Original Article

Antimicrobial efficacy of calcium silicate-based bioceramic sealers against *Enterococcus faecalis* and *Staphylococcus aureus* – An *in vitro* study

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Abstract

Context: The main objective of endodontic therapy is to achieve a sterile environment and three-dimensional filling of the root canals. Endodontic sealers play an important role in the obturation of the root canal system as they may provide a seal, which prevents the penetration of bacteria. Sealers are meant to entomb residual bacteria, prevent leakage of nutrients, and ideally possess antibacterial properties.

Aim: The aim of the study was to evaluate and compare the antimicrobial effectiveness of calcium silicate-based bioceramic root canal sealers against *Enterococcus faecalis* and *Staphylococcus aureus* using the agar diffusion method.

Materials and Methods: The materials were divided into four groups of nine samples each for both *E. faecalis* and *S. aureus*. Group I (control group) – MTA-Fillapex (Angelus), Group II – BioRoot RCS (Septodont), Group III – Bio-C Sealer (Angelus), and Group IV – Dia-Root Bio Sealer (Diadent). To evaluate the antibacterial activity of the samples, the agar diffusion method was utilized. To begin, a bacterial suspension was evenly spread onto sterile Petri dishes using sterile swabs. Four wells of 4 mm diameter were then punched, and the sealers were mixed following the manufacturer's instructions and placed onto the prepared wells. The Petri dishes were then incubated at 37°C for 72 h. After incubation, the zones of inhibition around each well were measured at 24 h, 48 h, and 72 h, and the resulting values were statistically analyzed.

Statistical Analysis Used: The data were analyzed using the ANOVA and *post hoc* Tukey's test.

Results: Calcium silicate-based root canal sealers showed significantly better efficacy than the control group. BioRoot RCS (3.44 ± 1.13 mm) showed maximum antibacterial effect against *E. faecalis* at 24 h, 48 h, and 72 h time intervals. Bio-C Sealer (16.44 ± 1.42 mm) showed maximum antibacterial effect against *S. aureus* at 24 h, 48 h, and 72 h time intervals. The effectiveness of root canal sealants eventually decreased over a period. Based on analysis using the ANOVA test, a statistically significant difference ($P \leq 0.05$) was observed between the mean values of antimicrobial efficacy of sealers against both *E. faecalis* and *S. aureus*.

Conclusions: The study's findings lead to the conclusion that BioRoot RCS showed a maximum antibacterial effect against *E. faecalis* and Bio-C Sealer showed maximum antibacterial effect against *S. aureus* at 24 h, 48 h, and 72 h.

Keywords: Antimicrobial efficacy; bioceramic sealers; calcium silicate; *Enterococcus faecalis*; *Staphylococcus aureus*

INTRODUCTION

The primary goal of endodontic treatment is to meticulously eliminate all bacteria and their by-products from the root canal system to ensure complete prevention of any potential

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Date of submission : 17.04.2024
Review completed : 04.05.2024
Date of acceptance : 23.05.2024
Published : 08.07.2024

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/jcde
	DOI: 10.4103/JCDE.JCDE_215_24

How to cite this article: Kirthiga M, Thomas G, Jose S, Adarsh VJ, Nair S. Antimicrobial efficacy of calcium silicate-based bioceramic sealers against *Enterococcus faecalis* and *Staphylococcus aureus* – An *in vitro* study. J Conserv Dent Endod 2024;27:737-42.

reinfection. This objective is achieved only through chemomechanical preparation using instrumentation and the use of chemical irrigants to eliminate bacteria. Although this process reduces the quantity of remaining microorganisms in the root canal, it does not entirely eradicate them.[1]

The endodontic microflora normally consists of both Gram-negative and Gram-positive bacteria and obligate anaerobes. *Enterococcus faecalis* is a facultative anaerobic bacteria that can endure extremely difficult conditions. It possesses several virulence factors that promote tissue invasion, influence immunomodulation, and result in toxin-mediated harm. These factors enable adhesion to host cells and extracellular matrix. Along with these factors, the presence of *Staphylococcus aureus* is one of the main reasons why root canal therapy fails. For this reason, a good root canal filler material with antibacterial properties would be helpful.[2]

