



**Research Article**

**COMPARATIVE EVALUATION OF FIVE DIFFERENT STERILIZATION METHODS OF PEDIATRIC ENDODONTIC FILES**

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**ABSTRACT**

**Aim:** To compare the efficacy of five different sterilization methods of pediatric endodontic files.

**Materials and Methods:** A total of 50 stainless steel K files were divided into five groups based on the sterilization methods followed – Group 1: Autoclave, Group 2: Glass bead sterilization, Group 3: Glutaraldehyde, and Group 4: Quitanet Plus (aldehyde-free solution) and Group 5: Sodium hypochlorite. In all the tested groups, the files were contaminated with *Enterococcus faecalis*. Before the experimental groups subjected to respective sterilization methods, presterilization colony counts were recorded. After that, the sterilized files were rinsed with distilled water and 100 ul of the diluted concentration was transferred and cultured onto the respective agar plates to determine the total microbial reduction.

**Results:** Group 1 (Autoclave) showed complete effectiveness in reducing the microbial count followed by Quitanet Plus, glass bead sterilizer, glutaraldehyde and Sodium hypochlorite.

**Conclusion:** Autoclave proved to be most efficient method and Glass bead was also closely acceptable compare to chemical methods of sterilization. Among chemical methods, Quitanet proved to be effective and can be used as alternative.

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**INTRODUCTION**

Infection control is stated as a major issue in medicine and dentistry because of concern over communicable disease transmitted in health care settings. (Punathil *et al.*, 2014) and 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) Because 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).

Autoclave works under the principle of utilizing heat in the form of saturated steam under controlled pressure and temperature. Although it is time-consuming, this method has several advantages such as cost-effectiveness, excellent microbial lethality, ability to be physically monitored and the lack of toxic residues, (Henderson *et al.* 1998).

Glass bead sterilizer, works by utilizing the principle of dry heat, and it is the rapid chairside sterilization technique and most commonly used method of sterilization of endodontic files. Large beads cannot transfer heat to the instruments so the beads used should be smaller than 1 mm in diameter. Furthermore, large air spaces between the beads prevents heat transfer (Sanofer A.*et al.* 2015).

Glutaraldehyde is the most commonly used agent for cold sterilization. It has a broad spectrum of bactericidal activity with pungent odor. Because of low surface tension it penetrates into blood and exudates and permits rinsing. However, contact with glutaraldehyde liquid as well as vapor severely irritate the eyes and burns the skin. Therefore, the need for safer chairside cold sterilization method is looked on as an alternative. (Vinay 2011)

Sodium hypochlorite is a potent disinfectant and has the ability to dissolve the organic material. Except titanium and some forms of stainless steel sodium hypochlorite corrodes most metals but when used in a ideal concentration and duration of exposure sodium hypochlorite serves as an disinfectant on the contaminated dental instruments.(Mark and selinger 1989). Sodium hypochlorite is also an hydrolyzing agent.(Pashley *et al.* 1985). It is bactericidal and proteolytic and its antimicrobial

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effectiveness of sodium hypochlorite, based in its high pH. The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lipidic peroxidation. (Dakin *et al* 1915) Quitanet Plus is recently introduced quaternary ammonium compound which comprises didecyldimethylammonium chloride and alkyl dimethyl benzyl ammonium chloride commonly called as “quats.”[Halebathiet *al* 2011). It is an aldehyde-free liquid used for cleansing of operatory instruments and disinfection. It has tuberculocidal, bactericidal actions and also acts against HIV (Kumar KV *et al* 2015).

Even though there are several techniques for the sterilization of pediatric endodontic instruments, studies comparing these were minimal. So, the aim of present study was to compare the effectiveness of five different sterilization methods (autoclave, glass bead sterilization, glutaraldehyde, Quitanet Plus, and sodium hypochlorite) on contaminated pediatric endodontic files.

