Effectiveness of Various Disinfectant Protocols on Orthodontic Pliers – An *In-Vitro* Study

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Abstract

Disinfection of the instruments used in orthodontic clinic is essential in order to prevent the transmission of disease to the practitioner, auxiliary staff and patients. Present study aims to evaluate the effectiveness of 2% Glutaraldehyde solution, quaternary ammonium compound based wipes and foam sprays on the disinfection of orthodontic pliers. **Methodology:** The efficacy of disinfection methods of orthodontic pliers used in everyday practice by orthodontists was evaluated using 30 sterile pliers. They were contaminated in vitro with bacteria commonly found in the oral cavity, *Streptococcus salivarius* [ATCC 13419], *Staphylococcus aureus* [ATCC 25923] *and Pseudomonas aeruginosa* [ATCC27853]. Following contamination of the pliers, each of ten pliers underwent one of the three disinfectant protocols in a solution of 2% Glutaraldehyde, alcohol-free wipes containing quaternary ammonium compounds and spraying a foam containing quaternary ammonium compound. The plier heads were spread on the surface of sterile blood agar plates after contamination and after disinfection. **Results:** 2% Glutaraldehyde solution, quaternary ammonium compound based wipes and foam sprays were found to be effective with regards to disinfection of orthodontic pliers. **Conclusion:** As per the results of our study, we conclude that 2% Glutaraldehyde solution, quaternary ammonium compound based wipes and foam spray were found to be effective with regards to disinfection of orthodontic pliers contaminated with standard strains of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus salivarius*.

Keywords: Glutaraldehyde, *Pseudomonas aeruginosa*, Quaternary Ammonium Compounds, *Staphylococcus aureus*, Sterilization, *Streptococcus salivarius*

1. Introduction

The oral cavity harbors a plethora of microorganisms more than 700 bacterial species or phylotypes, some of which can cause focal oral infections^[1]. Saliva carries high concentrations of potentially infective bacteria or viruses that can produce the common cold, herpes, tuberculosis, pneumonia, and Hepatitis B (HBV)^[2]. Also, blood is

known to transmit *Human Immunodeficiency Virus (HIV)*, the causative, agent of Acquired Immune Deficiency Syndrome (AIDS). According to a study, orthodontists have the second highest incidence of hepatitis B among dental professionals^[3].

The greatest danger for orthodontists and the staff are from puncturing of the skin with contaminated instruments or sharp edges of orthodontic appliance.

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The cuts or abrasions will allow microorganisms to enter into the body. The microorganisms can also spread by direct contact with a lesion, indirect contact through contaminated instruments or office equipment or inhalation of aerosols induced by hand pieces and ultrasonic cleaners; or while scrubbing of instruments^[4].

Dental procedures can provoke the introduction of oral microorganisms into the bloodstream or the lymphatic system. Even when the infectious agents remain at the primary oral site but bacterial toxins are liberated and can reach an organ or tissue via the bloodstream to cause a metastatic injury^[5]. Therefore, infection control in the orthodontic office is essential in order to prevent the transmission of disease to the practitioner, auxiliary staff and patients. Proper sterilization and disinfection of materials is mandatory to ensure infection control. Sterilization is a physical or chemical process that destroys all microorganisms, including spores^[6].

The pliers used in orthodontics are semi-critical instruments used to bend and cut wires inside and outside the mouth. Various protocols of disinfection are available. Traditionally chemical disinfectants such as Glutaraldehyde or alcohol based solutions are used for disinfection of instruments. Quaternary ammonium compound based disinfectants are also available. These chemicals also vary in their delivery method. Some require immersion in a solution for a given time period. Newer usage methods include a foam based spray to deliver the disinfectant as well as readymade wipes.

The objective of this study is to evaluate the effectiveness of various disinfectant protocols on the disinfection of orthodontic pliers. An in-vitro study has been chosen to test the efficacy of the traditional Glutaraldehyde immersion solution protocol and the quaternary ammonium based compounds via the foam spray and readymade wipes delivery method. The in-vitro study using specific microorganisms cultivated in the laboratory, helps reduce the confounding factors in the study by limiting the number of variables in the study. An in-vivo study using instruments exposed to patient's body fluids, would invariably have varying microorganisms of varying numbers number, which would make the evaluation more difficult, as the parameters of the study would be less standardized. The efficacy of the disinfectant protocol is to be determined by a simple microbiological count of the orthodontic pliers following disinfection.

2. Materials and Methods

The study was conducted for a period of 2 months from January 2017 to February 2017 as part of MDS thesis dissertation after obtaining institutional ethical committee clearance. 30 orthodontic pliers were used. They were contaminated with three different strains of bacterial culture suspensions and then disinfected separately with 2% Glutaraldehyde, alcohol-free wipes containing quaternary ammonium compounds and quaternary ammonium compound foam spray. Disinfectants used for this study were divided into three groups (Table 1).

