

Study the Effect of a Fixed Orthodontic Appliance on the Oral Microbial Cavity

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Abstract

Background: Orthodontics is that branch of dentistry specialty dealing with diagnosis, prevention, and correction of malocclusion aiming to enhance oral health, dental function, and esthetics. The presence of a fixed orthodontic appliance within the oral cavity can alter the microbial balance and so the composition of dental plaque. However, the presence of a fixed appliance alters the physiological characteristics of the oral cavity, causing impaired hygiene, a raise in dental plaque collection, and a delay in the spontaneous physiological processes of tooth cleaning that result in pathogenic bacteria colonization, which causes gingival inflammation, periodontal support degradation, and enamel surface alterations. **Objective:** The study's objectives were isolated, identification, and molecular detection of microbes associated with orthodontic appliance patients. **Materials and Methods:** A total of 88 study samples (68 case; 20 control), 29 males and 59 females, aged 14–35 years, underwent fixed orthodontics during the initial stage of treatment. The samples were cultured on enrichment media and selective media, including mitis-salivarius agar, Mitis-Salivarius Bacitracin agar, Lactobacillus MRS agar, Eosin Methylene Blue agar, and Mannitol agar. They cultured in aerobic and anaerobic (10% CO₂) conditions. **Results:** The positive samples were then purified and identified using morphological and biochemical tests. Among the microbial species gram-positive bacteria accounted for 82.3%, gram-negative bacteria 16.2%, and fungi 1.5%. Our results show that on one hand, gram-positive bacteria, *Streptococcus mutans* make higher percentage (48.5%) compared to other types. On the other hand, the *Escherichia coli* with 11.7% is the highest percentage of gram-negative bacteria. **Conclusion:** Bacteria colonization of orthodontic patients was found to be remarkably high compared to that of controls. Finally, the most common bacteria are *S. mutans* identify using PCR utilizing Sm.479.F/R primer combination.

Keywords: Bacteria, fixed orthodontic appliance, MSB agar, *Streptococcus mutans*

INTRODUCTION

Orthodontics is a dental specialty that focuses on treating and enhancing tooth and jaw abnormalities. This can entail using orthodontic tools such as braces to straighten teeth and rectify mismatched bites.^[1] Scientific studies have shown that the presence of fixed appliance in the oral cavity of orthodontic patients can change the composition of dental plaque. The structure of dental plaque, metabolism, and composition may change, leads to increase in the number of microorganisms.^[2] Dental plaque is thought to be one of the causes of oral cavity illnesses such as caries, gingivitis, periodontitis, and peri-implantitis.^[3] The use of fixed appliances alters the physiological characteristics of the oral cavity, causing impaired hygiene, a rise in dental plaque collection, and

a delay in the spontaneous physiological processes of tooth cleaning. Because of those reasons, an orthodontic biofilm grows and makes connections with many different parts of the mouth.^[4] Occupied by oral cavity bacteria after 1–2 days Predominant gram-positive cocci (*Streptococcus*) predominate over colonizing bacteria, which form 1–20 layers. The plaque is also home to filamentous and fusiform bacteria, as well as a tiny number of gram-negative cocci toward the apex. Aerobes and facultative anaerobes are the bacteria that

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live in this stratum (obligate anaerobes are very few).^[5] Fixed orthodontic appliances might hamper effective oral hygiene and pose a significant cariogenic challenge. Furthermore, due to the difficulties of maintaining dental cleanliness, orthodontic appliances may alter the subgingival microbiota since orthodontic accessories encourage bacterial plaque retention. That could lead to the growth of pathogenic bacteria, which can cause gum inflammation, loss of periodontal support, and changes to the surface of the enamel.^[6] Gingivitis is described as an inflammatory condition caused by microbes. Tooth decay is characterized as a dysbiosis produced by repeated sugar exposure. As a result, dental tissues become demineralized. There is considerable agreement that dental biofilm is the key biological determinant of illnesses development.^[7] Using brackets for orthodontics therapy is one of the factors that promotes tooth biofilm preservation owing to cleaning and plaque accumulation issues, and as a result, pH shifts and the development of caries and gingivitis are common.^[8] Using orthodontic appliances also enhances the retention of cariogenic bacteria, allowing them to proliferate and thrive, resulting in imponderables in the oral gram and demineralization around the bracket. *Streptococcus mutans* is recognized to be one of the most significant organisms engaged in cariogenic biofilm and to play a major pathogenic role in tooth decay pathogenesis due to its acid-genic and acidic characteristics.^[9] Patients with a fixed orthodontic therapy may experience an increase in gingival plaque formation as well as a periodontal microbial population.^[10] Some studies have found that the microbiology of the subgingival plaques of orthodontic patients has changed, and that the amount of periodontal-pathogens in the subgingival plaques has changed dramatically.^[11,12] This study aims to identify and molecular detection of microbes associated with orthodontic patients.

