

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AMONG GINGIVITIS IN PATIENT WITH ORTHODONTIC WIRES IN KUFA CITY /IRAQ.

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ABSTRACT

This study aimed to investigate the role of orthodontic wire on bacterial community in oral cavity as well as its effectiveness on *Staphylococcus aureus*. A total of 78 gingival swab samples have been collected from patient with orthodontic wires suffering from gingivitis and 71 samples were collected from healthy without orthodontic wire during four months from private dental clinics. The results of conventional isolation and identification of bacterial isolates as well as biochemical test showed that *S. aureus* was the commonest causative pathogens among acute gingivitis (46%) followed by *Granulicatella adiacens* (29.6%) where 36 isolates were isolated from acute and chronic gingivitis in comparison with 27 isolates from healthy. A mutation experiment was carried out to explain the role of orthodontic wires on *S. aureus*. The results revealed the same change has been obtained when using both of Nikle-titanium (NiTi) and stainless steel wire on *S. aureus* after 24-96 hr from incubation. Antibiotic sensitivity pattern of both original and treated isolates with NiTi and stainless steel wires explained increased in antibiotic resistance of *S. aureus* to bacitracin, ceftazidim, ogmentin, and erythromycin while other antibiotic remain sensitive such as cefotaxim and amikacin.

Key words: Orthodontic wires, *Staphylococcus aureus*, gingivitis.

INTRODUCTION

Periodontal diseases are a very common in all populations in which severe forms of periodontitis are rare worldwide (Albandar *et al.*, 1999). In some patients of periodontitis atypical subgingival periodontal pathogens may be found in high numbers such as the Gram positive facultative species *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and other staphylococci. *Parvimonas micra*, *Filifactor alocis*, *Eubacteria nodatum* and other *Eubacterium* species, and each of the three anginosus streptococci group species, *Streptococcus constellatus*, *Streptococcus intermedius*, and *Streptococcus anginosus* [Wade, 2013].

Staphylococcus aureus is a major causative agent of major human disease. In the oral tract, *S. aureus*, has been associated with dentoalveolar infections, and oral mucosal lesions and its colonization has been demonstrated from the tongue, saliva, mucosal surfaces, supragingival tooth surfaces and the periodontal pocket (Francis, 1995). Although *S. aureus* was believed to be a transient member of the oral microbial communities increasing evidence from several culturing surveys suggests that it is a common isolate from the oral cavity in healthy children and adults especially in saliva, supragingival plaque, and on the tongue (Peter *et al.*, 2010).

Several virulence factors have been produced from *S. aureus* which include (i) Cell wall associated proteins

and polymers, (ii) Toxins, and (iii) Extracellular enzymes (Peter *et al.*, 2010).

This study has been carried out to investigate the distribution of *S. aureus* in patient with orthodontic wires and suffering from gingivitis and the role of wire as a mutagen on bacterial isolates *in vitro*.

MATERIALS AND METHODS

Sample collection: A total of 78 gingival swab samples have been collected from patient with orthodontic wires and suffering from gingivitis whom admitted private dental clinics and 71 samples were collected from healthy without wire. All samples were collected from the mouth firstly by imprinting a sterile cotton swab across the gingival region in upper and lower gum. All samples were cultured on blood agar base and *Staphylococcus* 110, incubated aerobically at 37°C for 24-48 hr for primary isolation of bacteria.

Identification of bacteria: conventional methods have been used to identified bacterial isolates which include: cultural characteristics on selective and differential media, microscopic examination and biochemical tests (Macfaddin, 2000).

Antibiotic sensitivity tests: Disk diffusion method that described by Kirby was carried out to detect antibiotic resistance pattern of *S. aureus* to 6 antibiotics belong to five different class of antibiotic as mentioned in Table 1. The diameter of resulted inhibition zone was compared with CLSI (Bauer, 1966 & CLSI, 2010).

Table 1: Commercial antibiotics Disks.

Classes	Subclasses	Antibiotics	Symbol	Content (µg)
β-lactam/β-lactamase inhibitor combinations	Non	Amoxicillin/Clavuanic acide	AMC	30
		Cephems (parenteral)	Cefhalosporin III	
Macrolides	Non	Cefotaxime	CTX	30
		Ceftazidim	CAZ	30
Aminoglycosidase	Non	Erythromycin	E	15
		Amikacin	AK	10
Peptide	Non	Bacitracin	B	10

Mutation Experiment: Two types of orthodontic wires have been choosing to evaluate the mutagenic effect of orthodontic wire on four bacterial isolates that selective randomly. A method described by Lentino *et al.*, (1993) has been followed with some modifications that include using crushing orthodontic wire in which 0.95 mg/10 ml of stainless steel and 0.45 mg/10 ml of NiTi were added to BHI broth. The morphology of bacterial isolates treated with orthodontic wires as well as antibiotic resistance pattern were comparing with control after 24, 48, 72 and 96 hr.

