



## Fixed Orthodontic Appliance Associated With Change in Bacterial Diversity During First Stage of Active Orthodontic Treatment.

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### Abstract

Background: some researchers have noted that fixed orthodontic appliance (FOA) have stirring on oral hygiene that lead to high cariogenic challenge. Moreover, based on the difficulty of maintaining oral hygiene, it can also affect germs under the gums by orthodontic devices, These variables would probably lead to the colonization of pathogenic bacteria, which are responsible for inflammation of the gingival, destruction of the periodontal support and changes in the enamel surface. Objective: Isolation and identification of bacteria among orthodontic patients at progressive time during first stage of active orthodontic treatment and molecular identification of highly cariogenic bacteria *Streptococcus mutans* as well as an *in vitro* evaluation of antibiotic sensitivity/resistance for bacterial isolates. Material and Methods: Sixty-five patients treated with fixed orthodontic appliance (FOA) their age between 12-25 years. Imprint swab samples were collected between brackets on the tooth surface monthly at zero day, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> to be cultured aerobically and anaerobically. Bacterial isolated were identified in all age groups at progressive time. Molecular detection of *S.mutans* was performed using species specific primer Sm 479. The antibiotics sensitivity were done by use Kirby- Bauer disc diffusion method for bacterial isolates, the antibiotic selected was most common antibiotic used during orthodontic infection ( amoxicillin, amoxi-clav, cefotaxim, erythromycin and ciprofloxacin). Results: A total 186 bacterial isolates were obtained from 65 sample of tooth swabs in zero day (immediately after orthodontic device appliance) and 183 bacterial isolates were obtain at first month after wearing orthodontic device, while 195 bacterial isolates were obtained at second month after wearing the device, in the last visit (3<sup>rd</sup> month after put orthodontic device) 202 bacterial isolates were obtained, and all samples give positive bacterial culture as shows in table (2). Also the result shows that *Staphylococcus spp.* was the most bacterial isolates appear in patients with fixed orthodontic appliance (FOA). The study indicated neglected statistically significant difference over progressive time except alpha and beta streptococci and *P.auriginosa* according to Chi-square test. The bacterial isolated during this study include *Staphylococcus* (*S. epidermidis* and *S.aurus*), *Lactobacillus spp.*, *Streptococcus* (alpha, beta, and gamma), *E.coli*, *Klibsilla spp.*, *Enterobacter spp.*, and *P.auriginosa*. Species specific primer Sm 479F/R using polymerase chain reaction (PCR) indicated that *S.mutans* was detected and increased from 60% at zero day to 80% at 3<sup>rd</sup> month. The antibiotic sensitivity test recorded that ciprofloxacin and amoxi-clav shows high effective against bacterial isolates. Conclusion: The studies conclude that oral cavity colonized by large number of microorganisms that contribute in infection during orthodontic treatment. *Staphylococcus (aurus and epidermidis)* was the most common bacteria isolated from patients during initial stage of orthodontic treatment which appear in (81.5%) of the total isolates followed by *Lactobacillus spp.* and then *streptococcus spp.* which appear non-statistically significant difference ( $p \geq 0.05$ ) among progress with treatment. The most common gram negative bacteria in zero day is *E.coli* (20%), *Klibsilla* (18%), *Enterobacter spp.*(20.8) while *pseudomonas aeriginosa* (10.6) and also shows non-statistically difference except *P.auriginosa*, The flora exists in harmony with the host but this relation may be broken due to orthodontic device. The molecular detection of *S.mutans* by species specific primer shows increase in percentage from 60% in zero days to 80% in third month. All gram positive showed high level of susceptibility to amoxi-clav followed by ciprofloxacin and cefotaxime while gram negative show high sensitive to erythromycin followed by ciprofloxacin and cefotaxime.

**Keywords:** Fixed orthodontic appliance, Bacterial diversity, Antibiotic sensitivity, *S. mutans*.

## Introduction

The oral cavity can serve as a reservoir of some pathogens that can cause systemic infections [1]. As the oral cavity is colonized by natural micro flora, it is properly stable in the individual's composition result of a long-term relationship between bacteria and host [2]. It was reported that more than 400 types of bacteria found in the oral cavity, and some cause inflammation such as those seen in the periodontitis [15]. Some author has been reported that the presence of fixed orthodontic appliance inhibit oral hygiene and create new retentive area for plaque and debris which in turn predispose to increase carriage of microbes and subsequent infection [3,4].