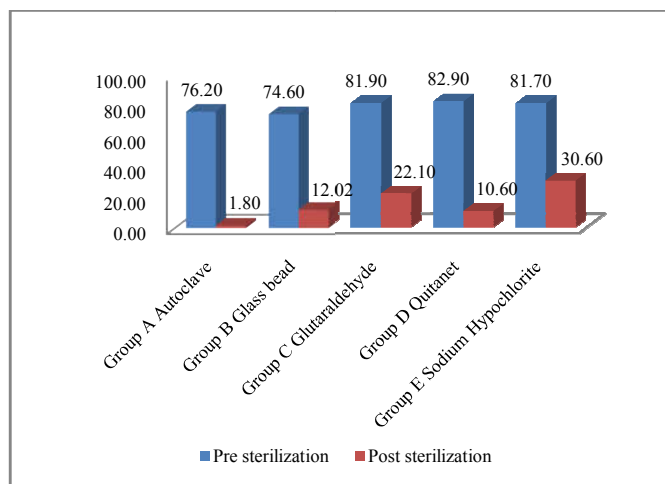
### MATERIALS AND METHODS

The study sample comprised 50 K files of 21 mm (Prime Dental Products Pvt. Ltd., Mumbai, India), is *an in vitro* microbial study done in the Department of Pedodontics and Preventive Dentistry. The study was evaluated the efficacy of various methods of sterilizing the pediatric endodontic hand files. The test microorganism used in the present study was *Enterococcus faecalis* (MTCC no. 452). The study sample were divided into five groups based on the method of sterilization-Group1: Autoclave, Group2: Glass bead sterilization, Group3: Glutaraldehyde (Merck specialities Pvt. Ltd. Mumbai, India), Group 4: Aldehyde-free solution (Quitanet Plus) (Septodont Healthcare India Pvt. Ltd., Maharashtra, India). Group 5: Sodium hypochlorite. All the files included in this study were presterilized in an endodontic instrument box by autoclaving for 30 min at 121°C at a pressure of 15 pounds for standardization. The presterilized files were placed in the test tubes containing bacterial broths and left for 24 h for contamination at 37°C, followed by transfer of these diluted concentrations (100 µl) onto the agar plates using spread plate technique. After incubating these agar plates for 24 h, they were subjected to colony count which served as presterilization values. Once these values were obtained, contaminated files in their respective groups were sterilized using the following methods: In Group 1, files were placed in the sterilization pouch and were subjected to autoclave at 121°C, for 15 min at 15 lb pressure; in Group 2, files wiped for 10 seconds with 2x2 gauzes soaked with surgical spirit and placed in the periphery of the glass bead sterilizer for 45 s at 240°C with beads of size 1–1.5 mm; in Group 3, files were placed in a sterile glass container containing 2% glutaraldehyde solution and left for 12hrs; in Group 4, files were placed in a sterile glass container containing aldehyde-free solution (Quitanet Plus) and left for 20 min (as per the manufacturer instructions) and in Group 5, files were placed in a sterile glass container containing 5.25% Sodium hypochlorite and left for 1 hour. After sterilization, the files were rinsed with distilled water and 100 µl of the diluted concentration was transferred onto the prepared Petri dishes and incubated at 37°C. Further, they were checked for growth of microorganisms After 24h and 72h

all were checked for growth of microorganisms. The colony forming units (CFU) were counted with the help of colony counter using the following formula: Number of colonies/dilution factor × volume plated.

### RESULTS

The postoperative samples of Group 1 showed complete sterilization followed by Group 4, Group 2, Group 3 and Group 5 (Table 1 and Graph 1). Group 1 samples had significant result. The remaining methods also proved effective as they showed statistically significant difference between pre- and post-sterilization values ( $P < 0.01$ ).



Graph 1 Intergroup comparison of sterilization methods

Table 1 Mean reduction in all five groups

		Mean	Mean difference	N	Std. deviation	t-value	p value
Group1: Autoclave	Presterilization	76.20		10	8.63		
	Poststerilization	1.80	74.40	10	3.50	21.932	<0.001
Group2: Glass bead sterilizer	Presterilization	74.60		10	7.43		
	Poststerilization	12.02	62.58	10	4.91	19.166	<0.001
Group3: Glutaraldehyde	Presterilization	81.90		10	6.79		
	Poststerilization	22.10	59.80	10	4.53	12.596	<0.001
Group4: Quitanet plus	Presterilization	82.90		10	6.47		
	Poststerilization	10.60	72.30	10	5.42	15.244	<0.001
Group5: Sodium hypochlorite	Presterilization	81.70		10	6.33		
	Poststerilization	30.60	51.10	10	5.14	29.085	<0.001

