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Comparative evaluation of antimicrobial efficacy of different endodontic sealers against Enterococcus Faecalis: An in-vitro study

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***Abstract*---**Aim: The aim and objective of this invitro study was to determine antimicrobial activity of root canal sealer (AH Plus, Sealapex, MTA Fillapex) against *Enterococcus faecalis*. Materials and Methods: Three different commercially available root canal sealers namely - AH-Plus (Dentsply), MTA Fillapex (Angelus, bioceramic sealer) and Sealapex sealer (Kerr) were evaluated for their antimicrobial potential against *Enterococcus faecalis (*E.faecalis)using agar diffusion method. Wells were formed in the agar plates by removing agar at equal distance and then, filled with the endodontic

sealers- AH Plus, Sealapex and MTA Fillapex. Standard antibiotic disc of co-trimoxazole was kept as a control. Petri dishes were inoculated at 37 ℃ at a time interval of 24 and 48 hours. The diameter of growth of inhibition zone were measured by using Antibiotic zone scale (HIMEDIA). The difference between the groups were evaluated by one - way ANOVA and intergroup evaluation was done by Tukey's post hic test. Result: AH-Plus sealer showed larger zone of inhibition as compared to sealapex and MTA Fillapex sealer against E.faecalis at 24 and 48 hours. Co- trimoxazole used as control exhibited the highest antimicrobial efficacy against E.faecalis at 24 and 48 hour. MTA Fillapex showed least antimicrobial action. Conclusion: The AH-Plus root canal sealer showed the better antibacterial efficacy against E.faecalis at 24 and 48 hours.

***Keywords*---**Agar diffusion test, Enterococcus faecalis, inhibition zone.

Introduction

The main objective of endodontic therapy is to eliminate all organic, inorganic debris and microorganisms along with the substrate that encloses microorganism from the root canal system and the prevention of reinfection.[1] The successful treatment is achieved by proper cleaning and shaping i.e. biomechanical preparation, disinfection and complete three-dimensional obturation of the root canal system.[2] One of the main reasons for pulpal and periapical diseases are presence of micro-organisms.[3] In, endodontics there is presence of a polymicrobial flora.[1] And most of the endodontic infections are mixed and polymicrobial with the presence of some facultative anaerobes, strict anaerobes and very rarely aerobes.[2] Endodontic failures are due to various reasons like intraradicular infections, extraradicular infection, foreign-body reactions, and true cysts. But most common cause of failure is by the presence of microorganism in the apical parts of root canals of obturated teeth. [4]

Most common microorganism perceived in endodontic failures is *Enterococcus faecalis*. *E. faecalis* is a gram-positive facultative anaerobe which can easily grow in the presence or absence of oxygen and can survive even in extremely harsh low nutrient environment. It can withstand high alkaline pH of 11.5 and can easily survive in root canals most commonly with periapical lesions without any support of other bacteria. Hence, it is called as monoinfection in root canal. Only by chemo-mechanical preparation of root canal system *E. faecalis* cannot be eradicated. According to Siren EK *et al*, it was observed that in root canal treated teeth with an inadequate seal the chances of presence of *E. faecalis* was very high.[4] Therefore, the use of root canal filling materials with antibacterial activity shows a beneficial effect to further reduce the number of remaining microorganisms and to eradicate any residual infection.[5]

Sealers are the materials which seal the voids or the space present between the gutta-percha itself and between the gutta-percha and the dentinal walls, since gutta-percha does not bind with the dentin. [5, 6] The sealers tested in this study are Bioceramic sealer, Epoxy resin sealer and Sealapex sealer.

AH plus sealer is epoxy resin sealer with good mechanical property, high radio opacity, low polymerization shrinkage, low solubility and high degree of stability. While Bioceramic sealer shows biological activity, are radiopaque and exhibits no shrinkage. Hence, when used with obturating systems, it exhibits inhibitory effect on the survival of bacteria. [7, 5] Sealapex is a calcium hydroxide containing noneugenol-based sealer, available as catalyst base system. It exhibits both antibacterial property as well as osteogenic cementogenic potential.

Agar diffusion test is a most common and standard test for antimicrobial activity of endodontic sealers. This method indicates potentiability of the sealer to eliminate microorganisms from the local microenvironment of the root canal system. [7,8]

The objective of this study is to evaluate antimicrobial efficacy of three different endodontic sealers against Enterococcus faecalis; on the bases of measuring the effect of close contact between test bacteria and tested material on kinetics of bacterial growth.

Materials and Methods

In this study, the endodontic sealers tested were as follows:
Group A- AH Plus (Dentsply, Germany), Group B – Sealapex (Kerr, USA), Group D – MTA Fillapex (Angelus, Brazil) and Co-trimoxazole (HIMEDIA, standard antibiotic disc) was used as control group C against Enterococcus faecalis.

