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# Biological Changes Associated with Fixed Orthodontic Appliances

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**Abstract**
Many studies have investigated the effects of fixed orthodontic appliances on periodontal health, dental caries, and microbial flora. However, none have examined the impact of different arch wires (Nickel Titanium (NITI) and Stainless Steel (SS)) ligated with elastomeric rings. This study assessed changes in periodontal health, caries development, and bacterial types and counts in 45 patients undergoing orthodontic treatment. The patients were treated with NITI arch wires for three months (Time 1), followed by SS arch wires for three months (Time 2). Clinical measurements (Gingival Index, Plaque Index, pocket depth, and caries development) and microbial changes were recorded at three stages: before bonding (Time 0), Time 1, and Time 2. Treatment with NITI arch wires at Time 1 significantly increased plaque, gingival bleeding, and pocket depth compared to baseline, likely due to NITI's flexibility and surface texture. Switching to SS arch wires at Time 2 resulted in decreased plaque and gingival bleeding due to SS's smoother surface. Despite these changes, caries incidence remained stable. Microbial analysis showed an increase in bacterial colonies from 2,171 at Time 0 to 4,046 at Time 1, dominated by gram-negative bacteria. At Time 2, the colony count decreased to 2,622, but gram-negative bacteria remained prevalent.

**Keywords:** Orthodontic treatment, Plaque accumulation, Gingival bleeding, Pocket depth, Dental caries, Bacterial colonization.

## 1. Introduction:

Numerous studies have mentioned that orthodontic patients who have treatment with permanent appliances frequently have poor oral hygiene because these appliances provide a suitable environment for the development of bacterial colonization that are hard to reach for optimal cleaning this can lead to Periodontium damages (Loe et al., 1965; Zachrisson and Zachrisson, 1972; Lundström et al.,1980; Huser et al., 1990; Forsberg et al., 1991; Boyd and Baumrind, 1992; Türkkahraman et al., 2005 and Alves de Souza et al., 2008). Forsberg et al. (1991) and Türkkahraman et al. (2005) mentioned that the fixed orthodontic appliances increase the colonization of *Streptococcus mutans* and *Lactobacilli*; while Lundström et al. (1980); Sallum et al., (2004) and Ristić et al. (2008) cited that fixed appliances may increase the growth of periodontal pathogenic bacteria such as *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Tannerella forsythia* which cause gingival inflammation in patient during treating with orthodontic. Moreover, several studies concluded that *Porphyromonas gingivalis*, *P. intermedia*, *P. nigrescens*, *Bacteroides forsythus*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Treponema denticola* are the more prevalent periodontal pathogenic bacteria associated with periodontal disease (Alves de Souza et al., 2008). On the other hand not all patients with periodontal disease suffers or have a periodontal pathogen species (Alves de Souza et al., 2008 and de Freitas et al., 2014) therefore several clinically studies have used plaque index, gingival index and pocket probing depth in orthodontic patients as a reference parameters for assessing periodontal health (de Freitas et al., 2014). Previous studies have primarily concentrated on the identification and colonization of particular bacterial species associated with orthodontic procedures, such as *A. actinomycetemcomitans* (Paolantonio et al., 1999 & Ristić et al., 2007), *F. nucleatum*, *P. gingivalis*, *P. intermedia* (Ristić et al., 2007), *S. mutans* and *Lactobacill* (Forsberg et al., 1991). In contrast, the majority of these studies focus on the oral biological changes caused by the types of ligatures used in orthodontic therapy. However, there is a lack of studies investigating the oral biological changes that may result from the different types of arch-wires used in orthodontic therapy.

Therefore the present study aimed to evaluate the changes in some oral biological characteristics, including the gingival index (GI), plaque index (PI), pocket depth (PD), dental caries index (DCI) and bacterial biofilm (both type and quantity) in patients undergoing orthodontic treatment with two types of arch-wires (Nickel-Titanium and Stainless Steel), ligated with elastomeric rings.

## 2. Materials and methods:

A total of Forty five patients (33 females and 12 males), aged between 20 and 35 years, undergoing fixed orthodontic treatment participated in the study. The participants were selected through a simple random sampling method from the Orthodontic Division of three major hospitals in Sana'a City: Al-Thawrah Hospital, Al-Gumhouri Teaching Hospital, and Al-Kuwait Hospital. All participants were in good general health, had permanent dentition, and were free from dental plaque, gingival index and pocket depth. They were also motivated to maintain good oral hygiene. Furthermore, none of the patients had used antibiotics in the three months prior to the baseline examination or during the course of the treatment. At baseline, none of the participants showed clinical signs of gingival inflammation. The study protocol was approved by the Research Ethics Committee of the Faculty of Science, Biological Sciences Department, and Sana’a University.

Fixed orthodontic technique:

One of the most commonly used auxiliaries, elastomeric rings, was employed to ligate two types of straight wire appliances: Nickel-Titanium arch wires (0.014 × 0.025 inch) and stainless steel arch wires (0.018 × 0.022 inch). These arch wires were bonded to the upper teeth, extending from the first molar on one side to the first molar on the opposite side, during both stage Time 1 (T1) and Time 2 (T2) of treatment (modified after Türkkahraman et al., 2005 and Ristić et al., 2007).

**Clinical and Microbiological procedures:**

Oral biological characteristics, including the gingival index (GI), plaque index (PI), pocket depth (PD), and bacterial biofilm (both type and quantity), were measured at Time zero (T0), before bonding with Nickel-Titanium (NITI) arch wires; Time 1 (T1), three months after bonding with NITI arch wires (before replacing them with stainless steel (SS) arch wires); and Time 2 (T2), three months after replacing NITI arch wires with SS arch wires. The total duration of the procedures was six months (modified after Türkkahraman et al., 2005 and Ristić et al., 2007).

