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Evaluation of the Effectiveness of Different Autoclaves on the Sterilization of Contaminated Rotary Endodontic Files

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Abstract
The aim of this study was to assess and compare the effectiveness of four accepted methods of endodontic instruments sterilization. The present study was performed on twenty five F2 Protaper files and they were tested for the efficacy of sterilization with different methods namely N class autoclave, B class autoclave, pressure cooker autoclave and glutaraldehyde. The biological indicator used was C albicans. Sterility of fresh files obtained from the manufacturer was also tested in the study. The study showed that the fresh files obtained from the manufacturers are sterile while remnants of microbial colonies was observed in rest of all the sterilization technique. N class autoclave was found to have highest efficacy and glutaraldehyde shows the least effectiveness in sterilizing endodontic instruments. Endodontic files, as supplied by the manufacturers to the endodontists are sterile.The study concluded that N class autoclave could be considered as a best method of sterilization in clinical practice while comparing with the rest of the study groups.
Keywords: *Sterilization, autoclave, rotary files.*

Introduction

Of the total 700 bacterial species residing in the oral cavity of human beings, each individual may harbor 100–200 species on an average(1). Most of the oral microorganisms are commensals, but some of these may become pathogenicand causes oral infections under certain situations. As the presence of microorganisms leads to endodontic infections, the success of endodontic treatment depends majorly on the complete eradication of those microbes from the pulp chamber and root canals(2). In root canals undergoing retreatment, in cases of failed endodontic therapy and in canals with persistent infections, the major microbial species identified are *E. faecalis* and yeast, mainly *C. albicans*(1)

Infection control is a major topic of concern in medical and dental health care settings.(3) Instruments that contact the 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. Endodontic files are

categorised as Critical Item and these instruments should be sterile before use. Absence of adequate infection control protocolmay transmit the pathogenic microbes via endodontic instruments. These pathogenic microorganisms may be sourced from within the root canal system or from the periradicular tissues.(4)

Most of the endodontic instruments are reused. During cleaning and sterilisation, it is of great importance to provide utmost care to prevent impairment of the instrument which mayinturn jeopardize the treatment success. In order to prevent the fracture of the material inside the root canal ,care must be given to monitor and control the number of uses of the endodontic instruments.(5)

Most of the endodontic instruments are sterilized using autoclaves .Autoclave works by utilizing heat in the form of saturated steam under controlled pressure and temperature. Even though it is time- consuming, this method has several advantages such as excellent microbial lethality, cost- effectiveness, lack of toxic residues, and the ability to be physically monitored. The most commonly used agent for cold sterilization is glutaraldehyde. It has a broad spectrum of biocidal activity with pungent odor. It penetrates into blood and exudates due to its low surface tension and permits rinsing.(2)

Even though there are various techniques for the sterilization of endodontic instruments, studies comparing these were scanty. The purpose of this study was to compare the sterilization efficacy of different autoclaves and cold sterilization method on contaminated endodontic files in our routine dental practice and recommend the effective method among them.

Aims and Objectives

The aim of this study was to assess and compare the effectiveness of four accepted methods of endodontic instruments sterilization. The different modes of sterilization used in the study includes

1. B class Autoclave **(**Figure1)
2. N class autoclave **(**Figure2)
3. Pressure cooker type autoclave **(**Figure 3)
4. Cold sterilization (with glutaraldehyde)

The present study had also evaluated the sterility of fresh endodontic file that we get from the manufacturer.

Materials and Methods

After obtaining the approval for the study design, this in vitro microbial study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Biogenix research centre TVM to assess and compare the effectiveness of various methods of sterilizing the endodontic files. The test microorganisms used in the present study was *Candida albicans*.

The present study was carried out on twenty five fresh F2 Protaper gold files (Dentsply Maillefer, Ballaigues, Switzerland) which were divided into five groups based on the method of sterilization – Group A: Fresh F 2 files from manufacture Group B: B Class autoclave Group C: N Class autoclave Group D: Pressure cooker type autoclave Group E:Glutaraldehyde

All the Protaper F2 files except the files included in Group A were presterilized in an endodontic instrument box by autoclaving for 30 min at 121°C at a pressure of 15 pounds for standardization.

Figure 1 - B Class Autoclave

Figure 2 – N Class Autoclave

Figure 3 – Pressure cooker autoclave

All the pre-sterilized files in Group B, C,D and E were contaminated with Candida albicans. Potato Dextrose Broth (PDB) was inoculated with 10 µl *Candida albicans* culture, ATCC 10231 and was grown for 48 to 72 hours. After the period of incubation, the file samples were kept in the culture and kept at room temperature for 5 days (Figure 4).