Table 1. List of the disinfectants used

Group	Brand (Manufacturer)	Composition		
A	Hospal G (PSK Pharma)	2% Glutaraldehyde solution		
В	Cleanisept Wipes (Dr. Schumacher)	Quaternary Ammonium Compound based wipes		
С	Zeta 3 foam (Zhermack)	Quaternary Ammonium Compound based foam spray		

Bacteria used to contaminate the pliers were Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus salivarius. The efficacy of disinfection methods of orthodontic pliers used in everyday practice by orthodontists was evaluated, using 30 sterile pliers (Figure 1), contaminated in vitro with bacteria commonly found in the oral cavity, Streptococcus salivarius [ATCC 13419], Staphylococcus aureus [ATCC 25923] and Pseudomonas aeruginosa [ATCC 27853]. The microorganisms were grown in three separate test tubes with Brain Heart Infusion (BHI) broth for Streptococcus salivarius and Alkali Peptone broth for the others, incubated at 37°C/48hrs. Contamination of orthodontic pliers was performed through a swab soaked in liquid culture (0.5Mc Farland turbidity standard) for 2 minutes. After drying of culture, the head of the contaminated pliers was spread on to one half of the sterile Blood agar plate. A swab directly from the broth was also spread on to other half of Blood agar plate serves as the control. This was to determine that sufficient quantity of bacteria was transferred and that they are viable.

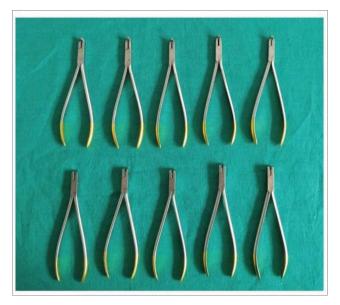


Figure 1. Orthodontic pliers- distal end cutters (Jaypee).

Following contamination of the pliers, the ten pliers underwent one of the three disinfectant protocols:

- Group 1: Immersion in a solution of Glutaraldehyde for 10 minutes.
- Group 2: Using alcohol-free wipes containing quaternary ammonium compounds for 1 minute.
- Group 3: Spraying a foam containing quaternary ammonium compound for 1 minute.

Following disinfection, the plier heads were spread on the surface of sterile Blood agar plates. The plates were then incubated at 37°C/48h and checked for bacterial growth (if any). The statistical calculations were performed using Systems software SPSS version 21.0. Mc nemar's test was used to assess the pre and post comparison and

Fisher's t test was used to find the association between the disinfectant protocols (Table 2).

3. Results

The present study has been planned to evaluate the effectiveness of different protocols of disinfectants. Orthodontic plier heads were contaminated with 3 different bacteria and were then disinfected using one of the three protocols.

Cultures were performed from the head of the contaminated plier and directly from the broth on each half of the sterile Blood agar plate and incubated for 37°C overnight. After incubation, the inoculated blood agar plates showed bacterial growth of 1.5x108 colony forming units which is proportional to 0.5 McFarland turbidity standard of the culture suspension used. (Figure 2 and Table 3).



Figure 2. Blood agar plate showing bacterial colonies from the contaminated plier and liquid culture.

Bacterial count following contamination and after disinfection of the pliers

Bacteria	Immersion in 2% Glutaraldehyde (10 minutes)		Quaternary Ammonium Compound based wipes (1 minute)		Quaternary Ammonium Compound based foam spray (1 minute)		p- value
	Dec.	Cont.	Dec.	Cont.	Dec.	Cont.	
Pseudomonas aeruginosa	10	0	10	0	10		0.002*¥
Staphylococcus aureus	10	0	10	0	10		0.002*¥
Streptococcus salivarius	10	0	10	0	10		0.002*¥
p- value	P > 0.05 €		P > 0.05 €		P > 0.05 €		

^{*}P < 0.05 – significant ¥ = mc nemar test € = fisher exact t test

Cultures from the head of the contaminated pliers after disinfection showed no bacterial growth (Figure 3 and Table 3).



Figure 3. Blood agar plate showing no bacterial growth from the disinfected plier.

