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Evaluation of Microbial Contamination of Different Orthodontic as Received Arch Wires from Manufacturers

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ABSTRACT

***Aims of study**: The present study was carried out to assess whether as received arch wires from manufactures are free of microbial contamination as well as to identify the bacterial count and types attached to arch wires. **Materials and Methods:** Eighty samples were included in this study consisting of two types of arch wires (nitinol and stainless-steel); they were from four companies (3 M, Ortho-Technology, Jiscop and G&H). The wires were inserted into plane tubes that contain 10 ml of BHI broth and tris-EDTA. Further 0.1 ml was withdrawn from plane tube and spread on agar plates. Moreover 16 plane tubes (8 tubes with brain heart infusion broth and 8 with tris-EDTA) without arch wires were considered as controls group. **Results:** Microbial sampling yielded growth from 7 of the 80 arch wires studied, the predominant bacteria isolated were staphylococci spp. No growth was recovered from 73 of the samples and from controls. The total viable count of bacteria in BHI reagent is more than that in Tris-EDTA reagent with statistically significant difference (P<0.05). Meanwhile this study found that the Jiscop company have more viable count as compared to other companies. However, there were no significant differences among them (P>0.05). In regard to the presence and distribution of bacteria according to types of wires, the stainless-steel wires have more viable count (49.58) than that in nitinol (47.42) but statistically not significant (P0.05). **Conclusion:** The arch wires received from manufacturer are often contaminated and therefore there is a need for routine disinfection of such items. This study found that the BHI more is effective in dislodging the bacteria from orthodontic arch wires than tris-EDTA. However, the stainless-steel arch wires were more contaminated than nitinol and most common contaminant were Staphylococci spp.*

Keywords: Arch wires, Contamination, *Staphylococci*, Brain heart infusion medium

INTRODUCTION

At present more people want orthodontic treatment to enhance their quality of life and obtain beautiful and healthy smile but placement of orthodontic appliances like brackets, tubes, band material, ligating materials and arch-wires inhibit the maintenance of a proper oral hygiene and are liable for microbial adhesion and create new retentive areas for plaque and debris, which in turn predisposes the patients to increased microbial burden and possibility of subsequent side effects such as gingival inflammation and white spot lesions [1]. The oral cavity is a natural habitat for a large number of microorganisms, this ecological niche can be a reservoir for opportunistic and pathogenic microorganisms that can pose a risk for cross-contamination and infection and may even cause systemic infections [2].

Cross contamination of non-serializable appliances in the dental clinics and laboratories may potentially be a health hazard to the members of the dental team. Although acceptable sterile techniques are applied to most of dental procedures however, disinfection of dental prosthesis has received inadequate attention. Generally, the pathways of contamination can be bidirectional, an infectious microorganism may be transferred from the patient to members of the dental team, but also vice versa, e.g. through the hands of the dental team [3].

Nosocomial infections caused by multi-drug resistant Gram-positive organisms such as staphylococcus aureus (S. aureus) and Enterococcal species are a growing problem in many health care institutions. Hands and instruments

used by health care workers serve as vectors for the nosocomial transmission of microorganisms [4]. The microbial contamination of the dental environment out of which some of the contaminated microorganisms such as *S. aureus* were epidemiologically important nosocomial pathogens [5].

It has been shown that improper disinfection of the dental environment can transmit infectious diseases and prove to be a health hazard to both dental personnel, as well as patients and this can prove to be fatal for immune deficient patients. The control of cross infection and biosecurity are issues of great importance to dental practice and in recent years have attracted greater interest of health professionals due to the spread of infectious diseases such as AIDS and Hepatitis B. Various studies revealed that diseases of this kind have led to a general awareness of the risks of contamination and have changed the habits of professionals in dental clinics [6]. Therefore, the present study was carried out to assess whether as received arch wires from manufactures are free from microbial contamination as well as to identify the bacterial count and prevalence attach to arch wires.

