Comparison of three different sterilization and disinfection methods on orthodontic markers

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ABSTRACT

Background: Marking pencils which are frequently used in orthodontics may cause microbial contamination. The purpose of this study was to evaluate and compare the effectiveness of three disinfection and sterilization methods (autoclave, glutaraldehyde solution, and Deconex spray) on orthodontic markers.
Materials and Methods: One hundred and twenty orthodontic markers were divided into four groups each 30 pencils: One control group and three groups for three different disinfection/sterilization methods. To evaluate the effectiveness of these methods, pencils were initially contaminated by common pathogen by immersing the pencils in a suspension containing 1.5×10^8 CFU/ml organisms. Then, the pencils were subjected to corresponding disinfection/sterilization methods, and the number of remaining microorganisms was calculated and compared with control group.
Results: In the control group, the mean number of *Escherichia coli* was significantly higher than the other two microorganisms (P = 0.01, P = 0.031). However, the mean numbers of *Staphylococcus aureus* and *Candida albicans* were not significantly different (P = 0.1). After sterilization with autoclave and glutaraldehyde, no microbial growth was observed, whereas after disinfection with Deconx spray some colonies of microorganisms still could be observed.
Conclusion: Autoclaving and glutaraldehyde solution are the best methods for disinfecting orthodontic markers.

Key words: Infection control, microbial contamination, orthodontics marker

INTRODUCTION

The purpose of the infection control is to minimize the risk of transmitting the disease from the patient to dentist, the dentist to the patient, from patient to patient and dental personnel, family, and finally to society. The nature of many dental procedures is so that the specific methods must be taken for preventing transmission of infection among dental personnel and patients. Since all infected patients cannot identified according to disease history, clinical examination and laboratory tests, all patients should be considered infectious, and avoidance contact with blood and body fluids from all patients should be seriously implemented. Orthodontics is the branch of dentistry that compared with other fields has minimal contact with the blood, however, when placing and removal of fixed appliances and forming wires or replacing of chains, ligatures, springs and modules, contact with the saliva of the patient is mandatory.[1] In practice, orthodontists generally focus their attention on the sterilization of pliers, headpieces, and other instruments.[2-5] Orthodontic marking pencils are not usually considered as a possible vector in the chain of infection.[6] These pencils are

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frequently used to mark on the archwires in the oral cavity when orthodontist decides to place a bend or attach a hook on them. Therefore, these markers may contaminate with saliva and transmit pathogens between patients. In this regard, several studies have been performed.[7-9] However, few reports have been published on the permanent marker (PM) pens and pencils. Ascencio *et al*. stated that marking pencils can transfer bacteria from contaminated archwires, and they used gas sterilization which is effective in killing bacteria but is also costly and difficult, making it impractical for orthodontic clinics.[6] Tadiparthi *et al*. also demonstrated that marker pens can act as fomites for nosocomial infection. Furthermore, it was proved that dry whiteboard markers and PM pens carry a significant risk of transmitting infection among patients, and they suggested using disposable markers for immunocompromised patients to prevent cross infection.[10] Thomas *et al*. concluded that marking pens may transmit pathogens from one patient to others.[8]

According to this documentation, the aim of this study was to evaluate and compare the effectiveness of three disinfection and sterilization methods (autoclave, glutaraldehyde solution, and Deconex spray) on orthodontic markers.

MATERIALS AND METHODS

Three common pathogen - a Gram-negative rod (*Escherichia coli*), a Gram-positive cocci (*Staphylococcus aureus*), and a fungus (*Candida albicans*) were grown to logarithmic phase in trypticase soy broth. One hundred and twenty marking pencils (White Wax Marker, Dentaurum, Germany) were classified into four different groups including a control group and three test groups (autoclave, glutaraldehyde 2%, and Deconex spray), so each group consisted of 30 pencils. Initially, suspensions with a concentration of 1.5×10^8 CFU/ml of organisms were prepared in liquid medium equal to 0.5 McFarland standard (Remel™, Thermoscientific, Lenexa, KS, USA). The optical density was 0.132 at 600 nm wavelength.[2]

Control Group

Within each 10 sterile test tubes, 4 ml of each microbial suspension poured pencils were immersed in bacterial suspensions. The pencils were air dried then the bacteria were washed and harvested by placing these pencils in 4 ml of sterile normal saline and by vigorous agitation. Ten microliters of this normal saline were inoculated on culture media and incubated at 37°C. After 24 h, the colonies were counted, and the numbers of remaining organisms were calculated per ml.

