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Study of Microbes on Tried-In Bands after Sterilization Using Current Protocols

Ujwala Paruchuri[1*] Triveni Venkatraman[2] Puvvula Navya[3] Y.N. Sasidhar[4] N. Satish Varma[5] Preetam Ravuri[6]

[1]Reader, Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.
[2]Orthodontics Practitioner, United Kingdom.
[3]Reader, Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.
[4]Professor, Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.
[5]Professor, Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.
[6]Senior Lecturer, Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.

ABSTRACT

Background: Molar bands are the most commonly used attachments on posterior teeth till date. Preformed bands are preferred as they save a lot of time. A possible concern with using such bands is the lack of cross infection control as several bands may have to be tried on the patient's teeth before finding the perfect match. This study was undertaken to compare the efficiency of the techniques in sterilization of tried-in orthodontic bands in a clinical situation and to study the growth of microbial cultures on such bands.100 bands to be used on 25 patients in all the four quadrants were sterilized in an autoclave and checked for microbial culture which was negative. They were then tried on the teeth in the subjects and rinsed thoroughly with water for three minutes. The bands were divided into groups of 30 each and sterilized using three different techniques, namely, chemical sterilization with Korsolex, hot air oven and autoclaving. They were then cultured in BHI broth followed by subculture in Mac Conkeys medium and blood agar for 48 hours to check for microbial growth. No microbial growth was seen on the bands of hot air oven group. It was found that the proportion of growth in the Korsolex group was significantly higher than the hot air oven group. ($p>0.05$) Subcultures of the groups where growth was seen showed the presence of Pseudomonas (72%), Staphylococcus aureus (18%) and Klebsiella (9%).

It was concluded that though cold sterilization is one of the cost and time effective methods, it has to be followed with correct dilution and time of immersion. Hot air oven proved to be the best method to sterilize tried-in bands.

Keywords: Molar bands, microbes, orthodontics.

INTRODUCTION

"Nothing worthwhile ever departs'. In spite of the tremendous developments in bonding; molar bands still are commonly used to retain orthodontic attachments on posterior teeth. Previously used stainless steel bands were anatomically crude & difficult to adapt to the teeth with out creating cement lines. Their popularity grew as manufacturers developed improved chrome alloys that allowed better adaptation. In the late 1960's Washbon introduced a molar band design that has remained the industry standard until now.[1] Today's bands have a reasonable performance record, but first molars still show the highest incidence of band failure. One reason is the convergence of forces of the 3 strongest muscles of mastication, namely the masseter temporalis, & internal pterygoids in the area of first molars & 2nd bicuspids.[2] For the same reason, bonding to 1st molars has enjoyed limited

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***Correspondence Dr. Ujwala Paruchuri.**
Department of Orthodontics, GITAM Dental College and Hospital, Visakhapatnam, India.
Email: Not Disclosed

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success. Using stronger adhesive may cause enamel damage or fracture. Preformed bands save a lot of time & work for the practitioners. They have been used by orthodontists for nearly half a century. When using preformed bands, due to variation in the size of the teeth, it is usually necessary to try in several bands before the appropriate size is selected.

A possible concern with using such bands is the lack of cross infection control. They come in contact with not only to saliva, but also microorganisms of the periodontal sulcus & blood stream. The same pathogens that find their way onto an endodontic file or periodontal probe may also find their way onto an orthodontic band. According to the classification outlined by Shaefer[3] (University of southern California school of dentistry), preformed bands are classified as semicritical instruments that touch the mucosa and they should be sterilized before reusing them. For protection of both the doctor & patient, sterilizationtechniques are of utmost importance in preventing the spread of infectious disease. This is of special significance in dentistry
because more microorganisms are found in the oral cavity than in any other part of the body. Matasa[4], in his study on the organisms present in plaque around orthodontic appliances found a variety of aerobic & anaerobic microorganisms. The analysis showed the presence of fungi & microbes belonging to different species of Streptococci, Staphylococci bacilli, Diphthe

With increasing number of adult patients & diverse lifestyles, orthodontists should employ proper sterilization
techniques in preventing the spread of infection & guard against their transfer. Sterilization destroys all microorganisms including viruses & spore forms & usually involves the use of heat. Disinfection destroys most microorganisms but not necessarily resistant spore forms. According to Thomson & Bogues[5] study on various sterilization methods, the descending order of usage of various techniques among orthodontists are cold disinfection, steam autoclave, Wash & scrub only, Central sterilization & Flaming in alcohol. Cold sterilization is the most common type of method used in orthodontic offices, but its effectiveness varied greatly with changes in pH and with presence of organic debris & saliva. The most frequently used liquid chemical agent is Gluteraldehyde. It provides a high level of disinfection in 10 minutes but it is caustic & has the possibility of causing skin sensitivity. Commercially available form of Gluteraldehyde is Korsolex. Korsolex contains a combination of Gluteraldehyde & commercially bound Formaldehyde. Mixtures of Gluteraldehyde & Formaldehyde are found to be 10 times more effective than either of the chemicals alone.