Preparation of the medium for Enterococcus faecalis

The E. faecalis (bacterial strain- ATCC 29212) was revived on blood agar medium plates and inoculated in brain heart infusion (BHI) broth. The plate was incubated at 37℃ for 24 hours. After 24 hours, from the isolated colonies inoculum of 0.5 McFarland concentration was prepared in normal saline solution and using Densichek (Biomerieux, USA) the bacterial concentration was checked.

Antimicrobial activity by agar diffusion test

Preparation of Mueller-Hinton agar in the petri-plates was carried out. A sterile cotton swab bud was dipped into standardized inoculum and was moved against the inner wall of the tube so as to remove the excess fluid. Between every streaking, the plate was rotated at 60˚ angle and was then kept for drying for atleast 10-15minutes. Afterwards, the syringe was taken and hub was cut. This modified syringe was used for forming the three wells in the petri plates. All the three root canal sealers used in the study were mixed under sterile condition and placed in one well each. Co-trimoxazole disc was placed in the petri plates. Petri plates were immediately incubated for 24hr at 35±2°C. The entire study was performed under sterile conditions. And was repeated for twenty times. The final plates were then incubated at 37˚C under aerobic condition for measuring the diameter of zone of inhibition at a time interval of 24 hours and 48 hours.

Inhibition zone reading

Antibiotic zone scale (HIMEDIA) was used to measure the diameter of zone of bacterial growth inhibition at 24 and 48 hours.

Statistical analysis

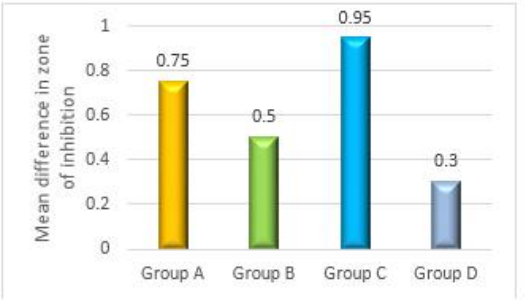
The data collect was subjected to further evaluation by statistical analysis using one-way ANOVA test to check the efficacy of the three different root canal sealers [Table 1] and intergroup comparison was tested by Tukey's post hoc test [Table 2]. $P < 0.05$ was considered statistically significant

Table 1
Statistical analysis ANOVA against Enterococcus faecalis at 24- and 48-hour hours

	After 24 hours		After 48hours			
STUDY GROUP	MEAN	SD	MEAN	SD	MEAN DIFFERENCE	p VALUE
GROUP A	9.20	0.77	8.45	0.68	0.75	<0.001
GROUP B	3.15	0.93	2.65	0.74	0.50	<0.001
GROUP C	27.75	0.64	26.8	0.52	0.95	<0.001
GROUP D	1.75	0.51	1.45	0.44	0. 0.30	0.01

Results

The mean diameter of growth inhibition zone for control group and for each group of endodontic sealer used are shown in Figure 1 and Graph 1. One-way ANOVA was used to evaluate the P- value and showed significant difference (P value <0.05) [Table 1]. AH Plus sealer (Group A) had the highest zone of growth inhibition [Figure 1 & Graph 1] in comparison to other two sealers. The value of all the sealers increases in the initial 24 hours and decreases after 48 hours. MTA Fillapex sealer showed lowest inhibitory effect against E. faecalis. While, co-trimoxazole (standard antibiotic disk) showed highest zone of growth inhibition against E.faecalis.



Graph 1: The above table and graph show the mean difference in the zone of inhibition (after 48 hours when compared to the zone of inhibition after 24 hours) for all the 4 groups.

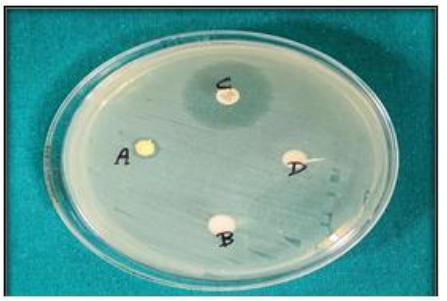


Figure 1: zone of inhibition exhibited by AH Plus sealer was higher than other two sealers. Whereas co-trimoxazole being the control group showed highest zone of inhibition among all the groups

Table 2
Statistical analysis Tukey post hoc test for 2- group comparison against Enterococcus faecalis

Treatment pair	Tukey HSD Q statistic	Tukey HSD p-value
Grp A vs Grp B	3.69	0.03 S
Grp A vs Grp C	4.26	0.007 HS
Grp A vs Grp D	3.85	0.03 S
Grp B vs Grp C	5.28	0.001 HS
Grp B vs Grp D	3.08	0.04 S
Grp C vs Grp D	5.68	0.001 HS