Results and Discussion

Identification of bacterial species: The result of microscopic examination and biochemical test of bacterial isolates that obtained from culturing of 149 gingival specimens collected from patient with orthodontic wires

for each gender: male (34 sample) and female (115 samples) with age group range from 17 onward showed that 63 (42.2%) isolates were belong to *S. aureus* where 27 (43%) from healthy and 36 (57%) from acute and chronic gingivitis (Table 2).

The results showed widely distribution of bacterial isolates among acute and chronic gingivitis in which it may represent a main cause of acute gingivitis in upper gum (25.3%). Also, the same results were obtained in lower gum where it occurs with a high percentage (20.6%) while in upper gum of chronic gingivitis it was 6.34% whereas in lower gum it was 4.76%. In state of healthy it appeared with a high dominance where the percentage of isolation were 23.8% in upper gum (Table2).

Table-2: Distribution of *Staphylococcus aureus* among gingivitis and healthy

Types of gingivitis	Locations	<i>S. aureus</i>		Total	
		No.	%	No.	%
Healthy	Upper	15	23.80	27	43.0
	Lower	12	19.04		
Acute	Upper	16	25.30	29	46.0
	Lower	13	20.60		
Chronic	Upper	04	06.34	7	11.0
	Lower	03	04.76		
Total		63	042.2	63	42.2

S. aureus are considered to be transient bacteria in the oral cavity and the amounts of it in oral specimens are (10^2 - 10^4 cfu/ml in saliva, 10^3 - 10^5 cfu/g in dental plaque) (Percival, *et al.*, 1991) Patients with periodontal disease represent possible reservoirs of these opportunistic bacteria in the oral cavity, they can also be an infection source to other individuals (Okada & Murakami, 1998) Previous study showed *Staphylococcus* spp. in 28.3% of individuals with age group 25-60 years and represented as the second most common subgingival *Staphylococcus* species (Slotes, *et al.*, 1999) while others found the prevalence of *Staphylococcus* spp. isolates in subgingival and oral cavity samples (Loberto, *et al.*, 2004). Our results indicated that dental devices serve as an artificial niche increasing the carriage of *S. aureus* in oral mucosa and subgingival plaque, this result was also recorded by previous study (Tonetti, 2005). *Staphylococcus* species present in low number when compared to those of viridans streptococci (10^4 - 10^6 cfu/ml in saliva, 10^7 - 10^9 cfu/g in dental plaque), oral staphylococcal that found in oral cavity of adult with age group from 20 to over 80 years old ranges from 60% -88% (Percival, *et al.*, 1991). The prevalence of *S. aureus* among infected site (periodontal disease) was higher than healthy subgingival sites (54 and 92%, respectively) (Murdoch *et al.*, 2004). The rates of *S. aureus* tend to increase with age which is possibly associated with xerostomia (decrease in saliva flow) and denture wearing although the composition and proportions of microflora in adult oral cavity are rather stable over long periods, the occurrence of staphylococci in denture plaque was found to be significantly

high which represent (88%) (Marsh, *et al.*, 2009). In the present study, the prevalence of *S. aureus* was higher in oral cavity and subgingival biofilm of patients with gingivitis who use dental devices.

S. aureus has many virulence factors that participate in destruction of the periodontal tissue. The pore forming toxins such as hemolysins, lipases and lecithinases can play a role in the destruction of tissue and inactivation of immune cells because of the lesional effects induced in the plasmatic membrane while the amylases can provide a nutritional advantage as well as play an important role in the constitution of the polysaccharide extracellular matrix of the biofilm in tooth whereas the DNAases production mediating the hydrolysis of the DNA which released after destruction of the cell the cell and also it help the bacterial cells in their synthesis by providing nucleotides to them and this enable them to compete with other bacteria for the colonization of a certain ecological niche (Obrien-Simpson, *et al.*, 2003). The extracellular proteolytic enzymes of *S. aureus* were capable of destruction proteins of the host and disturbing the immune response (Obrien-Simpson, *et al.*, 2003).