An antibacterial sealer must ideally flow into the untreated spaces in the root canal and hermetically seal off all the voids in the root canal along with the primary core material. Therefore, the sealers play a vital role in achieving a three-dimensional obturation of the canal space. In addition, certain sealers exhibit antibacterial properties, which are thought to be advantageous for limiting and inhibiting the growth of leftover germs. Endodontic sealers play a critical role in endodontic infection control by engulfing the residual bacteria and preventing the leakage of nutrients that lead to reinfection. An ideal root canal filling material should meet the technical and biological standards as well as have antimicrobial and antibiofilm activity to get rid of any remaining bacteria and biofilm following instrumentation and irrigation.[3]

Additional long-term methods to limit bacterial growth are through the use of intracanal medicaments and bio-sealers with antimicrobial properties. The term “bio” refers to any material which exerts action similar to the biological properties of living tissue. These materials can be either bioactive or bio-inert depending on their function. Bioactive materials are a type of material that exhibits interaction with surrounding natural tissues to induce the growth of more durable tissues. Examples are glass and calcium phosphate. Bio-inert materials such as zirconia and alumina are known for their ability to elicit minimal or no biological or physiological responses from surrounding tissues.[4] These newer calcium silicate-based sealers offer numerous advantages over traditional materials such as calcium hydroxide, zinc oxide eugenol, and resin-based sealers. Some of the advantages include biocompatibility, chemical inertness, dimensional stability, bio-inductive properties, a strong antibacterial effect due to high pH, and excellent sealing abilities.

Bioceramic sealers are commonly composed of a blend of materials, including zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various other filling and thickening agents. The hydrophilic nature of bioceramic sealers utilizes moisture within the canal to facilitate the setting reaction, while also minimizing any shrinkage that may occur during the setting process. The material is available in premixed calibrated syringes with intracanal tips, making it a convenient and reliable choice for endodontic procedures that require precision and accuracy.[5] This study aimed to evaluate the antimicrobial effectiveness of contemporary calcium silicate-based bioceramic sealers against *E. faecalis* and *S. aureus*.

MATERIALS AND METHODS

Four different types of sealers were used in this study, MTA-Fillapex (Angelus), BioRoot RCS (Septodont), Bio-C Sealer (Angelus), and Dia-Root Bio Sealer (Diadent). The sample size was calculated using the software G*Power version 3.1.

The sealers were divided into four groups:

- Group I (control group) – MTA-Fillapex (Angelus)
- Group II – BioRoot RCS (Septodont)
- Group III – Bio-C Sealer (Angelus)
- Group IV – Dia-Root Bio Sealer (Diadent).

This study utilized *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923) as the primary microorganisms, which were procured from “HiMedia.” The sealers were divided into four groups of nine samples each for both *E. faecalis* and *S. aureus*. The microorganisms were cultured in brain heart infusion broth for 24 h at 37°C. To confirm the purity of the cultures, a single colony of each *E. faecalis* and *S. aureus* was selected from the cultured plate and subjected to Gram’s staining. The morphology was confirmed by observing the stained cells under a microscope. The bacterial suspension was standardized by comparing it to a broth with a density equivalent to the 0.5 McFarland units of barium sulfate standard, which corresponds to 1.5×10^8 colony-forming units per milliliter. In total, 18 Muller Hinton Agar (MHA) plates were utilized in this study. Nine MHA plates were inoculated with the *E. faecalis* suspension using sterile swabs, while another nine plates received the *S. aureus* suspension.

Agar diffusion test

Microbiological assessments were performed in aseptic conditions within a laminar flow chamber. Each MHA plate was divided into four sections, and 4-mm diameter wells were made in each section by removing agar at equidistant points using a needle hub. The sealers were mixed according to the manufacturer’s instructions and poured into the wells. Petri dishes containing the sealers along with *E. faecalis* and *S. aureus* microorganisms were incubated for 72 h at 37°C in an incubator. Zones of inhibition were measured at 24h,48h and 72 h [Figure 1]. The size of the growth inhibition zone was measured in millimeters using a digital caliper, and the recorded values were noted. The point at which growth abruptly diminished, indicating complete growth inhibition, was considered the zone edge.