Discussion

'Disk diffusion test' also called as agar diffusion test was used most commonly to determine the antimicrobial potential of various endodontic sealers. This method helps in indicating which sealer has the maximum potential to eliminate microorganisms from the local microenvironment of the root canal system. [7,8] In this study, Enterococcus faecalis is used as the target organism as the most commonly recognized microorganism in failed endodontic therapy is Enterococcus faecalis.[7]

These are pathogenic microorganisms and is tolerant to most of the commercially available antibiotics.[9] E. faecalis is a gram-positive coccus and can survive in an atmosphere with low oxygen level and rich nutrients.[10,11] It was observed that E. faecalis increases in number in cases of failed root canal treatment as compared to the cases with primary infections. In cases with post endodontic pain, prevalence rate of Enterococcus faecalis is almost 90%.[10,12]Enterococcus faecalis can survive even after disinfection of root canals by using various intracanal medicaments like calcium hypochlorite solutions and irrigants.[13] Fluids present in periodontal ligaments can act as a nourishing medium for Enterococcus faecalis because of which it forms a protective biofilm against host resistance and

disinfecting agents.[14] So, it is very crucial to understand that the aim of endodontic therapy is to completely eliminate the prevalence of infection and prevention of reinfection of the endodontically treated tooth.

In this study three root canal sealers (AH Plus, Sealapex and MTA Fillapex) were used to evaluate the antibacterial action against Enterococcus faecalis at 24 and 48 hours. Co- trimoxazole being standard antibiotic disc was used as control group against E. faecalis and had showed the highest zone of inhibition followed by AH Plus, Sealapex and MTA Fillapex sealer. This bactericidal action is because of inhibitory action on the formation of folic acid.[5] The sequentially blocking up the folic acid enzyme causes inhibition of bacterial cell synthesis hence bacterial death.

In the present study, AH Plus sealer showed highest antimicrobial efficacy against E.faecalis followed by sealapex sealer and MTA Fillapex showed least antimicrobial action. AH Plus has two paste system, paste A contains epoxy resin and paste B has amines, when these two pastes are mixed together, the sealer reduces the cell viability.[1] The presence of Bisphenol-A-diglycidyl ether is responsible for the antimicrobial action.[15] It has good flowability because of which it can easily penetrate into the dentinal tubules, forming better hermetic seal. Therefore, enhances antimicrobial potential.[16] During the process of polymerization, formaldehyde is released resulting in sealers antibacterial action.[2] In the present study, AH Plus has better penetration action into dentinal tubules as compared to MTA Fillapex and Sealapex.This result could imply that these sealers contain more potent antibacterial inhibitors or may have better diffusion properties.

Whereas, Sealapex showed better action than MTA Fillapex because it has high dissociating action into calcium ions and hydroxyl ions[17] due to which alkalinity of environment increases. As the pH value increases, the enzymatic action which is necessary for the bacterial metabolism, growth and cell division decreases, leading to breakdown of cytoplasmic membrane.The potential release of calcium hydroxide ions in MTA Fillapex is less in comparison to sealapex due to which it has lower pH value resulting in lower antimicrobial action.[18] The results show that the value of zone of inhibition for all the 4 groups were higher at an interval of 24 hours in comparison to 48 hours and the mean difference of all the groups were statistically significant (p value < 0.05).

From this study it was also concluded that with increase in time antibacterial action of sealer decreases i.e., it was highest at 24 hours and lowest at 48 hours because diffusion ability of the freshly mixed sealer is more as compared to the set sealer.[5,1]

However, limitations associated with this study are that the results of agar diffusion method could be influenced by affinity and diffusion of the material to the culture medium as the material that diffuses easily mainly results in larger zone of inhibition of bacterial growth. Furthermore, agar diffusion method is not completely reliable due to its own constraints i.e. intensity of agar, condition in which plate is stored, incubation time and incapacity to differentiate between bactericidal and bacteriostatic actions. There are contemporary and more reliable

methods available to check antibacterial efficacy which can also be tried in the future to test the same.

Conclusion

Eradication of *Enterococcus faecalis* from the root canal is important for a successful endodontic treatment. Many studies have shown that biomechanical preparation and irrigation of root canal is not sufficient for elimination of microorganism. At this point comes the role of obturation of these decontaminated root canals with appropriate antimicrobial root canal sealers to prevent endodontic failures. Based on the results of the present study, it can be concluded that:

- AH plus (mean difference value = 0.75±0.55) has the highest antimicrobial efficacy amongst the three sealers used followed by Sealapex (mean difference value = 0.5±0.51) and MTA Fillapex (mean difference value = 0.30±0.47) sealers against *Enterococcus faecalis*.
- Co-trimoxazole (control group) has shown maximum antibacterial action against *Enterococcus faecalis*.
- Test sealers have shown bactericidal action when freshly mixed and their antibacterial action decreases with increase in time duration.

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Conflicts of interest
There are no conflicts of interest

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