**Clinical Parameters:**

Table 1 illustrates the clinical parameters—gingival index, plaque index, pocket depth, and dental caries index—collected according to the methodologies outlined by Wei and Lang et al. (1982), Parviainen et al. (2013), Kursheed et al. (2021), and Rathee and Jain (2024).

**Table1: Clinical Parameters.**

| | Clinical Parameters | | Symptoms |
|---|---|---|---|
| 1 | Gingival Index | 0 | Normal gingiva. |
| | | 1 | Mild inflammation - slight change in color, slight oedema. No bleeding on probing. |
| | | 2 | Moderate inflammation - redness, oedema and glazing. Bleeding on probing. |
| | | 3 | Severe inflammation - marked redness and oedema. Ulceration. Tendency to spontaneous bleeding. |
| 2 | Plaque index | 0 | No plaque in the gingival area. |
| | | 1 | A film of plaque adhering to the free gingival margin and adjacent area of the tooth, the plaque may only be recognized by running a probe across the tooth surface. |
| | | 2 | Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye. |
| | | 3 | Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface. |
| 3 | Pocket depth Index | 0 | Pocket depth is < 3.5 mm, no bleeding upon probing, and no calculus |
| | | 1 | Pocket depth is < 3.5 mm, bleeding on probing and no calculus |
| | | 2 | Pocket depth is < 3.5 mm, bleeding on probing and calculus present |
| | | 3 | Pocket depth ranged between 3.5-5.5 mm in depth |

| | | | |
|---|---|---|---|
| | | 4 | Pocket depth is >5.5mm |
| 4 | Dental caries Index | 0 | No or slight change in enamel translucency after prolonged air drying |
| | | 1 | First visual change in enamel (seen only after prolonged air drying or restricted to within the confines of a pit or fissure. |
| | | 2 | Distinct visual changes in enamel |
| | | 3 | Localized enamel breakdown in opaque or discolored enamel (without visual signs of dentinal involvement). |

**Plaque Samples:**

At Time Zero (T0), plaque samples were individually collected from each patient using sterilized cotton swabs and placed in plain tubes containing 4 ml of sterilized Peptone Water Buffer (PWB) transport medium. For bacterial colony counting, serial 10-fold dilutions of the transport medium containing plaque samples were prepared. Specifically, 0.1 ml of the transport medium with plaque samples was added to 0.9 ml of distilled water and mixed. Subsequently, 0.1 ml of this mixture ($10^{-1}$ dilution) was transferred to a tube containing 0.9 ml of distilled water and mixed ($10^{-2}$ dilution). This process was repeated until the desired dilution ($10^{-4}$) was achieved (Al-Nafaee et al., 2023 and Al-madani et al., 2024). About 0.1 ml from the $10^{-4}$ dilution was inoculated onto separate blood agar plates, which were then incubated for 48 hours at 37°C. After incubation, colony counts were conducted under a stereomicroscope. This procedure was repeated at Time 1 (T1) and Time 2 (T2) after the ligatures, elastomeric rings, and arch wires were carefully removed (Türkkahraman et al., 2005). For morphological diagnosis, a thin smear from each colony was prepared, stained with gram stain, and examined under a microscope. Microbial identification was conducted using the VITEK 2 Compact System at the National Center for Public Health Laboratory (NCPHL) in Sana'a, Yemen (Al-madani et al., 2024).

**Statistical analysis:**

To determine the significant changes in periodontal status in the 3 stages (T0, T1 and T2) the value of gingival index (GI), plaque index (PI), pocket depth (PD), dental caries index (DCI) of the three stages were subjected to one-way Anova test using Graph Pad Prism 6.01 program. If $P < 0.05$, then there is a significant changes in periodontal status among the 3 stages (T0, T1 and T2)

## 3. Results and Dissection:

According to Figure 1, the number of patients with plaque increases from 10 patients in Time 0 to 40 patients in Time 1 (Patients treated for orthodontics using NITI arch wires ligated with elastomeric rings). However, at Time 2 (T2), after the patients were treated with SS arch wires ligated with elastomeric rings, the number of patients with plaque decreased to 35 patients. Furthermore, Table 1 shows a significant increase in the mean plaque amount between T0 (0.2g) and T1 (1.1g), as well as between the mean plaque amount in T0 (0.2g) and T2 (1g). In addition, Table 2, illustrates a significant decrease in plaque index between T1 (1.1g) and T2 (1g). This result may be due to the fact that the orthodontic treatment increases the plaque amount in patients since the flexible nature and surface texture of NITI arch wires may lead to a higher plaque accumulation risk. In contrast, the SS arch wires are more rigid and smoother, likely to contribute to less plaque retention, and this agrees with the findings of Cardoso et al. (2015) and Mahindra et al. (2017), where they concluded that orthodontic arch wires increase the accumulation of dental plaque in orthodontic patients; furthermore, Kim et al. (2014) and Loveland (2017) mentioned that SS reduces adhesion of bacterial plaque when compared with the NITI which increases the adhesion of bacterial plaque and this causes accumulation of dental plaque.

Moreover, Figure 2 shows that the number of patients experiencing gingival bleeding increased from 0 at T0 to 35 at T1 and then decreased slightly to 33 at T2. Moreover, Table 2 demonstrates a significant increase in the mean of gingival bleeding index between T0 (0 ml) and T1 (0.96 ml), as well as between T0 (0 ml) and T2 (0.87 ml).

