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Microbial Analysis of Plaque Biofilm In Subjects Undergoing Orthodontic Treatment With Different Bracket Systems

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ABSTRACT

Objectives: To identify microorganisms isolated from patients wearing different types of fixed orthodontic appliances and evaluate the resistance of isolated bacterial strains to different antimicrobials.

Materials and Methods: Nineteen patients indicated to undergo fixed orthodontic treatment were chosen for the study and were divided into 3 groups -7 patients in group 1 (conventional metal brackets), 6 patients in group 2 (conventional ceramic brackets), and 6 patients in group 3 (Passive self-ligating metal system). Plaque biofilm formed around orthodontic brackets was collected, samples were plated onto brain heart infusion agar and mitis salivarius agar and were incubated. Similarly, samples were collected in blood broth and were streaked onto blood agar to evaluate the anaerobic colony counts. Results: Maximum aerobic and anaerobic bacterial count was seen in group 2 and least in group 3. Most prevalent microbial genera were streptococcus mutans showing predominantly sensitive strains with one strain from Group 3 which showed resistance against amikacin, cefazolin, cefixime, and cefazolin.

Conclusions: The number of aerobic and anaerobic CFU/ml is higher on the surface of ceramic brackets when compared to the conventional metal or the Passive self-ligating system. Streptococcus mutans was most prevalent bacterial strain amongst all aerobic bacteria isolated from all three groups.

Keywords: *Orthodontics ; biofilm; plaque biofilm; bracket*

INTRODUCTION

In a healthy oral cavity, microorganisms coexist with their host. But when changes occur in the normal oral environment the balanced flora changes and an imbalance and disease may result (1). Orthodontic appliances both removable and fixed can alter microbial colonization of the oral cavity. (2). These appliances when placed in the oral cavity encourage the formation and maturation of biofilm and also alter their composition, pH, carbohydrate content, and microbial populations of certain bacteria like Streptococci and Lactobacilli (3).

Specifically, a significant correlation between the surface free energy of a material and its plaque-retaining capacity has been established, with the higher energies showing a favorable effect on bacterial adherence (4). These changes normally appear one month after treatment begins and occur regardless of the type of device used. Fixed appliances have a greater influence on oral bacteria than removable appliances (5).

Studies by PS Stewart et al, showed that an orthodontic appliance in the oral cavity changes the biofilm quantitatively and qualitatively. This may lead to dental caries or periodontal problems which may have an impact on the patient's quality of life. Several clinical studies indicate that the nature of the used biomaterial significantly impacts biofilm formation in the short and long term. Especially the physicochemical properties of the surfaces are thought to be responsible for an influence on bacterial adherence and accumulation. (6) The microorganisms that accumulate around the brackets of a fixed orthodontic appliance can enter the patient's bloodstream after procedures in which the oral tissues are manipulated and cause transient bacteremia. (7)The impact of orthodontic therapy on the composition of dental biofilm has been investigated in prior research (Yejin Ren et al). However, there is very little evidence on the impact of using various bracket systems during fixed orthodontic treatment on the total aerobic and anaerobic CFU.

The aim of this comparative study was to evaluate the total microbial load of both aerobic and anaerobic bacteria and also Strepotococcus mutans from the plaque biofilms of patients undergoing orthodontic treatment with various bracket systems (conventional metal brackets, conventional ceramic brackets, and the passive self-ligation bracket system). In addition to this, an assessment of the antibiogram profile of the Streptococcus mutans isolated from the above groups for its sensitivity and resistance was performed.

MATERIALS AND METHODOLOGY

This prospective clinical study was performed in a university setting at the department of Orthodontics and Dentofacial Orthopedics, Saveetha Dental College and Hospital, Chennai between November 2021 till February 2022. The study was approved by the ethics committee and the IEC was given -SRB/SDC/ORTHO-2107/22/003.

The sample size and calculation were performed using the G- power 3.0.10 software and was based on a previous study performed by Pellissari et al (8). A power value of = 95 was calculated, and the sample size was calculated to be N = 19. These subjects were allocated into 3 groups based on the treatment indicated.

