Staphylococcus aureus count in salivary samples of patients treated with self ligating prescriptions: A comparative clinical study

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Abstract

Background: The oral environment provides the proper conditions for the colonization of a complex microbiota. Such changes can be brought about by the introduction of orthodontic appliance’s increased affinity of bacteria to the metallic surface because of electrostatic reactions, and causing retention areas & surfaces to which microorganisms can adhere. These factors strongly hamper orthodontic treatment and illustrate that the need for microbial control is even greater during orthodontic treatment than usual. This study was intended to assess the Staphylococcus aureus count in Salivary Samples of Patients treated with Self Ligating Labial and Self Ligating Lingual Orthodontic prescriptions.

Methodology: 20 healthy patients satisfying the criteria were recruited for the study. The lingual self-ligating prescription used was Ormco Generation 7 and the labial self-ligating prescription used was Damon 3 (Ormco Corporation, 1717 W. Collins Ave., Orange, CA 92867). Among these patients group I consisted of 10 patients having Self ligating Labial prescription and group II consisted of 10 patients having Self Ligating Lingual prescription. Salivary samples of all the 20 subjects were collected at various interval. All the collected sample were cultured and the microbes were isolated and identified according to their cultural Characteristics and Biochemical reactions.

Conclusion: The presence of microbial colonies associated with Self Ligating Lingual orthodontic appliance is more than count of Self ligating Labial orthodontic appliance.

Keywords: self ligating brackets, periodontal probing depth (PPD), bleeding on probing (BOP)

Introduction

The oral microflora provides habitat to at least 400 to 700 different types of bacteria. This microbiota host a complex and dynamic microbial community that is responsible for two major and highly prevalent infectious diseases: caries and periodontal disease [1]. When changes occur in the oral cavity, the microflora also change, causing a loss of balance and increasing the possibility of disease development [2]. Orthodontic treatment, with the use of fixed or removable appliances, causes specific alterations in the oral cavity [3] including pH reduction, increased accumulation of dental biofilm [4] and increased levels of microorganisms in saliva and biofilm [5,6].

Some opportunistic microorganisms, such as Staphylococcus and Candida species must be taken into consideration as probable pathogens, especially in individuals with different systemic impairments, e.g. diabetes mellitus, neutropenia, agranulocytosis, and AIDS [7,8]. It is well documented that orthodontic/orthopedic treatment increases the levels of yeasts and Staphylococcus species in the mouth [10,11]. The use of orthodontic appliances is a risk factor for gingival-periodontal diseases and dental caries, since they facilitate the accumulation of microorganisms, in terms of quantity altering the oral microbiota.

Staphylococcus aureus is a gram-positive, round-shaped bacterium that is a member of the Firmicutes and is frequent habitat in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Staphylococcus aureus has become one of the community and nosocomial pathogens of epidemiological concern. Fixed orthodontic appliances create new retention areas, which are suitable for bacterial colonization and lead to an increase in the absolute number and percentage of Staphylococcus aureus. A lot of studies have evaluated the effect of fixed orthodontic appliances on microbial
flora and periodontal status, however, their sample sizes were relatively low, and no additional periodontal evaluation was performed [3]. The advantage associated with self-ligating brackets are better hygiene, as they do not require wire ligatures, recognized as the focus of plaque formation. In literature, there are numerous reports about the periodontal and microbial effects of labial orthodontic treatment, but only limited research has been performed considering this question with respect to fixed lingual appliances [6].

All the above considerations, along with the growing trend towards the use of self-ligating brackets, seem to justify the present study which aims to comparatively evaluate the Staphylococcus aureus in Salivary Samples of Patients when orthodontic treatment is performed with Self Ligating Labial and Self Ligating Lingual Orthodontic prescriptions.

Materials and Methods
A Sample of 20 subjects and were divided into two groups. The first group (Lingual Group) consisted of 10 patients who were undergoing treatment with Lingual Self-ligating orthodontic therapy. The second group (Labial Group) consisted of 10 patients who were undergoing treatment with Labial Self Ligating therapy. The Lingual Self Ligating prescription used was Ormco Generation 7 and the labial Self-Ligating prescription used was Damon 3 (Ormco Corporation, 1717 W. Collins Ave., Orange, CA 92867).

Inclusion criteria
- Male and female patients between the age group of 12 and 35 years.
- Patients who had no clinical signs of gingival inflammation.
- Patients who had malaligned teeth and required fixed orthodontic therapy.

