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Antimicrobial activity of root canal sealers against some standard strains and clinical isolates

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Abstract

The aim of this study is to determine the antimicrobial effects of eight different canal sealants used in dentistry against five different standard strains and five clinical isolates by direct exposure test and agar diffusion test. In vitro antimicrobial effects of EndoSequence BC, Well Root ST, MTA-Fillapex, Ortho-MTA, AH Plus, 2 Seal, Sealapex, Adseal sealers to *S. aureus, P. aeruginosa, E. faecalis, C. albicans and S. mutans* (5 clinical isolates and 5 standard strains), were investigated by the direct exposure test and the agar diffusion test. In periods of 24h, 48h and 72 hours, zone of growth inhibition were measured. Results observed by agar diffusion test exhibited that Adseal sealer occurred the strongest antimicrobial activity to *S. aureus, P. aeruginosa, S. mutans*. In addition to this, 2 Seal sealer presented strongest antimicrobial activity to *E. faecalis and C. albicans*. All root canal sealers have been shown to have antimicrobial activity after 48 hours at the latest by direct exposure test. All sealers showed an antimicrobial effect by creating inhibition zones of different degrees against all microorganisms. All sealers have higher antimicrobial activity against clinical isolates than standard strains. In order for the root canal sealers to be effective against these microorganisms, they must be provided with a certain incubation period. In addition, reference studies should be made globally and periodically by the world dental authorities to determine the antimicrobial activities of these sealers. These studies should be taken as a reference by dentists at the application stage.

Keywords: Antimicrobial activity, agar diffusion test, direct exposure test, root canal sealers, dental sealers

Introduction

Bacteria in the root-canal system can give rise to endodontic pathologies, but also may be accompanied by yeasts and fungi. Destroying microorganisms in root canals increases the success of endodontic treatment. The microorganisms in the dentine tubules presence could be determined, despite irrigation solutions and intranasal drug administration pending the forming of the canal system. Therefore, to prevent possible infections, the used root canal sealers should have antimicrobial properties [1,2].

Microorganisms are the main cause of pulp and periapical diseases. The bacteria and fungi that keep the infected root canal create a natural habitat for themselves.

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Appearance of microorganisms again after the first treatment of root canal infections causes treatment failure. Among fungi, especially *C. albicans* has the ability to penetrate the dentin tubular structure and is found in the root canal system Thus, they change the oxygen pressure inside the canal. Thus, various intra-canal therapeutic drugs are recommended to control infection, especially in pediatric dentistry. In particular, facultative microorganisms such as *E. faecalis, S. aureus* and Gram negative microorganisms such as *P. aeruginosa* are the most resistant species in the oral cavity and may cause root canal treatment failure. *S. aureus* along with facultative anaerobic *S. mutans* may also be involved in the early stages of pulp infection. After a while, periapical lesion and pulp necrosis ocur [3].

These bacteria can play an important role in chronic periapical pathogenesis by retaining periapical periodontal tissue. The existing root canal materials may not provide a completely hermetic seal. Therefore, its antibacterial and antifungal properties should be effective in reducing the number of bacteria / fungi and preventing

periradicular contamination. The antibacterial properties of root canal sealers should help prevent the re-colonization and proliferation of microbial flora in the root canal system [4].

The aim of canal sealers is to prohibit the colonization of microorganisms, to prevent growth of the remnant microorganisms and efface the gaps between the canal walls and filling material [5,6]. However, the residual microorganisms in the dentin tubules are expected to be trapped by root canal sealers in the tubule [7].

According to recent studies, the antimicrobial effectiveness of many endodontic sealers has been tested [8,9]. Especially, antimicrobial activity of mineral trioxide aggregate (MTA) has been investigated. In many studies to date, antimicrobial effects of many endodontic sealers have been tested using agar diffusion test (ADT) and direct exposure test (DET) [8-12].

The aim of this research was to examine in vitro antimicrobial effects of eight particular root canal sealers against five standard strains and five clinical isolates.