Heat is the most reliable method of sterilization. It can be applied as dry heat or moist heat. The killing effect of dry heat is due to protein denaturation, oxidative damage & toxic effect of elevated levels of electrolytes[6]. Holtz[7] reported no bacterial growth of any type orthodontic instruments & bands in cassettes, after having been processed through hot air oven.

The lethal effect of moist heat is due to denaturation & coagulation of protein & its high penetrative power.[8] The advantage of steam lies in the latent heat liberated when it condenses on a cooler surface, raising the temperature of the surface. Many studies (Holtz & Fulford)[7,9] have reported decontamination of tried-in orthodontic molar bands by autoclaving.

The chemical sterilization, dry heat & autoclave are some of the common sterilization techniques present in all orthodontic offices. This study was undertaken to compare the efficiency of these techniques in sterilization of tried-in orthodontic bands in a clinical situation.

MATERIALS AND METHODS

The experimental sample comprised of 100 teeth to be banded in 25 patients. 100-preformed 3M UNITEK bands were procured and were autoclaved as received. Age group of the selected subjects ranged from 25-35years, with all four first molars present and had a plaque index of 2 or 3 according to Silness & Loe.[10] Sterilization of preformed bands: The 100 bands were wrapped in sterile gauze and sterilized in autoclave at 240° with 15 Pounds pressure for 40 minutes. Efficacy of the sterilization process with autoclave was checked periodically with standard indicators. 10 samples were randomly picked & cultured in BHI broth to check the efficacy of sterilization. The broth was incubated at 37°C for 2 days & checked for growth. No growth

was seen in any of the samples. Hence the autoclaved preformed bands were considered sterile & taken up for the study.

The autoclaved preformed orthodontic bands were tried in the patient's mouth and then washed thoroughly in running tap water for 3 minutes & then rinsed with distilled water for 1 minute. These bands were then sterilized using 3 different sterilization techniques.The 3 methods of sterilization used for the study are chemical disinfection using Korsolex, hot air oven & autoclave.

Korsolex:

30 bands which were tried in patient's mouth were washed under running tap water followed by a thorough
rinsing with distilled water were transferred with a sterile forceps into 5% diluted korsolex and left for 30 min following which were transferred into sterile containers procured from Artek laboratory with a sterile forceps and were sent to the microbiology laboratory for culture.

Hot-air oven:

30 bands which were tried in patient's mouth were washed under running tap water followed by a thorough rinsing with distilled water, were kept in hot-air oven in a glass bottle & heated to 320° F for 1 hour. Indicator strips were used to check the efficacy of hot air oven. Following the sterilization cycle these bands were transferred to sterile containers procured from Artek laboratory with a sterile forcepsand was sent to the microbiology laboratory for culture.

Autoclave:

30 bands which were tried in patient's mouth were washed under running tap water followed by a thorough rinsing with distilled water, were wrapped in sterile gauze and kept in a kidney tray for autoclaving. Steam sterilization uses saturated water vapour at 240° F with 15 pounds of pressure for 15 – 40 min.

Microbial culture:

The bands received were cultured in BHI broth at 37°C for 24 hours followed by subculture if growth is seen. Subculture was done using nutrient agar, Mac Conkeys medium and blood agar for 48 hours. After 48 hours, the plates were observed for the growth of organisms and the results were noted and tabulated

Statistical analysis:

Pearsons Chi-Square test was used to calculate the P value which is about 0.006.

Table 1: Results of culture in BHI for the 3 methods of sterilization.

Growth	Hotair oven (M1)	Autoclave (M2)	Korsolex (M3)
Present	0	3	8
Absent	30	27	22
Total sample size	30	30	30

Table 2: The significant groups at 5% level were.

Comparison of groups	P — value	Significant/non significant
M1 Vs M2	0.24	Not significant
M2 Vs M3	0.18	Not significant
M1 Vs M3	0.005	Significant

Inference: the proportion of growth in M3 i.e Korsolex is significantly higher than M1 (0.005). However no other contrasts are statistically significant. (P>0.05).