Exclusion criteria
- Smokers
- Pregnant or lactating women
- Patients who had undergone periodontal treatment, within three months of baseline examination.
- Systemic disorders that could influence periodontal and microbiologic conditions.
- Patients who had taken antibiotics on or before a period of three months of the initial examination.

The Salivary samples (6ml) were collected in a sterile test tube and were sent to the laboratory for microbial analysis. Subjects were instructed not to eat or to drink for at least one hour before sample collection. Subjects were made to sit on a dental chair, slightly bent forward and the unstimulated saliva was collected by having the subject spit into a sterile plastic graduated cup with 1-ml gradation marks. Salivary samples of all the 20 subjects were collected:
- Before beginning orthodontic treatment, (T0)
- 3 months after treatment,(T1)
- 9 months after treatment (T2)

All the collected sample were cultured aerobically on Macconkey agar and Chocolate Agar. After 24-48 hours, the microbes were isolated and identified according to their cultural characteristics, biochemical reactions and Microscopical appearance. Catalase test and Coagulase test were performed to distinguish between Gram positive and Gram negative bacteria.

Statistical analysis
The data was analysed using SPSS software and student t-test was used. Tables and graphs were generated using Microsoft Excel.

Result
Saliva samples were collected from the patients (T0, T1, T2). These samples were grown in selective media and Staphylococcus aureus were identified based on colony morphology and counts were taken using a semi-electronic colony counter.

![Fig 1: Strains of staphylococcus aureus on mconkey agar at T1](image1.jpg)

![Fig 2: Strains of staphylococcus aureus on mconkey agar at T2](image2.jpg)
Table 1: Difference in Staphylococcus aureus counts between different time intervals T0 T1 and T2 in Lingual and Labial group using Paired t test and ANOVA test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>T value</th>
<th>P value</th>
<th>F</th>
<th>P</th>
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<tbody>
<tr>
<td>Lingual group</td>
<td>T0</td>
<td>40</td>
<td>40.34</td>
<td>-2.322</td>
<td>0.04</td>
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<tr>
<td></td>
<td>T1</td>
<td>45</td>
<td>45.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>T0</td>
<td>42</td>
<td>42.06</td>
<td>-3.360</td>
<td>0.006</td>
<td>4.697</td>
<td>0.016</td>
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<tr>
<td></td>
<td>T2</td>
<td>47</td>
<td>47.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>46</td>
<td>46.50</td>
<td>-1.602</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>47</td>
<td>47.09</td>
<td></td>
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<tr>
<td>Labial group</td>
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<td>46.67</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>T0</td>
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<td>0.02</td>
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<tr>
<td></td>
<td>T2</td>
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<td>47.11</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>T2</td>
<td>47</td>
<td>47.76</td>
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</tbody>
</table>

Significant difference was observed in Staphylococcus aureus count between different time intervals within the lingual group (p=0.016). In labial group there was significant difference observed between time T0 and T2 (p=0.02) and between T1 and T2 (p=0.04).

Fig 2: Linear representation of the difference in Staphylococcus aureus count from T0 to T2

Table 2: Difference in Staphylococcus aureus counts between t0 and t2 using Paired t test

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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<td>47</td>
<td>18</td>
<td>4.60051</td>
<td>.68580</td>
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</tbody>
</table>

Paired samples statistics
**Fig 3**: Difference in Staphylococcus aureus counts between T0 and T2 using Paired t test

**Table 3**: Difference in Staphylococcus aureus counts between lingual and labial at different time intervals T0, T1 and T2 group using Unpaired t test

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>T0</td>
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<td>40.09</td>
<td>1.362</td>
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<td></td>
<td>Labial group</td>
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<td>T1</td>
<td>Lingual group</td>
<td>47</td>
<td>47.09</td>
<td>1.679</td>
<td>0.11</td>
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<tr>
<td></td>
<td>Labial group</td>
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<td>46.23</td>
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<td></td>
</tr>
<tr>
<td>T2</td>
<td>Lingual group</td>
<td>47</td>
<td>47.06</td>
<td>2.185</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Labial group</td>
<td>47</td>
<td>47.05</td>
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</table>

Significant difference was observed in Staphylococcus aureus counts between Lingual and Labial group only at time T2 (p=0.04)