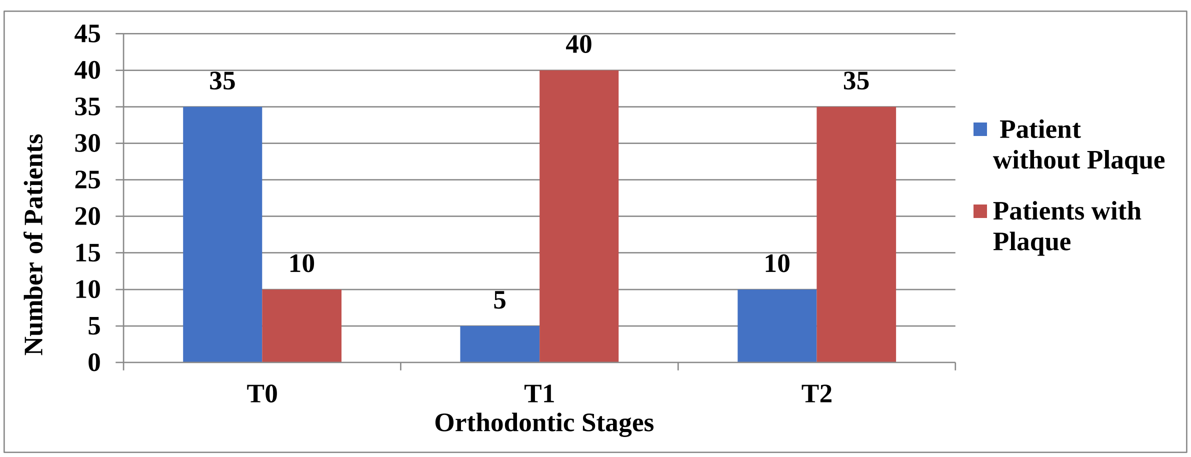


**Fig.1: Patients with Plaque during the Orthodontic Stages**

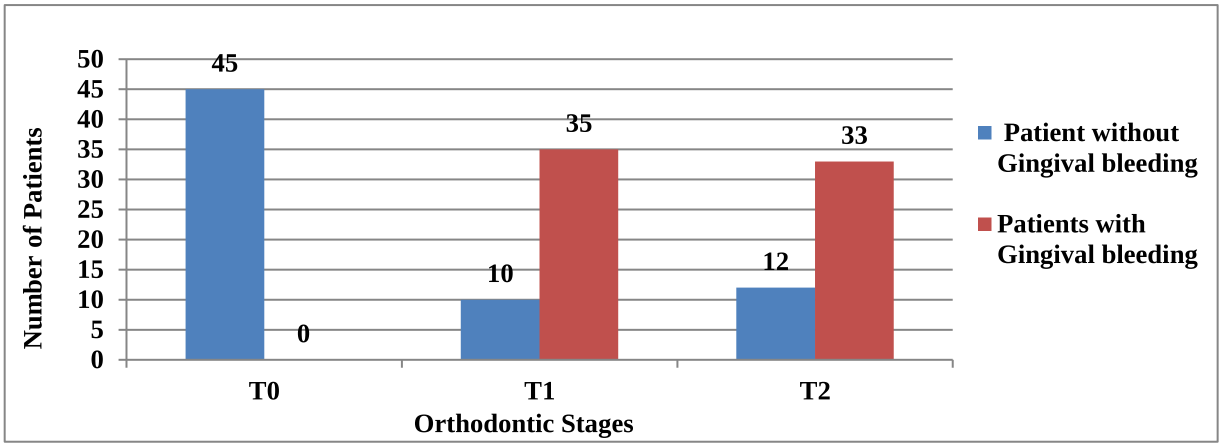


**Fig.2: Patients with Gingival Bleeding during the Orthodontic Stages**

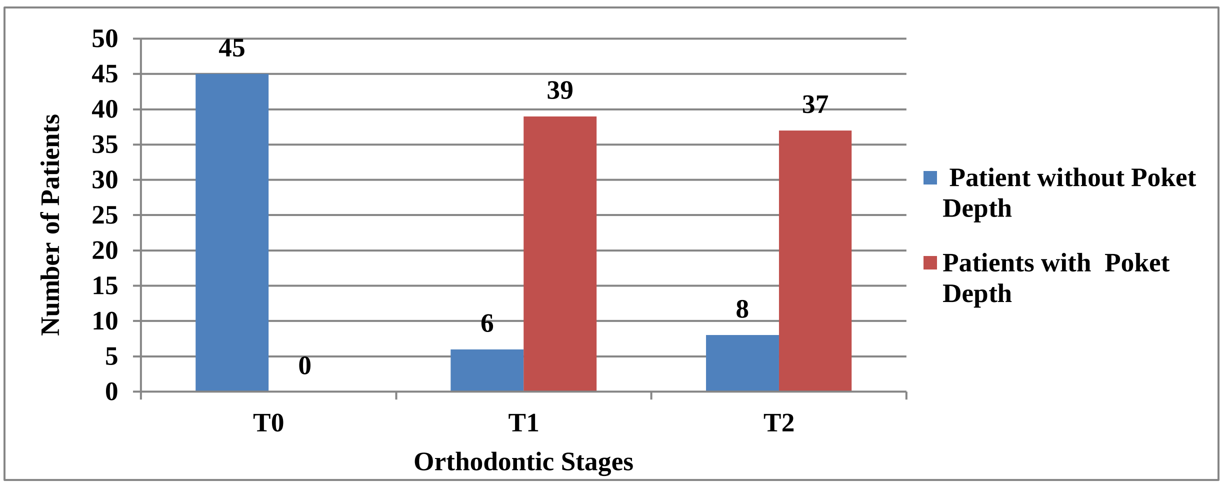


**Fig.**

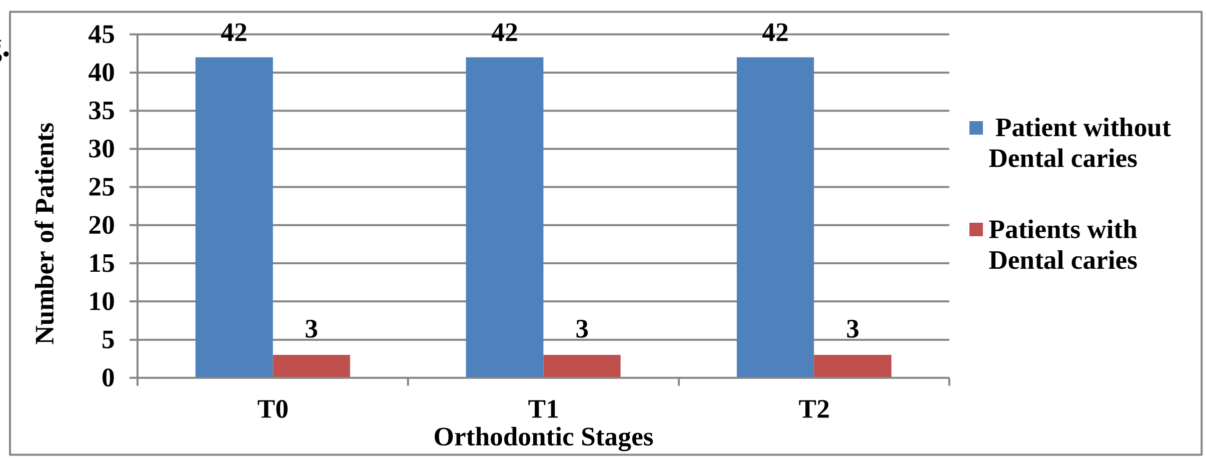


**Fig.4: Patients with Dental Caries during the Orthodontic Stages**

**Table. 2: Biological Parameters During the Orthodontic Stages**

| | Symptoms | T0 | T1 | T2 |
|---|---|---|---|---|
| **1** | **Plaque Index (gm.)** | 0 (0.2±0.4) 1 | 0 (1.1± 0.6) 2 T0 ****, | 0 (1 ± 0.6)2 T0 ****, T1 * |
| **2** | **Gingival Bleeding (ml.)** | 0 | 0 (0.96 ± 0.64) 2 T0 **** | 0 (0.87± 0.63) 2 T0 ****, T1 ns |
| **3** | **Pocket Depth(mm.)** | 0 | 0 (1.3 ± 0.8) 3 T0 **** | 0 (1.24± 0.8) 3 T0 ****, T1ns |
| **4** | **Dental Caries** | 0 (0.65 ± 0.3) 1 | 0 (0.089 ± 0.4) 2 T0 ns | 0 (0.089 ± 0.4) 2 T0 ns, T1 ns |

T0: Patients without arch-wires, T1: Patient with Nickel-titanium arch-wires combined with an elastomeric ring, T2: Patient with Stainless steel arch-wires combined with an elastomeric ring, Significant differences in Symptoms to T0 & T1, * :P< 0.05 : ****: P< 0.0001.

However, no significant difference was found between the mean gingival bleeding index at T1 (0.96 ml) and T2 (0.87 ml). This outcome is consistent with the findings of Mahindra et al. (2017), who noted that orthodontic appliance wearers tend to experience gingival bleeding.