All patients included in this study met the following inclusion criteria: 1) Patients with Angle's class I molar relationship (2) Patients with Tureskey scores ranging between 2- 3 indicating fair oral hygiene (3) Patients who were indicated for orthodontic treatment with fixed appliance. The patients excluded were those with (1) Severe malocclusions (2) missing mandibular anterior teeth (3) Indicated but not willing to undergo fixed orthodontic treatment (4) Patients who have been using antimicrobial mouthrinses in the recent past.

Based on the type of brackets that were indicated for treatment, the subjects were divided into 3 groups - Group 1: subjects who were allocated for treatment with a conventional metal bracket system (AO standard metal mini master standard edgewise brackets), Group 2: All subjects who were allocated for treatment with a conventional ceramic bracket system (Symetri clear ™ by Ormco) and Group 3: All subjects who were allocated for treatment with the passive self-ligating system (Damon ™ -Q, by Ormco). Before beginning treatment, the oral health of these patients was visually examined and a two-tone plaque-disclosing agent solution was used to evaluate the plaque accumulation around the brackets. The Tureskey plaque index scoring was used to select the appropriate samples (MA Yavan et al; SL Fischman et al). Only the patients with a Turesky score between 2-3 were selected for plaque sample collection. By the end of the scoring, 19 adult patients were selected, where Group 1 had 7 patients (n=7) , Group 2 had 6 patients (n=6) and Group 3 had 6 patients (n=6). The first plaque sample was collected just before beginning Fixed orthodontic treatment (T-0). To

collect the plaque biofilm the mandibular incisors were chosen. The choice of the mandibular anterior teeth as a site of index recording in this study was based on the shorter inter bracket distance, reduced crown width, and smaller overall tooth size, which contribute to excessive plaque retention relative to adjacent sites.(9) The patient was seated on the dental chair and with proper isolation protocols plaque collection was done. Insta SEE plaque disclosing agent was applied on the crowns of the teeth with a cotton pellet. Teeth surfaces with accumulated plaque appeared pink in colour and this stained plaque was collected using a sterile spoon excavator. After collection, the spoon excavator was directly inserted into the Eppendorf tube which contained freshly collected Tryptic digest broth (TDB). The Eppendorf tube was closed after plaque collection to avoid contamination.

Microbiological assay

3 samples were collected from each patient and 2 plaque scrapings were transferred to tryptic digest broth for the total aerobic microbial count and Streptococcus mutans count and one scraping to the sterile Brain Heart Infusion broth (BHI) for further microbiological processing. To measure the total anaerobic colony counts, lawn cultures were made onto sterile brain heart infusion agar and mitis salivarius agar, and the plates were incubated at 37℃ for 48 hrs (Fig 1). After incubation, the colonies were counted using a digital colony counter and were recorded as colony-forming units/ml (CFU/ml). Another sample was streaked onto the sterile Mitis Sanguis agar and was incubated at 37°C for 48 hrs for the total Streptococcus mutans count (Fig 3). After incubation, the colonies were identified by gram staining and preliminary biochemical assays.

Antibiogram profiling

The isolates were then subjected to antibiogram profiling using the antibiotics as recommended by the CLSI guidelines (2021) by Kirby Bauer antibiotic susceptibility profiling. Briefly, lawn cultures of the organisms were made onto sterile BHI agar and the antibiotic discs were placed onto the surface of the lawn, after which the plates were incubated at 37℃ for 24 hrs. The antibiotics included in the study were gentamycin, cefotaxime, cefoperazone-sulbactam, linezolid, amikacin, cefoperazone, clindamycin, vancomycin, amoxyclav, and cefixime. After incubation, the zone of inhibition around the discs was measured and was interpreted for sensitive, moderate, and resistant (Fig 7)

Detection of total anaerobic count

Similarly, the samples were collected in sterile anaerobic blood broth and were made as lawn cultures onto sterile anaerobic blood agar (Fig 2). The plates were incubated anaerobically using an Anaero GasPak system at 37℃ for 5-7 days after which the colonies were measured as earlier using the digital colony counter and the results were recorded as CFU/ml.

