



## Evaluation of Microbiological Colonisation on Various Types of Orthodontic Archwires - an in Vivo Study

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### KEYWORDS

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### ABSTRACT:

**Introduction:** Orthodontic archwires create an environment that promotes the colonization of oral microorganisms, potentially leading to dental issues like caries and periodontal diseases. **Objectives:** This study aims to evaluate the microbial adherence on different types of orthodontic archwires by comparing the Colony Forming Units (CFUs) of *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans*.

**Methods:** The study involved 120 orthodontic patients, divided into four groups based on the type of archwire used, all with the same cross-sectional size of 0.017 × 0.025 inches. The groups were:

- **Group I:** Aesthetic Coated Stainless Steel
- **Group II:** Stainless Steel (SS)
- **Group III:** Heat Activated Nickel Titanium (HANT)
- **Group IV:** Nickel Titanium (NiTi)

After a period of one month in the oral cavity, the archwires were retrieved and subjected to quantitative microbiological analysis.

- **Results:** For *S. mutans*, significant differences were observed between aesthetic coated SS and HANT (P=0.002), and NiTi (P=0.001).

- For *S. aureus*, a significant difference was noted between NiTi and aesthetic coated SS (P=0.01).

- For *C. albicans*, significant differences were found between aesthetic coated SS and HANT (P=0.0001), and NiTi (P=0.01).

**Conclusions:**All types of archwires showed significant microbial colonization, with *Streptococcus mutans* demonstrating the highest affinity for NiTi wires, followed by HANT, SS, and aesthetic coated SS. *Staphylococcus aureus* and *Candida albicans* exhibited varied colonization patterns. Overall, aesthetic coated SS archwires showed the least microbial colonization.



## 1. Introduction

During fixed orthodontic treatment, archwires play a crucial role in facilitating tooth movement, improving occlusion, and enhancing aesthetics. However, the placement of bands, brackets, and archwires creates new retentive surfaces that promote the accumulation of microbial dental biofilms. These biofilms can lead to iatrogenic decalcification of enamel, resulting in the formation of white spot lesions, which are early indicators of dental caries. These biofilms consist of dynamic and structured microbial communities embedded in a self-sustaining extracellular matrix. *Streptococcus mutans* is the primary bacterium responsible for dental caries, producing glucosyltransferases and other proteins that help form dental plaque and the biofilm matrix, thereby contributing to caries development. Additionally, *Candida albicans* and *Streptococcus mutans* form a symbiotic relationship within biofilms, enhancing their virulence. Many pathogenic bacteria exploit biofilm formation to mediate localized infections. The increasing prevalence of these bacteria in biofilms is a major factor in enamel demineralization and the deterioration of periodontal health.

Orthodontic archwires have evolved from traditional stainless steel to nickel-titanium alloys and, more recently, to aesthetic-coated archwires, driven by a growing demand for more aesthetically pleasing appliances. Archwires, with varying properties such as surface roughness, surface free energy, and surface topography, provide an environment conducive to microbial adhesion.

## 2. Objectives

To study and compare the microbiological colonization of *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* on four types of archwires with similar cross-sections: Aesthetic Coated Stainless Steel, Stainless Steel (SS), Heat-Activated Nickel-Titanium (HANT), and Nickel-Titanium (NiTi) in an in vivo setting.

## 3. Methods

The present study was carried out at the Department of Orthodontics and Dentofacial Orthopaedics, Government Dental College & Hospital, Ahmedabad. It was approved by the Institutional Ethical Committee (IEC). For this study 120 subjects undergoing fixed

orthodontic therapy at the Department of Orthodontics are divided into 4 equal groups based on the type of archwire of same cross section.

Group I - 30 subjects (0.017" \*0.025" Aesthetic Coated SS)

Group II - 30 subjects (0.017" \*0.025" Stainless SS)

Group III - 30 subjects (0.017" \*0.025" HANT)

Group IV - 30 subjects (0.017" \*0.025" NiTi)

After one month of the wires being in the oral cavity, they are retrieved, cut and subject to microbial analysis by colony counting of three microorganisms namely *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*.

