

The Effectiveness of Three Different Methods for Sterilization of the Endodontic Files (An in Vitro Study)

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Abstract

Background: Sterilization is the procedure by which all the vegetative or sport state microorganisms' threats are countered. The main aim of sterilization in the health care field is to eradicate the spread of existing infectious diseases and preventing any new infections. In dentistry, it primarily relates to reuse of instruments to prevent cross-infection and ensure optimal dental care. The complex miniature architecture of endodontic files makes cleaning and sterilization difficult, and some professionals have suggested single use for these instruments. **Aim:** The purpose of this study was to compare the effectiveness of different methods of sterilization for endodontic instruments: Autoclaving without a plastic bag, Autoclaving with a plastic bag, CO₂ laser, Diode laser, and Glass- bead sterilization. **Material and Methods:** The study was performed on 60 endodontic K-files, 21 mm long and size 50, divided into 6 groups, 10 for each, the first group were considered as control. The files contaminated with a homogenous spore suspension of *Bacillus thuringiensis*. Then, the contaminated files of the 6 groups sterilized by different sterilization methods: Autoclave, Autoclave with a plastic bag, glass-bead, Diode laser, and CO₂ laser. The sterilized files washed in 5ml of normal saline and then 0.1ml suspended solution powered in 8cm Petridis of nutrient agar and number of well isolated colonies were counted after 24 hours of incubation at 37 °C. **Results:** The study showed that the files sterilized by autoclave were 99.66 % sterile, while with a plastic bag give a 99.32 %sterile. Those sterilized by glass-bead were 96.74 % sterile and those with CO₂ laser were 93.85 %, and diode lasers were 84.24 % sterile. Statistical analysis of all sterilized groups showed a statistical significant difference between groups regarding their sterilization efficiency. Comparison of the sterilized groups with the control group about their efficacies in sterilization showed that the difference was statistically significant. **Conclusion:** Autoclave, with or without bag, is considered the best sterilizing technique. To achieve one hundred percent of sterilization the time of autoclave must be suitable to allow the killing of all microorganisms. For faster sterilization other methods could be used but with less effectiveness.

Aim:

The aim of this study was to investigate the efficacy of three methods for sterilization of endodontic instruments: Autoclaving (with plastic bags and without), laser sterilization (CO₂ and diode laser) and glass-bead sterilization.

Introduction

Scientifically, the bacteria were first demonstrated in the diseased dental pulp by Miller in 1894. The medical professionals worried and started to look at the infected dental pulp and oral tissue when William (1900) theorized that oral microorganisms could disseminate throughout the body, leading to systemic disease. Microorganisms induce a variety of infections and diseases in the human body and are largely ubiquitous in the nature of the contamination directly or indirectly lead to transmission of infections agents (Miller, 1999). The process of sterilization is designed to provide instruments that are free of microorganisms (Wittgow and Sabiston, 1977). There is no degree of sterilization as the instruments are considered sterile or not sterile. Re-sterilization is a process to reuse dental instruments for another patient without the dangerous of infection spread (Thoma, 1988). Endodontic files are tapered, small, slender instruments with approximately 25mm long and spiral cutting edges for cleaning and shaping of root canals (Morrison and Conrol, 2009). The term sterilization can only be applied to instruments and not to skin, where only antisepsis can be achieved (Sureshandra et al., 2010). Currently three methods are accepted for sterilization of endodontic instruments:

1-glass bead sterilizer: It is a common rapid technique of chair-side sterilization of small hand instruments, particularly endodontic files, in the dental clinic. It is usually used table salt, which consists approximately of 1 % sodium silicoaluminate, sodium carbonate or magnesium carbonate. So it pours more readily and does not fuse under heat. Glass beads can be used instead of salt, provided the beads are smaller than 1 mm in diameter because the larger size is not efficient in transferring heat to the endodontic instruments. The presence of large air spaces between the beads prevents transfer of the heat. The instruments can be sterilized in 5 to 15 seconds at a temperature of 437-465 F (260 °C). Several researchers from the 1950-1970 s achieved sterilization by the bead sterilizer within few seconds; nevertheless, there are no current evidence-based (Peretz 2009).