Scientific publications have shown that the presence of fixed devices in the oral cavity of orthodontic patients can alter the nature of the dental plaque.8 Alter the metabolism and composition of the dental plaque, leading to an increase in the population of bacteria, especially *Streptococcus* and *Lactobacillus* [8, 9].

FOA may interfere with oral hygiene practice and cover considerable parts of the tooth surfaces, so an increase of the total microbial population as well as an change microflora have been reported in relation to orthodontic treatment [10,11]. Moreover, based on the difficulty of maintaining oral hygiene, the sub gingival microbiota may also be influenced by orthodontic therapy. Based on the effort of maintaining oral hygiene, it can also affect the bacteria under the sub gingival by orthodontics appliances [12,13].

Since accessories orthodontic prefer to keep bacterial plaque. Such variables may lead to the colonization of Pathogenic bacteria, which are responsible for inflammation of the gingival, and the destruction of the periodontal support [12, 13], and changes in enamel surface [10]. The development of orthodontic devices creates an environment conducive to the accumulation of germs and waste food, which, in time, may cause tooth decay or exacerbate any periodontal disease already present [16].

The use of antibiotics as systemic treatment in the treatment of gum inflammation represents concepital shift in the field of gum towards the application of anti-infection treatment strategy, where the disease

specific associated bacterial pathogens, which is primarily targeted to suppress or eliminate the oral cavity of patients with gingivitis and inflammation of the gums [18].Antibiotics have shown to be effective in gingivitis and gingivitis treatment associated with orthodontic treatment include single drug regimens with tetracycline- HCL, aminoglycoside, doxycycline, amoxicillin, clindamycin, metronidazole, azithromycin and moxifloxacin, as well as combination drug regimens involving amoxicillin plus metronidazole, ciprofloxacin plus metronidazole [19].

System antibiotic administered can reach micro-organisms that cannot be reached to expand the scope of the instrument or is colonization deep slit the tongue, however, in determining whether the use of therapeutic antibiotic systemic treatment, it is essential to consider the possible benefits, and impact of negative, it includes the development of bacterial-resistant species [20].

Unfortunately, it led blind trust in the potential benefits of antibiotics to be used widely but is often inappropriate; Resistance of bacteria to one or more antibiotics is widely observed [17]. If the drug is in high concentration enough about bacteria, drugs are either prevalent bacterial reproduction (bacteriostatic) or kill bacteria actually (bactericidal) [18].

The occurrence of antibacterial resistance, an increase in antibiotic nights, the lowest inhibitory concentration (less concentration of antibiotic capacity to inhibit The growth of bacteria) for a given particular bacterial strain, the minimum concentration of inhibitor (MIC) of the same antibiotic for the wild population of bacteria strain itself is considered resistance [21].

Bacteria have many mechanisms of antibiotic resistance. A partial resistance type is not exclusive to one family of antibiotics; on the contrary, the altered microbial species may use a changed mechanism of resistance to the similar antibiotic agent [17]. Antibiotic resistance may occur if the bacteria collect an enzyme that can degrade from antibiotics [22]. Such as $\beta$ -lactamase thate hydrolyses the constitutive  $\beta$ -lactam ring of penicillins and cephalosporins.

In some formulations such as Augmentin, this mechanism of resistance is circumvented by combination of penicillin with clavulanic acid. The latter acts as a “decoy,” serving as the prime target for the  $\beta$ -lactamase and thereby protecting the penicillin's  $\beta$ -lactam core.

A second mechanism of resistance includes the alteration of the antibacterial target [23]. The bacteria may also take out antibiotics from the cells they initially entered [24]. This mechanism depends on the manifestation of inter membrane pushes, which eject medication. Finally, the target of antibiotics can be altered by genetic alteration (mutation), which decreases drug affinity to its substrate.

Often this mechanism is linked with resistance to erythromycin [46]. Studies have adopted *S. mutant* largely on agriculture for the identification and characterization of *S. mutans* in the oral cavity. The main limitation of the methods of culture is limited threshold of detection of *S. mutans* in clinical specimens.