Inclusion Criteria was patients undergoing fixed orthodontic therapy and with good oral hygiene. Exclusion Criteria was patients with any systemic diseases and patients undergoing any antibiotic therapy

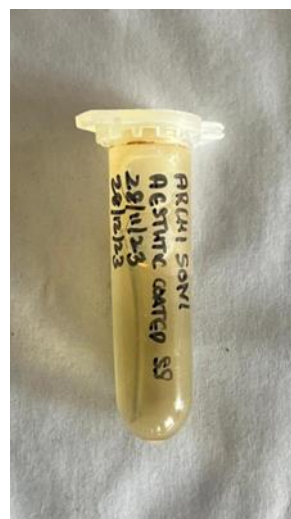


FIGURE 1: Wire in BHI Broth

### Methodology:

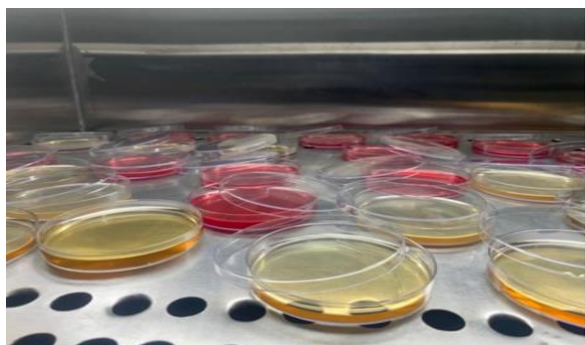
Cross section of 0.017" \*0.025" wires were inserted in patients undergoing Preadjusted Edgewise 0.022\*0.028" MBT fixed mechanotherapy and ligated using stainless steel ligatures. After the archwires were inserted, the participants individually received basic

instructions on oral hygiene and care regarding the orthodontic appliance. Patients were recalled after 4 weeks and wires were carefully retrieved so as to not dislodge the adherent biofilm. Posterior segments of



20mm were measured with divider and cut with a distal end cutter. Right posterior segment was subjected to biofilm measurement whereas the left posterior segment was subjected to quantitative microbial measurement.

#### Quantitative Microbiological Assessment:



**FIGURE 2: Pouring of culture media into Petri Dishes**

1. The left segment is dipped in an Eppendorf tube containing 2ml of Brain Heart Infusion (BHI) broth which acts as a microbial environment and transport medium. Patient details, date of insertion, date or retrieval and type of archwire is written on the respective tubes with a marker pen and cello taped in order to prevent it from wearing off.

2. The tubes are incubated for 36 hours in an incubator at 37-degree Celsius.

3. After incubation, the broth is then vortexed for even distribution of growth and is then subject to microbiological analysis.

4. Selective Culture media - Mitis Salivarius agar, Mannitol salt agar and Potato dextrose agar are prepared for the isolation of *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*.

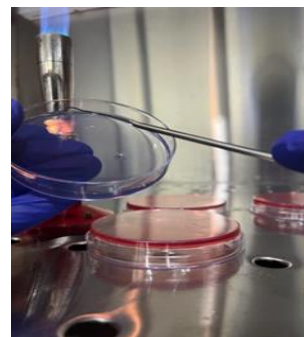
8. Pouring of agar into the petri dishes is done in the biosafety cabinet.

9. The prepared media are first diluted 3 times in BHI solution (6ml of BHI solution in 2 ml of sample), then cultured on the agar plates (mitis salivarius agar, mannitol salt agar, potato dextrose agar) and incubated at 37 degrees Celsius for 48 hours in an incubator.

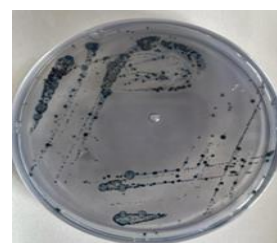
10. After the incubation period, all plates are evaluated to count Colony Forming Unit. For counting colonies, a

manual method is used, i.e., with the help of a magnifying glass, all colonies are counted and recorded.

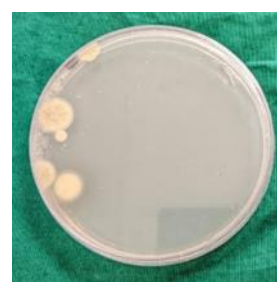
11. Data thus obtained is subjected to statistical analyses.