Materials and Methods

Microorganisms

In this research, the five standard strains and five clinical isolates were used for investigating the antimicrobial effect of the sealers; *Staphylococcus aureus (ATCC / 25923), Pseudomonas aeruginosa (ATCC 27853), Enterococcus faecalis (ATCC / 51299), Candida albicans (ATCC / 90028), Streptococcus mutans (ATCC / 25175)* and 5 clinical isolates that belong to the laboratory culture collection of bacteria and yeast species isolated from the root canal. Clinical isolates were isolated using conventional identification methods. These microorganisms were stored at -80$^{\circ C}$ in Micro-Bank beads (ProLab-Diagnostics, UK) until used.

Root Canal Sealers

The eight root canal sealers were used for experiments; EndoSequence BC Sealer, Well Root ST, MTA Fillapex, Ortho-MTA, AH Plus, 2 Seal, Sealapex, Adseal; Sealers was mixed in abide by the instructions of the manufacturers and under sterile conditions (Table 1). Thus, using two different methods, the antimicrobial effects of eight different root canal sealers were compared with the commonly used epoxy resin-based canal sealer.

Direct Exposure Test (DET)

DET was performed with a total of 50 sterilized absorbent-paper points (Tanariman Ind Ltd, Manacaru/Brazil/AM/) [13]. Each of the standard strains and clinical isolates were individually incubated at 24h for 37$^{\circ C}$ in 7ml brain heart infusion (BHI) (Difco-Lab, USA, MI). These strains were cultured on BHI Agar. These strains were accorded in saline suspension to recent concentration of 3x108 cells/ml. The mixture of microorganisms was prepared using one ml from each pure suspension.

Table 1. The tested root canal sealers

Material	Sealers	Manufacturer	Composition
Calcium silicate sealers	**EndoSequence BC Sealer**	ABD, Brasseler, Savannah, GA	Zirconium oxide, calcium silicate, calcium phosphate monobasic, calcium hydroxide, fillers and thickening agents
	Well Root ST	Vericom Co. Ltd, Tustin, CA, ABD	Calcium aluminasilicate component, zirconium oxide, fillers and thickening agents
Mineral Trioxide Aggregate (MTA) based sealers	**MTA Fillapex**	Angelus Solucoes Odontologicas, Londrina PR, Brezilya	Salicylate resin, natural resin, diluted resin, MTA, bismuth trioxide, nanoparticulate silica, pigments
	Orto-MTA	Angelus, Londrina, Parana, Brazil	Tricalcium silicate, dicalcium silicate, tetracalcium aluminoferrite, bismuth oxide, calcium carbonate, iron oxide,magnesium oxide, tricalcium aluminate, crystalline silica, residues (free magnesium oxide, calcium oxide and potassium and sodium sulfate components)
Epoxy-amine based sealers	**AH Plus**	Dentsply DeTrey GmbH, Konstanz, Almanya	Diepoxy resin, calcium tungstate, zirconium oxide, aerosol, 1-adamantane amine, TCD-diamine, dibenzyldiamine, aminoadamantane, pigments
	2 Seal	VDW GmbH, Münih, Almanya	
Calcium Hydroxide based sealers	**Sealapex**	Sybron-Kerr, Romulus, MI, ABD	Tricalcium phosphate bismuth trioxide zinc oxide, dioxide, silicon dioxide, titanium, Calcium oxide
Epoxy-amine based root canal sealers	**Adseal**	Meta Biomed Co. Ltd., Korea	BASE: Bismuth subcarbonate, zirconium oxide, glycol salicylate, calcium phosphate, epoxy oligomer resin, ethylene CATALYST: Bismuth subcarbonate, tri ethanolamine, calcium phosphate, zirconium oxide, calcium oxide, poly aminobenzoate

The sterilized paper points were dipped and removed in the microbial suspension for five minutes. These paper points placed in petri dishes are coated with saline or one of 8 different root canal sealers (control group). The paper points that were cut off contact with the sealers at intervals of 24h, 48h and 72h, and were added to Letheen broth (7ml), separately.