RESULTS

BioRoot RCS showed a maximum antibacterial effect against *E. faecalis*, followed by Bio-C Sealer and MTA-Fillapex, and the minimum zone of inhibition was shown by Dia-Root Bio Sealer. The effectiveness of root canal sealants eventually decreased over a period. The zone of inhibition was maximum during the first 24 h and minimum during the 72-h time interval [Table 1].

Against *S. aureus*, Bio-C Sealer showed the maximum zone of inhibition, followed by Dia-Root Bio Sealer and MTA-Fillapex, and the least amount of zone of inhibition was shown by BioRoot RCS [Table 1].

Statistical analysis of data

The results were statistically analyzed using IBM, SPSS (Statistical Package for the Social Sciences, IBM Co., Armonk, NY, USA) version 26 statistical software. One-way ANOVA test and Tukey's post hoc analysis were used to compare the mean zone of inhibition (in mm) between four groups at different time intervals. An ANOVA test was conducted on four distinct sealers to evaluate their efficacy against *E. faecalis* and *S. aureus* at different time intervals. The findings revealed a highly significant difference ($p \leq 0.05$) among the sealer groups for their antimicrobial activity against *E. faecalis* and *S. aureus* at three different time intervals [Table 2].

A graphical representation of the mean zones of inhibition at 24 h, 48 h, and 72 h against *E. faecalis* and *S. aureus* was represented in Figure 2.

DISCUSSION

In this study, four different sealers were used, namely, MTA-Fillapex (control group), BioRoot RCS, Bio-C Sealer, and Dia-Root Bio Sealer. The main focus of the study was

Table 1: Mean zone of inhibition for *E. faecalis* and *S.aureus* at three time points

Materials	24 h	48 h	72 h
Mean±SD of microbial inhibition in millimeters for *Enterococcus faecalis*			
MTA-Fillapex	13.89±0.78	13.56±0.88	13.00±1.00
BioRoot RCS	23.44±1.13	23.44±1.13	22.22±1.39
Bio-C Sealer	16.44±1.42	15.89±1.54	15.78±1.56
Dia-Root Bio Sealer	13.78±0.97	13.67±1.00	12.56±0.53
Mean±SD of microbial inhibition in millimeters for *Staphylococcus aureus*			
MTA-Fillapex	13.78±1.20	13.33±1.00	12.56±1.42
BioRoot RCS	13.56±1.51	12.89±1.36	12.22±1.39
Bio-C Sealer	19.22±1.39	19.00±1.50	18.33±1.22
Dia-Root Bio Sealer	15.67±1.12	15.56±1.01	14.78±0.97