2-autoclave: It is considered the most efficient method of sterilization due to the high efficiency of moist heat penetration than dry heat. It is useful to kill bacteria and microorganisms in medical equipment such as surgical instruments and else. Generally, steam sterilization denotes heat in an autoclave utilizing saturated

steam under a 15psi pressure approximately to achieve a chamber temperature of at least 121 °C. The time is measured after the material being sterilized reaches 121°C. All instruments are thoroughly washed in antiseptic solutions and wiped clean; they are packed into sterile pouches and vacuum sealed to prevent contamination (Jayanthi et al., 2010).

3-laser sterilization: Carbon dioxide lasers developed by Patal and Whisemant in 1964, it has a wavelength of 10.6mm and falls into the infrared range on the spectrum. CO₂ laser energy is greatly absorbed by tissues that are high in water content. When CO₂ laser is used in focused mode, its energy is dense, and it can perform a fine dissection. As the beam is defocused and widened, its impacts on the tissue change. Instead of a definitive action, the laser ablates the tissue by superficial vaporization of cells and coagulates blood vessels that are smaller than the diameter of the beam. Although, the diode laser comes in different wavelengths, the laser wavelengths of 810, 940, and 980 nm are the most common. The energy from these lasers targets pigments such as hemoglobin and melanin in the soft tissue, as a diode laser has high affinity for the pigments. The energy is delivered by a fiber in contact mode (Patal and Whisemant, 1964). *Bacillus thuringiensis* is a rod-shaped, gram positive and spore forming bacterium, which is highly resistant (American Dental Association, 2009). The main objective of this study was to investigate the effectiveness of various sterilization techniques applied to the used dental instruments including endodontic files.

Material and Methods

The study was performed using 60 endodontic K-files, 21 mm long and size 50. The files are divided into 6 groups, 10 for each group, first group were the control one, and the remaining groups were tested for efficacy of different sterilization techniques: Autoclaving, Autoclaving with plastic bag, Diode laser sterilization, CO₂ laser sterilization and glass-bead sterilization.

All the files included in this study were pre-sterilized in an endodontic instrument box by autoclaving for 30 minutes at 121°C at a pressure of 15 pounds, for standardization. Bacterial Isolates obtained from college of agriculture/University of Kufa were identified to the level of species using the traditional morphological and biochemical tests, according to the methods of (Holt et al., 1994 and MacFaddin 2000). The isolate was confirmed identification tests. To achieve a homogenous spore suspension of *Bacillus thuringiensis*, a tube containing 5ml normal saline was inoculated with a 1ml of spore suspension incubated for three days in 55°C. All the pre-sterilized files were contaminated with *Bacillus thuringiensis* for 5 minutes. After 5 minutes of immersion, the files were transferred to another sterile vacuum tube hood safety with the help of a sterile tweezers, following that, the files were dried in an incubator for 10 minutes at 37°C and stored in an endodontic instrument box till they were sterilized by different methods. The contaminated files were placed in an endodontic instrument box or sterile plastic bags, and subjected to autoclave at 121°C for 15 minutes at a pressure of 15 pounds for the first and the second group respectively. In the third group, the files were wiped for 10 seconds with 2x2 gauzes soaked with surgical spirit and placed in the periphery of the glass-bead sterilizer and sterilized for 45 seconds at 240°C. A special holder was used to hold the handle of the files and change the surface for exposure, while keeping the laser beam at 10 cm fixed distance away from the samples and then irradiated for 3 seconds per surface at 10 watts using CO₂ laser system. The laser beam was moved along the length of the instrument during the 3-second period, in the fourth group. The same for the fifth group except that irradiation was at the same distance and the same time.

After completion of file sterilization, the shaft of the instrument was removed from the handle by means of a sterile autoclave wire cutter and each file was washed in 5ml of normal saline and then 0.1ml suspended solution powered in 8cm Petridis of nutrient agar and number of well isolated colonies were counted after 24 hours of incubation in 37°C. The contaminated files in the control group were put by the same method described above without doing any sterilization.

