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Research Report

Effectiveness of various sterilization methods of contaminated post-fitted molar band

Anggia Tridianti, Krisnawati and **Nia Ayu Ismaniati**
Department of Orthodontics
Faculty of Dentistry, Universitas Indonesia
Jakarta-Indonesia

ABSTRACT

Background: *Molar band as anchoring device may be contaminated during the fitting process. Thus, decontamination process is essential to prevent cross-infection between patients.* ***Purpose:*** *The objective of this research was to determine the amount of bacteria in molar band post-fitted on the patient teeth, after previously undergone pre-sterilization using alcohol and ultrasonic cleaning bath followed by sterilization using dry heat oven and steam autoclave, in order to find the best method in decontamination of post-fitted molar band.* ***Methods:*** *Four molar bands which already fitted on one patient then divided evenly into two groups. The first group was pre-sterilized using alcohol. One of the bands then sterilized using dry heat oven, while the other one was sterilized using steam autoclave. The second group was pre-sterilized using ultrasonic cleaning bath. One band from this group then sterilized using dry heat oven and the other was sterilized using steam autoclave. The next step was to immerse all the bands in a phosphate-buffered saline solution. Using micropipette, the solution was retrieved and dropped upon a petri dish containing Brain Heart Infusion broth. The dish was then stored in an incubator for 24 hours, prior to counting the number of bacteria existed. The same methods were used to the rest of the patients, with total 128 molar bands from 32 patients.* ***Results:*** *There was a profound difference in numbers of bacteria found between those methods of sterilization. However, there was a non significant difference between the two groups which were at the alcohol-steam autoclave group and at the ultrasonic cleaning bath-steam autoclave group.* ***Conclusion:*** *This study showed that steam autoclave is better than for sterilizing molar band, as it left the minimal amount of bacteria in post-fitted molar band.*

Key words: *Molar band, dry heat oven, steam autoclave, sterilization*

ABSTRAK

Latar belakang: *Molar band merupakan suatu alat penjangkaran yang dapat mengalami kontaminasi selama proses fitting band, sehingga perlu dilakukan suatu proses dekontaminasi untuk menghindari terjadinya cross-infection pada pasien.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengetahui perbedaan jumlah bakteri pada molar band pasca fitting band setelah sterilisasi dry heat oven dan steam autoclave yang sebelumnya telah dilakukan pre-sterilisasi alcohol dan ultrasonic cleaning bath, sehingga dapat ditentukan metode sterilisasi yang terbaik dalam dekontaminasi molar band.* ***Metode****: Empat molar band yang telah melalui proses fitting band pada seorang pasien dibagi dalam dua kelompok. Pada kelompok pertama, dua molar band dilakukan pre-sterilisasi dengan alkohol, kemudian satu band dilakukan sterilisasi dengan dry heat oven dan satu band lainnya dengan steam autoclave. Kelompok kedua, dua molar band dilakukan pre-sterilisasi dengan ultrasonic cleaning bath, kemudian satu band dilakukan sterilisasi dengan dry heat oven dan satu band lainnya dengan steam autoclave. Molar band tersebut masing-masing kemudian dimasukkan ke dalam cairan phosphate-buffered saline, dengan micropipette cairan diambil dan dituangkan ke cawan petri yang berisi Brain Heart Infusion. Kemudian dimasukkan ke dalam inkubator selama 24 jam dan dihitung jumlah bakterinya. Metode yang sama dilakukan terhadap molar band lainnya, dengan total 128 molar band dari 32 pasien.* ***Hasil:*** *Terdapat perbedaan jumlah bakteri yang bermakna antara beberapa kelompok metode sterilisasi dan terdapat satu kelompok dengan perbedaan tidak bermakna, yaitu kelompok alcohol-steam autoclave dengan ultrasonic*

cleaning bath-steam autoclave. ***Simpulan****: Hasil penelitian menunjukkan bahwa steam autoclave merupakan metode sterilisasi yang terbaik pada molar band yang telah melalui proses fitting band karena menunjukkan jumlah bakteri yang paling minimal.*

Kata kunci: *Molar band, dry heat oven, steam autoclave, sterilisasi*

Correspondence: Anggia Tridianti, c/o: Departemen Ortodonsia, Fakultas Kedokteran Gigi Universitas Indonesia. Jl. Salemba Raya 4 Jakarta 10430, Indonesia. E-mail: myaidanna@yahoo.com

INTRODUCTION

Stainless steel molar bands are often selected as an component anchorage in orthodontic treatment, particularly if difficulties encountered when using bucal tubes which have to be bonded on the surface of the molar teeth. Thus, it may be detached accidentally from its place due to pressure of chewing.[1] In determining the suitable molar band, the process is often had to be carried out several times to find an appropriate size.[1,2]