SD: Standard deviation

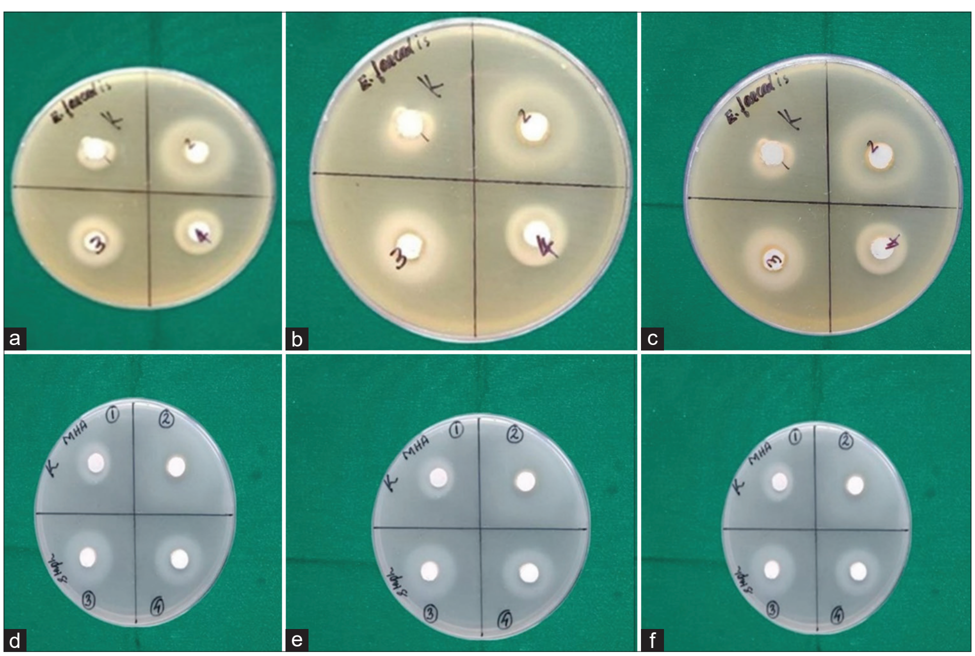

Figure 1: (a) Zones of inhibition for *Enterococcus faecalis* at 24 h, (b) Zones of inhibition for *E. faecalis* at 48 h, (c) Zones of inhibition for *E. faecalis* at 72 h, (d) Zones of inhibition for *Staphylococcus aureus* at 24 h, (e) Zones of inhibition for *S. aureus* at 48 h, (f) Zones of inhibition for *S. aureus* at 72 h

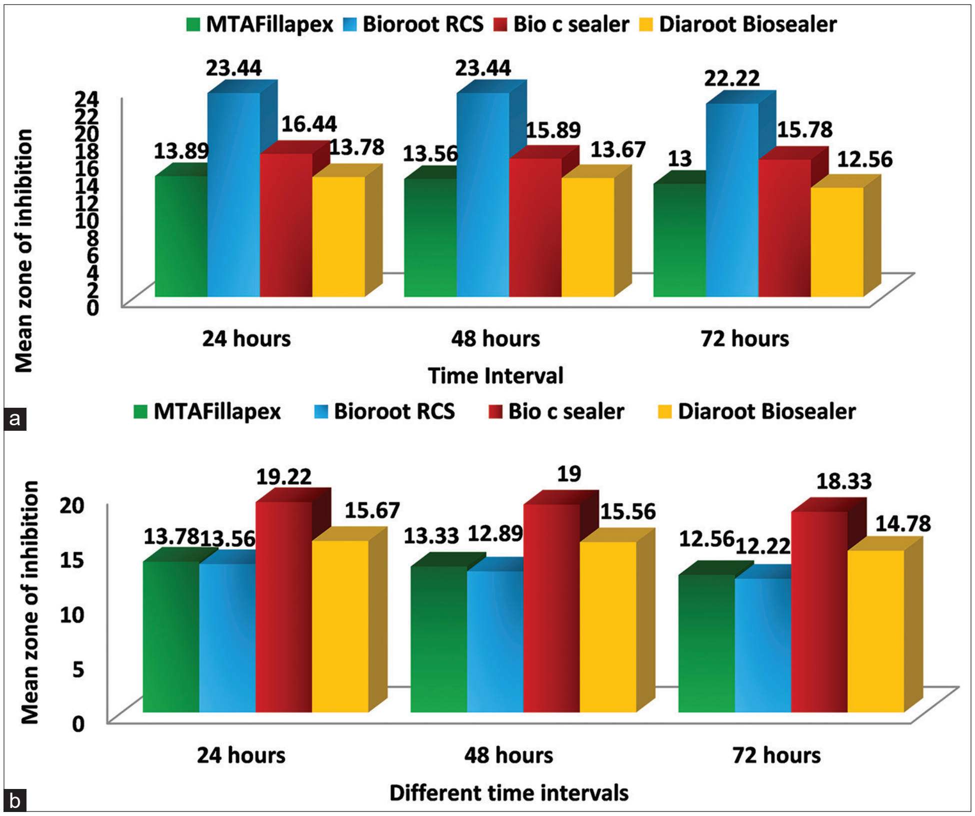

Figure 2: (a) Comparison of the mean zones of inhibition at 24 h, 48 h, and 72 h among the study groups for *Enterococcus faecalis*, (b) Comparison of the mean zones of inhibition at 24 h, 48 h, and 72 h among the study groups for *Staphylococcus aureus*