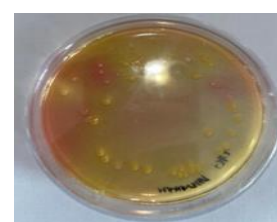
**FIGURE 3: Streaking of sample on the culture media**



**FIGURE 4: Staphylococcus Aureus on Mannitol Salt Agar**



**FIGURE 5: Streptococcus mutans on Mitis Salivarius Agar**



**FIGURE 6: Candida Albicans on Potato Dextrose Agar**



## Results

Table I, Graph I: Mean CFU for *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*, along with biofilm absorbance and concentration for aesthetic coated SS, stainless steel, HANT, and NiTi archwires (0.017\*0.025”):

- *S. mutans*: Aesthetic Coated SS: 153.36 ( $\pm 39.44$ ), SS: 176.33 ( $\pm 42.79$ ), HANT: 190.86 ( $\pm 33.62$ ), NiTi: 196.2 ( $\pm 38.53$ ).

- *S. aureus*: Aesthetic Coated SS: 12.26 ( $\pm 6.65$ ), SS: 13.5 ( $\pm 5.73$ ), HANT: 14.7 ( $\pm 6.94$ ), NiTi: 17.73 ( $\pm 6.70$ ).

- *C. albicans*: Aesthetic Coated SS: 1.22 ( $\pm 0.86$ ), SS: 2.93 ( $\pm 2.53$ ), HANT: 4.62 ( $\pm 3.51$ ), NiTi: 3.5 ( $\pm 2.86$ ).

Tables II, III, IV: Show significant differences among archwires for *S. mutans*, *S. aureus*, and *C. albicans* ( $P \leq 0.05$ ).

- For *S. mutans*: significant differences between aesthetic coated SS and HANT ( $P=0.002$ ) and NiTi ( $P=0.001$ ).

- For *S. aureus*: significant difference between NiTi and aesthetic coated SS ( $P=0.01$ ).

- For *C. albicans*: significant differences between aesthetic coated SS and HANT ( $P=0.0001$ ) and NiTi ( $P=0.01$ ).

## 4. Discussion

In the oral cavity, the orthodontic appliance creates surfaces and retentive sites with properties such as surface roughness and surface free energy, different to those of the natural oral hard and soft tissue structures. Oral biofilms formed on these surfaces cause demineralization of enamel, leading to white spot lesions which are the precursors of dental caries, gingivitis leading to periodontitis and even bacteremia.<sup>40</sup> Bjorn et al showed that white spot lesions are present even 5 years after fixed orthodontic treatment.<sup>41</sup> Today, on the basis of Variable modulus orthodontics that is, use of different materials with different moduli of elasticity while maintaining the same or similar cross section of archwires, is employed in orthodontic treatment.<sup>10</sup> Based upon this, orthodontic archwires of different materials are used which have different surface characteristics such as surface roughness and surface free energy which influences and increases the amount of plaque formation leading to biofilm adhesion and microbial colonisation

over them.<sup>7, 12</sup> The microorganisms in this biofilm are responsible for biocorrosion (microbiologically induced corrosion) of orthodontic appliances as they dissipate Fe, Ni, and Cr into the oral cavity, which can be a cause of metallic allergy.<sup>42</sup> Furthermore, biocorrosion from orthodontic archwires also leads to a vicious cycle in which there is increase in biofilm adhesion which in turn causes more corrosion, keeping this cycle intact.<sup>43</sup> The biofilm matrix consists of a multitude of opportunistic and pathogenic organisms, among them *Streptococcus mutans* plays a well-established role in initiating dental caries and enamel decalcification, commonly found in regions affected by white-spot lesions related to orthodontic appliances. This bacterium can generate exopolysaccharides (EPS) which is an essential part of biofilm. This EPS aids in the further increase of colonization of *S. mutans* and other cariogenic organisms on enamel and other surfaces, increasing the risk of dental decay.<sup>44</sup> *Staphylococcus aureus* is a major causative agent of many diseases and is associated with infections of the dentoalveolar complex, oral mucosal lesions and is also found to be the commonest pathogen in acute gingivitis in orthodontic patients.<sup>23</sup> Hibino et al showed that the most common candidal species in orthodontic patients was *Candida albicans* and also found that orthodontic patients can become candidal carriers.<sup>45</sup> Chandresh et al also observed that *Candida albicans* and *Streptococcus mutans* increase in patients undergoing orthodontic treatment.<sup>25</sup> Hence, knowledge regarding the growth of cariogenic bacteria in subjects with orthodontic appliances and biofilm adherence to orthodontic archwires can offer a better insight in prevention of white spot lesions and periodontal complications. As archwires of different materials are used according to treatment needs, information regarding affinity to microbiological colonisation will provide the clinician with the better choice in the microbiologic perspective as well, especially in patients with higher risk of dental caries and periodontal diseases. Therefore, this study was conducted to evaluate the amount of biofilm adhesion and microbial count of *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans* to various orthodontic archwires. For this study, 120 patients undergoing fixed orthodontic appliance therapy were selected and divided into 4 groups. 4 different orthodontic archwires Aesthetic coated Stainless Steel, Stainless Steel, Heat-Activated Nickel Titanium (HANT)



and Nickel Titanium(NiTi) of 0.017\*0.025 inch cross-section were placed in each group respectively. The wires are retrieved after 1 month, 2cm of the distal end was cut and subjected to microbiological analysis by evaluating the microbial count of *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*.