All tubes incubated for 48h at 37°C were macroscopically examined for the presence of turbidity. Therefore, all tubes were examined to verify the macroscopic results. The 100µl Recent Letheen broth was added to BHI broth (7ml) and incubated for 48h at 37°C. All results are recorded. Gram strains prepared from BHI culture were used to assess contamination. This test was repeated three times.

Agar Diffusion Test (ADT)

Antimicrobial activity of each of sealers was examinated by ADT [13]. First strains have been cultivated BHI broth. Three cavities were created on BHI agar plates with the help of a sterile metal spiral. After, 0.1 ml of pure suspensions each of microorganisms, adjusted to McFarland standard (0.5) were distributed on BHI Agar. However, the gaps were formed at a distance of 2.5 cm from each other, at a minimum distance of 1.5 cm from the plate edge, 4 mm deep and 4 mm in diameter. So, it was prepared separately for each sample. The sealers were filled in these cavities, and incubated for 48h at 37°C. Apart from that, positive and negative controls were be carried out inoculated and without inoculum by adding the bacterial suspension. The inhibition zones diameter of each of the sealers was determined. This test was repeated three times.

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Results

Antimicrobial activites of root canal sealers against mixed microbial cultures were determined by DET. Thus, it was observed Well Root ST and Sealapex were prevented the microbial growth from the 24th hour (Table 2). DET results shown that it is necessary to provide a certain time period for root canal sealers to demonstrate the potential for action against microorganisms present in endodontic infections. The millimetric measurements of the inhibition zones formed at 24, 48 and 72 hours of the canal sealers were tested by ADT, and the mean values of measurements were determined (Table 3 and 4). By the ADT, inhibition zone formation was provided by all materials. It was demostrated by DET that all root canal sealers (Well Root ST, Ortho-MTA, AH Plus, EndoSequence BC Sealer, 2 Seal, Sealapex, MTA Fillapex, Adseal) performed an overall antimicrobial effect to all microbial indicators after 48 h. The results supplied by ADT showed that

Table 2. The antimicrobial effect of sealers to mixed microbial cultures in DET

Sealers	Times		
	24 h	48 h	72 h
EndoSequence BCS	+++ *	- - - * *	- - -
Well Root ST	- - -	- - -	- - -
MTA Fillapex	+++	- - -	- - -
Ortho-MTA	+++	- - -	- - -
Adseal	+++	+++	- - -
2 Seal	+++	- - -	- - -
Sealapex	- - -	- - -	- - -
AH Plus	+++	- - -	- - -
Salin	+++	+++	+++

*: +++ growth presence= Positive result ; * *: - - - absence of growth=Negative result

Table 3. The inhibition zone diameters of standard strains in to ADT (in mm)

Sealers	Microorganisms				
	E. faecalis (ATCC 51299)	*S. aureus* (ATCC 25923)	*P. aeruginosa* (ATCC 7853)	*S. mutans* (ATCC 5175)	*C. albicans* (ATCC 90028)
EndoSequence BCS	6	9	9	5	6
Well Root ST	11	11	8	4	13
MTA Fillapex	10	9	8	9	6
Ortho-MTA	12	9	9	7	9
Adseal	2	14	12	10	13
2 Seal	13	12	11	9	15
Sealapex	3	8	9	9	8
AH Plus	2	10	10	8	11

Table 4. The inhibition zone diameters of clinical isolates in to ADT (in mm)

Sealers	Clinical isolates				
	E. faecalis	*S. aureus*	*P. aeruginosa*	*S. mutans*	*C. albicans*
EndoSequence BCS	6	10	10	7	8
Well Root ST	10	13	8	6	12
MTA Fillapex	10	11	9	10	7
Ortho-MTA	13	12	11	9	10
Adseal	4	14	13	12	14
2 Seal	15	13	12	11	16
Sealapex	4	9	10	10	10
AH Plus	2	11	10	10	10

Adseal sealer presented strongest antimicrobial effect to *S. aureus, P. aeruginosa, S. mutans*. In addition to this, the 2 Seal sealer presented the strongest antimicrobial activity to *C. albicans* and *E. faecalis*.