Furthermore, Figure 3 exhibits that the number of orthodontic patients suffering from pocket depth increased from 0 at T0 to 39 at T1 then decreased slightly to 37 at T2. Moreover, Table 2 shows a significant increase in the mean pocket depth index between T0 (0 mm) and T1 (1.3 mm), as well as between T0 (with a mean pocket depth of about 0mm) and T2 (with a mean pocket depth of about 1.24 mm). However, no significant difference was found between the mean pocket depth at T1 (0.96 1.3 mm) and T2 (1.24 mm). This result is compatible with the conclusions of Ristić et al. (2007), Van Gastel et al. (2008), and Liu et al. (2011), where they reported that orthodontic treatment increases the pocket depth. Additionally, Figure 4 and Table 2 illustrate that the number of orthodontic patients experiencing dental caries remains consistent across the three stages (T0, T1, and T2) of orthodontic treating, with only three patients affected in the three stages, and no significant differences were observed in dental caries index between these stages. This stability may be attributed to the effective oral hygiene education provided during orthodontic treatment, which plays a critical role in reducing the risk of caries (Duś-Ilnicka et al., 2024). In support of this, Doğramacı et al. (2019) have reported that orthodontic treatment itself is not considered a long-term risk factor for the development of dental caries.

**Table 3: Type and Colonies of Bacteria in Each Stage**

| Type of Bacteria | Time Zero | | Time One | | Time Two | |
|---|---|---|---|---|---|---|
| | No. of Bacteria | No. of Colonies | No. of Bacteria | No. of Colonies | No. of Bacteria | No. of Colonies |
| **Gram- Positive Cocci** | 7 | 880 | 7 | 1822 | 7 | 572 |
| **Gram-Positive Bacilli** | 1 | 19 | 0 | 0 | 0 | 0 |
| **Gram-Negative Cocci** | 0 | 0 | 0 | 0 | 1 | 6 |
| **Gram-Negative Bacilli** | 7 | 1272 | 8 | 2224 | 10 | 2044 |
| **Total Number of Bacteria** | 15 | 2171 | 15 | 4046 | 18 | 2622 |