The second plaque sample was collected in the same manner as described above, after 1 month of being under fixed orthodontic treatment (T-1), and the third plaque sample was collected after 3 months of starting FA (T-3).

Statistical Analysis

The collected data were tabulated in an excel sheet for descriptive analysis. The statistical analysis was performed using the IBM SPSS statistics software. Statistical tables 1 to 6 give a detailed statistical analysis of the intragroup and intergroup comparisons performed.

RESULTS

The data of the CFUs for each group were calculated and tabulated in Microsoft Excel (2022 version). It was later exported to SPSS (version 23) for statistical analysis. The descriptive statistics for each group were carried out using SPSS software.

Table 1 depicts descriptive and intragroup comparison of mean CFUs of Group 1 at different time intervals. The CFUs of aerobic, Streptococcus mutans and anaerobic bacteria increased at 3 months and the intragroup difference in CFUs at different time intervals was statistically significant (p=0.001). Table 2

depicts descriptive and intragroup comparison of mean CFUs Group 2 at different time intervals. The CFUs of aerobic, Streptococcus mutans and anaerobic bacteria increased at 3 months and the intra-group difference in CFUs at different time intervals was statistically significant (p=0.002). Table 3 depicts descriptive and intragroup comparison of mean CFUs of Group 3 at different time intervals - T0, T1, and T3. The CFUs of aerobic, Streptococcus mutans and anaerobic bacteria increased at 3 months and the intragroup difference in CFUs at different time intervals was statistically significant (p <0.005). Table 4 depicts the descriptive and intergroup comparative analysis of mean aerobic, Streptococcus mutans, and anaerobic CFUs, at different time intervals. The table shows a statistically significant difference (p<0.005) between the CFUs of all 3 groups at T0, T1, and T3 except for Streptococcus mutans count, which showed a statistically non-significant difference at T0 between all 3 groups.

Antibiogram profile of the Streptococcus mutans showed predominantly sensitive strains with one strain from Group 3 which showed resistance against amikacin, cefazolin, and cefixime, and another strain from group 3 which showed intermediate resistance to cefazolin. (Fig 4)

TABLE 1: Descriptive and intragroup comparative analysis of mean number of aerobic, streptococcus, and anaerobic CFUs in the conventional metal group (Group 1) at different time intervals.

Conventional metal (Group 1)	**Mean CFU/ML**	**Standard deviation**	**Chi-square value**	**p-value**
Aerobic				
Baseline	$1.74 * 10^{2}$	$5.72 * 10^{1}$	14.000	0.001*
1 month	$1.45 * 10^{7}$	$1.18 * 10^{7}$		
3 month	$1.32 * 10^{13}$	$2.28 * 10^{12}$		
Streptococcus mutans				
Baseline	4.29	5.34	14.000	0.001*
1 month	$2.87 * 10^{2}$	$1.99 * 10^{2}$		
3 month	$7.66 * 10^{9}$	$1.52 * 10^{10}$		
Anaerobic				
Baseline	$5.72 * 10^{2}$	$5.40 * 10^{2}$	14.000	0.001*
1 month	$6.14 * 10^{6}$	$4.81 * 10^{6}$		
3 month	$4.04 * 10^{9}$	$2.81 * 10^{9}$		

*p<0.05 is considered as statistically significant

TABLE 2: Descriptive and intragroup comparative analysis of mean number of aerobic, streptococcus and anaerobic CFU in ceramic bracket group (Group 2) at different time intervals

Ceramic (Group 2)	**Mean CFU/ML**	**Standard deviation**	**Chi-square value**	**p-value**
Aerobic				
Baseline	$2.51 * 10^{2}$	$1.15 * 10^{2}$	12.000	0.002*
1 month	$2.36 * 10^{10}$	$4.89 * 10^{10}$		
3 month	$1.46 * 10^{15}$	$1.97 * 10^{15}$		
Streptococcus mutans				
Baseline	$5 * 10^{1}$	$7.9 * 10^{1}$	12.000	0.002*
1 month	$5.05 * 10^{5}$	$9.31 * 10^{5}$		

