Research Article

Submitted: Jan 06, 2023
Accepted: Jul 10, 2023
Published: Aug 21, 2023

Efficacy of Different Sterilisation Methods of Endodontic Files: An In Vitro Study

Dr. Santosh Man Rajbhandari,[1] Dr. Anu Karmacharya,[2] Ms. Santwana Pandey,[3] Dr. Sunita Khanal[4]

[1,2]Department of Conservative Dentistry and Endodontics, Kantipur Dental College and Hospital, Basundhara, Kathmandu, Nepal

[3]Department of Microbiology, Kantipur Dental College and Hospital, Basundhara, Kathmandu, Nepal

[4]Department of Community and Public Health Dentistry, Kantipur Dental College and Hospital, Basundhara, Kathmandu, Nepal

Correspondence :

Dr. Santosh Man Rajbhandari. Email: santoshman.rajbhandari@gmail.com

ABSTRACT

Introduction: Sterilisation primarily relates to processing reusable instruments to prevent cross-infection. Various methods are used to sterilise these instruments. Precleaning and sterilisation of some instruments are difficult due to the complex architecture and small size endodontic files used for cleaning shaping of root canal systems.

Objective: To evaluate the efficacy on four modes of sterilising endodontic files in dental practice and to recommend the effective methods from among these.

Materials and Method: An analytical cross-sectional in vitro study was performed from 2022 February 18 to 2022 April 30, using Enterococcus faecalis (gram-positive cocci) as test microorganisms, on 125 K-files of size 25 with 21 mm length at department of Microbiology and department of Conservative Dentistry and Endodontics after institutional ethical approval from Kantipur Dental College. Efficacy of sterilsation was compared among different sterisation methods (B-class Autoclave, N-class Autoclave, Glutaraldehyde, and Glass-bead steriliser).

Result: Study showed group-A and group-B both had complete sterilisation followed by group-C and group-D for Enterococcus faecalis (gram-positive cocci). Out of four sterilisation techniques followed in this study, 100% results were noticed in group-A, group-B; while 99.97% in group-C, and 99.58% efficiency in group-D. These techniques were also effective as they showed statistically significant difference between pre and poststerilisation values ($P < 0.01$).

Conclusion: Both B-class and N-class autoclaves were most effective for sterilisation. Hence, they should be considered as gold standard methods to prevent cross-infection, reinfection, and for faster and alternative sterilisation. Other methods could be used but with less effectiveness.

Keywords: Autoclave; enterococcus faecalis; glass-bead steriliser; glutaraldehyde; gram-positive cocci; sterilisation.

INTRODUCTION

The bacteria were first showed with scientific evidence in diseased pulp by Miller in 1894.[1] In human oral cavity 700 bacterial species are present and 100-200 species individually harbour on average.[2] Oral microorganisms are also one

Citation
Rajbhandari SM, Karmacharya A, Pandey S, Khanal S. Efficacy of different sterilisation methods of endodontic files: An in vitro study. J Nepal Dent Assoc. 2023 Jan-Jun;23(36):16-20.

of the major causes of pulpal disease and aseptic technique is essential for endodontic success.[3] In dentistry, sterilisation primarily relates to processing reusable instruments to prevent cross-infection.[4] In endodontics various instruments like files, reamers, gates glidden drills, and peeso reamers are used for cleaning and shaping root canal system and to eliminate microorganisms from pulp canal space. Various sterilisation methods are used such as Dry Heat steriliser, Autoclave, Ethylene oxide gas, Glass-bead steriliser, Hot salt steriliser, etc.[5] Precleaning and sterilisation of some instruments are difficult due to complex architecture and small size.[6] Endodontic files are slender, tapered instruments with intricate topography, and spiral cutting edges.[7] Thus, in endodontic practice, main concerns are prevention of cross-contamination of infectious diseases among dental staff and patients as well as the successful endodontic treatment. Hence, this study was conducted with an aim to evaluate efficacy of four modes of sterilising endodontic files in dental practice and to recommend the effective methods from among these.