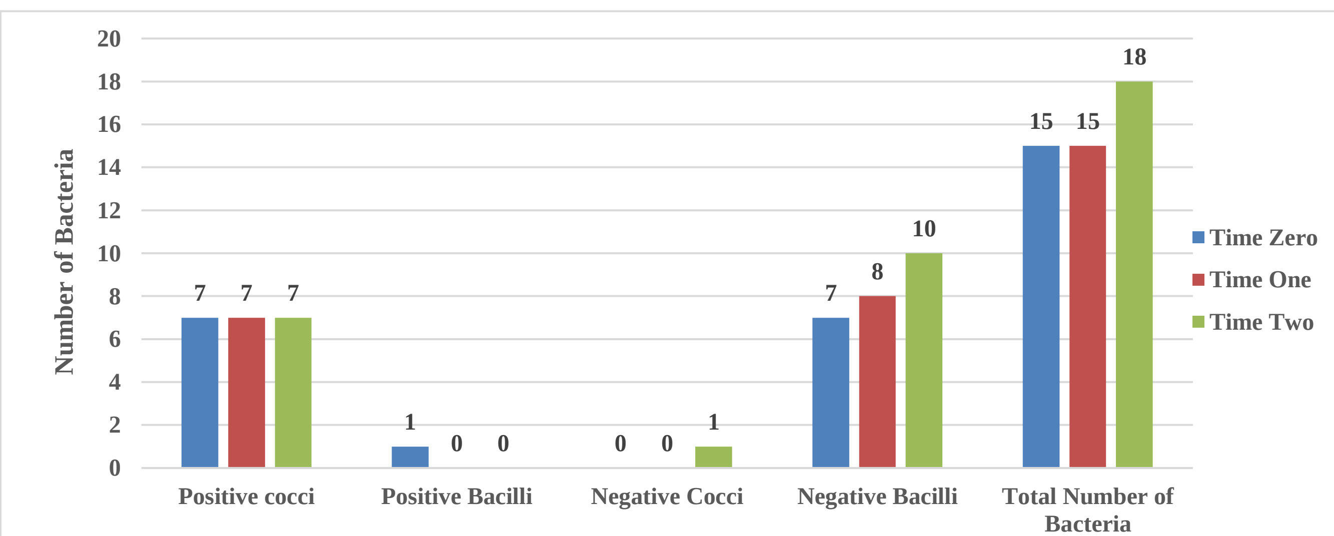


**Fig. 5: Type of Bacteria isolated from each Stage**

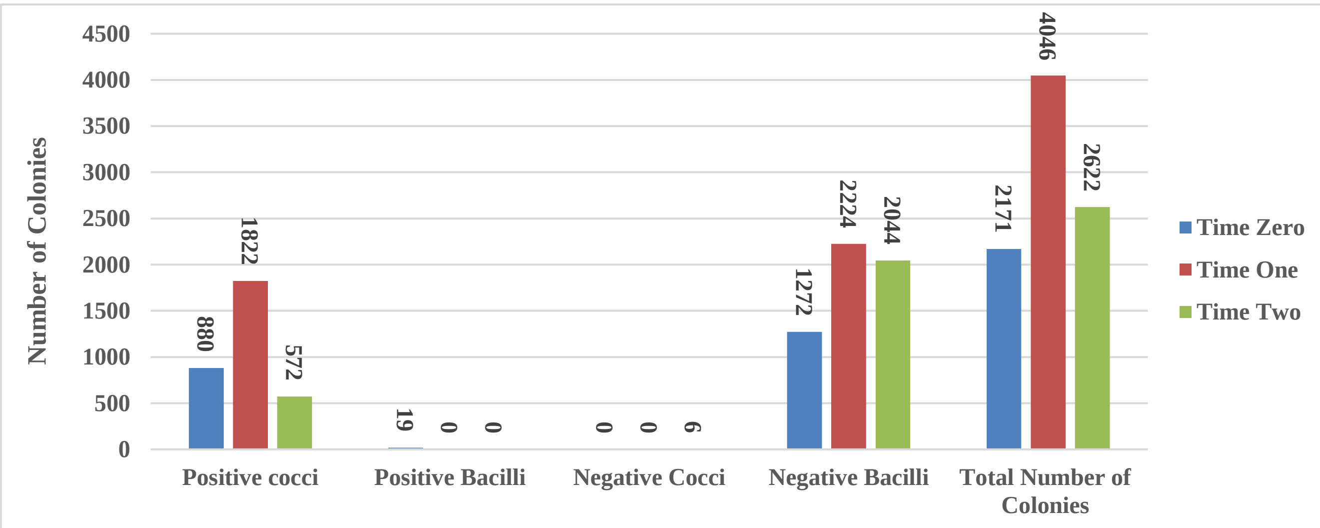


**Fig.6: Number of Bacteria Colonies in the three stages**

**Table 4: Positive and Negative Bacteria isolated from Orthodontic Patients and their Colony Count**

| Characters | | Bacterial species | Time Zero | Time One | Time Two |
|---|---|---|---|---|---|
| Gram - Positive | Cocci | *Aerococcus viridans* | 0 | 73 | 0 |
| | | *Gemella morbillorum* | 81 | 0 | 0 |
| | | *Kocuria kristinae* | 63 | 68 | 0 |
| | | *Kocuria rosea* | 222 | 0 | 0 |
| | | *Staphylococcus aureus* | 0 | 396 | 123 |
| | | *Staphylococcus epidermidis* | 0 | 0 | 80 |
| | | *Staphylococcus lentus* | 0 | 0 | 17 |
| | | *Streptococcus mitis* | 231 | 371 | 134 |
| | | *Streptococcus parasanguinis* | 0 | 443 | 0 |
| | | *Streptococcus pluranimalium* | 140 | 0 | 0 |
| | | *Streptococcus pseudoporcinus* | 68 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| | | *Streptococcus salivarius* | | 236 | 131 |
| | | *Streptococcus sanguinis* | 75 | 235 | 76 |
| | | *Streptococcus thoraltensis* | 0 | 0 | 11 |
| | **Bacilli** | *Rothia dentocariosa* | 19 | 0 | 0 |
| **Gram - Negative** | **Cocci** | *Neisseria animaloris* | 0 | 0 | 6 |
| | **Bacilli** | *Aeromonas hydrophila* | 70 | 0 | 300 |
| | | *Burkholderia cepacia complex* | 0 | 0 | 89 |
| | | *Enterobacter cloacae* | 209 | 239 | 227 |
| | | *Escherichia coli* | 0 | 197 | 189 |
| | | *Klebsiella oxytoca* | 0 | 246 | 154 |
| | | *Klebsiella pneumoniae* | 140 | 300 | 0 |
| | | *Leclercia adecarboxylata* | 0 | 0 | 300 |
| | | *Pantoea* spp. | 0 | 0 | 185 |
| | | *Pseudomonas aeruginosa* | 0 | 300 | 0 |
| | | *Raoultella ornithinolytica* | 155 | 159 | 297 |
| | | *Serratia ficaria* | 0 | 0 | 23 |
| | | *Serratia marcescens* | 173 | 477 | 280 |
| | | *Serratia odorifera* | 382 | 0 | 0 |
| | | *Sphingomonas paucimobilis* | 143 | 306 | 0 |

Based on Table 3and Figures 5 & 6, about 15 pathogenic bacteria were isolated and identified from 45 orthodontic patients in time 0, producing 2171 colonies among them, 8 gram-positive pathogenic bacteria (7 cocci- shaped bacteria and one bacilli-shaped bacteria) that generated 899 colonies and 7 gram-negative pathogenic bacteria (bacilli- shaped bacteria) which created about 1272 colony. Moreover the 8 gram-positive pathogenic bacteria includes 7 cocci- shaped bacteria which creates about 880 colonies (*Staphylococcus mitis*, *Kocuria rose*, *Streptococcus*