MATERIALS AND METHODS

A total of 88 study samples (68 case; 20 control), comprising 29 males and 59 females with the age ranging 14–35 years, underwent fixed orthodontic therapy during the initial stage of treatment. All samples were collected from the private orthodontic clinic and the specialized health center of dentistry in Babylon City, Iraq. The time span of study sample collection was from February to June 2022.

Specimen collection methods

The samples were taken from patients who had fixed orthodontic appliances for at least three weeks. A swab was taken between the orthodontic brackets attachment molar tube or band, the free gingiva margin from the first molar on both sides of the buccal for upper jaw by a sterilized micro applicator (bond micro brush swab). The bond brushes were used because they are the small in



Figure 1: Swab collection from patient

size which can reach a narrow area and also carry a good amount of sample as shown in Figure 1.

Bacterial diagnosis

The sample collected by bond brush swab placed in a plain tube containing 4ml of sterilized brain heart infusion broth (Candalab, Spain) was then cultured on blood agar (Acumedialab, USA), MacConky agar (Neogen, USA), other selective media such as mitis salivarius agar (Himedia, India), Mitis Salivarius Bacitracin agar (MSB agar), Lactobacillus MRS agar (Himedia, India), Eosin Methylene Blue agar (Himedia, India), and Mannitol agar (Himedia, India). They were incubated condition aerobic and anaerobic incubator candle jar at 37°C plus (10% CO₂) for 24–48 hours. A visual inspection is conducted for the determination of growth, fermentation, pigmentation, and colonial shape. The gram staining procedure was performed on the plates, demonstrating the development of colonies. Under a microscope, the colonies were examined to distinguish between gram-positive and gram-negative bacteria. In order to identify the bacteria in each sample based on their physical characteristics, biochemical tests were undertaken. Various gram-positive and gram-negative isolates were identified using the catalase test, citrate utilization test, coagulase test, indole test, lactose fermentation test, and oxidase test. MSB agar is a selective medium for growing *S. mutans*. It was made by adding selecting agents at the appropriate levels found for the Mitis–Salivarius Agar: 0.2 U/ml of bacitracin antibiotic and 20% (w/v) sucrose. MSB plates were incubated for 72h at 37°C in a 5% CO₂ environment. Then, up to 25 representative *S. mutans* colonies were chosen from MSB plates.

Genomic identification of *S. mutans*

The DNA was extracted from a mixture of *Streptococcus spp.* cultured on Mitis-Salivarius Bacitracin Agar (MSB)

using modified protocols described by the manufacturer (FAVORGEN). *S. mutans* isolates were confirmed by colony polymerase chain reaction using the species-specific pair of sm 479F/R^[13] to amplify 479bp DNA fragment with F: TCGCGAAAAAGATAAACAACA and R: GCCCCTTCACAGTTGGTTAG. The PCR mixture contain 1× PCR amplification buffer, master mix 10 µL (Promega, USA), 2 µL for each primer, 2 µL DNA template and nuclease free water 4 µL. The thermocycle condition for PCR follows; 95°C for 5 min, followed by 35 cycles of 95°C for 30s, 55°C for 30s and 72°C for 45s and finally 72°C for 5 min. The PCR product was separated on 2% agarose gel (Candalab, Spain).

Statistical analysis

The descriptive statistics, the statistical software for the social sciences, edition 22.0 (SPSS) program was used. Continuous variables were shown using means and standard deviations. To compare the *P* values of three groups, a chi-square calculator for independent samples was used. *P* values less than or equal to 0.05 were considered statistically significant, whereas *P* values higher than 0.05 were considered statistically insignificant.^[14]

Ethical consideration

The study was carried out in conformity with the ethical standards outlined in the Helsinki Declaration. Before taking the sample, the patients verbal and analytical consent was obtained. To obtain this permission, a local ethics committee evaluated and approved the study protocol, subject information, and consent form using document number 566 (containing the number and date in 30/01/2022).

RESULTS

The study includes 88 cases, of which 68 of them have fixed orthodontic appliances with 20 control group at different

times. Study subjects consist of 29 males and 59 females. Eighty-eight cases are divided into three age's groups: the first group includes 43 patients with six controls within the age of 20 years. The second group includes 20 patients with nine controls within the range of 21–30 years old. The last group includes five patients older than 31 years as shown in Table 1.