Morphology varies depending on the culture medium used; the high cost of the work unit. Moreover, the cultivation requires a viable sample, which makes its application in epidemiological studies and field and high productivity is a practical research [25].

Because the methods of traditional culture can limit population studies of *S. mutant* and its interaction with other bacteria in the oral cavity, it has been developed a number of DNA probe bases and primers. Several investigations or specific primers for some genes associated with virulence in targeted *S. mutans*, such as glucosyltransferases [26, 27], after extensive review studies, we found that many of the precursor tests PCR works well for pure cultures of the mutant. However, there was very little information about whether the targeted areas PCR- may be present in other bacterial species found in the same habitat as a mutant.

Or whether these primers can detect *S. mutans* mixed clinical specimens as well. In fact, some of these genetic traits may not be unique to *S. mutans* [40]. Patients undergoing orthodontic treatment have oral environmental changes that lead to increased numbers of mutant streptococci in the saliva and teeth plate of these patients [41].

The development of fixed orthodontic appliances on the teeth results in iatrogenic side effects. There is an increase in the size of the tooth plate as well as an increase in the number of bacteria and the concentration of carbohydrates in each milligram of plaque [42]. According to [42], the increase in the following *S. mutans* put orthodontic devices can be explained by an irregular nature of their surfaces, which promote the growth of these bacteria and acid generator acidity that prefer hard surfaces to grow on. *S. mutans* is part of the normal flora of the oral cavity.

When it becomes infected only under conditions that lead to repeated acidification and prolonged dental plaque [43]. *S. mutans* to adapt to the low pH of the environment, and thus, increasing the production rates of acid derived pH is still less than what led to the plaque carious which leads to tooth decay [44]. Patients undergoing orthodontic treatment have oral environmental changes that lead to increased numbers of streptococcus mutants in saliva and plaque [44]. Glans et al., 2003 reported The *S. mutans* colonizes 40-85% of patients with orthodontic devices.

The study of Scheie et al., was screened numbers of commensal bacteria and transient bacteria in anaerobic culture medium in the level of salivary, to investigate different bacteria during orthodontic treatment. Decreased the number of *S. mutans* and *lactobacilli* after 1 month of treatment and then increased to reach the initial level in 3 months [45].

## Materials & Methods

### Patients

A total of 65 patients (31 males and 34 females) who were treated with FOA were included in the study. Ages of patients ranged from 12 to 25 years. None of the patients had history of smoking, debilitating disease, antibiotic or steroid therapy.

### Sample Collection and Cultivation

Before the investigation, all individuals received oral hygiene instructions. Tooth swab and 2-3ml of saliva samples were taken from 65 patient which have fixed orthodontic appliance immediately after put the device and follow up monthly, this process was repeated for 4 times (at 0 day, 1st month, 2nd month, 3rd month).

The imprint swabs were collected between the brackets in tooth surface area around FOA. All samples were transported by sterile wooden stick swabs containing transport media to be cultured in the medical laboratory at the College of dentistry-Babylon University. Were cultured aerobically and anaerobically on blood agar, Macconkey agar, and an aerobically Mitis salivaris agar, Lactobacillus MRS Agar. After 24-48 hrs. All isolates were identified according to their culture characteristics, biochemical reactions and microscopically appearance as described by Collee *et.al* [7].

### Antibiotic Susceptibility Test of bacterial Isolates:

Selective antibiotics are most commonly used during orthodontic infection to show their effect on bacterial isolates from patients with orthodontic appliance. Antibiotic discs were supplied from (bioanalyses, Turkey). the disk diffusion test was performed using Kirby pour technique a pure culture of isolated microbes previously identified. It was determined the most effective antibiotic for each bacterial isolates as recommended by (Clinical and Laboratory Standards Institute, 2014).

In addition, 5 isolated colonies were grown on the blood agar plate to 5 mL of nourishing broth and incubated at 37 ° C for 18 hours and compared to a standard tube (0.5) McFarland standard tube. The use of a sterile swab to get the inoculum from the bacterial suspension, was tainted by the inoculum on Mueller Hinton agar plate and

leave to dry. The antibiotic discs were placed on the surface of the medium at intervals evenly spaced with flammable forceps and incubated for 24 hours at 37 ° C. Areas of inhibition were measured using a ruler and compared with the areas of inhibition identified (CLSI, 2014).