3 month	$7.05 * 10^{14}$	$1.17 * 10^{15}$		
Anaerobic				
Baseline	$8.82 * 10^{4}$	$1.01 * 10^{5}$	12.000	0.002*
1 month	$7.47 * 10^{8}$	$1.30 * 10^{9}$		
3 month	$1.57 * 10^{12}$	$2.08 * 10^{12}$		

*p<0.05 is considered as statistically significant

TABLE 3: Descriptive and intragroup comparative analysis of mean number of aerobic, streptococcus and anaerobic CFU in Passive self ligation metal group (Group 3) at different time intervals

Passive Self ligation metal (Group 3)	**Mean CFU/ML**	**Standard deviation**	**Chi-square value**	**p-value**
Aerobic				
Baseline	$1.66 * 10^{2}$	$0.56 * 10^{2}$	12.000	0.002*
1 month	$7.92* 10^{3}$	$6.88 * 10^{3}$		
3 month	$1.64 * 10^{9}$	$1.79 * 10^{9}$		
Streptococcus mutans				
Baseline	$2.17 * 10^{1}$	$3.86 * 10^{1}$	9.333	0.009*
1 month	$2.11 * 10^{2}$	$3.48 * 10^{2}$		
3 month	$1.30 * 10^{9}$	$1.82 * 10^{9}$		
Anaerobic				
Baseline	$2.02 * 10^{2}$	$1.36 * 10^{2}$	12.000	0.002*
1 month	$6.06 * 10^{3}$	$8.12 * 10^{3}$		
3 month	$2.82 * 10^{7}$	$7.83 * 10^{6}$		

*p<0.05 is considered as statistically significant

TABLE 4: Descriptive and intergroup comparative analysis of mean number of aerobic CFU , streptococcus CFU and mean number of anaerobic CFU between different groups at different time intervals

	P value at T0	P value at T1	P value at T3
Total aerobic microbial counts			
Group 1	0.069	0.000	0.002
Group 2			
Group 3			
Streptococcus mutans			
Group 1	0.596	0.002	0.072
Group 2			
Group 3			
Total anaerobic microbial counts			
Group 1	0.002	0.000	0.000
Group 2			
Group 3			

*p<0.05 is considered as statistically significant

DISCUSSION

Oral biofilms are diverse communities of adhering microorganisms, embedded in a self-produced matrix of extracellular polymeric substances and possessing a complex, spatially heterogeneous, and dynamic structure. (10) Surface roughness is one of the prime properties of any material placed in the oral cavity with respect to bacterial adhesion and biofilm formation, especially in supragingival regions.

This study has thus been performed to conduct a microbial analysis of plaque biofilm for aerobic bacterial CFU, streptococcus mutans, and anaerobic bacterial CFU in subjects undergoing orthodontic treatment with different bracket systems, namely the conventional metal bracket system, the ceramic brackets, and the Passive self-ligating metal bracket system. Different bracket types have different physical characteristics and clinical properties, affecting the amount of biofilm accumulation on the orthodontics device components, and consequently, plaque formation and gingivitis. (11) Conventional brackets- both metal and ceramic, are used with some other components such as the elastomeric and metal ligature to engage the metal wire inside the bracket slot. On the other hand passive self-ligating brackets do not require an elastomeric or wire ligature but have an inbuilt mechanism that can be opened and closed by the clinician to secure the archwire. (12) These additional components- like elastomeric or wire ligatures, which are used during the treatment with conventional brackets make it susceptible to a higher rate of plaque accumulation(13) leading to difficulty in maintaining oral hygiene and in turn, periodontal problems. (9) (14)

In this study, the plaque around the lower anterior teeth brackets was collected and subjected to microbiological testing. The plaque biofilm was collected from around the lower anterior brackets, as these are the areas of maximum plaque retention after the maxillary molar regions (9).