Figure 4- File samples kept in culture

Contaminated files were then removed from the culture medium and they were pouched separately. The corresponding groupof files were then sterilized with B Class, N Class, pressure cooker type autoclaves and with 2% glutaraldehyde respectively. After sterilization, files were collected and stored in sterile glass bottles with 15ml saline to maintain the viability of the organisms for further evaluations (Figure 5).

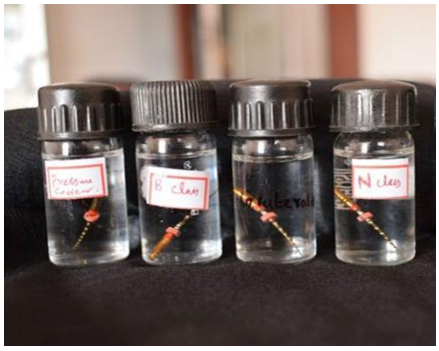


Figure 5- Files stored in 15ml saline to maintain the viability of organism

The Colony Forming Units (CFU) were determined after treatment. The resuspended files were vortexed and 10µl from each were swabbed on to Sabouraud Dextrose Agar (SDA) plates. The plates were incubated at room temperature for 48 hours. The plates were observed for the presence of Colony Forming Units. The CFUs were counted using a Digital Colony Counter (Figure 6) and was expressed in CFUs/ml. The Group A samples were kept in sterile PDB for 7 days to check its sterility After 7 days of incubation, the broth was visually observed for the presence of any turbidity. Further 10µl from it was swabbed on a SDA plate and was incubated at room temperature for 48 hours. The plates were thereafter observed for the presence of Colony Forming Units.

Figure 6- Digital Colony Counter

Statistical Analysis

Comparison of the mean CFU/ml in different sterilization techniques between groups and within groups were done using Analysis of Variance (ANOVA) and Tukey Post Hoc Test. Analysis of data was done using SPSS (Statistical Package for Social Science) software

Results

The study showed that the endodontic files in Group A (fresh F 2 files from manufacture) showed total sterility and rest of the samples contains remnants of microbial colonies (Figure 7).

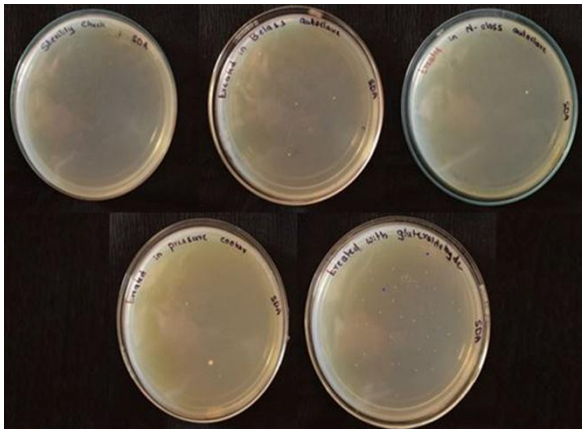
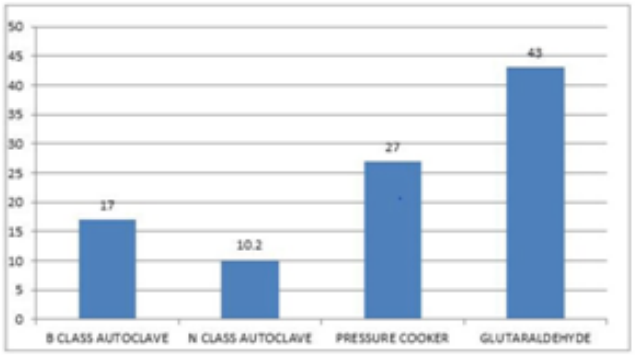


Figure 7 - Colony-Forming Units against Candida albicans with different sterilization methods

Graph 1 shows the Mean candidal CFU/ml in different sterilization techniques. Out of the four sterilization techniques followed in this study, superior results were noticed in Group C samples (N class autoclave) having least CFU/ml with a value of 10.2 CFU/ml. The samples disinfected in glutaraldehyde showed a high value of 43 CFU/ml.

Table 1 and 2 shows the comparison of mean CFU/ml in different sterilization techniques. Comparison of the different sterilization groups namely Group B: B Class autoclave, Group C: N Class autoclave, Group D: Pressure cooker type autoclave and Group E: Glutaraldehyde with regard to their efficacies in sterilization showed a statistically significant difference between each of the different sterilization techniques with $P < 0.05$. The statistical significant difference between Glutaraldehyde and N autoclave was found to be the largest while that between B class autoclave and N class autoclave was the smallest.