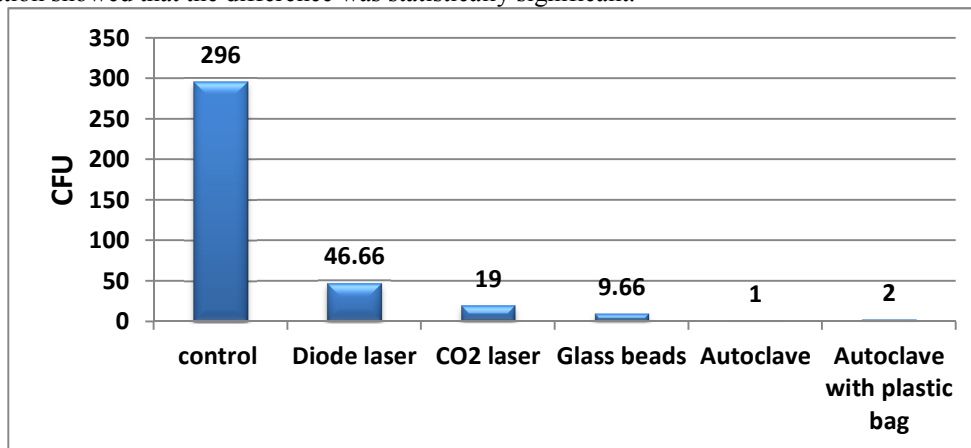
Result

The result showed that the endodontic files sterilized by autoclave in an instrument box at 121°C for 15 minutes at the study pressure of 15 pounds (first group) showed a sterility of 99.66%. These were the higher technique of sterilization. While the second group which sterilized by autoclave in a plastic bag under the same conditions showed 99.32%. The files subjected to sterilization by glass-bead sterilizer after wiping for 10 seconds with a 2x2 gauze soaked with surgical spirit and sterilized for 45 seconds at 240°C (third group) showed a sterilization to the range of 96.74%.

The CO₂ laser sterilization for 3 second per surface at 10 watts and 10 cm distance between the beam and the samples the (fourth group) showed 93.85% only. The (fifth group) of files sterilized by diode laser at the same conditions for the fourth groups showed 84.24%, which is the lowest value of sterility seen between all methods. The control group (sixth group) for which the files after contamination were not sterilized by any method, showed growth in all the plate, that means 0.00% of sterilization.

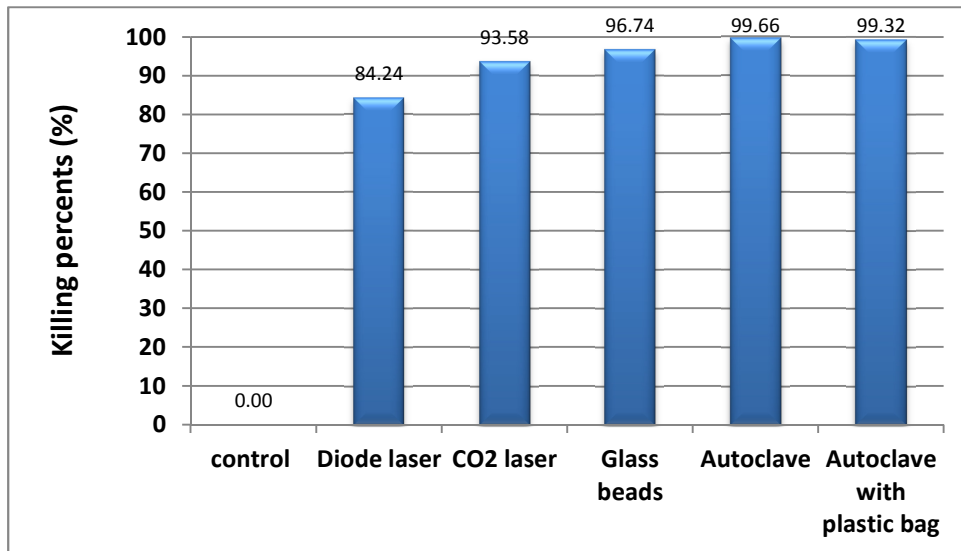
Statistical analysis of all groups showed a statistically significant difference between groups regarding

their efficacies in sterilization. Comparison of the sterilized groups with the control group about their efficacies in sterilization showed that the difference was statistically significant.