MATERIALS AND METHOD

This was an analytical cross-sectional in vitro study carried out in the department of Conservative Dentistry and Endodontics and department of Microbiology of Kantipur Dental College and Hospital at Basundhara, Kathmandu, Nepal. The study was performed from 2022 February 18 to 2022 April 30 after institution ethical approval (Ref. 2/022), on 125 K-files of 25 size with 21 mm length using convenience sampling technique. Twenty-five K-files were taken as control group and remaining 100 K-files were divided into four groups of 25 K-files each in four different modes of sterilisation: group-A: B-class Autoclave; group-B: N-class Autoclave; group-C: Glutaraldehyde, and group-D: Glass-bead steriliser. Glass-bead steriliser with glass beads size larger than 1.5 mm were excluded.

In this study, Enterococcus faecalis (gram-positive cocci) was used as test microorganism. Lyophilised forms of this bacterial species were activated by growing them on trypticase soy agar which favour the growth of Enterococcus faecalis (gram-positive cocci). All the 125 K-files were presterilised in an endodontic instrument box by Autoclaving for 30 minutes at 121°C pressure of 15 pounds, for standardisation to eliminate any bias. The presterilised K-files were placed in the test tubes containing bacterial broths and were left for 24 hours for contamination at 37°C and then were followed by transfer of these diluted concentration (100 µl) onto the agar plates using spread plate technique. The agar plates were incubated for 24 hours, they were subjected to colony count which served as presterilisation values (Figure 1). When these values were obtained then contaminated K-files were divided into four groups of 25 K-files each. Contaminated 25 K-files were kept for control group and 100 contaminated K-files were divided into four groups of 25 contaminated K-files each in four different modes of sterilisation:

group-A (B-class Autoclave): 25 contaminated K-files were placed in endodontic instrument box and were sterilised by B-class Autoclave at 121°C for 15 minutes at 15 pounds pressure.

group-B (N-class Autoclave): 25 contaminated K-files were placed in endodontic instrument box and were sterilised by N-class Autoclave at 121°C for 15 minutes at 15 pounds pressure.

group-C (Glutaraldehyde): 25 contaminated K-files were placed in a sterile glass container containing

Figure 1: Colony-forming units against Enterococcus faecalis (gram-positive cocci) before sterilisation.

2% Glutaraldehyde solution and were left for 20 minutes.

group-D (Glass-bead): 25 contaminated K-files were placed in the periphery of the glass bead steriliser for 10 seconds at 240°C with beads of size 1-1.5 mm.

After sterilisation, the files were rinsed with distilled water and 100 µl of the diluted concentration were transferred onto the prepared Petri dishes and were incubated at 37°C. Further, they were checked for growth of microorganisms after 24 hours and the colony forming units (CFU) were counted with the help of colony counter using the following formula: Number of colonies/dilution factor × volume plated.

The whole procedure was conducted by the principal investigator (SMR) to avoid bias in the result and the sample and calculation of the CFUs were evaluated by second investigator (AK). The values were subjected to statistical analysis using paired t-test (P <0.01).

RESULT

The result showed group-A and group-B both had complete sterilisation (Table 1, Figure 2) followed by group-C and group-D for Enterococcus faecalis (gram-positive cocci). Out of the four sterilisation techniques followed in this study, 100% results were noticed in group-A & group-B while group-C, and group-D had 99.97% and 99.58% efficiency respectively. These techniques were also effective as they showed statistically significant difference between pre and poststerilisation values (P <0.01).