The results of this present study show that the maximum colonization of aerobic and anaerobic colony counts was found around ceramic brackets (Group 2). These results are in disagreement with the study performed by Lindel et al (15) who concluded that ceramic brackets exhibit less long-term biofilm accumulation than metal brackets. The study also reports that out of all the aerobic bacteria found, the most prevalent aerobic bacteria was Streptococcus mutans which was in agreement with the results obtained by Komori et al (16) who in their study stated that among the facultative anaerobes, the proportion of Streptococcus was 44.4% in samples from molars with orthodontic bands.

According to some manufacturers, self-ligating brackets are less susceptible to plaque accumulation owing to the lack of metal and elastomeric ligatures used with them. (17) . The results of this study show that there was an insignificant difference in the total CFU/ml of aerobic, anaerobic, and streptococcus mutans counts obtained between the conventional metal and the Passive self-ligating metal bracket system surfaces. These results are in agreement with the study by Sunil et al who evaluated the plaque accumulated with metal and self-ligating orthodontic brackets in order to know which bracket type had a higher plaque retaining capacity and concluded that there was increased retention of plaque in metal brackets ligated with steel ligatures and comparatively less in self-ligating brackets at the base of the brackets. (18) However, there was a statistically significant difference in the total CFU/ml between the ceramic brackets and the Passive self-ligating metal bracket system, where the accumulation around the ceramic brackets was significantly higher. These results can be attributed to the fact that the ceramic brackets show an increased surface than the self-ligating bracket system thereby attracting more plaque biofilm. (19)

The eruption of the teeth during the first year leads to colonization by Streptococcus mutans and Streptococcus sanguis. These bacteria require a non-desquamating (nonepithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of Streptococci adhere strongly to the gums and cheeks but not to the teeth. Amikacin is one of the most effective antibacterial drugs for the treatment of dental caries (20) amikacin, cefazolin, and cefixime resistance of streptococcus mutans was this performed in the

study and was noted that the strains of Streptococcus mutans were predominantly sensitive to all three. In the current literature available, this study is the first of its kind to assess the antimicrobial resistance of bacteria isolated from different bracket systems.

LIMITATIONS

The results of this study should be adapted clinically with caution due to certain limitations associated with the study such as less sample size and consideration of only lower anterior teeth for plaque collection. Various other factors like the patient's oral hygiene and diet may affect the total plaque scoring, which might alter the results of the study.

There is scope for similar studies to be performed on more inclusive and larger samples in the future.

CONCLUSION

With the results of the performed study, it can be concluded that:

The total number of aerobic and anaerobic bacterial colonies is higher on the surface of ceramic brackets when compared to the conventional metal or the Passive self-ligating system.

There was no significant difference between the total colony counts of both aerobic and anaerobic bacteria around the conventional metal brackets and the Passive self-ligating bracket system.

Streptococcus mutans was the most prevalent bacterial strain amongst all the aerobic bacteria isolated from all the three bracket systems.

Resistant strains and intermediate strains of Streptococcus mutans were both observed in passive self-ligating bracket group.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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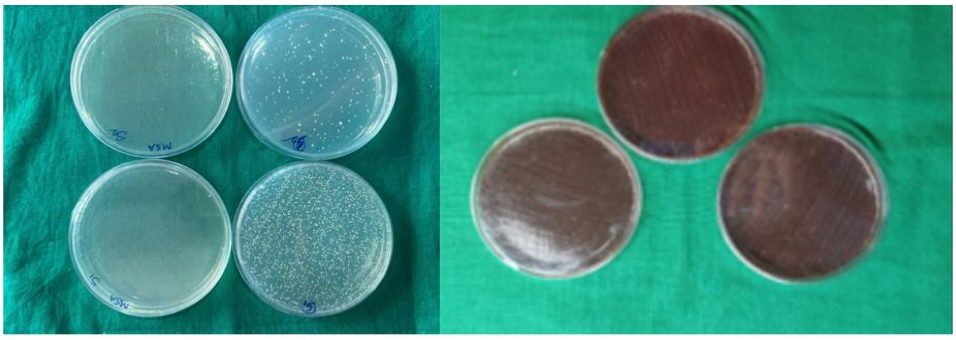