Autoclave Test Group

Similar to the control group, contamination step was performed and after 5 min, pencils air dried and autoclaved for 15 min at 121°C. Then pencils were placed in 4 ml of sterile saline and bacteria were washed and gathered by vigorous agitation. The numbers of bacteria were calculated by culturing it culture media.

Deconex Test Group

Similarly, contamination steps were performed and after 5 min, pencils were sprayed by Deconex (Borer, Switzerland) and after 15 min pencils were washed in 4 ml of sterile saline and bacteria harvested by vigorous agitation, and the number of remaining bacteria was counted.

Glutaraldehyde 2% Test Group

Similar to above steps the test procedure were performed, but the pencils were sprayed by glutaraldehyde 2% (Behsa, Iran) and after 30 min the pencils were washed in 4 ml of sterile saline and bacteria harvested after vigorous agitation. The number of bacteria was calculated similarly.

RESULTS

In this study, three different methods of sterilization/disinfection (autoclave, Deconex solution, glutaraldehyde) for marking pencils were evaluated. Three type of microorganism (*E. coli*, *S. aureus*, *C. albicans*) were used in our four groups (one control and three experimental groups).

In two groups (autoclave and glutaraldehyde), no microorganism was remained after sterilization/disinfection procedure. Therefore, these groups were considered complete and fully sufficient.

Control Group

In the control group, the mean population of bacteria and *C. albicans* which settled after contamination was significantly different. Tukey test showed that the mean number of *E. coli* was significantly greater than *C. albicans* and *S. aureus* ($P = 0.01$, $P = 0.031$, respectively) There was no significant difference between the population of *S. aureus* and *C. albicans* ($P = 0.1$) [Table 1]. This shows that *E. coli* could contaminate markers much greater than Gram-positive bacteria and fungi.

Deconex Group

In this group, similarly Mann–Whitney U-test confirmed that the mean number of *E. coli* which settled on pencils was significantly greater than the mean number of *C. albicans* and *S. aureus* ($P = 0.026$, $P = 0.003$) and there was no significant difference between the number of *S. aureus* and *C. albicans* ($P = 0.125$) [Table 2].

As it could be seen in Table 3, the numbers of bacteria and *C. albicans* after treatment with Deconex were reduced compared to control group.

As the ideal sterilization/disinfection method should reduce the number of bacteria to zero. In the control group, the numbers of three microorganisms were significantly greater than zero [Table 4].

In Deconex group, the numbers of three microorganisms were not significantly greater than zero [Table 5]. Hence,

Table 1: The number of bacteria in control group

Bacteria	Number	Mean	SD	Minimum	Maximum	Result (ANOVA) (*P*)
Escherichia coli	10	163.4	142.694	43	500	0.029
Staphylococcus aureus	10	61.3	32.225	38	150	
Candida albicans	10	82	13.960	56	100	

SD – Standard deviation; ANOVA: Analysis of variance

Table 2: The number of bacteria in Deconex group

Bacteria	Number	Mean	SD	Minimum	Maximum	Result Kruslkal–Wallis (*P*)
Escherichia coli	10	10.9	19.301	0	60	0.004
Staphylococcus aureus	10	0.2	0.632	0	2	
Candida albicans	10	1.2	4.909	0	15	

SD – Standard deviation

Table 3: The comparison of bacteria between Deconex and control group

Bacteria	Group	Number	Mean	SD	Result independent *t*-test (*P*)
Escherichia coli	Control	10	163.4	142.694	0.008
	Deconex	10	10.9	19.301	
Staphylococcus aureus	Control	10	61.3	32.225	<0.001
	Deconex	10	0.2	0.632	
Candida albicans	Control	10	82	13.960	<0.001
	Deconex	10	2.1	4.909	