Mutation experiment: To evaluate the effect of orthodontic wire on bacteria isolates 4 isolates of *S. aureus* have been selected randomly. The result of 24 hr of incubation of *S. aureus* on brain heart broth containing NiTi wire showed no change in the appearance of culture media and the same result was obtained with broth containing stainless steel wire in comparing with control broth as showed in figure (1-A) While the result of 48 h of incubation of *S. aureus* in

broth containing stainless steel wire broth appeared the same of control but NiTi wire broth cause discoloration of the culture media as showed in figure (1- B). After 72

and 96 hr from incubation of *S. aureus* both NiTi and stainless steel wire broth cause discoloration of brain heart media as showed in figure 1- C and D.

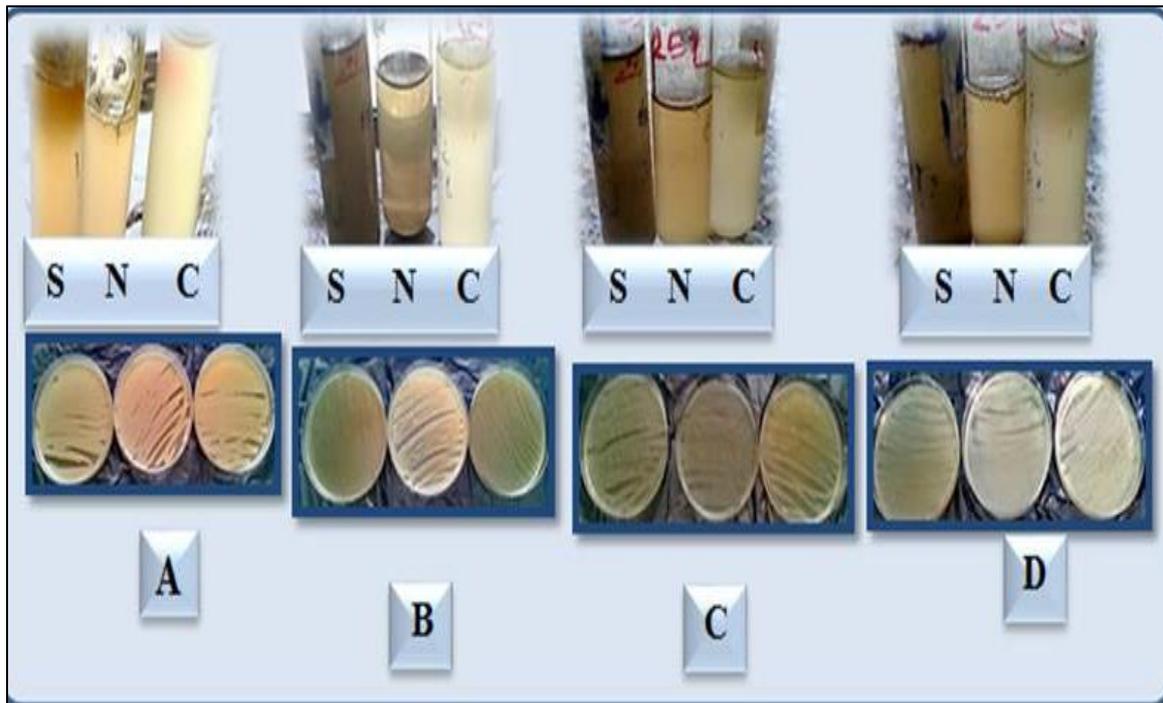


Figure- 1: Morphological characteristic of treated *Staphylococcus aureus* grow on Brain Heart Infusion Agar. A- after 24 hr. of incubation period. B- after 48 hr from incubation period. C- On 72 hr. of incubation period. D- On 96 hr. of incubation period. N- treated with Nikle-titanium wire, S- treated with stainless steel wire, C- control

Many appliances are available either fixed or removable in accordance with the main purpose of the treatment (Chung, *et al.*, 2004). Orthodontic fixed appliance therapy is the commonest mode of treatment and the most commonly used orthodontic materials are brackets, tubes, band material, ligating materials and arch-wires, these materials facilitate the microbial adhesion and greatly inhibit oral hygiene and provide new retentive areas for plaque and debris which in turn predisposes the wearer to increased microbial burden and possibility of subsequent infection Magno, *et al.*, 2008).