The microbial isolate diagnosed Microscopically and biochemically. A microbial swab was isolated from patients after three weeks of installing a fixed orthodontic appliance. Noticeably all the samples gave a positive microbial culture. The control samples were taken in swab from healthy people without orthodontic appliances *Staphylococcus epidermis* isolated from 40% cases while the other 60% samples had no growth. Sixty eight of eighty eight cases were diagnosed with positive microbial growth samples from microbial swabs, shown in Table 2. Twenty samples of them were used as controls which were taken from healthy people without orthodontic appliances as shown in Table 3. The microbial swabs were divided into three groups based on age (<20 years, 21–30 years and >30 years).

Detection of *S. mutans* by a specific gene

S. mutans isolates were identified using conventional methods such as colonial morphology, microscopic examination, and biochemical tests before being subjected to DNA extraction and PCR assays. The concentration and purity of extracted DNA were determined by using nanodrop. DNA bands were confirmed by gel electrophoresis. The results demonstrated that thirty-three isolates had a detectable *Sm479F/R* gene with band 479 pb. The gel was photographed by using gel documentation to save the image as shown in Figure 2.

Table 1: Distribution of cases with fixed orthodontic treatment according to gender and age groups

			Patients	Control	Total	P value
Gender	Male	No.	19	10	29	0.06
		%	27.9	50	33	
	Female	No.	49	10	59	
		%	72.1	50	67	
	Total	No.	68	20	88	
		%	100	100	100	
Age	<20 year	No.	43	6	49	0.015
		%	63.2	30	55.7	
	21–30 year	No.	20	9	29	
		%	29.4	45	33	
	>30 year	No.	5	5	10	
		%	7.4	25	11.3	
	Total	No.	68	20	88	
		%	100	100	100	

Significant level < 0.05

Table 2: Distribution of microbial isolates from the patients with fixed orthodontic appliances according to gram stain and age groups

Microbial			No. (%)	<20 years	21–30 years	>31years	
Gram-positive bacteria no. 56 (82.3%)	<i>Streptococcus mutans</i>	No. (%)	33 (48.5)	19 (28)	9 (13.2)	5 (7.35)	
	<i>Lactobacillus ssp</i>	No. (%)	12 (17.6)	8 (11.7)	4 (5.9)	0 (0.0)	
	<i>Staphylococcus aureus</i>	No. (%)	5 (8.62)	3 (4.4)	1 (1.47)	1 (1.47)	
	<i>Streptococcus salivarius</i>	No. (%)	4 (5.88)	4 (5.88)	0.0	0.0	
	<i>Streptococcus pyogenes</i>	No. (%)	1 (1.47)	1 (1.47)	0.0	0.0	
	<i>Streptococcus vestibularis</i>	No. (%)	1 (1.47)	1 (1.47)	0 (0.0)	0 (0.0)	
Gram-negative bacteria no. 11 (16.2%)	<i>Escherichia coli</i>	No. (%)	8 (11.7)	4 (5.8)	4 (5.9)	0 (0.0)	
	<i>Klebsiella pneumoniae</i>	No. (%)	2 (2.9)	1 (1.4)	1 (1.47)	0 (0.0)	
	<i>Pseudomonas aeruginosa</i>	No. (%)	1 (1.47)	0 (0.0)	1 (1.47)	1 (0.0)	
Fungi no. 1 (1.5%)	<i>Candida spp</i>	No. (%)	1 (1.5)	1 (1.5)	0 (0.0)	0 (0.0)	
	Total	No.	68	42	20	6	
			%	100	100	100	
			% of total	83.6	61.7	29.4	8.9

Table 3: Distribution of microbial isolates from control group (non-orthodontic) according to gram stain and age groups

Microbial			No. (%)	<20 years	21–30 years	>31 years
Gram-positive Bactria (40%)	<i>Staphylococcus epidermis</i>		8 (40)	1	4	3
No growth (60%)	No growth		12 (60)	0	0	0
Total	No.		20	1	4	3
	%		100	5	20	15