Results of subculture in Mac Conkey, Nutrient agar & Blood agar:

Sub culture of the autoclaved samples:

Sample — 1

Gram -ye rods, Indole +ve, Citrate —ve, TSI non-reactive, Oxidase +ve. Organism — Pseudomonas.

Sample — 2

Mucoidal, Indole +ve, Citrate —ye, TSI non-fermentive, Oxidase +ve. Organism — Pseudomonas.

Sample — 3

Mucoidal, grey irregular, Indole +ve, Citrate +ve, TSI non-fermentive, Oxidase +ve. Organism — Pseudomonas.

Sub culture of the Korsolex samples:

Sample — 1

TSI, Citrate — non reactive, Indole —ye, Oxidase +ve,ahemolysis colony. Organism- Pseudomonas.

Sample — 2,3,4,5

Gram -ve rods, Indole -ve, Citrate —ve, TSI non-reactive, Oxidase +ve. Organism — Pseudomonas.

Sample —6,7

Coagulase +ve, catalase +ve. Organism — Staphylococcus aureus.

Sample — 8

TSI, Citrate — non reactive, Indole —ve, Oxidase +ve. Gram —ve bacteria. Organism- Klebsiella.

Table 3: Tabulation based on organism sub- culture:

Pseudomonas	Staphylococcus aureus	Klebsiella
8	2	1
72%	18%	9%

DISCUSSION

The results of the study indicate complete absence of growth in all the 30 samples tested with hot air oven. Among the samples tested with autoclave 3 exhibited growth while 27 had no growth. Both the above methods had no statistically significant growth. The results of the Korsolex group indicated presence of growth in 8 samples, which was statistically significant when, compared with hot air oven. Dry heat is well-suited method of sterilization and is shown to be most effective in various studies. According to Rubo[11], dry heat causes oxidative destruction of bacterial protoplasm and it is effective against spores also. Similar results have been reported by Hohlt and Miller[7] on sterilization of bands. The efficacy of the auto clave has been well documented and no statistically significant growth was exhibited in the present study by autoclaving. However 3 samples exhibited growth. The results of the subculture of these 3 samples indicate the presence of Pseudomonas. 8 samples indicated growth in Korsolex. On subcultures, 5 samples indicated the presence of Pseudomonas. Thus Pseudomonas was present in 72% of the subcultures. Pseudomonas is an aerobic, nonsporing, gram -ve bacilli & it is an important cause of hospital infetions. It is a nonspecific opportunistic microbe present in the oral flora commonly contaminating the dental offices". It is known to exhibit high degree of resistance to antiseptics & disinfectants. Hence this could explain the presence of this organism in the present study. Pseudomonas is also shown to survive best in wet environment, which explains the presence of organism in the autoclaved +ve samples.

The organism subculture indicated an 18 % of Staphylococcus aureus (a total of 2 samples). Staphylococci are extremely hardy organisms & survive in adverse environment for a long time. They are non-sporing, gram +ve cocci present occasionally in the mouth, which are highly resistant to the action of disinfectant. Thus, two samples sterilized in korsolex demonstrated the growth of these organisms. Pseudomonas & Staphylococcus have been identified in and around orthodontic appliances in a study by Matasa. 9% of the subculture samples indicated the presence of Klebsiella. Klebsiella is the 2nd most populous member of the aerobic bacterial flora of the intestine & also an important cause of nosocomial infections. Staphylococcus was also identified as the most common bacterial species by Barker et al in their study.[12]

5% solution of korolex recommended for standard disinfection was used in the present study. The sterilization might be much more effective if the emergency disinfection percentage that is 10 % or the immersion time of 1 hour is followed. However the efficacy has to be evaluated & appropriately applied to ensure effective sterilization.

SUMMARY AND CONCLUSION

This study highlights the importance of sterilization of tried-in orthodontic bands. Though cold sterilization is one of the cost & time effective methods, it has to be followed with proper attention to dilution & time of immersion. The organisms identified are Pseudomonas, Staphylococcus & Klebsiella, which are known to have definite pathogenic effects on entering the blood stream. As orthodontic bands are frequently exposed to saliva & blood, the importance of sterilization of these bands is significant.

Further research is required to investigate the efficacy of sterilization techniques to reduce occupational hazards & provide optimal infection control in orthodontic office.

1. Sterilization in hot air oven & autoclave showed no significant growth & hence, can be chosen as a method of sterilization of tried-in preformed bands.
2. 5% korsolex for 30min demonstrated significant growth hence; cold sterilization should be used with caution.
3. Subcultures indicated 72% of Pseudomonas, 18 % of staphylococcus aureus, & 9% of Klebsiella.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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