Discussion

After determining that the bacteria are the primary agent in the etiology and pathogenesis of periapical tissue diseases, it is obligatory to suffuse the shaped root canal hermetically, and to inhibit the spread of antigens and bacteria from root canal system to periapical region.

However, root canals with complex anatomical structures are not always possible to do this. [14].

Therefore, root canal sealers material should prevent infection and re-infection. Thus, antimicrobial agents were added to the channel sealers to prevent the growth of microorganisms. In general, all channel sealers contain some antibacterial agents and exhibit bactericidal or bactericid activity immediately after insertion in the canal [14].

Today, there are many studies investigating the antimicrobial activities of channel sealers with different techniques and different microorganisms. Therefore, it is not easy to compare the results of the research. [9]. The most important difference of this study from other studies was revealed efectives of eight different root canal sealers against five different microorganisms isolated often from sealers root canal treatments and persistent apical periodontitis. In our study, antimicrobial activities of canal sealers were different from each other and from the results of other studies.

The antimicrobial effect of a root canal material is thought to vary depending on the penetration capacity of the test material into the environment and the type of microorganism analyzed [15]. Therefore, in this research, since the materials whose antimicrobial activities were examined were used in root canals, strains of microorganisms generally existed in root canal flora were used.

There are many methods to investigate antimicrobial activity. However, these techniques also have advantages and disadvantages. ADT is widely used. With ADT, the antimicrobial activity of these materials can be easily compared directly, and variables can also be easily controlled in ADT. ADT does not give information about the viability of microorganisms and does not exhibit the bacteriostatic and bactericidal effects of root canal materials. The ADT necessitates watchful standardization of inoculum, size, medium content, density, agar viscosity and number of specimens per plate [4,16]. In our research, the ADT test results obtained with clinical isolates are similar to the ADT test results of standard strains. However, all sealers have higher antimicrobial activity against clinical isolates than standard strains. In according to ADT, the strongest inhibition zones were observed between EndoSequence BCS and *S. aureus*, Well Root ST and *C. albicans*, MTA Fillapex/Ortho-MTA and *E. faecalis*, Adseal and *S. aureus*, 2 Seal and *C. albicans*, Sealapex and *P. aeruginosa*, *S. mutans*, AH Plus and *C. albicans*.

DET is associated with direct contact with microorganisms and substance effectiveness. This method is very easy to use in the laboratory and is independent of other variables. However, due to the qualitative results of antimicrobial activity with this method, it is difficult to interpret the results [16].

According to the DET test results of our study, while Well Root ST and Sealapex showed antibacterial activity after 24h, and all of the sealers except Adseal after 48h showed completely antibacterial activity. Adseal showed antibacterial activity 72h after test. The results depictured that, by the DET, all root canal filling materials exhibited antimicrobial efficacy to *E. faecalis, S. aureus, S. mutans, P. aeruginosa* and *C. albicans*. In a study conducted in Taiwan, using the ADT, it was reported that AH plus showed moderate effect against *S. aureus* in the end of a 48h period [4].

According to a study by Huang et al with ADT, the mixed RealSeal and AH Plus was not exhibited antimicrobial effect to *E. faecalis*, but exhibited antimicrobial effect to *E. coli* (3.17mm) and *C. albicans* (3mm). In addition, Freshly mixed MTA demonstrated antimicrobial effect to *C. albicans* (3.5mm) [17].

According to a study by Dalmia et al, Sealapex was demostrated the highest antimicrobial activity to *E. faecalis* while MTA Fillapex was demostrated least. Moreover, the efficacy of the sealers to *E. faecalis* was decreased with time. Mean diameters of inhibition zones of AH Plus, Sealapex, MTA fillapex were detected respectivelly 9mm, 14.66mm, 6.66mm after 24h [18].