Table 3. Amount of pliers investigated according to the kinds of bacteria and protocols (with p-values)

Name of the Disinfectant	Bacterial count after contamination of pliers	Bacterial count after disinfection of pliers
2% Glutaraldehyde	1.5x 10^8 colony forming units (CFU/ml)	0
Quaternary Ammonium Compound based wipes	1.5x 10^8 colony forming units (CFU/ml)	0
Quaternary Ammonium Compound based foam spray	1.5x 10^8 colony forming units (CFU/ml)	0

4. Discussion

Communicable diseases pose a major threat in routine dental practice. Infection control in the dental office is essential in preventing the transmission of disease to the practitioner, auxiliary staff and patients. Traditional sterilization methods such as autoclaving require 15 to 20 minutes followed by a cooling period before the instrument can be used. This results in increased waiting periods between appointments as each set of instruments has to undergo sterilization. Alternatively, the orthodontist could invest in several sets of instruments at a greater cost to themselves^[7]. Repeated autoclave cycles several times a day are likely to corrode instruments especially those that are not made of stainless steel^[8]. Orthodontic instruments such as orthodontic pliers are semi-critical instruments (items that contact mucous membrane) used to bend and cut wire inside and outside the mouth^[9]. In our study, we evaluated the efficacy of three disinfection protocols: Immersion in a solution of 2% Glutaraldehyde for 10minutes, using alcohol-free wipes containing quaternary ammonium compounds for 1 minute and spraying a foam containing quaternary ammonium compound for 1 minute.

As per the results of our study, we conclude that all the three disinfectants were proved to be effective, since after contamination of the pliers, colony count was 1.5x 108 colony forming units (CFU/ml) and after disinfection colony count was found to be zero (i.e. they were able to decontaminate all the pliers contaminated with each of the three bacteria used). 2% Glutaraldehyde (preferably alkaline over acidic) has been shown to be effective as a chemical sterilant (exposure time of 6 to 10 hours)[10]. It is sporicidal after 7 to 10 hours of exposure. Glutaraldehyde is considered the disinfectant of choice for sterilizing medical and dental equipment and is the most popular high level disinfectant used in dentistry[11]. Glutaraldehyde has a broad range of action and rapid lethal activity^[12]. The recommended contact time is 10 minutes. The use of 2% Glutaraldehyde for a period of 20-45 minutes is said to correspond with high level disinfection. Studies have shown that an immersion period of 30 minutes was sufficient to decontaminate pliers^[6]. In our study, a 10-minute immersion was shown to be sufficient to decontaminate pliers. Healthcare workers were shown to be more than 8 times more likely to be allergic to Glutaraldehyde than their non-health-care working peers. Allergic contact dermatitis from Glutaraldehyde often causes permanent dermatitis which frequently forces patients to leave their jobs. Therefore, there has to be an emphasis on improving safety standards and barrier protection^[13]. Aside from issues with toxicity, 2% Glutaraldehyde are said to cause corrosion and rusting of stainless steel instruments^[4].

Quaternary Ammonium Compounds (QAC) have been discussed in scientific literature as far back as 1947. QACs act by reducing surface tension between bacteria and an object^[3], initiating autolysis and disrupting the bacterial cell wall including inducing leakage of intracellular constituents^[14]. One of the disadvantages of QACs is their reduced effectiveness in the presence of organic matter. Cotton, air or heavy bacteria can prevent the contact of the disinfectant with the cell wall^[3]. QACs have shown incompatibility with other chemical substances in the dental office such as other disinfectants which can lead to them neutralizing each other. While P. aeruginosa is considered to be less sensitive than S. aureus to QACs^[15], this was not seen in our study where QAC were equally effective in completely decontaminating pliers contaminated by both these bacteria.

Based on our study and the review of literature, 2% Glutaraldehyde is an effective method of disinfecting orthodontic pliers, however in the interest of a shorter disinfection time (10 times shorter) and reduced allergenicity, the QAC products we tested could be used as low to intermediate level disinfectants as orthodontic pliers are seen as semi-critical instruments. Based on the review of literature, we recommend mechanical cleaning with distilled water to remove any organic matter such as saliva[16-18] (preferably using an ultrasound bath), followed by disinfection using the QAC spray or foam. It should be emphasized that disinfection is not to be viewed as a replacement to sterilization. Critical instruments must be sterilized and non-critical and semi-critical instruments should be sterilized when possible. Manufacturer guidelines should be followed to avoid corrosion and dulling of instruments^[19].

Apart from improving methods of disinfection and sterilization, improved knowledge, attitude and practice regarding infection control is needed among healthcare workers^[20] especially orthodontists who have traditionally been laxer with regards to infection control^[21-23].

5. Conclusion

This study concludes that 2% Glutaraldehyde solution, quaternary ammonium compound based wipes and foam spray were found to be effective with regards to disinfection of orthodontic pliers contaminated with Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus salivarius. However one of the limitations of our study was that we were unable to determine which disinfectant protocol was most effective. Hence we suggest a follow up study to compare which of the three disinfectants is most effective as the present study indicates that there is a statistical significance with respect to the effectiveness between the three disinfectant protocols.

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7. Conflict of Interest

Nil

8. References

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