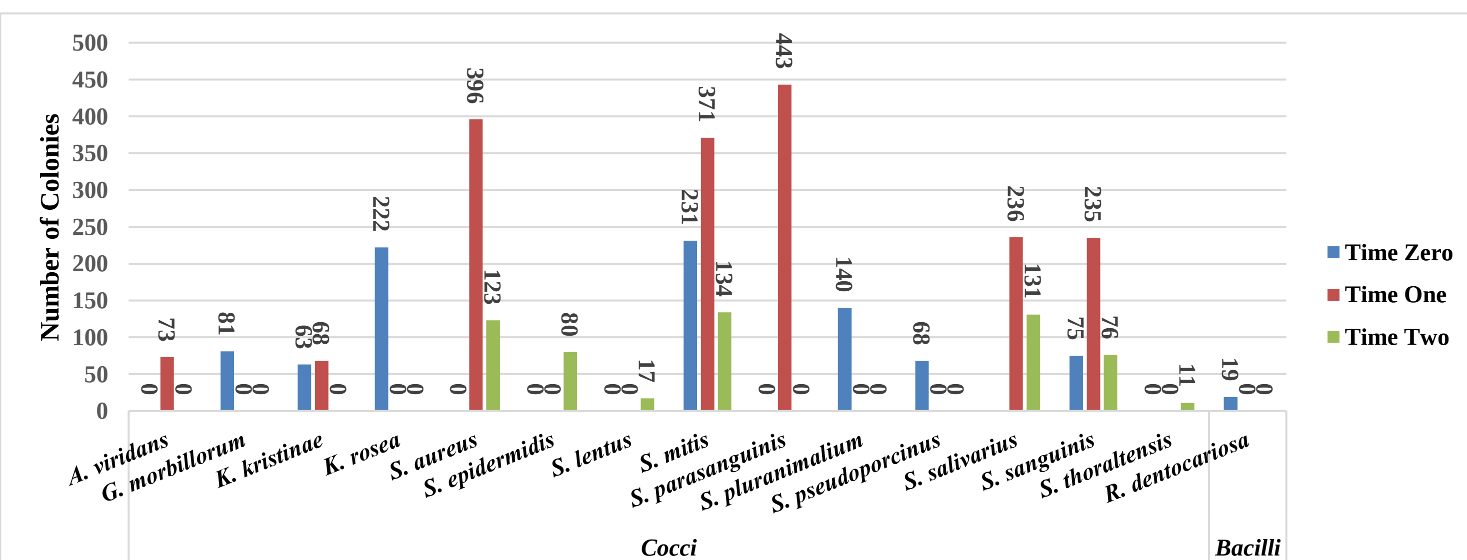


**Fig.7: Type of Gram-Positive bacteria isolated from Orthodontic Patients and their Colony Count**

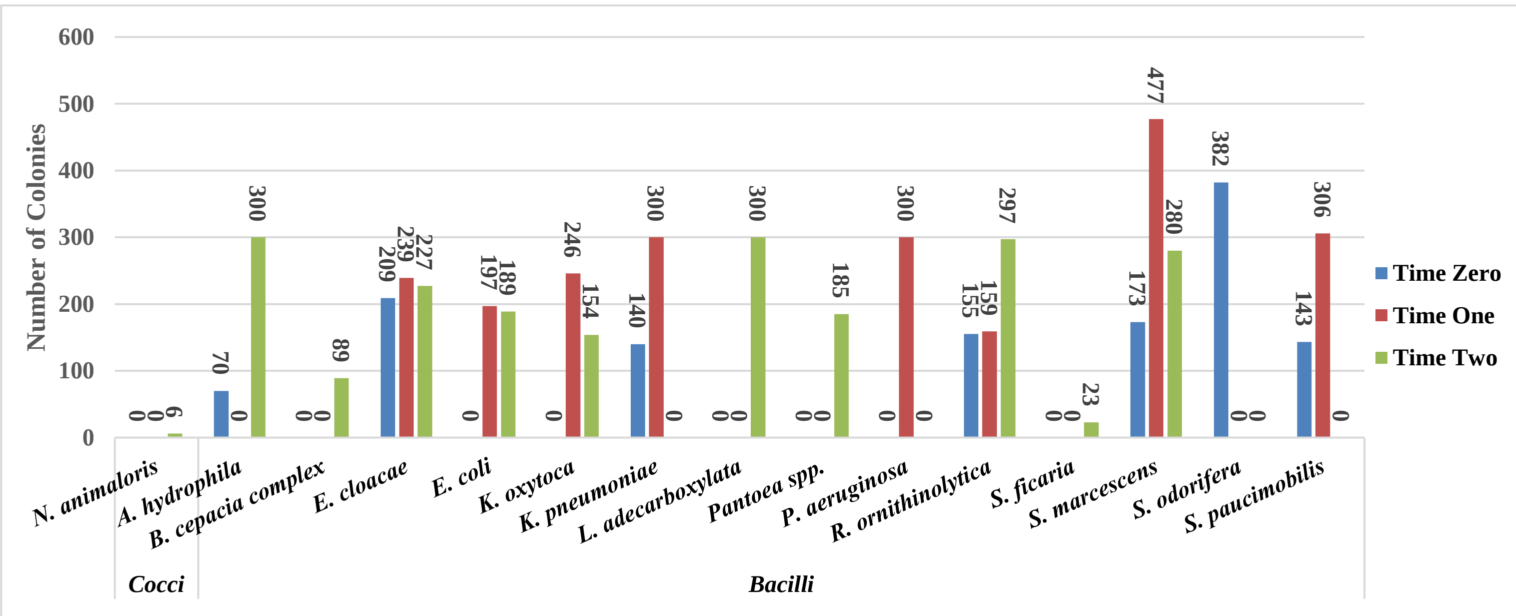


**Fig.8: Type of Gram-Negative bacteria isolated from Orthodontic Patients and their Colony Count**

*pluranimalium*, *Gemella morbillorum*, *Streptococcus sanguinis*, *Streptococcus pseudoporcinus* and *Kocuria kristinae*, with respective colony counts of about 231, 222, 140, 81, 75, 68 and 63) and one bacilli-shaped bacteria (*Rothia dentocariosa*) which produced 19 colonies (Table 3, 4 and Fig. 5, 6, 7 & 8). In contrast, the gram-negative pathogenic bacteria comprises 7 bacilli-shaped bacteria which generates 1272 colony (Table 3 and Fig. 5 & 6) including *Serratia odorifera* with 382 colonies followed by *Enterobacter cloacae* (209 colonies), *Serratia marcescens* (173 colonies), *Raoultella ornithinolytica* (155 colonies), *Sphingomonas paucimobilis* (143 colonies), *Klebsiella pneumoniae* (140 colonies) while *Aeromonas hydrophila* produces the lowest colony number of about 70 colonies (Table 4 and Fig. 7 &8). In contrast, about 15 pathogenic bacteria were isolated and identified from 45 orthodontic patients treated with NITI archwires ligated with elastomeric rings in Time 1, generating a total of 4046 colonies (Table 3and Figures 5 & 6), among them 8 gram-negative bacilli-shaped bacteria, creating about 2224 colonies (*S. marcescens* with 477 colonies, trailed by *S. paucimobilis* with 306 colonies, *K. pneumoniae* with 300 colonies, *Pseudomonas aeruginosa* with 300 colonies, *Klebsiella oxytoca* with 246 colonies, *E. cloacae* with 239 colonies, *Escherichia coli* with197 colonies while, *R. ornithinolytica* produced the lowest number of colonies 