Gholamhoseini et al determined that MTA-Fillapex had antibacterial activity to *E. faecalis* by showing inhibition zone 12mm, but not effect against *S. aureus* [19].

A study conducted by the ADT in Turkey, antimicrobial effect of MTA Fillapex and AH plus to *C. albicans, E. faecalis, P. aeruginosa, S. aureus* were examined in 24h, 48h, 72 hours. AH plus exhibited the highest activity against *P. aeruginosa*. MTA Fillafex showed lower activity than AH plus for all bacteria MTA Fillafex and AH plus showed a gradually decreasing activity over time period. In the end of 48 hours, it was reported zone diameters for MTA Fillafex and AH plus were found respectively 5.47-6.46mm for *S. aureus*, 8.43-19.41mm for *P. aeruginosa*, 5.36-6.43mm for *E. faecalis* and 5.37-6.31mm for *C. albicans* [20].

In another study, it was reported that AH plus caused a significant decrease in cell proliferation and had a strong antimicrobial activity [21,22].

This effect may be associated with the minimum amount of formaldehyde or the release of bisphenol A diglycidyl ether [23].

According to some studies, MTA Fillapex occured antimicrobial effect to *E. faecalis* and *S. aureus* at 48. and 72. hours respectively. However, it has been exhibited that MTA Fillapex has antimicrobial effect to *E. faecalis* and still maintains antimicrobial effect after 7 days [20,24,25].

The antimicrobial effect of this substance is thought to be due to its high pH, hydrophilicity property and active calcium hydroxide release [26,27].

According to a study in Bangladesh, calcium hydroxide based root canal sealers and MTA demostrated the lowest activity to *P. aeruginosa* while the highest activity to *S. auerus* by the ADT method. These two substances showed no antimicrobial activity to *E. faecalis*. The antimicrobial activity of MTA based canal sealer was found lower than that of calcium hydroxide based canal sealer. Thus, zone diameters for MTA based and calcium hydroxide based canal sealers were reported to be 15-25mm for *S. aureus*, 8.5-12.5mm for *P. aeruginosa*, and 13-16mm for *C. albicans*, respectively [28]. In a study performed in Turkey, MTA Fillapex has shown satisfactory results in ADT against *S. aureus, E. faecalis* and *C. albicans* [29].

In another study performed in Turkey, AH Plus has larger inhibition zone than MTA Fillapex and Sealapex. Sealapex has less antibacterial effect on *E. Faecalis*. The results demostrated that eugenol based root canal sealers have significant antibacterial effects [30].

In study in Bangladesh, Sealapex had a minimum antimicrobial activity against *C. albicans* (12mm) over a 24h interval. It occured the highest antimicrobial activity to *E. faecalis* (14mm). Sealapex showed increasing activity over time over a period of 24h, 48 hours and five days [31].

In India, in a study conducted with the ADT, the antimicrobial effect of Bioseramic-BC sealer (13mm) to *E. faecalis* throughout 24h and 48h times period was markedly lower than that of Ah plus (25mm). It is believed that the antibacterial effect of BC sealers can result from collocate of high pH and active calcium hydroxide diffusion [32]. In another study in Brazil, the results indicate that fresh Bio-C Sealer does not inhibit *S. mutans* growth, but exhibits antibacterial activity against *E. faecalis, S. aureus, P. aeruginosa* and *E. coli* [33].

According to another study mentioned in India, Sealapex has been found to have more antimicrobial effect to *E. faecalis* than AH plus. These sealers showed a decreasing effect over time period. Furthermore, the zone diameters of AH plus and Sealapex were reported to be 20mm and 12mm, respectively, over a 24h period [34]. In a study performed by London, AH Plus has larger inhibition zone than MTA Fillapex and Sealapex. Sealapex has less antibacterial effect on *E. faecalis*. The results demostrated that eugenol based root canal sealers have significant antibacterial effects [35].