LSD (P 0.05) = 1.707

Figure (1): Number of colonies is forming units (CFU) of *B. thuringiensis* spores grown on an agar plate inoculated with 0.1ml of spore suspension prepared from rammer sterilized by different sterilization methods.



LSD (P 0.05) = 10.498

Figure (2): killing percent for *B. thuringiensis* spores by different sterilization methods

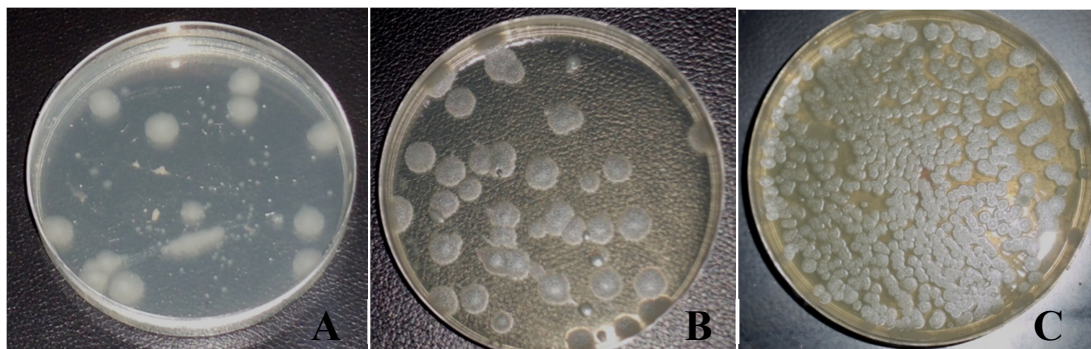


Figure (3): Number of colonies forming units (CFU) of *B. thuringiensis* spores grown on agar plate inoculated with 0.1ml of spore suspension prepared from rammer sterilized by (A) CO₂ laser, (B) Diode laser (C) control treatment.

Discussion

Many methods have been advocated sterilization of endodontic instruments, the spores of *Bacillus thuringiensis*, used to contaminate the files in this study are heat-resistant bacterial spores used in many of the previous researches. Steam autoclaving and glass-bead sterilizers are among the commonly recommended methods of sterilization.

Boyd and Hoeri (1996) stated that moist heat kills microorganisms by coagulation of proteins. However, coagulation occurs only when overkill conditions are attained. Less drastic changes such as inactivation of enzymes, changes in nucleic acids and cytoplasmic membrane alterations probably kill microorganisms before coagulation occurs. The present study indicated that a complete sterilization was possible by autoclaving the instruments in an endodontic box or a plastic bag also give a good result. This is significantly similar to the findings from studies done by other researchers like (Rajkumar and Lakshminarayanan, 2001; Hurtt and Rossman, 1993; Velez et al., 1998). Normally, the autoclave gives 100% sterilization for 45 minutes (Travis and O'Callaghan, 1998). The inability to achieve this percentage in this study might be due to the autoclave time used in this study which is just 15 minutes.

This study also showed that sterilization by glass-bead sterilizer was up to only 96.74% and that total sterility was not found even after sterilizing for 45 seconds at 240 °C. Incomplete sterilization was in the range of 3%, the present study result was contradictory to that of previous research done by Rajkumerand Lakshminarayanan, (2001), but it was the same as that of the research done by (Hurtt and Rossman, 1993) who performed the study with salt instead of glass-beads.

A multitude of factors are to be considered for sterilization of endodontic instruments with laser, the present study used CO₂ laser since it is commonly used nowadays in the dental office for endodontic instruments as researches done by (Nammour and Majerus, 2001 and Hooks et al, 1988). However, the basis of this study used the beam of laser with 10 cm away from the samples, the result gives only 93.58% of sterilization which concluded that increased the distance between the source and the instruments will decrease its effect because this will scatter the radiation. The same for the diode laser which found to be capable of sterilizing at the lowest energy level (84.24%).

Conclusion

To sum up, autoclave, with or without bag, is considered the best sterilizing technique. To achieve one hundred percent of sterilization the time of autoclave must be suitable to allow the killing of all microorganisms. Endodontic instruments should be sterilized effectively before use on different patients to prevent cross-contamination. For faster sterilization other methods could be used but with less effectiveness.

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