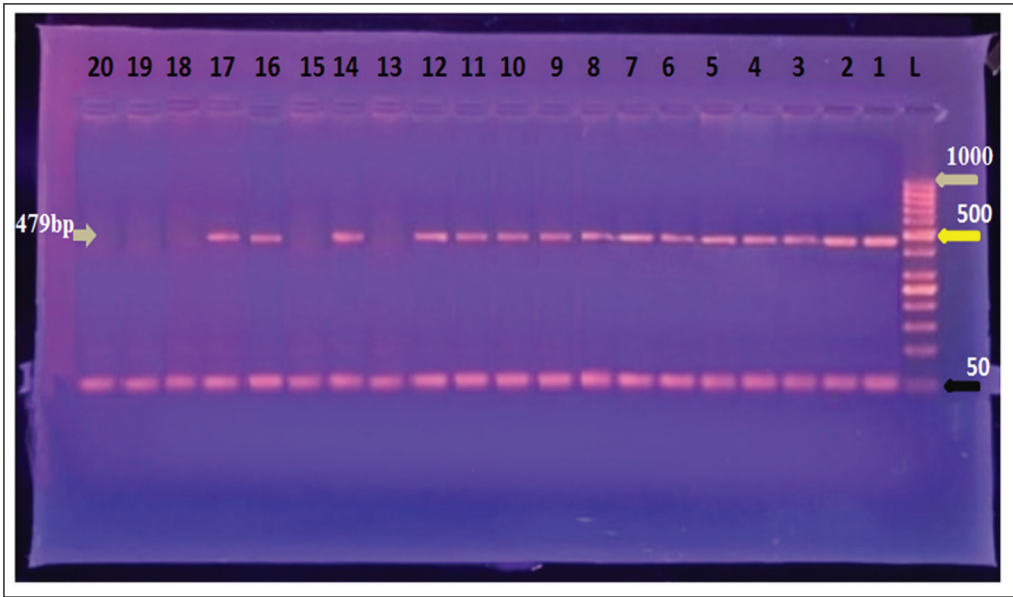


Figure 2: Conventional PCR for detection gene. 2% Agarose gel electrophoresis at 85 volt for 45 min for Sm479F/R primer PCR. Products visualized under UV light after staining with ethidium bromide. L: Marker DNA Ladder, lanes (13, 15, 18, 19, and 20) only negative. The size of the product is 479bp molecular weight fragment DNA

DISCUSSION

In this study, the results indicated that the prevalence of females (72.1%) is higher than males (27.9%) with *P* value = 0.06. This result is in good agreement with that found by AL-Gunaid *et al.*^[15] That might be attributed to the fact that females place a higher value on attractiveness compared to males. The patients were selected from

ages 14–35 because most of their permanent teeth were complete. This agreed on with Olkun and Sayar^[16] and partially agreed on with Shimpo *et al.*^[17] who found that the age of patients ranged 13–35 years. With a significant correlation, age groups 20 have a higher prevalence (63%) of patients than other age groups. Because the teeth shift as a result of pressures exerted on them throughout time,

many orthodontic issues are just as readily correctable in adults as they are in children and adolescents. Adults' bone tissue is denser than that of youngsters, so therapy may take a bit longer. The relationship between the age groups and prevalence of patients was in a reversible relationship. Almansob *et al.*^[18] reported that there was a tendency for older individuals to use orthodontic appliances more than did younger subjects, in disagrees with our finding.

The percentage of gram-positive bacteria isolated from patients' oral cavities was 82.3%, the percentage of gram-negative bacteria was 16.2%, and the percentage of fungi was 1.5% as shown in Table 2. This finding is consistent with the findings of Samiappan *et al.*^[19] who revealed that the majority of microorganisms isolated from orthodontic brackets were gram-positive bacteria with 21 isolates, while gram-negative bacteria of seven isolate. Results recorded that gram-positive bacteria *S. mutans* in patients with fixed orthodontic appliance were predominant (48.5%) of fixed orthodontics [see Table 2] and found that *Lactobacillus spp.* was (17.6%) and *Staphylococcus aureus* was (8.62%). *Streptococcus salivarius* was (5.88%) and *Streptococcus pyogenes* with *Streptococcus vestibularis* was (1.47%). While this result was slightly agreed on with Jing *et al.*^[20] which observed a rise in the pathogen *S. mutans* as a change in the oral bacterial ecology of patients undergoing long-term permanent orthodontic treatment, that was also agreed by Shirozaki *et al.*^[21] who reported that the *S. mutans* contamination levels are similar in the different orthodontic ligature types. Also, Farhadian *et al.*^[22] reported that *S. mutans* is the main microbe that causes caries and that may make orthodontic patients more likely to get caries. The current results showed that *Staphylococcus spp.* in patients with fixed orthodontic appliances was predominant (8.62%) in fixed orthodontic as shown in Table 2. This finding was supported by Pellissari *et al.*^[23] and partially supported by Abbas *et al.*^[24] whose demonstrated that *S. aureus* bacteria are the most common type of gram-positive aerobic bacteria found in the saliva of people wearing fixed orthodontic appliances, accounting for 38% of the total 15 specimens. Exhibited *Staphylococcus spp.* (69.5%) in the oral cavity. Because *S. aureus* can form biofilms and live in certain places, it has been able to develop ways to stay alive, such as ways to avoid the body's defenses and make antibiotics less effective.^[25] The presence of *S. aureus* in the oral cavity of orthodontics patients and the usage of prostheses were linked to the bacteria's presence. This emphasizes the significance of proper dental hygiene, as *S. aureus* can cause dangerous systemic infections through the oral cavity, and the use of prostheses was linked to the bacteria's presence.^[26]