In another study conducted in Iran, it was recorded that calcium hydroxide exhibited the highest antimicrobial effect to *S. aureus* (7mm), and MTA showed the most antimicrobial effect against *E. faecalis* (5.5mm) by ADT [36].

In a study performed in Brazil, it was determined by ADT that MTA-based substance and Sealapex were exhibited highest antimicrobial effect to *E. faecalis* (14mm), but lowest antimicrobial effect to *S. aureus* (8mm) and *P. aeruginosa* (8mm) [37].

Conclusion

When it was evaluated the results of previous research on this subject and the results of this study, some important results appear. These substances have been known to effect mostly cytoplasmic membranes of bacteria. Because membrane structures of bacteria are similar, these sealers may effect on anaerobic, aerobic, Gram negative and Gram positive bacteria.

It was observed that Calcium silicate, Mineral Trioxide Aggregate, Epoxy-amine and calcium Hydroxide strengthen its antimicrobial properties by covering all pulpal tissue. According to these data, root canal occluders should be exposed for a certain period of time to show their potential against microorganisms that cause endodontic infections. However, global and periodic reference studies are required to determine antimicrobial activities.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
Afyonkarahisar Health Sciences University clinical studies Ethics Committee Approved (Date: 06.07.2018, Meeting no: 2018/175 Decision: 18)

References

1. Card SJ, Sigurdsson A, Ostravik D. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod. 2002;28:779-83.
2. Johnson WT, Gutmann JL. Obturation of the cleaned and shaped root canal system. In: Cohen S, Hargreaves KM, eds, Pathways of the Pulp. St Louis: Mosby. 2006;358-99.
3. Gomes, BPFA, Pedroso JA, Jacinto R, et al. In vitro evaluation of the antimicrobial activity of five root canal sealers. Brazil Dent J. 2004;15:30-5.
4. Lai CC, Huang FM, Yang HW et al. Antimicrobial activity of four root canal

sealers against endodontic pathogens. Clin Oral Invest. 2001;5:236-9.

5. Patel DV, Sherriff M, Ford TR, et al. The penetration of realseal primer and tubliseal into root canal dentinal tubules: a confocal microscopic study. Int Endod J. 2007;40:67-71.

6. Siqueira JF JR, Rôças IN, Santos SR, et al. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod. 2002;28:181-4.

7. Kokkas AB, Boutsioukis ACh, Vassiliadis LP, et al. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. J Endod. 2004;30:100-2.

8. Arora R, Rawat P, Bhayya DP. A comparative evaluation of antimicrobial efficacy of three endodontic sealers: Endoflas FS, AH Plus and sealapex against Enterococcus faecalis an in vitro study. J Dent Med Sci. 2014;13: 90-3.

9. Cobankara FK, Altinoz HC, Ergani O, et al. In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod. 2004;30: 57-60.

10. Stowe TJ, Sedgley CM, Stowe B, et al. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial properties of tooth-colored pro root mineral trioxide aggregate. J Endod. 2004; 30:429-31.

11. Hasheminia M, Razavian H, Mosleh H, et al. In vitro evaluation of the antibacterial activity of five sealers used in root canal therapy. Dent Res J. 2017;14:62.

12. Shree MK, Ganesh B, Ranı L. Endodontic sealers with antibiofilm properties. J Contempor Issue Business Government. 2020;26:2.

13. Amorim LT, Toledo OA, Estrela CRA, et al. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. Brazilian Dent J. 2007;17:317-22.

14. Grossman L. Antimicrobial effects of root canal cement. J Endodon. 1980; 6:594.

15. Willerhausen I, Callaway A, Briseno B, et al. In vitro analysis of the cytotoxicity and antimicrobial effect of four endodontic sealers. Head Face Med. 2011;7:15.

16. Estrela C, Estrela CRA, Bammann LL, et al. Two methods to evaluate the antimicrobial action of calcium hydroxide paste. J Endod. 2001;27:5-7.

17. Huang Y, Li X, Mandal P, et al. The in vitro antimicrobial activities of four endodontic sealers. BMC Oral Health. 2019;19:118.

