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## RESEARCH ARTICLE

# COMPARATIVE EVALUATION OF EFFECTIVENESS OF STERILIZATION OF ENDODONTIC FILES BY DIFFERENT METHODS WITH AND WITHOUT DISINFECTION BY ULTRASONICS

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**ABSTRACT**

**Aim:** To assess and evaluate the effectiveness of sterilization of endodontic files by different methods (Autoclave, Diode laser and Glutraldehyde) with and without disinfection by ultrasonics cleaner.
**Methodology:** The study was performed on 280 K-files to test the sterilization efficacy of autoclave, glutaraldehyde and diode laser with and without ultrasonics. Standard bacterial isolates of *Bacillus stearothermophilus, Enterococcus faecalis, Streptococcus mutans* and fungi *Candida albicans* were obtained and the pre-sterilized files were contaminated. All the endodontic files randomlydivided into seven groups were subjected to sterilization by different methods of sterilization. The test tubes were kept for incubation at 55$^{O}$C for 21 days and checked for growth. Presence of turbidity in a test tube indicated the presence of bacteria and that the particular file was not sterilized completely.
**Results:** It was observed that in Group I turbidity was observed in all test tubes. No turbidity was found in any of the test tubes in Group II a and Group III a thereby indicating total sterility. The files of Group II b showed turbidity in 4 test tubes and Group III b showed turbidity in 2 test tubes (5%). Files of Group II c showed turbidity in 6 test tubes while Group III c showed turbidity in 5 test tubes. The subculture from turbid tubes of various groups were identified by various bacteriological tests.
**Conclusion:** It can be concluded that to achieve one hundred percent of sterilization, autoclave is a suitable method for killing of all microorganisms.



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## INTRODUCTION

Infection control is a major issue in medicine and dentistry because of concern over communicable disease transmitted in health care settings. (Punathil *et al*., 2014) Microorganisms cause a variety of infections and diseases in the human body and are largely ubiquitous in nature. The composition of microflora of root canals has been the focus of considerable research over the years. In the primary endodontic lesions black-pigmented bacteria (BPB) are the species which have frequently been isolated from the infected root canals. Microbiological findings from filled root canal with persistent periapical disease have shown a high proportion of *enterococci*, ranging from 29% to 77%. (Peciuliene *et al*., 2008) Since microorganisms have been shown to be the major cause of endodontic pathology and sterilization of endodontic instruments is a mandatory step for maintaining asepsis in endodontics. (Punathil *et al*., 2014) Over the years, several methods have been used to sterilize endodontic instruments. These include steam autoclaves, dry heat ovens, unsaturated chemical vapor sterilizers, and ethylene oxide gas sterilizers. The American Dental Association recommends that all instruments, burs, mirrors, bands, and other devices used in intraoral treatments be sterilized by one of these four methods. (Punathil *et al*., 2014) Autoclaving is generally accepted as the method of choice to render contaminated instruments safe for reuse. (Vickery *et al*., 2000) Currently steam sterilization using portable autoclaves is recommended for sterilizing dental instruments for use in critical sites but it has been reported that some practitioners are still utilizing high-grade disinfection such as buffered alkaline glutaraldehyde (Gibson *et al*., 1995) although this is now recommended for use in semi-critical areas only. A range of lasers is now available for use in dentistry. diode lasers can be used for a multitude of dental procedures that include soft tissue surgery, periodontal pocket therapy, periimplantitis, but can also be used for certain applications like endodontics, disinfection and laser-assisted tooth whitening. (Pirnat, 2007) Disinfection of instruments also plays a critical role. Ultrasonic offers an increase in shelf life of endodontic instruments. Ultrasonic cleaning consists immersing the instruments in distilled water or ultrasonic solution and subsequently subjecting it to high frequency pulses, which result in specific regions of alternating pressure.

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Steam bubbles are thus formed in the low pressure zones, which ultimately burst in the high-pressure zones thereby creating cavitations that aid in cleaning the file surface. (Peciuliene *et al*., 2008) There has been very little evaluation of the efficacy of cleaning procedures used for contaminated endodontic files. Hence this in vitro study has been under taken with a view to investigate the effectiveness of a technique for sterilizing endodontic files and to find out the efficacy of amethod to obtain optimum sterilization of endodontic files against microorganisms commonly encountered in the root canal system.

## MATERIALS AND METHODS

The study was performed on 280 K-files, 21 mm long and of size 40. All the files included in the study were presterilized and placed in autoclavable endodontic instrument boxes for standardization to eliminate any bias.