Table 2: Comparison of groups based on zone of inhibition for *E. faecalis* and *S. aureus* at three time points

ANOVA						
Time points	Comparisons	Sum of squares	df	Mean square	*F*	*p**
Comparison of groups based on *Enterococcus faecalis* inhibition zones at three time points						
24 h	Between groups	556.67	3	185.56	152.69	0.000
	Within groups	38.89	32	1.22		
	Total	595.56	35			
48 h	Between groups	586.97	3	195.66	144.49	0.000
	Within groups	43.33	32	1.35		
	Total	630.31	35			
72 h	Between groups	536.22	3	178.74	126.17	0.000
	Within groups	45.33	32	1.42		
	Total	581.56	35			
Comparison of groups based on *Staphylococcus aureus* inhibition zones at three time points						
24 h	Between groups	185.56	3	61.85	35.77	0.000
	Within groups	55.33	32	1.73		
	Total	240.89	35			
48 h	Between groups	210.53	3	70.18	45.73	0.000
	Within groups	49.11	32	1.53		
	Total	259.64	35			
72 h	Between groups	213.64	3	71.21	44.39	0.000
	Within groups	51.33	32	1.60		
	Total	264.97	35			

*Considered significant at $p \leq 0.05$ based on One-way ANOVA

to evaluate the antimicrobial efficacy of these sealers against *E. faecalis* and *S. aureus*. *E. faecalis* is a type of Gram-positive facultative anaerobic bacteria that can thrive in environments with or without oxygen and is commonly detected in endodontic failures. It can easily survive in root canals and can resist a high alkaline pH of 11.5.[6] A study conducted by Byström *et al*. has shown that *E. faecalis* was the species examined that was most resistant to calcium hydroxide.[7] Its prevalence ranges from 40% to 77%, and its highly invasive nature enables it to infiltrate dentinal tubules and adhere to collagen, making it difficult to eliminate. This presents a significant challenge in the field of endodontic treatment.[8,9] *S. aureus* is a microorganism that has demonstrated significant resistance to treatment after repeated root canal procedures; the act of leaving the root canal open during root canal therapy is a significant etiological factor that contributes to the development of primary endodontic infections and chronic infections that occur between treatment sessions.[10] Hence, we chose these two organisms for our study. It is not possible to completely eliminate bacteria from the root canal system, and therefore, root canal filling comprising a core and a sealer with antimicrobial activity is employed to reduce microorganisms and prevent infections. Bioceramic-based endodontic sealers have gained popularity in recent years due to their biocompatibility, stability, and minimal postoperative pain, and they also possess the ability to promote biomineralization.[11,12]

Antibacterial action was assessed using the agar diffusion test (ADT). The ADT refers to a method in which an inhibition zone, also known as a halo, forms around the item being tested on the agar plate. The size of the zone corresponds to the antibacterial effect of the sealer. It has several advantages, including simplicity, affordability, and ease of use, allowing for the testing of a vast number of microorganisms and antimicrobial agents, and leading to straightforward interpretation of results.[13] In this study, BioRoot RCS showed maximum antibacterial effect against *E. faecalis*, which is in accordance with a study conducted by Abduljabbar and Abumostafa[14] and Arias-Moliz and Camilleri.,[15] where they found that the antimicrobial activity being the highest for BioRoot RCS. There are no studies in the literature that have been done with BioRoot RCS against *S. aureus.* Siboni *et al*., in 2017, stated that the antimicrobial efficacy of BioRoot RCS is due to the release of mineralizing ions, such as $Ca{+}{+}$, which bind to physiologically active ions and promote the nucleation of apatite precursors on the material surface, demonstrating bioactivity.[16] In our present study, the least zone of inhibition has been shown by BioRoot RCS against *S. aureus* compared to other sealers, and this can be explained by the size of the zone of inhibition. This size is determined by two crucial factors. First, the level of sealer diffusion in a specific medium is a significant factor in determining the size of the inhibition zone. Second, the toxicity of the sealer to particular bacterial strains significantly impacts the size of the inhibition zone. Furthermore, the amount of diffusion of the sealer is influenced by three key factors: the size of the sealer, its rate of release from the matrix, and its hydrophilicity. Because of the above-mentioned factors, the test results may differ.[14]