18. Dalmia S, Gaikwad A, Samuel R, et al. Antimicrobial efficacy of different endodontic sealers against Enterococcus faecalis: An in vitro study. J Int Soc Prevent Communit Dent. 2018;8:104-9.

19. Gholamhoseini Z, Alizadeh S, Bolbolian M. In vitro evaluation of antimicrobial activity of three bioceramic endodontic sealers against Enterococcus faecalis and Staphylococcus aureus. Ann dental specialty. 2018;6:261-3.

20. Gürel M, Demiryürek EÖ, Özyürek T, et al. International antimicrobial activities of different bioceramic root canal sealers on various bacterial species. Int. J. Appl. Dent. Sci. 2016;2:19-22.

21. Zhang H, Shen Y, Ruse ND. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009; 35:1051-5.

22. Al-Hezaimi K, Al-Hamdan K, Naghshbandi J, et al. Effect of white-colored mineral trioxide aggregate in different concentrations on Candida albicans in vitro. J Endod. 2005;31:684-6.

23. Slutzky-Goldberg I, Slutzky H, Solomonov M, et al. Antibacterial properties of four endodontic sealers. J Endod. 2008;34:735-8.

24. Morgental RD, Vier-Pelisser FV, Oliveira SD, et al. Antibacterial activity of two MTA-based root canal sealers. Int Endod. J 2011;44:1128-33.

25. Kuga MC, Faria Q, Weckwerth PH et al. Evaluation of pH, calcium release and antibacterial activity of MTA Fillapex. Rev odontol UNESP. 2013;42:330-5.

26. Lovato KF, Sedgley CM. Antibacterial activity of endosequence root repair material and Pro Root MTA against clinical isolates of Enterococcus faecalis. J Endod. 2011;37:1542-6.

27. Zarrabi MH, Javidi M, Naderinasab M, et al. Comparative evaluation of antimicrobial acitiviy of three cements: new endodontic cement (NEC), mineral trioxide aggregate (MTA), and portland. J Oral Sci. 2009; 51:473-2.

28. Banu MLA, Bashar AKM, Howlader MR, et al. Antimicrobial efficacy of mineral trioxide aggregate against therapy resistant endodontic microorganisms. J Bangladesh Coll Phys Surg. 2015;33:140-5.

29. Türkyılmaz A, Erdemir A. Antibacterial and antifungal activity of MTA based root canal sealer versus epoxy resin based and methacrylate resin based sealers. Int Dent Res. 2020;10:66-72.

30. Duzyol E, Aydogdu S, Duzyol M , et al. Antimicrobial activity of root canal sealers on facultative gram-positive cocci. Int J Dent Sci Res. 2020;8:80-2.

31. Chowdhury R, Alam S, Rubby G, et al. City Dent Coll. 2013;10:27-30.

32. Zhang H, Shen Y, Ruse ND, et al. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod. 2009;35:1051-5.

33. Barbosa VM, Silva AP, Silva MO, et al. Antibacterial activity of a new readyto-use calcium silicate-based sealer. Brazilian Dent J. 2020;31:611-6

34. Arora R, Rawat P, Bhayya DP. A Comparative evaluation of antimicrobial efficacy of three endodontic sealers: Endoflas FS, AH Plus and sealapex against Enterococcus faecalis an in vitro study. IOSR-JDMS. 2014;13:90-3.

35. Bose R, Ioannidis K, Foschi F, et al. Antimicrobial effectiveness of calcium silicate sealers against a nutrient-stressed multispecies biofilm. J. Clin. Med. 2020;9:2722.

36. Asgary S, Kamrani FA, Taheri S. Evaluation of antimicrobial effect of MTA, calcium hydroxide, and CEM cement. IEJ. 2017;2:105-9.

37. Estrela C, Bamman NLL, Estrela CRA, et al. Antimicrobial and chemical study of mta, portland cement, calcium hydroxide paste, sealapex and dycal. Braz Dent J. 2000;11:3-9.