacilli* counts in saliva by means of selective agar in a dip-slide

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techniques culture (CRT® bacteria, Ivoclar Vivadent clinical).

The purpose of this study is to investigate if there is a difference in numbers of *S. mutans* and *Lactobacilli* CFU in saliva from test subjects before fixed orthodontic appliance placement and during treatment.

**MATERIALS AND METHODS**

This study was approved by the Institutional Review Board of the UMDNJ. Written informed consent was obtained from the parents or legal guardians of all participating subjects. A clinical trial was performed on 64 patients selected from a group of patients seeking treatment at the New Jersey Dental School, Department of Orthodontics, in Newark, New Jersey. A convenience sample of potential participants was collected using 32 patients who presented for a screening appointment and 32 patients who were having their brackets placed. The following were the selection criteria for the study sample: 1) patients that required orthodontic treatment with straight wire metal orthodontic brackets, 2) children, 12 to 15 years old, 3) no periodontal disease, 4) no mental handicap, 5) no physical handicap that restricts free movement of the hands, 6) less than 5 dental restorations, 7) more than 26 teeth and 8) no use of antibiotics for two weeks. The individuals in the experimental group were sampled prior to the placement of orthodontic appliances and after two months. The control group was tested at the beginning and at the end of the two month trial but without orthodontic appliances.

Subjects were given a questionnaire at both visits. The questionnaire obtained demographic data (gender and race), dietary information (snacking habits, frequency of ingestion of fermentable carbohydrates, sugar and non-sugar containing drinks and milk products) and oral hygiene information (brushing, flossing habits, mouthwash and water Pik® use). Data on hygiene and nutrition were obtained for the purpose of

ruling out any potential differences that might affect the CFUs between the control and experimental groups.

One calibrated examiner collected the data. Salivary collection and bacterial culture followed manufacturer’s instructions. Both bacteria were ranked for CFU density: 1 = 0 < 10<sup>4</sup>, 2 = 10<sup>4</sup> < 10<sup>5</sup>, 3 = 10<sup>5</sup> > 10<sup>6</sup> and 4 = >10<sup>6</sup>. All plaque index (PI) measurements were performed utilizing disclosing agent on the lingual surfaces of the second premolars. Lingual surfaces were used because buccal surfaces had appliances on them and it would have been impossible to accurately assess plaque accumulation. Plaque was scored using a modified Turesky modification of Quigley and Hein Index.<sup>8</sup>

**RESULTS**

Over the two month period there were no significant differences in brushing, flossing, mouth wash, and Water Pik® use between the test and control groups. In addition, no significant changes were found among both groups with regard to snacking patterns and nutritional habits from the baseline to the end of the 2 month study period.

Initial analyses revealed that the two groups (with appliance and without appliance) were initially different in their plaque indices (PI) (Table 1). At the onset of the study the appliance group had a lower incidence of plaque than did the no appliance group (28% vs. 39%, p = 0.001). It was also noted that the groups were initially different with respect to the number of *Lactobacilli* and *S. mutans* CFU (Table 1). The group with appliance had significantly higher mean *Lactobacilli* CFU than those without the appliance (2.9 vs. 1.8, p = 0.0003). *Streptococcus mutans* in the appliance group exhibited a higher mean of CFU’s compared to the control group but was not statistically significant (2.9 vs. 2.5, p = 0.09).

Due to pre-test differences in plaque scores and numbers of *Lactobacilli* colony forming units among

**Table 1:** Raw scores for plaque (PI), *S. mutans* (SM), and *Lactobacilli* (LB) colony forming units (CFU) among 64 orthodontic patients by appliance group over time.

	Control group (No appliance)				Experimental group (With appliance)				Pre-test differences  p
	Baseline		2 Month Follow-up		Baseline		2 Month Follow-up		
	mean	(SD) +	mean	(SD) +	mean	(SD) +	mean	(SD) +	
Plaque (PI) %	39	16	34	11	28	6	30	11	0.001
SM CFU	2.5	1.2	2.8	0.9	2.9	0.9	3.3	0.8	0.09
LB CFU	1.8	1.1	2.3	1.1	2.9	1.2	3.5	0.7	0.0003

Note: significance was determined at p < 0.05

**Table 2:** Adjusted mean, controlling for plaque and pre-test CFU counts

	Control group (No appliance)	Experimental group (With appliance)	p
SM CFU	3.1	3.0	0.66
LB CFU	2.6	3.3	0.0036

Note: significance was determined at  $p < 0.05$

the treatment and control groups, an analysis of covariance was conducted to evaluate if the groups were different at the two-month follow-up. An analysis of covariance (ANCOVA) using a generalized linear model with plaque and *Lactobacilli* CFU at the baseline for covariates was performed. The appliance group showed significantly higher *Lactobacilli* CFU counts than the control group at the 2 month follow-up (3.3 vs. 2.6,  $p = 0.0036$ ) (Table 2). The same statistical analysis was applied to *S. mutans*, but instead the covariates were plaque and *S. mutans* CFUs at baseline. The appliance group and no appliance group had no difference in *S. mutans* CFU counts at the 2 month follow-up (3.0 vs. 3.1,  $p = 0.66$ ) (Table 2).

## DISCUSSION

The aim of this study was to determine whether the CFU capability of *Lactobacilli* and *S. mutans* was affected by orthodontic bracket placement. The study found a positive change in *Lactobacilli* CFU numbers with orthodontic bracket placement and no change in *S. mutans*' CFU scores with brackets. The present research detected an increase in *Lactobacilli* CFU's among subjects wearing orthodontic appliances. This observation was in agreement with previous studies<sup>3,9</sup>. Brackets, similar to proximal surfaces of teeth or margins of restorations may act as substratum on which pellicle formation may build allowing the attachment of *Lactobacillus* species.<sup>9-11</sup> The *Lactobacilli* do not depend on pioneer bacteria for adhesion; however, these bacteria can take advantage of the pioneer bacteria's lattice network, which is a form of mechanical retention and source of nutrients.<sup>10</sup>

This study found no statistically significant differences with regard to CFU scores of *S. mutans* between the subjects with or without orthodontic appliances. Fournier *et al*<sup>2</sup> found results similar to this with respect to *S. mutans* CFU accumulation. The bacterium, *S. mutans*, is a successor microorganism which binds to initial colonizing species of *Streptococcus* such as *S. gordonii*, *S. sanguis*, *S. mitis*, *S. oralis*, and *S. sobrinus* through lectin interactions. This process thus plays an important role in the *S. mutans* adherence and colonization.<sup>9</sup> Failure of the adherence of the colonizing pioneer bacteria on the surface of the stainless steel bracket would interfere with growth of *S. mutans*. Therefore, this study showed that the pioneer organisms' colonization on the orthodontic appliance was

inhibited leading to no significant increase in *S. mutans* population in either study group. Additionally, the increased incidence of *Lactobacilli* observed in this study might have resulted in a very low pH environment which might have further inhibited growth of the facultative aerobic *S. mutans* in the oral microenvironment.

A limitation of this study should be noted. Of concern was the pre-test difference in plaque indices and CFU numbers found in the two groups. The researcher could not ascertain the reasons for this difference. Additional research should be performed using a cross-over design where patients serve as their own control. Another design could pair control subjects to experimental subjects with common CFU and plaque indices.

## CONCLUSIONS

- 1) A higher number of colony forming units of *Lactobacilli* were associated with the group wearing orthodontic appliances after two months.
- 2) There was no difference in the levels of colony forming units of *Streptococcus mutans* between the control group and the group wearing orthodontic appliances.

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