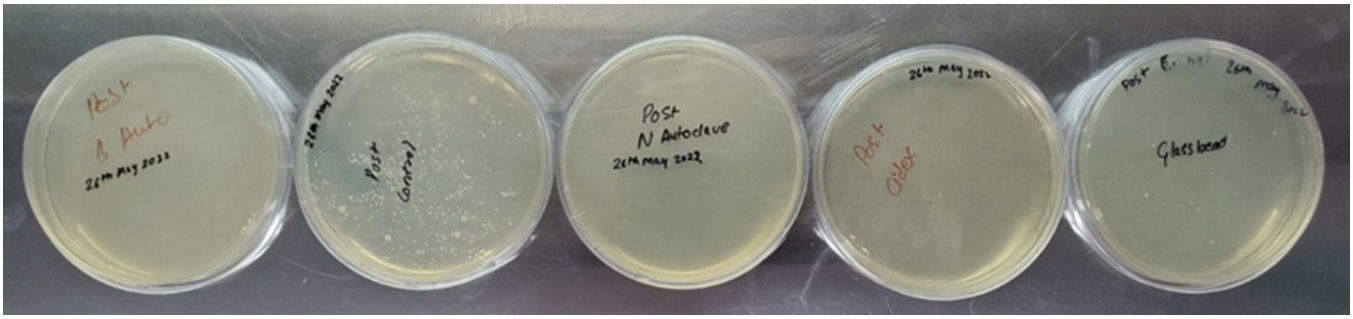

Figure 2: Colony-forming units against Enterococcus faecalis (gram-positive cocci) after sterilisation with different methods.

Table 1: Mean microbial reduction in all the four groups (N = 5).

Group		Mean ± Standard deviation	Standard error of mean
Group-A	pre	513.44 ± 109.28	48.872
	post	-	-
Group-B	pre	388.96 ± 107.908	48.258
	post	-	-
Group-C	pre	630.56 ± 73.960	33.076
	post	0.14 ± 0.054	0.024
Group-D	pre	366.24 ± 71.056	31.777
	post	1.52 ± 0.432	0.193

DISCUSSION

There are 700 bacterial species present in the oral cavity of human beings and 150 microbial species have been isolated from the infected root canal, among them Enterococcus faecalis (gram-positive cocci) is one of the most commonly isolated and resistant bacteria in primary and secondary root canal infections. It has prevalence rate of 24% to 77% in root canal infection.[2,8] So, Enterococcus faecalis (gram-positive cocci) was chosen as test microorganism in the present study.

The success of root canal treatment depends on cleaning, shaping, disinfection of infected root canal which accomplished by canal instrumentation, and proper obturation of root canal. So, the main aim of sterilisation of endodontic file is to prevent cross contamination to patient during endodontic treatment as well as for achieving successful endodontic treatment.[9] There are basically, three principal methods that have been advocated for sterilisation of endodontic instruments: steam under pressure (autoclave), dry heat, and chemiclave.[10] In addition, recently various wavelength LASERs are also available for sterilisation of endodontic instruments but not widely used.

Recently, steam sterilisation are available in two different principal sterilisation technique in market: B-class Autoclave and N-class Autoclave but, in both steam sterilisation there are uses of pressurised chamber at 15 pounds pressure of steam per square inch with hot steam (250°F or 121°C) at approximately 20 minutes of exposure time but only difference is that, instruments are dried within B-class Autoclave. Boyd stated that moist heat generally kills microorganisms by coagulation of proteins. However, coagulation occurs only when over kill condition are attained. Less drastic changes such as inactivation of enzymes, changes in nucleic acids, and cytoplasmic membrane alterations kill microorganisms before coagulation occurs. In previous studies in different microorganisms, both types of autoclaves have 100% success rate for sterilisation. This process prevents cross-infection and increases the success rate of endodontic treatment.[10] The study of Eldik et al. (2004) stated that, no bacteria were detected from files subjected to steam sterilisation. Hurtt et al. (1993) and Malathi et al. (2017), Kumar et al. (2015) also showed that steam autoclave had complete sterilisation.[11-14] In present study, both steam autoclaving the instruments in endodontic box also showed complete 100% sterilisation of all samples microorganisms so result of this study is agreement with other previous studies.

Glutaraldehyde solution is also commonly used as Chemiclave for killing bacteria along with spores. In this study, contaminated files were immersed in this solution for 20 minutes and showed significant sterilisation. In present study, Glutaraldehyde solution showed 99.97% of microbial clearance which is higher than the result of study done by Venkatasubramanian et al. (80%) and Raju TB et al. (80%) though instruments are kept immersed in solution for 12 hrs.[10,15]

Glass bead sterilisation is also used commonly for sterilisation of endodontic instruments. It is mainly used for chair side sterilisation of working ends of endodontic files and reamer. This works on the principle of intense dry heat physically scraping contaminants off their surface. In present study, the sterilisation by glass bead steriliser showed 99.58% effectiveness at 240°C for 10 seconds which is also similar to the result showed by the study of Sanofer et al.[16] But the result of present study are not in agreement with the results of study by Venkatasubramanian et al. (2010) and Raju et al. (2013) which showed only 90% effectiveness at 240°C for 45 seconds.[10,15]

Further studies with larger sample are required to evaluate the detrimental effects of endodontic files following sterilisation to emphasise the efficient sterilisation methods without damaging the working efficacy of instruments.