MATERIALS AND METHODS

Eighty samples were included in this study consisted of two types of arch wires (nitinol and stainless-steel), they were from four companies (3M, OrthoTechnology, Jiscop and G&H). The wires were cut in to four pieces by sterilized wire cutter then these pieces of the arch wires from both groups of each company were inserted into plane tubes that contain 10 ml of brain heart infusion broth and tris-EDTA buffer solution, and then samples were homogenized by Vortex mixer for one minute. Moreover 16 plane tubes (8 tubes with brain heart infusion broth and 8 with tris-EDTA) without arch wires were considered as controls group.

Further 0.1 ml was withdrawn from plane tube and spread by using sterile microbiological spreader on agar plates. The samples were cultured on blood agar and MacConkey agar to quantify the number of bacteria. The blood agar plates were incubated aerobically incubation for 48 hours, at 37°C and an aerobically by using a gas pack supplied in an anaerobic jar for 48 hours, at 37°C. While MacConkey agar plates were incubated aerobically for 48 hours at 37°C. After incubation, microbial counts were recorded by colony counter taking in consideration the dilution factor and expressed as colony forming unit. All colonies with different morphologies, colors, sizes were identified and then stained with a Gram-stain and examined under a light microscope and biochemical tests were performed to confirmed types of bacteria.

Statistical analyses

Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version 21). Statistical analyses can be classified into two categories: descriptive analysis for nominal variables and inferential analysis.

RESULTS

Out of the 80 samples screened in this study (forty inserted in Tris-EDTA and other forty of samples inserted in BHI broth), the microbial growth was observed in 7 of samples. No growth was recovered from 73 of the samples and no growth of microorganism was also from Tris-EDTA and BHI samples without the arch wire (control). Gram-positive bacteria which included Staphylococcus species were mostly isolated on blood agar plates that incubated an aerobically.

Table 1 Descriptive and statistical test of total viable count among reagents

Reagent	Min.	Max.	Mean	SE	Median	MR	Statistics	P-value (Wilcoxon test)
Tris-EDTA	0.00	107	2.44	2.23	0.00	44.5	2.292	0.022*
BHI	0.00	105	11.25	4.3	0.00	52.5		
*Significant at P<0.05								

The current results revealed that the total viable count of bacteria in BHI reagent (52.50) is more than that in Tris-EDTA reagent (44.50) with statistically significant difference between them (P<0.05), as clearly shown in Table 1. On the other hand, within all companies' wires the total viable count of isolated bacteria recorded in BHI reagent was more than that recorded in Tris-EDTA but with statistically no significant differences between reagents (P>0.05) (Table 2).

Table 2 Descriptive and statistical test of total viable count among reagents within companies and wires

Company	Wires	Reagent	Min.	Max.	Mean	SE	Median	MR	Z	P-value
3M	Stainless Steel	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	6	1	0.317[$]
		BHI	0.00	10	1.67	1.67	0.00	7		
	Nitinol	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	6.5	0.00	1.00[$]
		BHI	0.00	0.00	0.00	0.00	0.00	6.5		
Jiscop	Stainless Steel	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	5	1.892	0.059[$]
		BHI	0.00	72	15.83	11.67	1.5	8		
	Nitinol	Tris-EDTA	0.00	7	1.17	1.17	0.00	5.83	0.843	0.399[$]
		BHI	0.00	100	33.33	21.08	0.00	7.17		
G&H	Stainless Steel	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	6	1	0.317[$]
		BHI	0.00	26	4.33	4.33	0	7		
	Nitinol	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	5	1.897	0.058[$]
		BHI	0.00	102	17.33	16.93	0.5	8		
OrthoTechnology	Stainless Steel	Tris-EDTA	0.00	107	18.33	17.74	0.00	7	0.631	0.528[$]
		BHI	0.00	105	17.5	17.5	v	6		
	Nitinol	Tris-EDTA	0.00	0.00	0.00	0.00	0.00	6.5	0.00	1.00[$]
		BHI	0.00	0.00	0.00	0.00	0.00	6.5		

