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ORIGINAL RESEARCH

Comparative Evaluation of the Efficacy of Two Methods of Sterilization for Rotary Nickel-Titanium Files: An *in vitro* Study

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ABSTRACT

Sterilization in endodontic practice is a must as the major motive of an endodontist is to combat endodontic diseases by eliminating the causative microorganisms. Owing to the design of the endodontic files, it is very conducive for the microorganisms and debris to be anchored onto them. So, the aim of this study was to investigate the sterilizing efficacy of two methods of sterilization using rotary nickel-titanium files: Autoclave and chemical sterilization using two solutions (Quitanet Plus and GlutraMil) and to check whether any sterilizing technique could match the standard of autoclave and which of the two chemical sterilizing agents were more efficient. The rotary endodontic files contaminated with *Bacillus stearothermophilus* spore suspension, were sterilized by the two methods and their efficacy was checked by immersing the contaminated files in test tubes containing thioglycollate medium. The results of the study showed that the files sterilized by autoclave were 100% sterile and those sterilized with GlutraMil showed 75% sterility with Quitanet showing only 25% sterility. So, the study concluded that autoclave could be used as a reliable method of sterilization in clinical practice but GlutraMil and Quitanet Plus cannot be relied upon completely.

Keywords: Nickel-titanium endodontic instruments, Sterilization, *Bacillus stearothermophilus,* GlutraMil, Quitanet plus.

INTRODUCTION

Microorganisms have long been recognized as the cause for pulpal and periapical diseases and the success of endodontics depends on the eradication of these from the pulp chamber and the root canals. Instruments that contact sterile areas of the body, enter the vascular system or penetrate the oral mucosa are classified as 'critical items' and must be sterile before use.[1] This classification includes the endodontic files. Mechanical debridement of the microorganisms requires the usage of the endodontic files extensively. The advent of rotary instrumentation has revolutionized the way that endodontics is being done. No longer do we need to be stuck in tedious appointments straining your wrist and fingers. Owing to their frequent reuse, following a strict sterilization protocol is essential as the risk of cross-infection is higher. Various methods have been proposed for this purpose, namely the autoclave, dry-heat sterilization, glass-bead sterilizer, laser, chemical sterilization, etc.

In recent times, the reuse of the endodontic files has been scrutinized. In 2007, the Department of Health in the UK recommended that endodontic files and reamers be treated as single use instruments (Fig. 1). The heat of sterilization may act to fix residual debris onto the surface of the file, resulting in a viscous cycle if the file is reused on multiple occasions.

Fig. 1: Label denoting single usage

As a result, many manufacturers labeled endodontic files as single-use devices.[2]

Recommendations concerning cleaning and sterilization procedures should be based on scientifically obtained and clinically relevant data, and be justifiable, achievable, and consistent with known risks.[3] Unfortunately, there is little research information available on which to base infection control procedures.[4] There are several practices, protocols, and guidelines to manage sterilization processes in endodontic offices.[5]

The purpose of the present study was to compare the sterilizing efficacy of two methods of sterilization using rotary nickel-titanium files: Autoclave and chemical sterilization, using two solutions (Quitanet plus and GlutraMil) and to check whether any sterilizing technique could match the standard of autoclave and which of the two chemical sterilizing agents were more efficient.

MATERIALS AND METHODS

A total of 30 new rotary NiTi files from the manufacturer Protaper (Tulsa—DENTSPLY, USA) of standardized size F4 and of length 21 mm (Fig. 2); two chemical sterilizing solutions namely Quitanet plus (Specialities Septodont Pty Ltd) (Fig. 3) and GlutraMil (Mil Laboratories Private Limited, Mumbai, Maharashtra) (Fig. 4) were used for the study. All the files were placed in an endodontic instrument box and were sterilized at 121°C for 15 minutes at 15 psi in an autoclave (Fig. 5).

These files were divided into the following four experimental groups:

Group 1: 8 F4 files sterilized by autoclave

Group 2: 8 F4 files sterilized by GlutraMil

Group 3: 8 F4 files sterilized by Quitanet plus

Group 4: Control group – six files –subjected to no sterilization

The bacterial spore suspension was prepared by immersing the commercially available *Bacillus stearothermophilus* strips (SGMstrip® spore strip Biological Indicator) (Fig. 6) into thioglycollate medium (HiMedia Laboratories) and incubated at 55°C for 7 days (Figs 7 and 8). The presence of growth in the test tube was tested by Gram's stain which showed the Gram positive *Bacillus stearothermophilus*.