### Molecular Identification of *S. mutans*:

DNA was extracted according to protocols recommended by manufacturer (Bioneer, Korea) from mixed bacterial culture on Mitis Salivaris Agar (MSA) and cultured on Brain Heart Infusion broth (BHI). PCR technique done using species specific primer Sm479F: 5'- TCGCGAAAAGATAAACAAACA-3' and Sm479R: 5'-GCCCTTCACAGTTGGTTAG-3'. The reaction was conducted as follows: 94°C for 3 min, followed by 40 cycles of 95°C for 30 s, 55 °C for 30 s, and 72°C for 59 s, then finally 5 min at 72°C for extension. The PCR amplicons were evaluated using 2% agarose gel at 100 volt for 50 minute. In TBE (Tris, Borat- EDTA) buffer and stained with ethidium bromide solution (5 micrograms / ml). It was arrested on the final pictures of the gels by the digital camera.

### Results and Discussion

Patients were grouped according to age and gender as shown in (Table 1). The majority of gram positive bacteria isolated in all duration of treatment with FOA were *Staphylococcus* spp., *Lactobacillus* spp. and *Streptococci* as shown in (Table 2). Cultures yielded high prevalence of gram positive than gram negative bacteria in different durations of FOA treatment (Table 3).

Table 1: Distribution of patients with fixed orthodontic treatment according to Gender and age

Age group	Gender		No. of male and female	%
	Male	Female		
12-15	17	16	33	50.8%
18-25	14	18	32	49.2%
Total No.	31	34	65	100%
Total %	47.7%	52.3%		100%

Table 2: Frequency and percentage of bacterial isolated from patients with fixed orthodontic treatment at different time and non-orthodontic control group

Type of bacteria	0 day	frequency	1 <sup>st</sup> m	frequency	2 <sup>nd</sup> m	frequency	3 <sup>rd</sup> m	frequency	control	Frequency	X <sup>2</sup>	P value
<i>Lactobacillus</i>	72.3 %	47	73.8 %	48	76.9 %	50	75.4 %	49	84.2%	16	57.19	0.000
<i>Staph. aureus</i>	24.6 %	16	27.7 %	18	26.2 %	17	30.8 %	20	15.8%	3	16	0.003
<i>Staph. epidermidis</i>	56.9 %	37	38.5 %	25	49.2 %	32	44.6 %	29	63.2%	12	22.7	0.000
$\alpha$ -hemolysis <i>Streptococci</i>	44.6 %	29	56.9 %	37	55.4 %	36	52.3 %	34	57.9%	11	28.6	0.000

β – hemolysis streptococci	1.5%	1	1.5%	1	3%	2	3%	2	0	0	2.38	0.66
γ- hemolytic Streptococci	16.9 %	11	12.3 %	8	15.4 %	10	15.4 %	10	0	0	11.8	0.01
<i>E.coli</i>	20%	13	21.5 %	14	21.5 %	14	29.2 %	19	26.3%	5	9.81	0.04
<i>Klebsilla spp.</i>	18.5 %	12	20%	13	23.1 %	15	32.3 %	21	21.1%	4	14.4	0.006
<i>Enterobacter</i>	20.8 %	13	21.5 %	14	24.6 %	16	23.1 %	15	21.1%	4	10.7	0.03
<i>p.auriginosa</i>	10.6 %	7	7.8%	5	4.6%	3	4.6%	3	5.3%	1	4	0.21

p≤0.05

**Table 3: Percentage of gram positive and gram negative isolates from patients with fixed orthodontic appliance**

Bacterial type	Zero day	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	control
G-positive	75.8%	75.3%	75.4%	71.3%	80.8%
G-Negative	24.2%	24.7%	24.6%	28.7%	19.2%
Total no.	G+	141	137	147	144
	G-	45	45	48	58

It is well known that wearing orthodontic appliances leads to increase carriage of oral bacterial population which may cause gingivitis and periodontitis which may cause complications in the orthodontic working. However, the present study was designed to identify the predominant bacteria associated with FOA.