SD – Standard deviation

Table 4: The comparison of bacteria in control group with zero

Bacteria	Number	Mean	SD	Result one sample test (*P*)
Escherichia coli	10	163.4	142.694	0.006
Staphylococcus aureus	10	61.3	32.225	<0.001
Candida albicans	10	82	13.960	<0.001

SD – Standard deviation

Table 5: The comparison of bacteria in Deconex group with zero

Bacteria	Number	Mean	SD	Result one sample test (*P*)
Escherichia coli	10	10.9	19.301	0.108
Staphylococcus aureus	10	0.2	0.632	0.343
Candida albicans	10	2.1	4.909	0.209

SD – Standard deviation

using Deconex is not an appropriate method for disinfection markers. Figure 1 showed the mean number of microorganisms in groups.

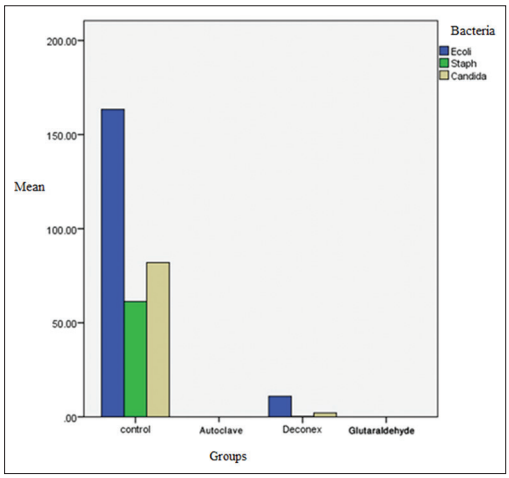

Figure 1: The number of bacteria in each groups

DISCUSSION

This study compared the efficacy of three disinfection/sterilization procedures on marking pencils contaminated with *E. coli*, *S. aureus,* and *C. albicans*. The results of the study showed that after disinfection of marking pencil by autoclave and glutaraldehyde solution all the microorganisms were killed while in disinfection with Deconex all three microorganisms had a little growth that was not significant. Based on the result of our study, all three procedures were acceptable for disinfecting of marking pencils in orthodontic offices.

Woo *et al*. evaluated the compliance with infection control procedures among California orthodontists and they concluded that orthodontists still need improvement in all aspects of their infection control procedures.[11] Ascencio *et al*. evaluated the effect of different disinfection procedures on contaminated marking pencils. The tip of pencils was wiped with either sterile gauze or gauze treated with IodoFive disinfectant. They concluded that only gas sterilization completely killed bacteria which are an expensive procedure. However, the pencils were used in this study were not autoclavable.[6] In our study, glutaraldehyde solution completely destroyed microorganisms, and this disinfectant can be used instead of gas sterilization. Terzic *et al*. evaluated the efficacy of autoclave in the sterilization of surgical marking pencils and they reported that no microorganisms were cultured after using autoclave which was in agreement with the results of our study.[12] Venkatasubramanian *et al*. compared the efficacy of four different disinfection methods on endodontic files. They used *Bacillus stearothermophilus*. They reported that

autoclave and CO_2 laser carried out sterilization completely while glutaraldehyde was not able to sterilize endodontic files completely. In our study, glutaraldehyde showed acceptable disinfection characteristics and this difference could be as a result of different types of bacteria, different solutions, and different subjects who were evaluated.[13] Camilla *et al*. evaluated the effect of different disinfection methods on orthodontic pliers. They reported that glutaraldehyde was an acceptable disinfectant agent which it was in agreement with our study.[14]

Parnia *et al*. examined the effect of different disinfecting agents on contaminated impression materials. They reported that Deconex was an acceptable disinfecting agent which was similar to our study.[15] It should be noted that every patient should be considered infectious. Therefore, this study was performed on contaminated markers to examine which infection control procedures are the best for marking pens and pencils in orthodontic clinics. However, the results do not reflect accurately the extent to which the sterilizing and disinfecting methods are reliable methods to be applied in-clinic.

CONCLUSION

Based on our result, autoclave and glutaraldehyde solution were the best methods for disinfection of orthodontic marking pencils. The remained bacterial contamination after disinfecting by Deconex solution was no significant, and therefore, this agent could be a proper substitute if the two aforementioned methods were not available.

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Conflicts of Interest
There are no conflicts of interest.

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