All the presterilized files were contaminated with this suspension in a sterile Petri-dish for 5 minutes (Fig. 9). Following this the files were transferred to another sterile Petri-dish using a sterile tweezer and were dried in an incubator for 10 minutes at 37°C and were stored in an endodontic instrument box (Fig. 10) until sterilized by other methods.

The eight contaminated files in group 1 were placed in an endodontic instrument box and subjected to autoclave at 121°C for 15 minutes at a pressure of 15 psi.

The eight contaminated files in group 2 and group 3 were placed in separate sterile containers containing the GlutraMil and Quitanet plus solution respectively, and were left in it for 12 hours (Figs 11 and 12).

Fig. 2: Protaper nickel-titanium rotary endodontic files

Fig. 4: GlutraMil solution

Fig. 3: Quitanet plus

Fig. 5: Autoclave

Fig. 6: Biological indicator strips

Fig. 9: Files contaminated in spore suspension

Fig. 7: Thioglycollate medium

Fig. 10: Files stored in endodontic box

Fig. 8: Incubator

Fig. 11: Files placed in Quitanet plus solution

After completion of the sterilization of the files as described above, the files from all the four groups were transferred with a sterile tweezer to separate test tubes containing thioglycollate medium.

The six contaminated files in group 4, which is the control group, were not subjected to any sterilization cycle.

The test tubes containing the files were labeled and were incubated at 55°C for 7 days. The date of incubation was noted on. After completion of the incubation, the test tubes were checked for the presence of turbidity in them. The presence of turbidity in a test tube indicated the presence of *Bacillus stearothermophilus* and that the particular file was not sterilized properly. The

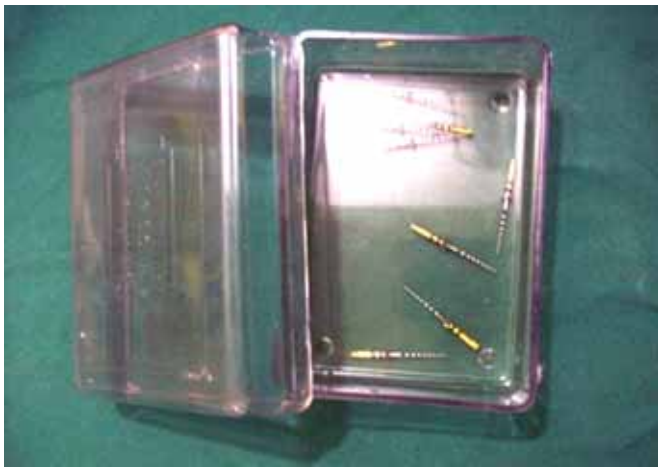


Fig. 12: Files placed in GlutraMil solution

growth of the organism was confirmed by Gram's stain which showed the Gram positive *Bacillus stearothermophilus*.

RESULTS

The study showed that the endodontic files sterilized by autoclaving at 121°C for 15 minutes at a pressure of 15 psi showed total sterility.

The files subjected to chemical sterilization by GlutraMil solution showed the presence of turbidity in two out of the eight test tubes. Incomplete sterilization to the range of 25% was observed by this method.

The files subjected to chemical sterilization by Quitanet plus solution showed the presence of turbidity in six out of the eight test tubes. Incomplete sterilization to the range of 75% was observed by this method.

The control group showed turbidity in all the test tubes as they were not sterilized by any method (Figs 13A to D).