Previous work reported new data on the duration of salivary microbial changes induced by the placement of fixed orthodontic appliances they noted the success of antimicrobial preventive measures for orthodontic patients with proper timing. Such measures should be applied between weeks 6 and 12 of orthodontic therapy which is the time where *Streptococcus mutans* and *Lactobacillus* spp. increase in the saliva in which their increase significantly in 6 months after the insertion of fixed orthodontic appliances (Peros, *et al.*, 2011) The negative effect on microbial flora can occur at long-term utilization of orthodontic appliances and so increase the risk of new carious lesions (Topaloglu, *et al.*, 2011).

Currently, NiTi alloys compose of 55% nickel and 45% titanium (Roach, 2007). NiTi arch wires gained popularity more than due to their elasticity of 20% higher than stainless steel alloys (Chaturvedi and Upadhyay, 2010) but also has a disadvantage which include a decrease in mechanical properties due to

corrosion processes (Cai, *et al.*, 2010). To avoid the corrosion process NiTi arch wires were covered with Teflon based materials, composite resins, hydrogenated carbon or zirconium dioxide, which restricted corrosion and restrict the release of Ni by 80% without alter the mechanical properties of the archwires (Elayyan, *et al.*, 2000). Some oral clinical manifestations in orthodontic patients such as gingival hyperplasia and periodontitis might be associated with an inflammatory response elicited by the corrosion of orthodontic appliances and the subsequent release of nickel (Genelhu, *et al.*, 2005), the alteration in the composition of surface NiTi arch-wires after intra-oral exposure for 1–6 months due to the occurrence of amorphous precipitates and micro-crystalline particles in proteinaceous biofilm which lead to high affinity of *S. aureus* for titanium surfaces (Eliades, *et al.*, 2000).

Stainless steel arch wires have been used as orthodontic wires with a wide range of applications in both the fixed and removable appliances (Brantley, *et al.*, 2002).

Studies on it showed that the smoothness of their surface is responsible for the decrease in the count of *Streptococcus* colonies on it where the adhesion ability in the coated and non-coated group was increased by the extended incubation time and was the highest after three hours of incubation (Yu, *et al.*, 2011) So, we conclude that extended incubation time increased the adhesion of cariogenic *Mutans streptococci* (Amini *et al.*, 2012).

The action of microbial colonization is twofold either take up and metabolize metals from alloys or microbial byproducts with the metabolic processes may alter the conditions of the microenvironment, *i.e.*, decreasing the pH and therefore contributing to the initiation of the corrosion process) (Palaghias, 1985).

Aerobic, facultative and anaerobic bacteria favoring the corrosion process Aerobic bacteria utilize the simple sugar then enter glycolysis and TCA cycle releasing carbon dioxide (Gerhard, 1985). The facultative bacteria enter the fermentative pathway utilizing the simple sugars and produce organic acids, alcohols and CO₂, the formation of organic acids cause reduction of pH thereby it favoring corrosion. facultative in the anaerobic zone utilize the lactate as carbon source and reduce sulphate to sulphide then Sulphide combines with iron to form ferrous sulphide as the corrosion, The sulphide produced by sulfide reducing bacteria (SRB) enters into the interface of the anaerobic and facultative zones where it gets oxidized by sulphate oxidizing bacteria to sulphate, Sulphuric acid is also formed which reduces the pH and cause tooth decalcification and corrosion of metallic implants because of its corrosive nature The low pH offer favourable environment for aerobic microbes such as iron oxidizing bacteria (Maruthamuthu *et al.*, 2005). The metal ions, MnO₂,

FeO, Fe₂O₃ combine with the end-products of the bacteria along with the chloride ion in the electro-lyte (saliva) to form more corrosive products like ferric chloride (FeCl₃), manganese chloride (MnCl₂), etc. This leads to metal leaching with subsequent release of nickel and chromium into the body and then decalcification of teeth (Christopher, *et al.*, 2004).

Antibiotic sensitivity test

Antibiotic resistance pattenen was detected for both origin isolates and mutated isolates to explain the effect of orthodontic wire on increasing or decreasing of antibiotic resistance manner of isolates. The result of 24 hr of incubation of *S. aureus* showed that all origin isolates were sensitive to cefotaxim, bacitracin, ogmentin, erthromycin and amikacin except one of them which showed multiple drug resistant as showed in Table 3. on the other hand all origin and mutated isolates showed resistance to ceftazidim and all mutated isoltes were sensitive to cefotaxim while one mutated isolate treated with stainless steel wire was resistant to bacrtracin and amikacin as well as two isolate of it showed resistant to erthromycin and ogmentin wherase all isolate treated with NiTi wire were sensitive to bacitracin, ogmentin, erthromycin, and amikacin with exception that one of isolates appear as multidrug resistant.