**Discussion**

Self-ligating brackets have attracted a lot of attention in Orthodontics in recent years, which explains the various designs developed by manufacturers of orthodontic material. Self-ligating brackets have important benefits that may contribute to the efficiency of lingual orthodontic treatment: reduced friction and faster alignment, improved oral hygiene, improved clinical efficiency and time saving. These properties of self-ligating brackets address the main difficulties of the lingual technique. Self-ligating brackets provide secure, full archwire engagement, maximizing the potential long range of action of low elasticity modulus wires. This enables precise control of rotation, tip, and torque. Activation range is increased and fewer appointments are needed for activation \(^9\). Very low friction has been clearly demonstrated with Labial Self Ligating brackets \(^10\)–\(^13\) and with low friction the net tooth-moving forces are lower and more predictable. The reciprocal forces are correspondingly smaller, leading to better anchorage control \(^14\).

In a study conducted by Pellegrini et al.\(^15\) with the objective of assessing accumulation of bacterial plaque in self-ligating and conventional brackets, the study concluded that active self-ligating brackets are less likely to accumulate dental plaque when compared to conventional brackets. Nevertheless, it is speculated that active self-ligating brackets allow better hygiene, as they do not have locks or clips completely closing the bracket slot and forming a fourth wall (buccal) similar to molar tubes. Passive brackets, on the other hand, present a buccal wall and, for this reason, could cause plaque accumulation inside the bracket slot.

Depending on the type of brackets used, different microbial trends were found in a study conducted by Mummolo et al.\(^16\). The authors collected saliva samples from 60 patients, divided into three groups of 20 patients each, self-ligating, conventional and untreated control group in order to assess *Lactobacillus* spp and *S. mutans*. The assortment of the various species of bacteria change over time during the orthodontic treatment, and seems to show different trends, depending on the type of orthodontic device and comparatively reduced count was seen in self ligating.
All above considerations, along with the different results found in the studies previously cited and the growing trend towards the use of self-ligating brackets, seem to justify the present study which aims to comparatively evaluate the Staphylococcus aureus count in Salivary Samples of Patients when orthodontic treatment is performed with Self Ligating Labial & Lingual Orthodontic appliance and is first of its kind. In the literature, there are numerous studies about the periodontal and microbial effects of labial orthodontic treatment, but only limited research has been performed considering this question with respect to fixed lingual appliances and Self Ligating prescriptions. In the present study all the individuals were treated with self-ligating brackets. Self Ligating Labial brackets featured a low count of staph.aureus count in the salivary samples when compared with patients treated with Self Ligating Lingual brackets. These results suggest that the use of labial self-ligating brackets predisposes a reduction in dental plaque retention on the tooth surface. However, against this evidence, no significant differences were found at the site in terms of white lesion development or formation, which depends more on oral hygiene conditions and less on the bracket type or ligature used.

Since the advent of lingual orthodontic appliances in the 1970s, recent years have witnessed a marked increase in the demand for lingual orthodontic appliances among orthodontic patients seeking esthetic improvement. Lingual orthodontic appliances enjoy esthetic advantages over conventional labial orthodontic appliances. Moreover, in a study by von der Veen et al. (17) it has been claimed that lingual appliances bear a lower risk of caries than fixed labial appliances, on comparing White Spot Lesions which is in agreement with the present study.

Many studies (1,8, 18-25) have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status however, only a few studies (26-30) have evaluated the type of ligation as an additional factor. The wires are secured/ligated to the bracket by three methods, ligation with steel wires, ligation with elastomeric ligatures and self-ligating brackets. Many studies documented; suggest that the elastomeric ligatures increase the retention of dental biofilm compared with the two other methods. The limitation of the present study was the unaccountability of the socio-demographic status of the subjects and compliance in following the oral hygiene instructions. Since the Self ligating orthodontic appliance is gaining popularity among adults and adolescents due to its great esthetic benefits, it is crucial that the limitations concerning the detrimental effects on oral hygiene be overcome.

Conclusion
Based on the data recorded from the present study, the following conclusions can be drawn:
(A) The microbial load of Staphylococcus aureus species increased significantly in Salivary samples during the progression of fixed orthodontic treatment in both the groups.
(B) The presence of Staphylococcus aureus species in salivary samples associated with Self Ligating Lingual orthodontic appliance surpasses the count of Self Ligating Labial orthodontic appliance which indicates that oral hygiene instructions should be more reinforced in self ligating lingual group and different brushing techniques should be advised and demonstrated to patients for better Cleaning of lingual and palatal surfaces.

References