Overall, on all archwires, the microorganism showing maximum adhesion is *Streptococcus mutans* followed by *Staphylococcus aureus* and the least adhesion is by *Candida albicans*. These findings are concurrent with the study by **Pritish Polke et al.**,<sup>32</sup> in which *Streptococcus mutans* has the highest affinity for adhesion to orthodontic archwires and *Candida albicans* has the least affinity.

Aesthetic Coated SS showed the lowest adhesion of *Streptococcus mutans* followed by Stainless Steel and HANT, whereas the highest adhesion was found on NiTi archwires. These findings are concurrent with the findings of the study by **In-Hye Kim et al.**,<sup>18</sup> which showed that NiTi(7.70±3.38) archwires had significantly higher *Streptococcus mutans* colony count (expressed as log<sub>10</sub> CFU) than the Stainless Steel archwires (4.70±1.34) and the Aesthetic Coated archwires had the lowest SM adhesion. ANOVA test was carried out to evaluate whether the difference was significant. A statistically highly significant difference was observed in the mean value of CFUs of *Streptococcus mutans* on all the archwires with a (P=0.002). Since the results showed Statistically significant difference between all the archwires, Post Hoc Tukey's test was carried out in conjunction with ANOVA to find out which of the readings are significantly different from one another. The Post hoc Tukey's test revealed that a statistically very significant difference was found between Aesthetic Coated SS and HANT archwires (P=0.002) and a statistically highly significant difference between Aesthetic Coated SS and NiTi (P=0.001). In the study by **In-Hye Kim et al.**,<sup>18</sup> the Aesthetic wires showed a significantly lower SM adhesion than NiTi archwires.(P<0.05). Similar findings were observed in studies done by **Aliaa Abdul Rhman Al-Lami et al.**,<sup>20</sup> **Reem A Rafeeq et al.**<sup>26</sup> and **Renea Radovic et al.**<sup>37</sup> which showed NiTi archwires to have highest adhesion of *Streptococcus mutans* in comparison to the Aesthetic coated and SS archwires. Increased adhesion to NiTi archwires can be attributed to its high surface roughness which is explained by **Atia Yousif et al.**<sup>22</sup> and **Vincenzo**

**D'Anto et al.**,<sup>46</sup> making NiTi responsible for the increased count of *Streptococcus mutans* in comparison to SS which has a smoother surface. The reduced adhesion of *Streptococcus mutans* to the Aesthetic coated SS can be attributed to the coatings present on it. Reduction in surface roughness of coated archwires reduces plaque accumulation onto them. This was observed in the study done by **Seyed Hamid Raji et al.**<sup>19</sup> who compared coated and uncoated archwires. Conflicting results by **Sevil Samadzadeh et al.**<sup>35</sup> observed more *Streptococcus mutans* adhesion on stainless steel archwires(0.016\*0.022") as compared to NiTi archwires(0.016\*0.022") in an invitro study. The increased release of metal ions from stainless steel increased the surface energy of the archwires, may lead to greater colonisation. In the study by **Kleist Christian Costa Lima et al.**,<sup>27</sup> the archwires, fully(rodium) coated NiTi, partially(teflon) coated NiTi, uncoated NiTi and SS with cross section of 0.016\*0.022", were compared in vivo for *Streptococcus mutans* adhesion. It was found that coated NiTi had the highest adhesion of *Streptococcus mutans* (11.80±0.82) (expressed in logarithmic scale) followed by uncoated NiTi (9.28±2.13), uncoated SS (8.15±1.37) and least was on partially coated-NiTi (7.01±0.79). The finding that uncoated NiTi has higher *Streptococcus mutans* adhesion in comparison to uncoated SS is similar to the present study. Whereas, the finding that the aesthetic fully coated archwire accumulating most microorganisms is contrary to the present study. This may be due to different materials investigated(rhodium, teflon, stainless steel, and nickel titanium), as the initial adhesion of bacteria to surfaces also depends on the composition of the solid substratum.