The second frequent bacteria are *Lactobacillus spp.*, which was found in patients with FOA. This result agrees with Al-Lehaibi *et al.*^[27] who found that substantial differences in colony rates of *S. mutans* and *Lactobacillus spp.*, with number of colonies being more *S. mutans* than

Lactobacillus spp. colonies over the three months at 10² dilution, and their colony mean values were significantly higher ($P = 0.05$) especially during the period between the 1st and 2nd months. While nearby Marda *et al.*^[28] which found that the insertion of an orthodontic appliance makes it harder to eliminate dental plaque using a toothbrush and floss. They found an increase in cariogenic oral bacteria in T0 and T2, particularly *Streptococcus mitis*, *Streptococcus sobrinus*, and *Lactobacillus*. These results were statistically significant (P value = 0.021, 0.016, 0.031). *Lactobacillus* was 53% in the oral cavity. The result shows no significant change in *Lactobacillus spp.*

Streptococcus salivarius was the 3rd predominant bacterial with 5.88% found in patients during orthodontic treatment, and this result agrees on with Gomez *et al.*^[29] The mentioned acid-base physiology of *S. salivarius* plays an important role and the presence of orthodontic appliances can produce an increase in bacteria if associated with metal alloy. When gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (11.7%, 2.9%, and 1.47%, respectively) were identified in the present study, *E. coli* and *K. pneumonia* were found to be the most frequently detected gram-negative bacteria (11.7%, 2.9%), which is consistent with Lima *et al.* and Pellissari *et al.*^[23,30] who found *E. coli* is especially common in the mouth as a successional community after antibiotic treatment as suggested by Chen *et al.*^[31] Also, people with weakened immune systems and people in hospitals are more likely to get the bacteria. In contrast to our results, Al-Oebady *et al.*^[32] proposed that *P. aeruginosa*, *Klebsiella spp.*, and *Enterobacter* species were isolated from fixed orthodontic patients (*P. aeruginosa*). Four-month bacterial growth was more than one-month bacterial growth and both were greater than the maximum six-month bacterial growth time. It is difficult for germs to persist in the oral cavity over time. The current study found that 1.5% of the collection samples from fixed orthodontic patients were isolated *Candida spp.*, which agrees with Lucchese and Bondemark,^[4] who found the prevalent microbial genera identified in group 1 were *Candida* (12.0%). Yang *et al.*^[33] reported that in the saliva and occlusal plaque of the fixed orthodontics appliance group, a tendency toward greater *Candida spp.* levels was identified, while the control group included higher levels of *S. mutans*. Moreover, for *S. mutans* levels, both the fixed orthodontic and control groups demonstrated a positive connection between saliva and occlusal plaque regarding *Candida spp.*

The results of this study indicate that the Sm.479.F/R primer set contains these characteristics and might be used for slightly elevated epidemiological investigations of *S. mutans* infection as well as for better understanding of microbiological role of *S. mutans* in carious lesions.^[13] Wang *et al.*^[34] reported that the effects of *S. mutans* on caries in the permanent teeth of several residents of a north China town varied. The ratios of *S. mutans*

to total bacteria detected in saliva by real-time PCR utilizing Sm479F/R and 16S RNA primers were shown to correlate strongly with the prevalence of dental caries in the same cohort. These tests may be useful for identifying a person's dental caries risk. According to the findings of this investigation, the Sm.479.F/R primer combination is highly specific and sensitive for identifying *S. mutans* in either pure or mixture DNA samples.

CONCLUSION

There is no significant statistical gender difference; however, females tend to be predominant over males regarding study sample rate. The number of orthodontic patients under 20 years age group is significantly higher than that in the other age groups. While controls give one type of bacteria that is *S. epidermis*, the fixed orthodontic patients have many types of microbials such as gram-positive bacteria, gram-negative bacteria, and fungi. The most common isolated bacteria were *S. mutans* determined by PCR. Microbial isolate from orthodontic patients were found to be high compared to controls.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

The authors declare that there is no competing interest.

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