**Table 1. Endodontic files divided into groups**

| S.No. | Group | Subgroup | n | Mode of sterilization |
|---|---|---|---|---|
| 1. | Group i | Group I | 40 | Control group |
| 2. | Group ii | Group II a | 40 | Endodontic files sterilized with Autoclave |
| 3. | | Group II b | 40 | Endodontic files sterilized with Diodelaser |
| 4. | | Group II c | 40 | Endodontic files sterilized with Glutraldehyde |
| 5. | Group iii | Group III a | 40 | Endodontic files disinfected in an Ultrasonic Cleaner and sterilized with Autoclave |
| 6. | | Group III b | 40 | Endodontic files disinfected in an Ultrasonic Cleaner and sterilized with DiodeLaser |
| 7. | | Group III c | 40 | Endodontic files disinfected in an Ultrasonic Cleaner and sterilized with Glutraldehyde |

### Bacterial Broth Preparation

Standard bacterial isolates of *Bacillus stearothermophilus, Enterococcus faecalis, Streptococcus mutans* and fungi *Candida albicans* were obtained and used as source for contamination of the endodontic files. To achieve homogenous suspension a test tube containing 5 ml peptone water was inoculated with 1 ml of eachspore suspension &incubated at $55^0$C for 48 hrsat which time period 100% sporulation was achieved. All the pre-sterilized files were contaminated with homogenous suspension of bacterial isolates and fungi in a sterile Petridish for 5 minutes by a standard technique. (Venkatasubramanian *et al*., 2010) After 5 minutes of immersion, the files were transferred to another sterile Petri dish under vacuum hood safety with the help of a sterile tweezer, following which the files were dried in an incubator for 10 minutes at 37°C and stored in endodontic instrument boxes. The contaminated files were randomly divided into 7 groups and arranged in 7 autoclaved endodontic boxes (n=40) using sterile tweezers till they were subjected for predesigned study.

**GROUP I:** control group- 40 files in control group were not subjected to any sterilization and disinfection protocol.

**GROUP II a:** autoclave- The contaminated files (n=40) were placed in an endodontic instrument box and subjected to autoclave at 121ºC for 15 minutes at a pressure of 15 pounds.

**GROUP II b:** diode laser-Representatives (n=40) of this group were sterilized using diode laser. The files were held by handle using a tweezer to 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 laser system.

**GROUP II c:** glutraldehyde (chemiclave) :- The contaminated files in this group were placed for 12 hours in a sterile plastic container containing 2.4% glutaraldehyde at pH=8.3 solution as per manufacturer's guidelines.

**GROUP III a:** sterilization in an autoclave combined with ultrasonic cleaner -Endodontic files (n=40) in this group were subjected to disinfection in an ultrasonic cleaner for 2 cycles of 1 minute each (as per manufacturer's guidelines) followed by autoclave. Ultrasonic cleaning consists of disinfecting the instrument in an ultrasonic cleaner by subjecting it to high frequency pulses for 180s creating cavitations that aid in cleaning the file surface. After ultrasonification, the files were transferred to an endodontic instrument box and subjected to autoclave at 121ºCfor 15 minutes at a pressure of 15 pounds.

**GROUP III b:** sterilization using diodelaser combined with ultrasonic cleaner-Endodontic files (n=40) in this group were subjected to disinfection in an ultrasonic cleaner for 2 cycles of 1 minute each (as per manufacturer's guidelines) followed by sterilization using Diode laser.

**GROUP III c:** sterilization using 2.4% glutraldehyde combined with ultrasonic cleaner-Endodontic files (n=40) in this group were subjected to disinfection in an ultrasonic cleaner for 2 cycles of 1 minute each followed by sterilization using 2.4% glutraldehyde solution (as per manufacturer's guidelines). After ultrasonification, the contaminated files in this group were placed in a sterile plastic container containing 2.4% glutaraldehyde solution and were left in it for 12 hours.

### Subculture and identification

After completion of sterilization of the files by different sterilization modes, the shafts of the files were removed from the handle by means of a sterile autoclaved wire cutter and each file was placed in individualtest tube containing thioglycollate medium with the help of a sterile tweezer for observing the growth of bacteria and fungi. The test tubes were placed in test tube holding stand and labelled with their respective groups and kept for incubation at 37°C for 7 days and subsequently 21 days. Presence of turbidity in a test tube indicated the growth of the contaminated bacteria and or fungi and indicated that the particular file was not sterilized completely. Turbidity comparable with Mc farland (0.5N $BaSO_4$) were observed. The culture isolates were identified by colour morphology, gramstaining using light microscope biochemical tests.