159 colonies) and 7 gram-positive cocci-shaped bacteria with about 1822 colony including *Streptococcus parasanguinis*, *Staphylococcus aureus*, *S. mitis*, *Streptococcus salivarius*, *S. sanguinis*, *Aerococcus viridans* and *K. kristinae* that creates 443, 396, 371, 236, 235, 73 and 68 colonies correspondingly (Table 4 and Figures 7 & 8). On the other hand Table, Figures 5 & 6, exhibited that about 18 pathogenic bacteria were isolated and identified from 45 orthodontic patients treated with SS archwires ligated with elastomeric rings in Time 2, creating a total of 2622 colonies among them 11 gram-negative bacteria (one cocci-shaped bacteria and 10 bacilli- shaped bacteria) that creates 2050 colony and 7 gram-positive bacteria (comprises of 7cocci-shaped bacteria) that generates 572 colony. Moreover the 11 gram-negative bacteria includes one cocci-shaped bacteria (*Neisseria animaloris*) that creates 6 colonies (Table 3, 4, fig 5, 6& 8) and 10 bacilli- shaped bacteria that generates 2044 colonies among them; *A. hydrophila* (300 colonies ) *Leclercia adecarboxylata* (300 colonies), *R. ornithinolytica* (297 colonies), S. *marcescens* (280 colonies), *E. cloacae* (227 colonies), *E. coli* (189 colonies), *Pantoea* sp. (185 colonies), *K. oxytoca* (154 colonies), *Burkholderia cepacia* complex (89 colonies), *Serratia ficaria* (23 colonies) (Table 3, 4, fig 5 , 6 & 8). However, the 7 gram-positive bacteria comprises of cocci-shaped bacteria (*S. mitis* that creaets 134 followed by *S. salivarius*, *S. aureus*, *Staphylococcus epidermidis*, *S. sanguinis*, *Staphylococcus lentus* and *Streptococcus thoraltensis* that creates 131 colonies, 123, 80, 76, 17 and 11colonies accordingly) that generated 572 colonies (Table 3, 4, fig 5, 6 & 7)