Fig 1(a) Fig 1(b)

FIGURE 1: Microbial count calculated at T0 recorded as colony forming units/ml
a. Aerobic bacterial counts b.Anaerobic bacterial counts

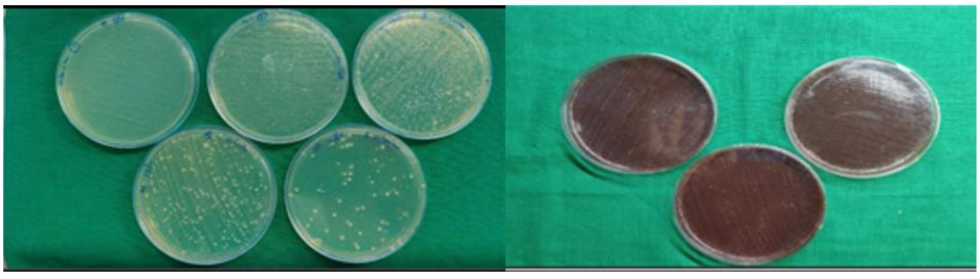

Fig 2(a) Fig 2(b)

FIGURE 2: Microbial count calculated at T1 recorded as colony forming units/ml
a. Aerobic bacterial counts b.Anaerobic bacterial counts

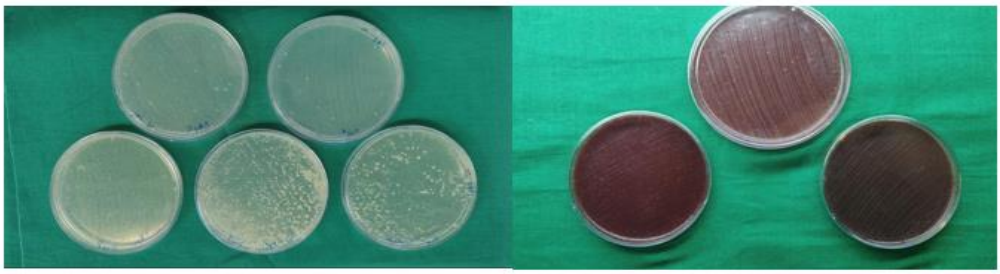

Fig 3(a) Fig 3(b)

FIGURE 3: Microbial count calculated at T3 recorded as colony forming units/ml
a. Aerobic bacterial counts b.Anaerobic bacterial counts

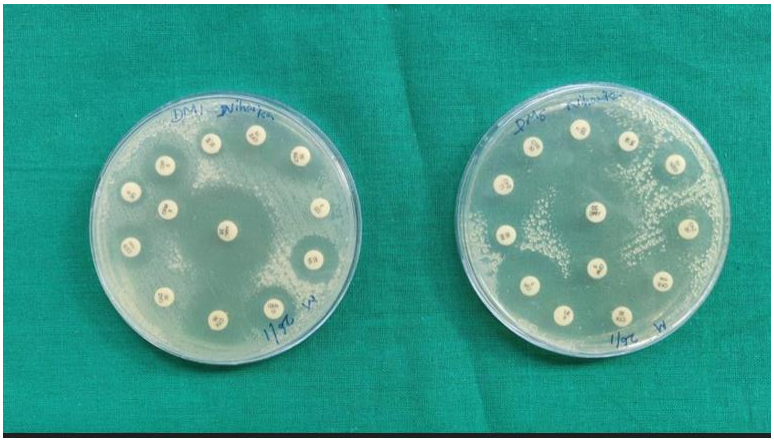

FIGURE 4 showing the antibiogram profile of Streptococcus mutans evaluated as per CLSI guidelines.

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