## OBSERVATIONS AND RESULTS

Under the limitations of the study after 21 days following observations were made:

1. The control group (Group I), in which the files after contamination were not disinfected & not sterilized by any method, turbidity was observed in all test tubes meaning 0.00% sterilization. (Figure 1).
2. Endodontic files sterilized only by autoclaving in an endodontic instrument box at 121°C for 15 minutes at a

pressure of 15 pounds (Group II a) and also the files which were first disinfected in an ultrasonic cleaner and sterilized with autoclave in an endodontic instrument box at 121°C for 15 minutes at a pressure of 15 pounds (Group III a) showed no turbidity in any of the test tubes, thereby indicating total sterility. (Figure 2,3).

3. The files on sterilization by Diode laser (Group II b) showed turbidity in 4 test tubes (10%) showing 90% sterility. (Figure 4). The files sterilized by Diode laser after disinfecting in an ultrasonic cleaner (Group III b) showed turbidity in 2 test tubes (5%) and no turbidity in 38 test tubes showing 95% sterility. (Figure 5).
4. The endodontic files sterilized by immersing in glutraldehyde for 12 hours (Group II c) showed turbidity in 6 test tubes (15%) and no turbidity in 34 test tubes indicating thereby 85% sterility. The files disinfected in an ultrasonic cleaner followed by sterilization by immersing in glutraldehyde for 12 hours after disinfecting in an ultrasonic cleaner (Group III c) showed turbidity in 5 test tubes (12.5%) and no turbidity in 35 test tubes showing thereby sterilization up to only 87.5%. (Figure 6,7).

followed by Candida albicans (Table 3). The Colony forming units isolates from turbid tubes were between $10^3$& $10^5$ CFU/ml as depicted in Table 4. Results clearly depict that in group II a where autoclave was used alone and III a where it was used conjugation with an ultrasonic cleaner exhibited complete elimination of the entire bacterial specimen.


**Figure 1. Group I showing 100.0% Turbidity**

**Table 2. Comparison of the growth of the micro organism (Turbidity) after 21 days in the test tubes containing endodontic files after sterilization by different methods**

| | | Growth | | No Growth | | p Value | |
|---|---|---|---|---|---|---|---|
| GROUP | n | Number | Percentage | Number | Percentage | Different Groups Vs Control Group | Among the groups |
| Group I | 40 | 40 | 100 | 00 | 00 | | |
| Group II a | 40 | 00 | 00 | 40 | 100 | | |
| Group III a | 40 | 00 | 00 | 40 | 100 | | |
| Group II b | 40 | 4 | 10 | 36 | 90 | | |
| Group III b | 40 | 2 | 5 | 38 | 95 | | |
| Group II c | 40 | 6 | 15 | 34 | 85 | 0.0048 | |
| Group III c | 40 | 5 | 12.5 | 35 | 87.5 | | 0.51183 |

**Table 3. The growth of different microorganisms isolated from the turbid broth in different groups of endodontic files**

| GROUP | GROWTH OF MICROORGANISMS IN NUMBER OF TUBES | | | |
|---|---|---|---|---|
| | *Bacillus stearothermophilus* | *Enterococcus faecalis* | *Streptococcus mutans* | *Candida albicans* |
| GROUP I | 40 | 40 | 40 | 40 |
| GROUP II a | 0 | 0 | 0 | 0 |
| GROUP III a | 0 | 0 | 0 | 0 |
| GROUP II b | 2 | 0 | 0 | 2 |
| GROUP III b | 2 | 0 | 0 | 0 |
| GROUP II c | 3 | 0 | 1 | 2 |
| GROUP III c | 3 | 0 | 0 | 2 |