According to the results presented in Table, 3 & Fig.6, there was a significant increase in the number of bacterial colonies between Time 0 and both Time 1 and Time 2. At Time 0, the total number of colonies was 2171 which increased to 4,046 at Time 1, when patients were treated with NITI arch wires ligated with elastomeric rings. However, the number of colonies decreased from 4046 (in Time 1) to 2050 at Time 2, when patients were treated with SS arch wires ligated with elastomeric rings. These findings align with the with the results of Türkkahraman et al. (2005) and Mavani et al. (2016), were they reported that fixed orthodontic appliances lead to increased plaque accumulation and bacterial colonization in orthodontic patients when compared to those without fixed appliances. Moreover, Kim et al. (2014) and Mulimani and Popowics (2022) indicated that NITI arch wires have the highest free surface energy, resulting in the greatest bacterial adhesion, whereas stainless steel arch wires exhibit comparatively lower bacterial adhesion and, consequently, a lower number of colonies. Furthermore, previous findings presented in Table 3 & Figure 6 show that the number of gram-negative bacterial colonies was significantly higher than that of gram-positive bacterial colonies, particularly in patients undergoing orthodontic treatment. This observation is consistent with the work of Velliyagounde et al. (2022), were they reported that patients wearing orthodontic appliances had a higher prevalence of pathogenic gram-negative microorganisms in their oral cavities.

Additionally several previous studies support the results in Tables 4 and Figures 7 &8, which indicate that *A. hydrophila* (Hoceini et al., 2016 and Al-madani et al., 2024), *E. cloacae* (Zaatout, 2021 and Al-madani et al., 2024), *G. morbillorum* (Vasishtha et al., 1996 and Al-madani et al., 2024), *K. kristinae* (Ananieva et al., 2018 and Al-madani et al., 2024), *K. pneumoniae* (Zaatout, 2021 and Al-madani et al., 2024), *K. rose* (Lu et al., 2015 and Al-madani et al., 2024), *R. dentocariosa*, *R. ornithinolytica* (Al-madani et al., 2024), *S. mitis* (Zaatout, 2021 and Al-madani et al., 2024), *S. odorifera* (Bogacz et al., 2019 and Al-madani et al., 2024), *S. pluranimalium* , *S. pseudoporcinus* (Al-madani et al., 2024) were isolated from the dental plaque of patients receiving dental care. However, some earlier studies recorded that *E. cloacae* , *E. coli*, *K. oxytoca*, *P. aeruginosa* (Pellissari et al., 2021), *N. animaloris* (Kouvelis et al., 2021), *R. ornithinolytica* (Derafshi et al., 2017), *S. aureus* (Pellissari et al., 2021), *S. marcescens* (Garg et al., 2015), *S. mitis*, *S. parasanguinis* (Pellissari et al., 2021), *S. salivarius* and *S. sanguinis* (Kouvelis et al., 2021), were isolated from different parts of orthodontic patients oral cavity including the area around the brackets were the biofilm is presented. Moreover, based on Table 4 and Figures 7 & 8 *A. hydrophila*, *A. viridans*, *B. cepacia* complex, *K. kristinae*, *K. pneumoniae*, *Pantoea* sp., *S. epidermidis*, *S. ficaria*, *S. lentus* and *S. thoraltensis* were isolated from orthodontic patients while previous studies mentioned that they were isolated from patients receiving dental care (Dhotre et al., 2016; Hoceini et al. 2016; Zawadzki et al., 2017; Ananieva et al., 2018; Bogacz et al., 2019; Zaatout, 2021; Al-madani et al. 2024 & Al-Taii et al., 2024) this agrees with conclusion of Liu et al. (2023) were they mentioned that Patients wearing orthodontic appliances had microorganisms commonly found in the oral cavity of orthodontic patients in Time 0 (patients not wearing orthodontic appliances) on the other hand table 4 & Figure 8 Illustrate the isolation of *L. adecarboxylata* from the oral cavity of orthodontic patients in T2 this may be linked to that *L. adecarboxylata* was transport, to the patients oral cavity by drinking the poor quality drinking water (Keren et al., 2014) which is due to the ongoing conflict in Yemen which compromised water treatment and sanitation infrastructure, potentially leading to the contamination of drinking water sources by *L. adecarboxylata*

## 4. Conclusion:

Orthodontic treatment using NITI arch wires (at Time 1) led to a significant increase in plaque accumulation, gingival bleeding, and pocket depth compared to the baseline measurements (at Time 0). This increase can be attributed to the flexibility and surface texture of NITI arch wires, which promote greater plaque retention. However, when switching to stainless steel (SS) arch wires at Time 2, there was a slight decrease in plaque accumulation and gingival bleeding, likely due to the smoother, more rigid surface of the SS arch wires. Despite these changes, the incidence of dental caries remained stable across all orthodontic three stages. Additionally, the results indicate a significant change in bacterial colonization in orthodontic patients throughout the different stages of treatment. At Time 0, 15 pathogenic bacteria were identified, producing a total of 2,171 colonies. During Time 1 (when NITI arch wires were used), the number of bacterial colonies increased to 4,046, with a predominance of gram-negative bacteria. However by Time 2, after switching to SS arch wires, the number of colonies decreased to 2,622, though Gram-negative bacteria still prevailed. Notably, the prevalence of gram-negative bacteria was significantly higher, with *S. marcescens* showing the highest number of colonies (477) at Time 1. However, *A. hydrophila* and *L. adecarboxylata* exhibited highest colony counts of about 300 (individually) at Time 2. The isolation of *L. adecarboxylata* from orthodontic patients at Time 2 may be linked to external factors, such as contaminated drinking water, especially in areas with poor sanitation.

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