Age of persons ranged from 10-30 years (Table 1) females consisted 52.3% while the remaining were males, however, it is well known that females take care of their expression more than males so they visits orthodontic clinic in larger number. Many investigators supported these facts [8, 9]. The duration of wearing appliances, play an important role in causing problems. In the present study patients were grouped into two groups growing (12-15) and non-growing (18-25) (Table 1).

The existence of orthodontic appliances in the mouth of patients can stimulate many environmental changes e.g. drop of pH, increase of carbohydrates, accumulation and retention of bacterial plague [10]. Bacterial cultures revealed growth of many types of bacteria in different time. The majority of

gram negative bacteria were found to be like *Klebsiella*, *E. coli* and *Enterobacter*. While gram positive bacteria isolates were prescribed as *Staphylococcus epidermidis*, *S. aureus*, and *Lactobacillus spp.* and α, β and γ Hemolytic Streptococci (Table 2).

The present study highlights the importance of maintenance of a good oral hygiene during treatment with FOA include the use of some antibiotics to eradicate the pathogenic microorganisms in addition to proper use of tooth brushes and medical tooth paste to maintain a good general health to all patients with FOA in all periods of wearing this appliance. Further future studies on the Colony Forming Unit and determination the strain of bacterial isolates by PCR is recommended.

**Identification of *S. mutans* by PCR**

*S. mutans* was identified in 60% of patients (Figure 1) which have orthodontic appliance and increased after three month to 80% as compared with non-orthodontic group which appear 66%. This result was agreeing with and very close to the result of (Collee et al., 1; Nikawa et al., ; Ogaard et al.).



Figure 1: Gel electrophoresis of PCR product of specificity Sm479F/R primers by PCR. DNA amplification was observed from the *S. mutans* isolates numbered (1, 2, 3, 6, 7, 10, 11) were positive

In this study, patients with *S. mutans* to identify the proportion of the time this is due to the increased provision of an enabling environment for the accumulation of bacteria in addition to the formation of new surfaces for the consistency of bacteria inside the mouth [60]. Several clinical studies have said that dental caries is largely associated with an increase in bacterial acidity ratios and acidity generator, especially mutant *S.* which is able to mineralize enamel [61, 62, 63].

As the oral organs are in direct contact with enamel and ivory teeth, the mutant *S.* may be on the surface of the tooth an opportunity to join the oral organ. Moreover, most devices are used orally for several months or more, and this can increase the possibility of opportunistic infections.

Above all, it was necessary to check whether mutant cling to the organs by mouth or not in the study. As a result, the oral device can be prone to dental decay from time to time, [46]. Some author suggested that in addition to a quantitative change, metal banding resulted in a qualitative change characterized by an increase in percentage of *S. mutans* during fixed orthodontic treatment [65].

Several factors, such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves and the ability to synthesize extracellular polysaccharides are present in dental caries [61, 67]. So that *S. mutans* was the main cariogenic microorganism and may increase risk of Caries associated with orthodontic treatment.

In this study Amoxicillin shows 70% resistance against *S. aureus* and 80% resistance against, *Lactobacillus spp.*, *Streptococcus*, in another hand *E. coli* and *Klebsilla spp.* shows 80% resistance, while *Enterobacter spp.* 50% resistance and 60% resistance to *pseudomonas aeruginosa*. Amoxi-clav (Amoxicillin/ clavulanic acid) also shows 40% resistance to *S. aureus* and 50% resistance against *S. epidermidis*, *Lactobacillus spp.* and *Streptococcus*, in another hand *E. coli* shows 60% resistance and *Klebsilla spp.* shows 50% resistance, while 20% of *Enterobacter spp.* resistance and 10% of *pseudomonas aeruginosa* resistance. Cefotaxim belong to cephalosporin group which shows 60% of *S. aureus* resistance and 70% of *S. epidermidis* resistance, and 60% to *Lactobacillus spp.* also