Molar bands are quite expensive, thus orthodontists choose not to throw it out if the size does not fit to a patient, as it may be suitable for others. In the process of fitting, the molar bands usually come in contact with contaminated saliva or blood, that caused by the injury to the gums on the subgingival area. These areas contain the anaerobic gram-negative bacteria, such as *Porphyromonas gingivalis* and *Actinobacillus actinomicetemcomitans*.[2,3] While supragingival plaque composed of gram-positive bacteria, such as *Streptococcus sanguis, Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius* and *Lactobacillus.* These bacteria play an important role in dentistry, as they are sources of mouth diseases, such as caries and periodontitis.[4]

Inadequate cleaning process and sterilization may result in remaining of potentially harmful blood-borne agents or contaminants on the orthodontic band surface. The contaminated molar bands can lead to a spreading of diseases or causing cross-infection to other patients, such as subacute bacterial endocarditis, herpes, hepatitis B, hepatitis C and HIV. Such diseases have a high mortality rate, thus it is necessary to do preventive action to avoid transmission of pathogenic microorganisms from infected individuals.[5]

Sterilization is a process of destroying all forms of life, including spores. On the other hand, disinfection is a process to destruct most of microorganisms but it does not include spores. Usually, this process needs a solution like phenol, alcohol, chlorine or iodine that is applied to the instrument.[6,7] Health workers should always use personal protective equipment (PPE) such as disposable gloves, masks and goggles. It has to be done as a form of infection control so there would be no cross-infection to other patients.[6,8]

There are three stages in the decontamination process, which are pre-sterilization stage, sterilization stage and storage stage.[9,10] Recommendation for the initial cleaning process include removal the contaminant by hand, the use of disinfectant enzyme-based cleaning solution or alcohol, the use of instrument washer such as ultrasonic cleaning bath, and then followed by drying it using hot air or a sponge, this step is important to avoid any damages on the instruments during the sterilization process. Methods of sterilization can be done using steam autoclave, chemical, dry heat ovens, boiling water, salt or glass bead sterilizer, and hyperbaric gas (ethylene oxide) sterilization.[6,10,11]

According to surveys, most of orthodontists in the United Kingdom always clean and disinfect the band prior to reuse on other patients. Due to the availability of various types of sterilization method, until today there is no reliable method to deliver the best results in decontamination.[9] The purpose of this research is to find the best method in decontamination of post-fitted molar band.

MATERIALS AND METHODS

On this research, there were 128 molar bands that came from 32 patients where each of them used 4 bands. The four molar bands that came from the first patient divided evenly into two groups. The first two groups were pre-sterilized using alcohol, one molar band then sterilized using dry heat oven at 150° C for 20 minutes (group A), while the second one was using steam autoclave with a temperature at 130° C for 1 hour (group B). The last two groups were pre-sterilized using ultrasonic cleaning bath, one molar band then sterilized using dry heat oven at 150° C for 20 minutes (group C), and the other one was applying steam autoclave at 130° C for 1 hour (group D).

The molar bands in all groups were soaked in a medicine bottle containing 5 ml phosphate-buffered saline (PBS), then they were inserted into a shaker for 30 minutes, and left at room temperature for 15 minutes. Ten µl of PBS was taken with a micropipette and poured onto a petri dish, contained with Brain Heart Infusion agar. The PBS solution was then swiped to spread it out evenly. The dish was then placed into an incubator for 24 hours with CO_2 in it. The same methods were also used to the other 31 patients. The number of bacteria that appeared on the media was then calculated.

The researcher also calculated the bacteria from 5 new molar bands that were taken straight out of the box and 5 molar bands that have gone through random fitting process, without applying any decontamination process. These procedure were conducted to examine the amount of bacteria from new molar band before fitting and the amount of bacteria prior to decontamination.

In addition of this research, another examination has been applied on randomly taken molar bands, to determine availability of gram positive or negative bacteria from the molar bands. This examination was performed by staining the bacteria using crystal violet 10% liquid and self-ranin liquid. The bacteria were then viewed under microscope.

Data that have been obtained were processed to see its normality. By using the Shapiro-Wilk test, abnormal distribution can be observed. The Kruskal-Wallis test was also performed to see if all values have significant difference between each other. To examine comparative difference between two groups, the Mann-Whitney tests was done.

Table 1. The number of bacteria from molar band before contaminated and prior to decontamination

No	5 new molar band taken out of the box (CFU/ml)	5 molar bands taken from 5 randomized patients who have not been through decontamination (CFU/ml)
1	1	45
2	1	32
3	0	49
4	2	41
5	1	38

RESULTS

From 5 new molar bands that were taken directly from the box, the number of bacteria that was found was 0 CFU/ ml at the minimum, while at the maximum was 2 CFU/ ml. The number of bacteria on molar bands, which were taken directly from the patients without conducting any decontamination process, at the minimum was 32 CFU/ ml, and at the maximum was 49 CFU/ ml (Table 1).

The results showed significant differences between the groups and there was one group that has a non-significant difference, it was the group which applying method of alcohol-steam autoclave with ultrasonic cleaning bath-steam autoclave (group B-D). Which means, the two groups of sterilization method provides equally good results in the decontamination of bacteria on the molar band (Table 2 and 3).