The colony count for *Staphylococcus aureus* is lowest for Aesthetic Coated SS, followed by Stainless Steel and HANT and is highest for Nickel Titanium. ANOVA test showed a statistically very significant difference of colony counts of *staphylococcus aureus* between all the four archwires.(P=0.014). Tukey's post hoc analysis showed a statistically highly significant difference only between NiTi and Aesthetic coated SS(P=0.01). In the study by **Pritish Polke et al.**,<sup>32</sup> it was found that there was no statistically significant difference between Aesthetic coated SS and Stainless Steel. These findings are in accordance with the present study. Increased adhesion of *Staphylococcus aureus* to NiTi archwires can be due to corrosion induced nickel release from



orthodontic appliances which could modify the behaviour of bacteria in the oral cavity due to which there is increased adaptation of *Staphylococcus aureus* to NiTi orthodontic archwires. This was observed by **Andrej Pavlic et al**<sup>31</sup> Similar results were seen in an in vitro experiment by **Manar Hussain Abbas et al.**,<sup>23</sup> in which there was a similar change in adhesion of *S. Aureus* on NiTi and SS archwires after incubation of 24-96 hours.

Lowest Candidal growth was seen on Aesthetic Coated SS followed by SS, NiTi and HANT. Similar findings were found in study by **Saloom HF et al**<sup>16</sup> who found that appliances with more aesthetic appearances such as coated archwires and brackets showed less candidal growth in comparison to appliances with more metallic components. ANOVA analysis found that the readings of all the archwires were statistically highly significant from one another ( $P=0.0002$ ). Post Tukey analysis showed that there was a statistically highly significant difference between aesthetic coated SS and HANT ( $P=0.0001$ ) and a statistically highly significant difference between aesthetic coated SS and NiTi group with a ( $P=0.01$ ). No significant difference was found between HANT and NiTi archwires as well as Aesthetic coated SS and SS archwires. **Harikrishnan et al.**<sup>17</sup> observed no significant difference between teflon coated ligatures and stainless steel ligatures for the adhesion of *Candida albicans*. In the study conducted by **Prithish Polke et al.**,<sup>32</sup> significant difference was found between SS and Aesthetic Coated SS ( $P=0.01$ ) which wasn't found in the present study. This may be attributed to the loss of coatings of Aesthetic coated SS during orthodontic treatment due to low pH, mechanical stress and brushing. This was observed by **Arata Ito et al.**<sup>47</sup>

## 5. Summary And Conclusion

Orthodontic archwires are an integral and active component of the fixed orthodontic appliance but are prone to and act as retention sites for plaque formation leading to biofilm adhesion and microbiological colonisation. This puts the patient at risk for increase in incidence of dental caries and periodontal diseases. The conclusions of the study are:

1. All the archwires show significant amount of microorganisms on them.

2. *Streptococcus mutans* had the highest affinity for retention on all the archwires followed by *Staphylococcus Aureus* and *Candida Albicans*.

3. *Streptococcus mutans* had highest affinity for NiTi wires, followed by HANT, SS and Aesthetic coated SS wires. HANT and NiTi archwires showed significant difference with Aesthetic coated SS.

4. For *Staphylococcus aureus*, NiTi archwires showed highest amount of adhesion followed by HANT, SS and Aesthetic Coated SS. However the difference is statistically not significant except between NiTi and Aesthetic coated SS wires.

5. For *Candida albicans*, HANT archwires showed highest amount of adhesion followed by NiTi, SS and Aesthetic coated SS. Both HANT and NiTi archwires shows significant difference with Aesthetic coated SS wires.

Hence, it is concluded that, in order to provide a more wholesome orthodontic treatment, keeping the patients' overall prognosis in mind, NiTi and HANT archwires, being the wires with more amount of microbial colonisation should be used for the most optimum and shortest period of time especially in patients with higher risk of dental caries and periodontal complications. Wire progression to wires with lesser biofilm adhesion and microbial colonisation such as to Aesthetic coated SS and SS at the earliest is recommended.

**Limitations Of The Present Study:** In the present study, microbiological evaluation was done by placing and retrieving archwires for the period of 1 month. Long-term studies would be more conclusive. On the Aesthetic coating SS archwires, coatings may peel during intervention or in areas where tooth brush can reach easily, can affect the outcome.