### DISCUSSION

Root canal infection is a dynamic process with diverse microbes such as Gram-positive facultative cocci, lactobacilli, *Fusobacterium nucleatum*, *Actinomyces*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *E. coli*, *E. faecalis*, and *Candida*, with *Actinomyces* which are dominating at various stages of disease process. Even though about 500 bacterial species are recognized as normal inhabitants of oral cavity, only 150 microbial species have been isolated from the infected root canals. In the present study, *E. faecalis* was chosen as the test microorganisms because 29.77% *E. faecalis* have been isolated in primary and secondary endodontic infections.

*E. faecalis*, a Gram-positive facultative anaerobic spherical bacterium, can survive in very harsh environments including extremely alkaline pH (9.6) and salt concentrations. It has been reported to be associated with persistent infections and failed endodontic therapies nine times more than the cases of primary

infections [narayana *et al* 2010). The classic endodontic triad for the success of root canal treatment includes canal instrumentation, i.e., cleaning along with shaping, disinfection, and obturation, out of which canal instrumentation is commonly accomplished by endodontic files. Thus, sterilization of these contaminated instruments is imperative for achieving success in the endodontic treatment. [Carrotte P 2004]

In this study, autoclave (Group 1) showed complete sterilization of all the samples for *E. faecalis* microorganism. The results were in similar with the studies conducted by Hurtt and Rossman and Rajkumar and Lakshminarayanan. However, contrary results were noticed in the study by Schug-Kosters *et al.* who stated that there is failure of hot steam to reach all the intricate parts of endodontic instruments resulting in incomplete sterilization.

Quitamet Plus (Group 4) is an aldehyde-free solution which acts by disruption of intermolecular interactions within the cell membrane of microorganisms, thereby compromises the cellular permeability and induces leakage of cellular contents. In the present study, Quitamet Plus showed potent disinfectant next to autoclave. However, a study by Halebathi *et al.* showed that Quitamet Plus was least effective in sterilizing the endodontic files as it may be due to different sterilization protocols followed during the study.

Glass bead sterilizer (Group 2) showed incomplete sterilization of the samples for tested organism despite using smaller sized (1–1.5 mm) beads, which results in better conduction of heat. Moreover, intense dry heat damages spore and vegetative forms of bacteria. The results of the present study were same to the study conducted by Raju *et al.* The incomplete efficacy of dry-heat sterilization was due to its low penetrating ability into the microbes compared to moist heat. The outcome of glutaraldehyde (Group 3) sterilization in this study was undesirable because none of the files showed complete sterility. Glutaraldehyde acts by denaturation of proteins and alkylation of nucleic acids of bacteria. The other mode of action involves cross-linking of proteins at outer and inner layers of bacterial cell that leads to inhibition of enzyme activity, transport, and synthesis of RNA, DNA, and proteins. The results were close to the studies conducted by Venkatasubramanian *et al.*, Kumar *et al.* who stated that incomplete sterilization of endodontic files with glutaraldehyde might be due to some unknown resistant factor of bacteria to that chemical.

Sodium hypochlorite (Group 5) showed incomplete sterilization compared to other methods. The efficacy of Sodium Hypochlorite as tissue dissolving and disinfecting agent depends on its concentration and time of exposure. Naocl was used as it removes the organic debris completely during cleaning procedure. The strength of Naocl solution and duration for which the instrument should be exposed to Naocl must be balanced against potential damage to the instrument by corrosion. ( Punathil S *et al*)

To evaluate the detrimental effects of pediatric endodontic files following sterilization to emphasize the effective sterilization method without damaging the working efficacy of instruments further studies with larger sample are recommended.

## CONCLUSION

Autoclave proved to be most efficient method and Glass bead was also closely acceptable. Among chemical methods, Quitamet proved to be a effective and can be used as an alternative.

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