Table -3: Antibiotic resistance pattern of *Staphylococcus aureus* (origin and mutated isolates) to certain antibiotic after different incubation period

Incubation period (hr)	No. of isolates	Cefotaxim			Bacitracin			Ceftazidim			Ogmentin			erthromycin			Amikacin		
		C	N	\$	C	N	\$	C	N	\$	C	N	\$	C	N	\$	C	N	\$
24	1	R	S	S	R	S	R	R	R	R	R	S	S	S	S	R	S	S	R
	2	S	S	S	S	S	S	R	R	R	S	R	R	S	S	S	S	S	S
	3	S	S	S	S	S	S	R	R	R	S	S	S	R	R	R	S	S	S
	4	S	S	S	S	S	S	R	R	R	S	S	R	S	S	S	S	R	S
48	1	S	S	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S
	2	S	S	S	S	S	R	R	R	S	R	R	R	S	S	R	S	S	S
	3	S	S	S	R	S	R	S	R	S	R	R	R	R	S	R	S	S	S
	4	S	S	S	S	R	S	R	R	R	R	S	R	S	S	S	S	S	S
72	1	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
	2	S	R	S	S	R	R	R	R	S	R	R	R	S	R	R	S	S	S
	3	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	S	S	S
	4	S	S	S	R	R	R	R	R	R	S	R	R	S	R	R	S	S	S
96	1	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R
	2	S	S	S	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R
	3	S	S	S	R	R	R	R	R	S	R	R	R	R	R	R	R	S	R
	4	S	S	S	R	R	R	R	S	S	S	R	R	S	R	R	S	S	S

N=Nikle-titanium wire, \$=Stainless steel wire, C=control, R=Resistant, S= Sensitive.

After of 48 hr of incubation of *S. aureus*, all origin and mutated isolates were sensitive to cefotxim and amikacin while all origin isolate were sensitive to bacitracin and erthromycin with ecption of one was resistant to them as well as all origin isolate showed resistant to ogmentin and ceftazidim while one isolate appear as which was sensitive to these antibiotic wherase two isolate mutated with stainless steel wire showed resistance to bacitracin, ceftazidim and ogmentin and erthromycin and two was sensitive to them. most isolate mutated with NiTi were sensitive to bacitracin and erthromycin and resistant to ogmentin and ceftazidim as

showed in Table 3. After 72 hr incubation of *S. aureus* all origin and mutated isolates were resistant to ogmentin and sensitive to amikacin two of origin isolate was resistant to bacitracin, ceftazidim and erthromycin. Three of isolates mutated with stainless steel wire were sensitive to cefotaxim, ceftazidim, and one was resistant to it while all isolate mutated mutated with stainless steel wire were resistant to bacitracin and erthromycin. All isolate mutated with NiTi were resistant to bacitracin, ceftazidim and erthromycin where all isolate mutated with NiTi were sensitive to cefotaxim with ecption of one was resistan to it. Finally After 96 hr. of incubation

of *S.aureus* all origin and mutated isolates showed resistant to ogmentin and erthromycin and sensitive to cefotaxim. Three of origin isolate were resistant to bacitracin and amikacin and one was sensitive to them while all origin isolate were resistant to ceftazidim. All mutated isolate were resistant to bacitracin as showed in Table 3. Three isolates mutated with stainless steel wire were resistant to ceftazidim and one was sensitive compared with isolates mutated with NiTi were three isolate showed resistant to it and one was sensitive while two isolate mutated with stainless steel wire were sensitive to amikacin and two was resistant compared with isolates mutated with NiTi wire where three of them showed sensitive to it and one was resistant.

Previous results reported that *S. aureus* isolated from patients with periodontal disease showed antimicrobial resistance rates of 92.6% to ampicillin, 90.7% to penicillin, 11.1% each to oxacillin and cefotetan, and 5.6% to erythromycin (Kim., 2012) while others reported that *S. aureus* isolated from the nasal cavity of students and hospital clinical staff showed resistance rates of 90% to penicillin, 43% to tetracycline, 37% to erythromycin, 10% to cephalothin, and 13% to clindamycin and vancomycin (Jung and Lee, 1998) this may have been related to the fact that nasal cavity is more favorable than the oral cavity for the survival of *S. aureus*. In contrast, others reported that resistance rates of *S. aureus* in patients with acute oral infections were 100% to penicillin, 68.1% to oxacillin, and 88.1% to erythromycin (Kim, 1996).

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