For this study, 0.017\*0.025 rectangular cross section was taken. Different cross sections can change the outcome. The standardization of eating habits is not possible which may affect the outcome of the study.

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**Table I, Graph I: Mean of Colony Forming Units(CFU) of *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans* and Biofilm Absorbance and Biofilm Concentration of Aesthetic Coated SS, Stainless Steel, Heat Activated Nickel Titanium and Nickel Titanium archwire of 0.017\*0.025” cross section.**

	Aesthetic Coated SS		Stainless Steel		HANT		NiTi	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Streptococcus Mutans</b>	<b>153.36</b>	<b>39.44</b>	<b>176.33</b>	<b>42.79</b>	<b>190.86</b>	<b>33.62</b>	<b>196.2</b>	<b>38.53</b>
<b>Staphylococcus Aureus</b>	<b>12.46</b>	<b>6.65</b>	<b>13.5</b>	<b>5.73</b>	<b>14.7</b>	<b>6.94</b>	<b>17.73</b>	<b>6.70</b>
<b>Candida Albicans</b>	<b>1.22</b>	<b>0.86</b>	<b>2.93</b>	<b>2.53</b>	<b>4.62</b>	<b>3.51</b>	<b>3.5</b>	<b>2.86</b>



TABLE II shows Comparison of ‘Streptococcus Mutans’ on different 0.017\*0.025” archwires - Aesthetic coated SS, Stainless Steel, HANT, and NiTi

	Mean	SD	
Aesthetic Coated SS	153.36	39.44	
Stainless Steel	176.33	42.79	
HANT	190.86	33.62	
Niti	196.2	38.53	
ONE WAY ANOVA TEST P VALUE	0.002*		
Groups	Difference	95% Confidence Interval	P value
Aesthetic Coated SS vs Stainless steel	22.97	-3.0987 to 49.0387	0.10
Aesthetic Coated SS vs HANT	37.5	11.4313 to 63.5687	0.002*
Aesthetic Coated SS vs Niti	42.84	16.7713 to 68.9087	0.001*
Stainless Steel vs HANT	14.53	-11.5387 to 40.5987	0.46
Stainless Steel vs Niti	19.87	-6.1987 to 45.9387	0.19
HANT vs Niti	5.34	-20.7287 to 31.4087	0.95

TABLE III Comparison of ‘Staphylococcus Aureus’ on different 0.017\*0.025” archwires - Aesthetic coated SS, Stainless Steel, HANT, and NiTi

	Mean	SD	
Aesthetic Coated SS	12.46	6.65	
Stainless Steel	13.5	5.73	
HANT	14.7	6.94	
Niti	17.73	6.70	
ONE WAY ANOVA TEST	0.014*		
Groups	Difference	95% Confidence Interval	P value
Aesthetic Coated SS vs Stainless steel	1.04	-3.3489 to 5.4289	0.92
Aesthetic Coated SS vs HANT	2.24	-2.1489 to 6.6289	0.54
Aesthetic Coated SS vs Niti	5.27	0.8811 to 9.6589	0.01*
Stainless Steel vs HANT	1.20	-3.1889 to 5.5889	0.89
Stainless Steel vs Niti	4.23	-0.1589 to 8.6189	0.06
HANT vs Niti	3.03	-1.3589 to 7.4189	0.27



**TABLE IV Comparison of 'Candida Albicans' on different 0.017\*0.025" archwires - Aesthetic coated SS, Stainless Steel, HANT, and NiTi**

		Mean	SD	
Aesthetic Coated SS		1.22	0.86	
Stainless Steel		2.93	2.53	
HANT		4.62	3.51	
Niti		3.5	2.86	
ONE WAY ANOVA TEST		0.0002*		
Groups	Difference	95% Confidence Interval		P value
Aesthetic Coated SS vs Stainless steel	1.71	-0.2203 to	3.6403	0.10
Aesthetic Coated SS vs HANT	3.4	1.4697 to	5.3303	0.0001*
Aesthetic Coated SS vs Niti	2.28	0.3497 to	4.2103	0.01*
Stainless Steel vs HANT	1.69	-0.2403 to	3.6203	0.10
Stainless Steel vs Niti	0.57	-1.3603 to	2.5003	0.86
HANT vs Niti	1.12	-3.0503 to	0.8103	0.43