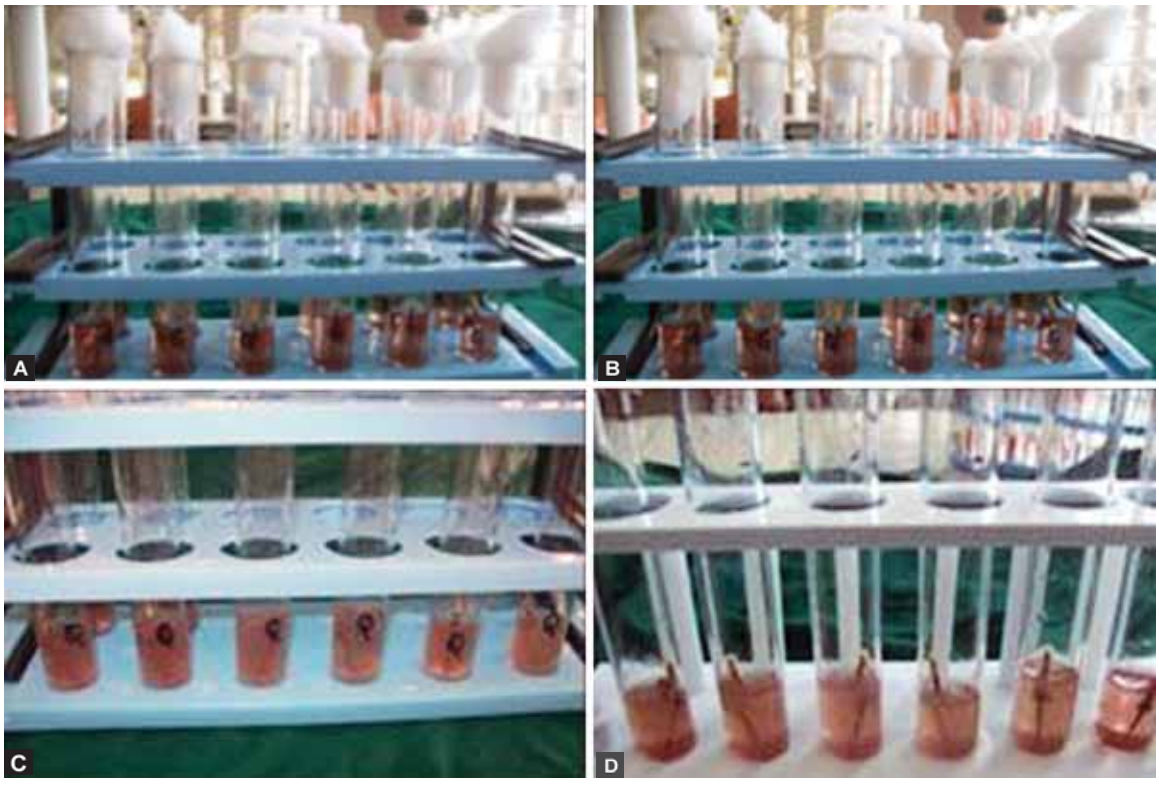
GROUPS

Statistical analysis of the four sterilized groups showed a statistically significant difference between groups with regard to their efficacies in sterilization (Fig. 14).

DISCUSSION

There are no reported cases of accidental cross-infection subsequent to dental treatment but the current concern over the risk of iatrogenic transmission of prion diseases has contributed to the view that consideration should be given to treating endodontic instruments as single-use.[6] However, it is extremely important to consider that highly specific cross-infection control measures in dentistry are required only for patients with, or at notable risk of, prion diseases. Hence, there seems to be no scientific evidence for the single use of endodontic instruments on the basis that prion diseases may be transmitted via contaminated files. Nevertheless, concerns have been raised regarding the safety of multiple use files because of an inability to efficiently clean them.[6]

At present, only a few dental instruments cannot be sterilized, and these are either disinfected or disposable.[5] The complex miniature architecture of dental burs and endodontic files makes precleaning and sterilization difficult. For decades, clinicians have searched for the ideal chairside sterilization method and several different mediums have been used.[7] Sterilization provides a method of instrument recycling that can



Figs 13A to D: (A) Files in test tubes after autoclave sterilization; (B) files in test tubes after GlutraMil sterilization; (C) files in test tubes after Quitanet plus sterilization; (D) files of the control group

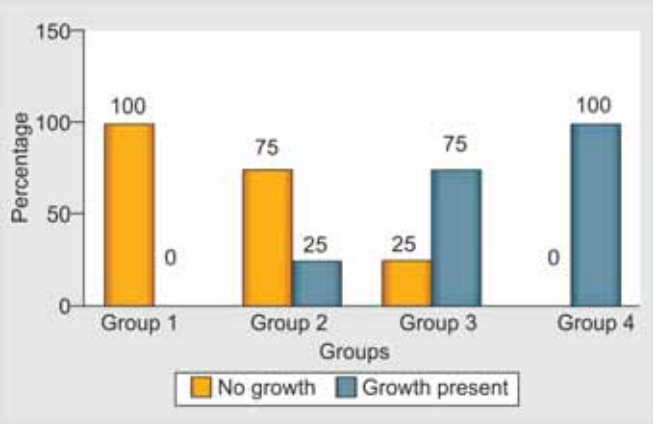


Fig. 14: The comparison of the four groups

be monitored and documented to show that conditions for control of disease transmission were established.