**Table 4. The growth of different microorganisms (CFU/ml) isolated from the turbid broth in different groups of endodontic files**

| Group | GROWTH OF MICROORGANISMS IN NUMBER OF TUBES | | | |
|---|---|---|---|---|
| | *Bacillus stearothermophilus* | *Enterococcus faecalis* | *Streptococcus mutans* | *Candida albicans* |
| Group I | >1.35X10$^5$CFU/ml (n=40) | >1.55X10$^5$CFU/ml (n=40) | >2.12X10$^5$CFU/ml (n=40) | >0.82X10$^5$CFU/ml (n=40) |
| Group II a | - | - | - | - |
| Group III a | - | - | - | - |
| Group II b | >0.32X10$^3$CFU/ml (n=2) | - | - | ≥1.15X10$^3$CFU/ml(n=2) |
| Group III b | ≥1.0X10$^3$CFU/ml(n=2) | - | - | - |
| Group II c | >1.8X 10$^5$ CFU/ml (n=1) ≥1.2X10$^3$CFU/ml(n=2) | - | ≥2.2X10$^3$CFU/ml(n=1) | >2.0X 10$^5$CFU/ml (n=2) |
| Group III c | >1.52X 10$^5$ CFU/ml (n=1) ≥1.210$^3$CFU/ml(n=2) | - | - | >1.0X 10$^5$ CFU/ml (n=1) ≥1.2X10$^3$CFU/ml(n=1) |

Statistical analysis of the sterilized group of endodontic files using Chi-square test showed statistically highly significant difference in growth of microorganisms in the endodontic files after sterilization with different methods as compared to control group ($p \leq 0.0048$) (Table 2). The subculture from the turbid tubes of various groups were identified by various bacteriological techniques like gram staining. Biochemical tests showed growth mostly of Bacillus stearothermophilus

## DISCUSSION

The presence of microbes inside the canal is the main reason for post-treatment infection. Therefore, the maintenance of the disinfection obtained during the treatment is imperative. (Nabeshima *et al*., 2011) Endodontic files have a lot of importance in endodontic treatment.