[$]Not significant at P>0.05

Among important bacteria identified in this study was *Staphylococcus epidermidis*, and *Staphylococcus aureus*. The present result showed 5 out of 80 samples were contaminated with of *Staphylococcus epidermidis*, four of them were recognized by BHI reagent distributed in to all colonies count and one case was identified by Tris-EDTA reagent with no significant differences (P=0.362) (Table 3). Contaminated with *Staphylococcus epidermidis* among companies showed that 2 cases in for Jiscop and other case in OrthoTechnology and one case for 3M while other two last colonies count for Jiscop company but with statistically no significant association (P=0.390) (Table 4). All the five cases of contamination with *Staphylococcus epidermidis* in this study were recorded in stainless-steel wires and there is no growth of this bacteria in nitinol wires with statistically significant association (P=0.022) (Table 5).

Table 3 Association between contaminations with *Staphylococcus epidermidis* among reagent

Contamination status		Reagent		F.E.P.T	df	P-value	Total
		Tris-EDTA	BHI				
With Contamination	No.	1	4	1.899	1	0.362[$]	5
	%	20	80				100
Without Contamination	No.	40	35				75
	%	53.4	46.6				100

[$]Not significant at P>0.05; F.E.P.T= Fisher's exact probability test=1.899; df=degree of freedom=1

Table 4 Association between contamination with *Staphylococcus epidermidis* among companies

Contamination status		Manufacturer Company				F.E.P.T	df	P-value	Total
		3M	Jiscop	G&H	Ortho Technology				
With Contamination	No.	1	3	0	1	3.283	3	0.39[$]	5
	%	20	60	0.00	20				100.00
Without Contamination	No.	19	17	20	19				75
	%	25.33	22.68	26.66	25.33				100.00

[$]Not significant at P>0.05; F.E.P.T=Fisher's exact probability test=3.283; df= degree of freedom=3

Table 5 Association between contaminations of *Staphylococcus epidermidis* among the wires

Contamination status		Wires		F.E.P.T	df	P-value	Total
		Stainless Steel	Nitinol				
With Contamination	No.	5	0	5.275	1	0.022*	5
	%	100	0				100
Without Contamination	No.	35	40				75
	%	46.67	53.33				100

*Significant at P<0.05; F.E.P.T=Fisher's exact probability test=5.275; df=Degree of freedom=1

Only 2 cases of contamination were identified with *Staphylococcus aureus* in tris-EDTA one case was identified by BHI with no statistically significant association was found (P=1.00) (Table 6).

Table 6 Association between contaminations with *Staphylococcus aureus* among reagents

Contamination status		Reagents		F.E.P.T	df	P-value	Total
		Tris-EDTA	BHI				
With Contamination	No.	2	1	0.344	1	1.00[$]	3
	%	66.67	33.33				100
Without Contamination	No.	38	39				77
	%	49.35	50.65				100
[$]Not significant at P>0.05; F.E.P.T=Fisher's exact probability test=0.344; df=degree of freedom=1							

Regarding the distribution of *Staphylococcus aureus*, Table 7 showed that 3 cases were contaminated in companies distributed in to 2 cases in OrthoTechnology and 1 case in Jiscop (33.33%) with no statistically significant association was found (P=0.616).

Table 7 Association between contaminations with *Staphylococcus aureus* among companies

Contamination status		Company				F.E.P.T	df	P-value	Total
		3M	Jiscop	G&H	Ortho Technology				
With Contamination	No.	0	1	0	2	2.974	3	0.616[$]	3
	%	0.00	33.33	0.00	66.67				100.00
Without Contamination	No.	20	19	20	18				77
	%	25.81	24.72	25.81	23.66				100.00
[$]Not significant at P>0.05; F.E.P.T=Fisher's exact probability test=2.974; df=degree of freedom=3									

The contamination with *Staphylococcus aureus* among wires in this result only 2 cases were found to be recorded contamination one in stainless steel wires and one case in nitinol wires (33.33) in 7 colonies with no statistically significant association was found (P=1.00) (Table 8).