Graph 1 - Mean candidal Colony-Forming Units/ml in different sterilization techniques

Table 1 Comparison of Mean CFU/ml In Different Sterilization Techniques using ANOVA

ANOVA*					
Colony-Forming Units / ml					
	Sum of Squares	DF**	Mean Square	F	P*** value
Between Groups	3045.400	3	1015.133	118.729	0.000
Within Groups	136.800	16	8.550		
Total	3182.200	19			
*ANOVA – Analysis Of Variance, **DF – Degree of freedom ***P – Probability					

Table 2 Comparison of Mean CFU/ml In Different Sterilization Techniques using Post Hoc test

POST HOC TEST				
(I)Streilization Type	(J) Streilization Type	Mean Difference (I-J)	*Std. Error	**p value
B Class Autoclave	N Class Autoclave	6.80000	1.84932	.010
B Class Autoclave	Pressure Cooker	-10.00000	1.84932	.000
B Class Autoclave	Glutaraldehyde	-26.00000	1.84932	.000
N Class Autoclave	Pressure Cooker	-16.80000	1.84932	.000
N Class Autoclave	Glutaraldehyde	-32.80000	1.84932	.000
Pressure Cooker	B Class Autoclave	10.00000	1.84932	.000
Pressure Cooker	Glutaraldehyde	-16.00000	1.84932	.000
*Std Error – Standard Error **p Value – Probability Value				

Discussion

The endodontic instruments according to their nature could be either disposable or reusable through sterilization processes. Reusable instruments act as a source of infection for the professionals, and if sterilization and disinfection procedures are not done properly, patients may be exposed to an infectious risk too.[6]

The main methods of sterilization of endodontic files and reamers have been reported to be application of steam under pressure in a steam autoclave, application of dry heat in a sterilizing oven, and sterilization by chemical vapour.[7] The latter 2 methods are considered unreliable and are of limited use.[8]

- Sterilization – "the process by which an article, surface or medium is freed of all microorganisms either in vegetative or spore state."
- Disinfection – "destruction or removal of all pathogenic microorganisms which give rise to infection but not necessarily their spore forms."[9]

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.[2] It has been demonstrated that fungi have a role in endodontic treatment failure.*C albicans* has a greater role in the failure than others. That is why *C albicans* was chosen as the biological indicator in the present study.*C albicans* is the fungal species most commonly detected in oral cavity. *C albicans* forms blastospores or chlamydospore and survives in a wide range of pH values.

Conversion of *C albicans* from an innate micro-organism to a pathogenic one depends on minor changes in various pathogenic characteristics such as:

- Adhesion factor(thigmotrophism)
- Hypha formation
- Proteinase secretion
- Phenotypic switching phenomenon[10]

Prokaryotic and eukaryotic cells respond to a sudden change of temperature by the increased production of an array of proteins called heat-shock proteins (HSPs). These HSPs may be induced by heat stress or may be constitutive proteins whose production is markedly increased as a response to such stress. At elevated temperatures, yeast cells of *Candida albicans* synthesize nine heat-shock proteins (HSPs) with apparent molecular masses of 98, 85, 81, 76, 72, 54, 34, 26 and 18 kDa. Production of HSPs has been associated with an increase in thermo tolerance, i.e., an enhanced ability of organisms to survive exposure to otherwise lethal temperatures[11]. This could be accounted as a reason for the presence of remnant microbial colonies in the sterilized study groups.

The different modes of sterilization compared in the present study were B class autoclave, N class

autoclave, pressure cooker type autoclave and glutaraldehyde.

Class N autoclave has no vacuum function. Air removal is performed by gravity displacement. The class B autoclave is defined by a presterilization vacuum cycle. Prevacuum for cold air removal and post vacuum for air removal. The class B is considered to be the highest class of autoclave and can be used to sterilize all loads including solids, type A hollow instruments, type B hollow instruments, porous loads and wrapped instruments. Drying performed by compressor or natural venting requires longer sterilization time and drying time. The presence of a postvacuumcycle component at the end of the sterilization interval is designed to facilitate drying, thereby providing the clinician with dryer instrument packages at the end of the process.[12]

Pressure cooker type autoclave works by utilizing heat in the form of saturated steam under controlled pressure and temperature. Even though it is time- consuming, it has several advantages such as excellent microbial lethality, cost- effectiveness, lack of toxic residues, and the ability to be physically monitore.[2] In pressure cooker type autoclave, sterilization cycle is fast and can achieve 121 degree celcius within 20 minutes.[13]