Sterilization protocol goes hand in hand with decontamination, which includes cleaning, disinfection and sterilization. Sterilization is a process to render an object free from viable organisms including bacterial spores and viruses. So, sterilization is an "all-or-none" phenomenon.[8] We need to sterilize endodontic instruments because microorganisms are the major cause of endodontic disease and therefore an aseptic technique is to be followed.

The various methods available for sterilization include steam sterilization by autoclave, dry heat sterilization and chemical sterilization. In the present study, the sterilizing efficacy of two methods of sterilization using rotary nickel-titanium files was investigated: Autoclave and chemical sterilization, using two solutions (Quitanet plus and GlutraMil) and to check whether any sterilizing technique could match the standard of autoclave and which of the two chemical sterilizing agents was more efficient.

The invention of autoclave by Charles Chamberland in 1879 revolutionized the practice of sterilization. To date it is one of the best methods for sterilizing endodontic instruments. It sterilizes the equipment by subjecting them to a high pressure saturated steam at 121°C or more at 15 psi for 15 minutes.

Quitanet plus (Specialities Septodont Pty Ltd) is a chemical sterilizing solution containing alkyl dimethyl benzyl ammonium chloride and didecyl dimethyl ammonium chloride. GlutraMil (Mil Laboratories Private Limited, Mumbai, Maharashtra) is a chemical sterilizing solution containing 2.45% w/v alkaline glutraldehyde and has shown proven efficacy in the presence of organic matter. In the present study, autoclave and the above two chemical solutions were compared in their efficacy of sterilization of rotary nickel-titanium files.

In this study we have used the commercially available *Bacillus stearothermophilus* strips (SGMstrip®) to test the efficiency of the sterilization methods used. It is commonly used as a challenge organism for sterilization validation studies and periodic check of sterilization cycles. SGMstrip is a conventional paper strip biological indicator. It contains bacterial spores on a filter paper carrier sealed within a convenient, peel-open envelope. The biological indicator is to be aseptically cultured in soybean casein digest medium/ thioglycollate medium, and incubated at the appropriate temperature for 7 days.

Thioglycollate Broth (HiMedia Laboratories) is used in detecting microorganisms in normally sterile materials, and is an alternative to certain products that are turbid or cannot readily culture because of the viscosity. This broth was used in this study to incubate the organism *Bacillus stearothermophilus* and check for the presence of turbidity in the medium.

The present study utilized the rotary nickel-titanium files as they are taking on the hand instruments and are now widely used. The rotary files demonstrate a significantly greater tendency to retain cultivable bacteria which may result in greater retention of biological debris on them, protecting the bacteria from the antibacterial mechanisms. The aggressive action of the rotary files induces the packing of biological debris with the U-shaped flute design adding on the retention.[9]

In a busy private practice setting it is not possible to achieve acceptable sterilization results owing to ignorance and hurried manual attempts to save time.[10] So, the need for rapid chairside sterilization is looked upon as an alternative. So in the present study two chemical sterilizing solutions were compared for their sterilizing efficiency.

Steam autoclave sterilization kills the microorganisms by coagulation of proteins. In the present study, this method achieved 100% sterilization of the endodontic files, hence proving the best method of sterilization for these instruments.

The files subjected to sterilization by GlutraMil solution showed 75% sterilization and, therefore, cannot be relied upon completely for sterilization of these instruments.

The files subjected to sterilization by Quitanet plus solution showed only 25% sterilization and this method cannot be relied upon sterilization of these instruments. Further studies need to be carried out to prove the efficacy of these solutions as a sterilizing agent.

CONCLUSION

As the saying goes, "Cleanliness is next to Godliness"; similarly cleanliness in our noble profession plays an important role. Sterilization forms a part and parcel of dental practice due to the risk of cross-infection.

The dental personnel need to be trained regarding the sterilization protocols and though the time and efforts are more in achieving an aseptic field, the required measures need to be taken for the success of endodontics.

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