It is noteworthy to mention that MTA-Fillapex has been extensively researched and analyzed across numerous studies, emerging as the most commonly investigated bioceramic material within the current literature. In our present study, MTA-Fillapex showed a minimum zone of inhibition against *E. faecalis* and *S. aureus*. This might be due to the presence of salicylate resin in MTA which should not be considered a tricalcium silicate sealer, with the mineral trioxide aggregate constituting just a small part.[17] The result we got from this study is similar to a study conducted by Dalmia *et al*., in 2018.[18] However, it is contradictory to a study conducted by Torabinejad *et al*. who observed that MTA was effective against a few facultative bacteria but had no effect against anaerobic bacteria, *Escherichia coli, S. aureus, Bacillus subtilis,* and *E. faecalis*.[19]

Bio-C Sealer showed the second-most antibacterial effect against *E. faecalis* and the maximum zone of inhibition against *S. aureus*. The results we obtained from our study is similar to a study conducted by Barbosa *et al*. where they found that Bio-C Sealer exhibited antibacterial effects against *E. faecalis, E. coli, Pseudomonas aeruginosa*, and *E. faecalis* strains, but not against *Streptococcus mutans*. Despite the sealer's short setting time and high solubility, the considerable release of calcium hydroxide may further contribute to the antibacterial properties of fresh Bio-C Sealer.[20] Whereas the results are contradictory to a study conducted by Raouf *et al*., where they found that Bio-C Sealer demonstrated the best antibacterial activity against *E. faecalis* but was weaker against *S. aureus*, whereas MTA-Fillapex showed maximum antibacterial effect against *S. aureus*.[21]

In this *in vitro* study, Dia-Root Bio Sealer shows minimal antibacterial effect against *E. faecalis*. El-sayed *et al*. stated that the antibacterial activity of DiaRoot BioAggregate might be attributed to the presence of more calcium hydroxide and hydroxyapatites, which are powerful antibacterial inhibitors when mixed with fluids.[22] There are no studies in the literature that have been done with Dia-Root Bio Sealer against *S. aureus*. In this study, the antimicrobial effect of Bio-C Sealer was the maximum against *S. aureus*, followed by Dia-Root Bio Sealer, MTA-Fillapex, and BioRoot RCS.

It has been observed from the present study that the antimicrobial activity of all four sealers eventually decreases with time. It was the maximum at 24 h and the minimum at 72 h. The present study has shown that BioRoot RCS has a maximum antibacterial effect against *E. faecalis* at 24 h, 48 h, and 72 h, followed by Bio-C Sealer, MTA-Fillapex, and Dia-Root Bio Sealer. Therefore, based on the findings of this study, Bio-C Sealer can be the sealer of choice in cases of persistent root canal infections. It is important to note that this study is not conclusive as it was conducted *in vitro* and not *in vivo*. The presence of dentin can significantly modify the antimicrobial efficacy of sealers. Therefore, further clinical studies are required to be conducted *in vivo* to validate the effectiveness of these sealers.

CONCLUSIONS

The study's findings lead to the conclusion that all the sealers exhibited antibacterial effects on the test microorganisms. BioRoot RCS showed maximum antibacterial effect against *E. faecalis* and Bio-C Sealer against *S. aureus* at 24 h, 48 h, and 72 h, respectively.