CONCLUSION

The present study showed, both of B-class autoclave and N-class steam autoclave give 100% complete sterilisation whereas Glutaraldehyde solution and Glass-bead steriliser showed 99.97% and 99.58% sterilisation respectively. So, this study suggests that, both B-class and N-class autoclave

are most effective for sterilisation and mandatory to be considered as gold standard methods to prevent cross-infection and reinfection and for faster and alternative sterilisation, other methods could be used but with less effectiveness.

Conflict of interest: None.

REFERENCES

1. Miller WD. An introduction to the study of the bacterio-pathology of the dental pulp. Dent Cosmos. 1894;36:505-27. [PubMed | Full Text]
2. Henderson B, Wilson M. Commensal communism and the oral cavity. J Dent Res. 1998;77:1674-83. [PubMed | Full Text | DOI]
3. Kakehashi S, Stanley H, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965;20(3):340-9. [PubMed | Full Text | DOI]
4. Rajkumar K, Lakshminarayanan L. The effectiveness of two commonly used methods of sterilising endodontics. J Indian Dent Assoc. 2001;72:245-8. [Full Text]
5. Council on Dental Materials, Instruments and Equipment, Council on Dental Practice, Council on Dental Therapeutics. Infection control recommendations for the dental office and the dental laboratory. J Am Dent Assoc. 1988;116(2):241-8. [PubMed | Full Text | DOI]
6. Whitworth CL, Martin MV, Gallagher M, Worthington HV. A comparison of decontamination methods used for dental burs. Br Dent J. 2004;197:635-40.[PubMed | Full Text | DOI]
7. Smith A, Dickson M, Aitken J, Bagg J. Contaminated dental instruments. J Hosp Infect. 2002;51:233-5. [PubMed | Full Text | DOI]
8. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis (gram-positive cocci): Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006 32(2):93-8. [PubMed | Full Text | DOI]
9. CH Miller. Infection control and office safety sterilisation: Disciplined microbial control. Den Clin North Am. 1991;35:339-55. [PubMed | Full Text]
10. Venkatasubramanian R, Jayanthi, Das UM, Bhatnagar S. Comparison of the effectiveness of sterilising endodontic files by 4 different methods: An in vitro study. J Indian Soc Pedod Prev Dent. 2010;28:2-5. [PubMed | Full Text | DOI]
11. Van Eldik D, Zilm P, Rogers A, Marin P. Microbiological evaluation of endodontic files after cleaning and steam sterilisation procedures. Aust Dent J. 2004;49(3):122-7. [PubMed | Full Text | DOI]
12. CA Hurtt, LE Rossman. The Sterilisation of Endodontic Hand Files. J Endod. 1996;22:321-2. [PubMed | Full Text | DOI]
13. Yenni M, Bandi S, Avula SS, Margana PG, Kakarla P, Amrutavalli A. Comparative evaluation of four different sterilisation methods on contaminated endodontic files. J Health Res 2017;4:194-7. [Full Text]
14. Kumar KV, Kiran Kumar KS, Supreetha S, Raghu KN, Veerabhadrappa AC, Deepthi S. Pathological evaluation for sterilisation of routinely used prosthodontic and endodontic instruments. J Int Soc Prev Community Dent. 2015 May-Jun;5(3):232-6. [PubMed | Full Text | DOI]
15. Raju TB, Garapati S, Agrawal R, Reddy S, Razdan A, Kumar SK, et al. Sterilising endodontic files by four different sterilisation methods to prevent cross-infection-An in vitro study. J Int Oral Health. 2013;5:108-12. [PubMed | Full Text]
16. Sanofer A. Glass bead steriliser used as chair side sterilisation in the dentistry. J Pharm Sci Res 2015;7:466-7. [Full Text]