2% glutaraldehyde for 20 minutes is used for disinfection.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 transport, enzyme activity, and synthesis of RNA, DNA, and proteins.[2] In order to achieve sterility by glutaraldehyde exposure, 8–12 hours are required. The problems with the use of solutions based on glutaraldehyde are both the toxicity of the products and the time required to achieve sterility.[6]

In the present study, the fresh files from the manufacturer does not contain any microbial growth and could be considered as sterile. However candidal growth was observed after sterilization in groups B,C,D and E.Sterilization with N class autoclave was most effective with least colonies and sterilization with glutaraldehyde was least effective with more number of colonies. The outcome of glutaraldehyde sterilization in this study was unsatisfactory. According to this study, sterilization of endodontic instruments using N class autoclave could be considered as most reliable. Even after following the strict sterilization protocol using different technique, microbial remnants were present in each technique. Hence the reuse of endodontic instruments especially in case of retreatments should be considered judiciously.

Further studies with larger samples are recommended to evaluate the detrimental effects of endodontic files following sterilization to emphasize the efficient sterilization method without damaging the working efficacy of instruments.

Conclusion

Sterilization of instruments is significant to ensure optimal patient care and for the eradication of existing infectious diseases and preventing any new infections. In contemporary endodontic practice, the instruments directly come in contact with tissues, blood and tissue fluids, saliva and gingival crevicular fluid which may seep through the rubber dam if not properly placed. Proper sterilization of the used instruments is necessary for infection control.

The files that we get from the manufacturer does not contain any microbial growth since there is no turbidity in the sample. Even though westerilize using an autoclave, there is growth of candidal colonies in the sample of files, which means reusing of endodontic files is not advocated especially in cases of retreatment and endodontic failure conditions in which candidal contamination is there. Out of B class, N class and pressure cooker type autoclaves, N class autoclave is more effective then B class and then pressure cooker type. Chemical sterilization is weak when compared to autoclaves

References

1. Narayanan Ll, Vaishnavi C. Endodontic microbiology. J Conserv Dent. 2010;13(4):233.
2. Yenni M, Bandi S, Avula SS, Margana PG, Kakarla P AAC evaluation of four different sterilization methods on contaminated endodontic files. CJHR 2017;4:194-7. Health and Research.
3. Raju TBVG, Garapati S, Agrawal R, Reddy S, Razdan A, Kumar SK. Sterilizing Endodontic Files by four different sterilization methods to prevent cross-infection - An In-vitro Study. J Int oral Heal JIOH [Internet]. 2013;5(6):108–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24453454%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3895727
4. Eldik D Van, Zilm P, Rogers A, Marin P. A SEM evaluation of debris removal from endodontic files after cleaning and steam sterilization procedures. Aust Dent J. 2004;49(3):128–35.
5. Lunelli SFLF, De Melo TAF, Cord CB. Analysis of cleaning, sterilization, storage process and frequency of endodontic instrument replacement of endodontists from the city of Caxias do Sul/RS. Rsbo. 2017;1(1):11.
6. Dioguardi M, Sovereto D, Illuzzi G, Laneve E, Raddato B, Arena C, et al. Management of Instrument Sterilization Workflow in Endodontics : A Systematic Review and Meta-Analysis. 2020;2020.
7. Enabulele JE, Omo JO. Sterilization in endodontics : Knowledge , attitude , and practice of dental assistants in training in Nigeria – A cross - sectional study. 2018;106–10.
8. Laneve E, Raddato B, Dioguardi M, Gioia G Di, Troiano G, Muzio L Lo. Sterilisation in Dentistry : A Review of the Literature. 2019;2019.
9. RAJARAM SBMM. Endodontic files: Are routine sterilization procedures effective? Endodontology. 2009;75(1):47–50.
10. Naglik JR, Challacombe SJ, Hube B. Candida albicans Secreted Aspartyl Proteinases in Virulence and Pathogenesis. 2003;67(3):400–28.
11. H. MLZAD. Thermotolerance and the Heat-shock Response in Candida albicuns. 2019;(1989).
12. Varghese K, Abraham JP, Jaysa JJ. An in vitro Evaluation of the Efficiency of Various Disinfection and Sterilization Methods to Decontaminate Dental Handpieces. 2018;50–3.
13. Part H, Spallanzani L, Chamberland C, Papin D. Operation of the autoclaves. 1908.