The bacterial determination on result showed that 4 out of 5 sample were found as Gram negative bacterial (Table 4).

Table 4. Staining the bacteria to determine the gram positive or negative bacteria

Randomly taken sample	Staining results
1	Blue
2	Red
3	Red
4	Red
5	Red

Note: Red: Gram negative; Blue: Gram positive

Table 2. The number of bacteria at minimum, maximum from each method of sterilization

Methods of sterilization	Number of samples	Minimum number of bacteria (CFU/ml)	Maximum number of bacteria (CFU/ml)
Alcohol+dry heat oven (A)	32	10	31
Alcohol+steam autoclave(B)	32	0	7
Ultrasoniccleaning bath+dry heat oven (C)	32	6	18
Ultrasoniccleaning bath+steam autoclave (D)	32	0	6

Table 3. Post-Hoc analysis of the 2 sterilization methods groups were compared

Comparison of two groups of methods of sterilization	*p*
Alcohol-dry heat oven compared to alcohol-steam autoclave (group A-B)	.000*
Alcohol-dry heat oven compared to ultrasonic cleaning bath-dry heat oven (group A-C)	.000*
Alcohol- dry heat oven compared to ultrasonic cleaning bath-steam autoclave (group A-D)	.000*
Alcohol-steam autoclave compared to ultrasonic cleaning bath-dry heat oven (group B-C)	.000*
Alcohol-steam autoclave compared to ultrasonic cleaning bath-steam autoclave (group B-D)	.182
Ultrasonic cleaning bath-dry heat oven compared to ultrasonic cleaning bath-steam autoclave (group C-D)	.000*

* $p < 0.05$ means that there is a difference

DISCUSSION

Among 4 groups that have undergone process of decontamination, the minimal amount of bacteria of 0 CFU/ml, was found in a steam autoclave alcohol group (group B) and the ultrasonic cleaning bath with a steam autoclave group (group D), which showed similar results with the brand new molar bands that were taken out straight from the box. While the maximum number of bacteria, found in the group of alcohol-dry heat oven (group A), that was 31 CFU/ml (Table 2), not much in difference when compared to the minimal number of bacteria on the molar bands which has not been through decontamination process of 32 CFU/ml (Table 1).

There are significant differences between the 2 groups of sterilization method, between group A and B, between group A and C, between group A and D, between group B with C, and between group C with D. While there were no significant differences found between group of alcohol-steam autoclave (group B) with the ultrasonic cleaning bath-steam autoclave (group D). This is due to the two groups using a steam autoclave sterilization method that have minimum number of bacteria 0 CFU/ ml.

Some studies showed that steam autoclave sterilization is the method of choice by health workers because it provides the best results in eliminating all forms of microorganisms.[9-11] According to Dowsing and Benson,[9] most orthodontist in the United Kingdom were using a conventional steam autoclave sterilization as a method for the prevention of cross-infection on the orthodontic instruments. As for the sterilization of fitted-in molar bands, they used different types of steam autoclave. In addition to conventional steam autoclave they also used vacuum-phase autoclave.

There are many different opinions about the best way of sterilizing orthodontic instruments. Some experts argue that dry heat oven is better than steam autoclave, which can cause corrosion on the instrument, thus reducing its effectiveness in cutting the wire, and also cause corrosion on the joint. Vendrell RJ *et al*.,[12] conducted a study to compare the effects of steam autoclave with dry heat oven at ligature cutting pliers, the results of this study showed that both methods of sterilization are equally effective and does not cause rust and corrosion, as long as the orthodontic instruments are made of stainless steel.

In this research, the other examination had been done to determine what kind of bacteria on the molar band if the bacteria are gram positive or negative. Under the microscope, the results obtained from 5 samples there were 4 red colors and 1 blue color. The red color were formed because the bacteria bind to self-ranin liquid which indicates bacteria are gram-negative. While the blue color was formed because the bacteria bind to the crystal violet 10% liquid which indicates the bacteria are gram-positive. Based on these samples the majority of bacteria that have been founded are gram-negative.

The gram-negative bacteria are anaerobic bacteria, commonly found in subgingival plaque, which can lead to periodontitis.[3,4] This finding fits with a research conducted by Huser *et al*,[13] which stated that the usage of molar bands can increase the number of *fusobacterium, spirocheta* and *spirilla,* that are usually found on periodontitis. The steam autoclave sterilization provides the best result in decontamination of post fitted molar band, assuming gram-negative bacteria also. The decontamination prevents cross-infections between patients as in Dowsing and Benson's research.[9] Furthermore, according to BDA Advisory Service[10]and McCarthy *et al*.,[11] the steam autoclave sterilization is the method of choice for orthodontic instrument decontamination. However, this method is not recommended for ligature cutting plier or joint pliers that is not made of stainless steel.

Based on this research, any method of sterilization combined with steam autoclave gave the best result in reducing bacteria, so it can be concluded that effective method in decontamination of post fitted molar band is the steam autoclave.

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