70% of *Streptococcus* resist, in another hand *E. coli* shows 10% resistance and *Klebsilla spp.* shows 40% resistance, while 20% of *Enterobacter spp.* Resistance to and 30% of *pseudomonas aeruginosa* resistance. Ciprofloxacin which belong to Fluoroquinolones group and shows 50% of *S. aureus* resistance and 40% of *S. epidermidis* resistance, also 30% of *Lactobacillus spp.* resistance, while 20% of *Streptococcus* resistance, in another hand *E. coli* shows 40% resistance, *Klebsilla spp.* 20%, 10% of *Enterobacter spp.* Resist and 60% of *pseudomonas aeruginosa* resist. Erythromycin belong to the Macrolides group shows 80% resistance to *S. aureus* and 90% resist to *S. epidermidis*, 80% resist to *Lactobacillus spp.*, 80% resistant to *Streptococcus*, while 20% resist to *E. coli* and 30% resist to *Klebsilla spp.*

Also 40% of *Enterobacter spp.* Resist and 60% resist to *Pseudomonas aeruginosa* (shows in chapter four). It has been found that there is clear variation in resistance, and Most of the isolates showed resistance to one or more of these antibiotics. Most of these isolates were found to be highly resistant to the beta-lactam group. This result is almost identical with those obtained by Clarke *et al.*, and Mukhopadhyay *et al.*, who investigated that these bacteria produce beta-lactamases that mediate the resistance of beta-lactam and cephalosporin, and also by limiting the permeability of these intracellular antibodies by changes in outer membrane proteins (porins). This result of high resistance to amoxicillin is nearly compatible with that of Kehinde *et al* (2004).

Who found that *S. aureus* were resistant exclusively to ampicillin and cloxacillin ( $\beta$ -lactam antibiotics), and in agreement with Humphreys *et al.*, (2004) who reported that resistance of *S. epidermidis* to  $\beta$ -lactams is mediated by  $\beta$ -lactamase production under chromosomal control. Al-Saedi, who found that 98.2% of *K. pneumoniae* were resistant to amoxicillin due to the production of  $\beta$ -lactamase.

Macrolides antibiotics such as Erythromycin showed that all bacteria isolates were highly resistant to Erythromycin that agree with Gladstone *et al.*, who found that Erythromycin has only very limited use in the treatment of gram negative infections. Erythromycin had not developed resistance

and was given as and the alternative patients who are allergic to penicillin are widely used for the prevention of adenocarcinitis associated with dental procedures [53]. Its resistance may be because of gaining one of the genes 21ert. This code for the metaphysic methylase performs a methylase of the adenine (nitrogen base) still in RNA 23S, preventing the binding of erythromycin to 50S ribosomes [54]. Also, Fluoroquinolones antibiotics such as ciprofloxacin showed that all bacteria isolates were variable resistant to ciprofloxacin that agree with Ali *et al*(2010).

Who found that *Escherichia coli* (30%), *Staphylococcus aureus* (33%), *Klebsiella pneumonia* (14%) and *Pseudomonas aeruginosa* (14%) resistance. High resistance to antibiotics might be due to the development of resistance of bacteria because of misuse of antibiotic especially in our country where any person can take antibiotic without doctor prescription.

In addition, the lack of control on all animal products and meat, especially poultry, antibiotics are given in a preventive manner and sent to the market and are saturated with antibiotics and other drugs, so they are factories of resistant bacteria and constitute a threat to human life.

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## Conclusion

The study has arrived at the following conclusions:

- Oral cavity colonized by large number of microorganisms that contribute in infection during orthodontic treatment. *Staphylococcus* spp. was the most common bacteria isolated from patients during orthodontic treatment which appear in (81.5%) of the total isolates followed by *Lactobacillus* spp. and then *streptococcus* spp. which appear no statistically significant difference ( $p \geq 0.05$ ) during progress with treatment
- The most common gram negative bacteria in zero day is *E.coli* (20%), *Klibisilla* (18%), *Enterobacter* spp. (20.8) while *pseudomonas aeruginosa* (10.6) and also shown no statistically significant difference.
- All gram positive showed high level of susceptibility to Amoxi-Clav followed by ciprofloxacin and cefotaxime while gram negative sho high sensitive to Erythromycin followed by ciprofloxacin and cefotaxime.
- The molecular detection of *S.mutans* by species specific primer show increase in percentage from 60% in zero days to 80% in third month.



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