**Figure 2. 0.0% Turbidity in Group II a**


**Figure 3. 0.0% Turbidity in Group III a**

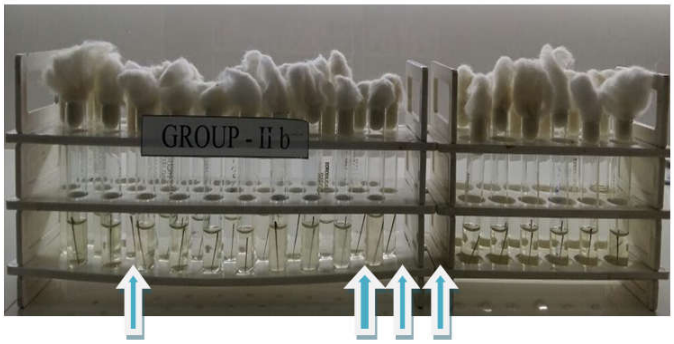


**Figure 4. Turbidity observed in 4 test tubes in Group II b**

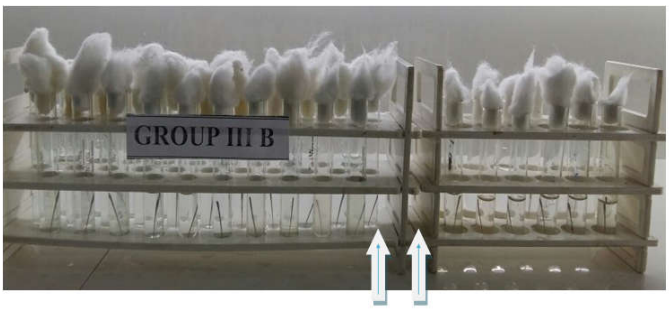


**Figure 5. Turbidity observed in 2 test tubes in Group III b**

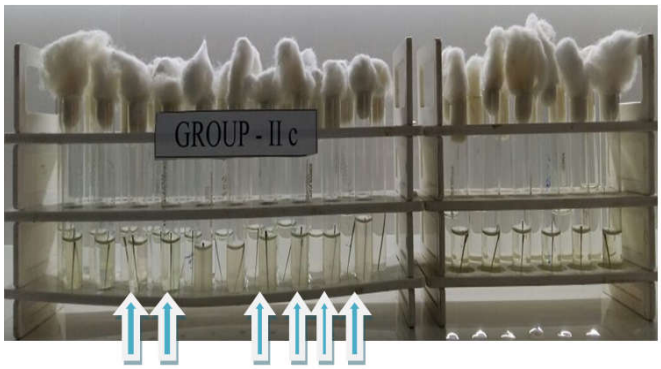


**Figure 6. Turbidity observed in 6 test tubes in Group II c**

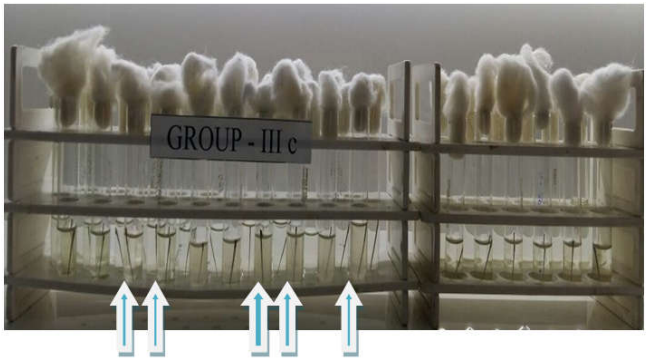


**Figure 7. Turbidity observed in 5 test tubes in Group III c**

Organic debris adheres tenaciously to these instruments after clinical use. Hence, to remove the organic matter and debris completely from the files, effective disinfecting procedure must be carried out to achieve sterilization. (Punathil *et al*., 2014) There are many methods to sterilize and disinfect instruments. Autoclave, chemiclave, glass bead sterilizer, glutraldehyde, lasers, ultrasonics are amongst the most commonly employed methods to achieve sterilization. In this study, effectiveness of autoclave, diodelaser and glutraldehyde were evaluated because according to Palenick *et al*. (1986) they are the most popular sterilization methods. There has been limited investigation of the efficacy of current cleaning procedures for endodontic files. To the best of our knowledge, this is the first study where such a large number of files (280) were used for the experiment. Results of our study were in accordance with the ones conducted by Raju *et al*. (2013), Al Jamell *et al*. (2014), Kuritani *et al*. (1993) and Venkatasubramanian *et al*. (2010) who in their separate researches inferred that autoclave as the most superior method for absolute elimination of microorganisms whereas Sajjanshetty *et al*. (2014) conducted an invitro study to evaluate the effect of various sterilization methods on dental burs and concluded that none of the methods including autoclave and ultrasonics were capable to achieve absolute sterilization making their observations contradict our findings. In the present study, diode laser exhibited near perfect sterilization which was also observed by Al-Jamell *et al*. (2014) who in their study observed that diode laser provided 84.24%. There are a lot of laser systems like argon, diode, $CO_2$ and NdYAG that can help in achieving sterilization. Diode laser was used in the present experiment as it is one of the more commonly used mode of laser in the dental office. Glutraldehyde presented with insufficient sterilization of endodontic files. Our findings were in agreement with (Gennaro *et al*., 2004) Hurtt *et al*. (1996) and Venkatasubramanian *et al*. (2010) who in their separate researches concluded that glutraldehyde was incapable of providing complete sterilization.

Ultrasounds have been utilized for long time as an effective means to clean surgical instruments and, in particular, dental devices before sterilization. (Gennaro et al., 2006) Ultrasonic cleaning has been shown to be effective in removing dried blood and saliva from the dental instruments and remains an important system that enhances dental personnel safety during instrument handling. (Sajjanshetty *et al*., 2014) In this study, we included bacterial isolates of *Bacillus stearothermophilus, Enterococcus faecalis, Streptococcus mutans* and *Candida albicans*, which are both highly resistant to the standard local root canal medicaments and frequently associated with therapy-

resistant apical periodontitis. Al-Jamelletal (2014) used homogenous spore suspension of Bacillus thuringiensis & Hurtt *et al*. (1996) used Bacillus stearothermophilus. Punathil *et al*. (2014) and sierra and bucher (1971) used heatresistant bacterial spore of Bacillus subtilis. Limitation of the study includes inability to assess the effectiveness of current sterilization methods against the entire oral microflora including all aerobic and anaerobic gram positive and gram negative bacteria, fungi and viruses also. Although autoclave is the gold standard for sterilization, it cannot be used as a chair side sterilization method. Sterilization is defined as the "complete destruction of all forms of microbial life". Therefore, sterilization is an "all-or-none" phenomenon. According to this experiment, autoclave with or without ultrasonic treatment of instruments inserted fulfills this requirement.

**Conclusion**

It can be concluded that, endodontic hand files should be completely sterilized between use on different patients. This may be best accomplished by steam autoclaving. To achieve one hundred percent of sterilization the time of autoclave must be suitable to allow the killing of all microorganisms. Although autoclave is the gold standard for sterilization, it cannot be used as a chair side sterilization method. Further research is required to find a faster and improved method of chair side sterilization of endodontic hand files and feasibility ofsingle use endodontic files. Additional cleanings regimes such as ultrasonic cleaning may enhance the effectiveness.

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