Bio-C Sealer showed promising results against both the test organisms, thereby proving its worth as a recommended sealer in the retreatment of infected root canals.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. *In vitro* antimicrobial effectiveness of root canal sealers against *Enterococcus faecalis*: A systematic review. J Endod 2016;42:1588-97.
2. Seelan RG, Kumar AA, Emil Sam RJ, Maheswari SU. Antimicrobial efficacy of different root canal sealers by using real-time polymerase chain reaction: An *ex vivo* study. J Conserv Dent 2015;18:474-8.
3. Kapralos V, Koutroulis A, Ørstavik D, Sunde PT, Rukke HV. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. J Endod 2018;44:149-54.
4. Al-Haddad A, Che AB Aziz ZA. Bioceramic-based root canal sealers: A review. Int J Biomater 2016;2016:9753210.
5. Mangat P, Dhingra A, Muni S, Bhullar HK. To compare and evaluate the antimicrobial activity of three different root canal sealers: An: *In vitro*: Study. J Conserv Dent Endod 2020;23:571-6.
6. Pallavi P, Bansal A, Kukreja N, Kaur N, Chhabra S, Ahuja J. Comparative evaluation of antimicrobial efficacy of different endodontic sealers against *Enterococcus faecalis*: An *in-vitro* study. Int J Health Sci 2022; 6(S1):9838-45.
7. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Dental Traumatology. 1985;1:170-5.
8. Shetty A, Anand KV, Rai N, Shetty A, Pradeep K, Kumar AA. *In vitro* comparative study of antimicrobial efficacy of endodontic sealers against common pathogens in the dental pulp. J Conserv Dent 2023;26:216-20.
9. Tiwari S, Murthy CS, Usha HL, Shivekshith AK, Kumar NN, Vijayalakshmi L. A comparative evaluation of antimicrobial efficacy and flow characteristics of two epoxy resin-based sealers-AH plus and perma evolution: An: *In vitro*: Study. J Conserv Dent Endod 2018;21:676-80.
10. Zan R, Kutlu G, Hubbezoglu I, Sumer Z, Tunc T, Mutlu Z. Bactericidal effects of various irrigation solutions against *Staphylococcus aureus* in human root canal. J Istanb Univ Fac Dent 2015;49:19-26.
11. Castillo-Villagomez P, Madla-Cruz E, Lopez-Martinez F, Rodriguez-Delgado I, Flores-Treviño JJ, Malagon-Santiago GI, *et al.* Antimicrobial effectiveness of root canal sealers against *Enterococcus faecalis*. Biomater Investig Dent 2022;9:47-51.
12. Arul B, Varghese A, Mishra A, Elango S, Padmanaban S, Natanasabapathy V. Retrievability of bioceramic-based sealers in comparison with epoxy resin-based sealer assessed using microcomputed tomography: A systematic review of laboratory-based studies. J Conserv Dent 2021;24:421-34.
13. Panwar D, Sidhu K, Bhushan J, Kakkar V, Mehta M, Sharma J. Evaluation of antimicrobial efficacy of nanocurcumin-coated gutta-percha against *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent 2023;26:160-4.
14. Abduljabbar SM, Abumostafa A. Antimicrobial effect of different calcium silicate – Based bioceramic endodontic sealers against *Enterococcus faecalis*: An: *In vitro*: Study. Saudi J Oral Sci 2021;8:48-52.
15. Arias-Moliz MT, Camilleri J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. J Dent 2016;52:30-6.
16. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG. Properties of bioroot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. Int Endod J 2017;50 Suppl 2:e120-36.
17. Kumar A, Kour S, Kaul S, Malik A, Dhani R, Kaul R. Cytotoxicity evaluation of Bio-C, CeraSeal, MTA – Fillapex, and AH plus root canal sealers by microscopic and 3-(4, 5 dimethythiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. J Conserv Dent 2023;26:73-8.
18. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial efficacy of different endodontic sealers against *Enterococcus faecalis*: An *in vitro* study. J Int Soc Prev Community Dent 2018;8:104-9.
19. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. J Endod 1995;21:403-6.
20. Barbosa VM, Pitondo-Silva A, Oliveira-Silva M, Martorano AS, Rizzi-Maia CC, Silva-Sousa YT, *et al.* Antibacterial activity of a new ready-to-use calcium silicate-based sealer. Braz Dent J 2020;31:611-6.
21. Raouf SH, Bakr DK, Ahmed UM, Amin BK. *In vitro* evaluation of the antibacterial effects of MTA- fillapex and BIO-C® sealer at different time intervals. Cell Mol Biol (Noisy-le-Grand) 2023;69:116-9.
22. El-Sayed MA, El-Mahdy RH. *In-vitro* comparative study of diadent bioaggregate and other retrofilling materials: Dye penetration and antibacterial activity. Dent J 2012;58:1115.