Table 8 Association between contamination with *Staphylococcus aureus* among the wires

Contamination status		Wires		F.E.P.T	df	P-value	Total
		Stainless Steel	Nitinol				
With Contamination	No.	2	1	0.344	1	1.00[$]	3
	%	66.67	33.33				100
Without Contamination	No.	38	39				77
	%	49.36	50.64				100
[$]Not significant at P>0.05; F.E.P.T= Fisher's exact probability test=0.344; df=degree of freedom=1							

DISCUSSION

In orthodontic treatment the disease may be transpose either through direct interaction with contaminate instrument or material, use the material directly from manufacture packing or utilized the instruments without appropriate sterilization or disinfection [7,8]. Orthodontic arch wires used for alignment the teeth and come in contact with mucous membrane and sometime cause tear of mucosa, therefor the orthodontic arch wires consider semi-critical instrument and must be sterilized before used [9,10].

The samples of the present study consist of two types of maxillary orthodontic arch wires (stainless-steel and nitinol) from four companies: 3M United company, OrthoTechnology Company, G&H Wire company and Jiscop company.

The outcomes of the investigation show that bacteria were existing on arch wires as received from the manufacture. Thus, these arch wires are not sterilized, and this approve the outcome of prior studies of dental burs [11], endodontic files [12], orthodontic molar tubes [13] and orthodontic pliers [14]. The level of contamination was found to be negative and can be considered to be minor since these arch wires are located in the oral environment, which has approximately 5×10^8 bacterial cells per milliliter.

Most bacteria recognize in the present study refer the contamination of unused orthodontic arch wires associated to hand hygiene or probable from aerosols created from the mouth during packaging of arch wires. In a healthy oral environment, the relationship of the host with microorganism is equilibrium and complex [15].

In present study, the orthodontic arch wires exhibited microbial contamination. And the arch wires have vital clinical effects as come in contact with oral mucosa and consequently to the blood stream establishing potential source of cross contamination. The orthodontic arch wires are providing in individual sealed packages. So that microbial contaminations show in as received orthodontic arch wires may be associated with transfer of microorganism throughout manufacturing procedure, handling, or transportation.

The result show in the present study that different types of bacteria are present, the *Staphylococcus epidermidis* and *Staphylococcus aureus* is most bacteria isolated, this mean skin contact during manufacturing or packaging is the most common cause of contamination, this result partial agree with study performed by Azeredo Fabiane, et al. [14] determine bacterial contamination of orthodontic pliers and found various types of *Staphylococcus* species (*Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and estimate the band removal plier is high contamination and the plastic material in the tip of plier encourage bacterial contamination. The result agrees with the findings of dos Santos Gerzson, et al. that found most bacterial contaminate the orthodontic bracket were *Staphylococcus aureus* and *Staphylococcus epidermidis* [16].

But it partially agrees with Barker, et al. who investigated bacterial contamination of different orthodontic material that found large amount of *Staphylococcus epidermidis* and founded the most source of contamination through skin contamination [17]. And other type of bacteria found was *Streptococcus sanguinis*. It resident bacteria in the oral flora in healthy people, its inculpates as contributing agent in infective endocarditis. And the existing this type of bacteria has special attention since the may hit the soft tissue and consequently to the blood stream and become potential area for cross contamination. In general, the present of microorganism greater than normal level maybe lead to systemic complication beside to caries and periodontal disease [18,19] occasional the *Staphylococcus epidermidis* is causative agent of endocarditis when there's complication of dental treatment with present of bacteremia [20] and there is relationship some type of orthodontic process like orthodontic banding and bacteremia [21,22].

The pathogenic capacity of *Staphylococcus aureus* related to combination between the effect of extracellular factor and toxin, collected with invasive properties. The *Staphylococcus aureus* can cause endocarditis, osteomyelitis, meningitis, or lung infection [23]. The presence of these respiratory pathogens in the biofilm can work as a reservoir for microorganism associated with nosocomial pneumonia [24].

CONCLUSION

From the result of present study, not all materials that received from manufacturer are free from contamination and needed to effective method for sterilization and disinfection to avoid cross-contamination among the patients and the arch wires must be sterilized by suitable method before clinical used.

DECLARATIONS

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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