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Original Article

Evaluation of Antimicrobial Efficacy of Different Endodontic Sealers Against Root Canal Microorganisms: An *In Vitro* Study

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Abstract

Objective: The present study aimed to evaluate the antimicrobial efficacy of endodontic sealers that are - MTA Fillapex, AH26, Apexit Plus, Zinc Oxide Eugenol (ZOE), and Zinc oxide (ZO) + Thyme oil against *E. coli, P. aeruginouosa, S. aureus*, and *E. faecalis*.

Materials and Methods: The antimicrobial tests were performed using the Agar diffusion Method. Microorganisms were inoculated with Muller Hinton Agar, and Five holes were punched and filled with respective sealers: MTA Fillapex (Group 1); AH26 (Group2); Apexit Plus (Group 3); ZOE (Group 4) and ZO+ Thyme oil (Group 5). These plates were then incubated, zone of inhibition was checked after 24 hours. The data was statistically analyzed using one-way ANOVA and Tukey's Test ($p<0.05$).

Results: All the sealers showed some level of antimicrobial efficacy against test microorganisms with the highest antimicrobial activity exhibited by Zinc oxide + Thyme oil followed by AH26, MTA Fillapex, Apexit Plus, and least by ZOE.

Conclusion: Zinc oxide + Thyme oil exhibited the highest antimicrobial efficacy against all test microorganisms.

Keywords: Antimicrobial efficacy, endodontic sealers, pediatric endodontics, thyme oil, zinc oxide eugenol

Introduction

Microbial infection in root canals is polymicrobial with a predominance of strict anaerobes, some facultative anaerobes, and some aerobes.[1] Recent studies have associated the presence of fungi, along with other microbes to be the main pathogenic factors in pulpal and periapical infection.[2,3] Sundqvist et al[2] recovered numerous species of anaerobic bacteria from failed root canal systems, some of these bacteria include *E. Faecalis, streptococcus sp., S. aureus*, and *Fusobacterium nucleatum*.[2]

The major goal of root canal treatment is the elimination of microorganisms from the root canal system and preventing subsequent reinfection.[4] Lin et al[5] and Sequeira et al[6] have demonstrated that a part of root canal space often remains untouched during cleaning and shaping regardless of technique and instrument employed. Love et al[7], Molander et al[8] and Sundqvist et al[2] reported the presence of the microorganism in areas, such as the isthmus, apical delta, canal space irregularities, and dentinal tubules even after thorough chemo-mechanical preparation of the root canal system.

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The use of root canal filling material with antimicrobial activity will help to achieve this goal hence antimicrobial agents are incorporated into root canal sealers to enhance their antimicrobial efficiency and aid in the repair process of periapical tissues.[9,10]

Materials and Methods

For the study standard strains of *E. Coli (ATCC 25922)*, *P. aruginosa (ATCC 27853), S. aureus (ATCC 25923)*, and *E. faecalis (ATCC 29212)* were procured. Five test materials were taken and divided into groups, MTA Fillapex (Group A), AH26 (Group B), Apexit Plus (Group C), Zinc Oxide Eugenol (Group D), Zinc oxide + Thyme oil (Group E). The sealers were prepared in strict compliance with the manufacturer's recommendation, and for mixing Zinc oxide and Thyme oil the powder-liquid ratio was, 0.2 mg of powder which was mixed with 0.07 cc oil. Mixing powder and liquid was done using a spatula on a dry glass slab.

Fresh bacterial colonies were suspended in nutrient Agar slants and their turbidity was adjusted to 0.5 Mc Farland standard, corresponding to approximately 1.5×108 cfu/ml. To achieve a lawn of bacterial growth, the bacterial suspension was evenly distributed onto the surface of Muller Hinton Agar plates using sterile swabs.

After inoculation, five wells of diameter 6mm were punched at equidistant points and then filled with freshly mixed sealers. All the plates were kept at room temperature for 2 hours for pre-diffusion of the sealers, later these plates were incubated at 370 degrees Celsius for 24 hrs.

After 24 hours, the zone of inhibition around each one of the wells was measured with the help of a metal scale (Fig. 1-4).

Results

A zone of inhibition by five endodontic sealers against four bacterial strains was measured. Maximum zone of inhibition was recorded for zinc oxide thyme oil with mean value of zone of inhibition found to be (35.75±3.892 mm) followed by AH 26 with the mean value of zone of inhibition to be (23.90±1.483 mm), followed by MTA Fillapex (17.00±2.596 mm), followed by Apexit Plus (12.85±2.661 mm) and the least zone of inhibition was shown by zinc oxide eugenol with the mean zone of inhibition to be (11.25±1.860 mm). Statistical analysis was done using one-way ANOVA showed that there was a statistically significant difference among the study groups (p-value <0.001).

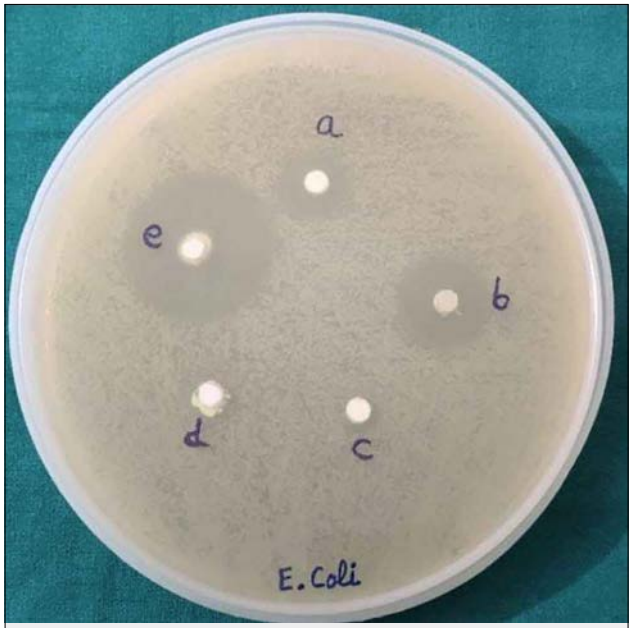

Figure 1. Zones of inhibition representing antibacterial activity of test materials; a, b, c, d, e, against *e. coli*

Figure 2. Zones of inhibition representing antibacterial activity of test materials; a, b, c, d, e, against *p. aeruginosa*

Discussion

The rationale for endodontic treatment is to eradicate the infection in the root canal and peri radicular tissue and also to prevent its subsequent reinfection.[11–14]

Chemo-mechanical preparation is undoubtedly one of the most important steps in endodontic treatment.[9] However, it has been demonstrated that a part of root

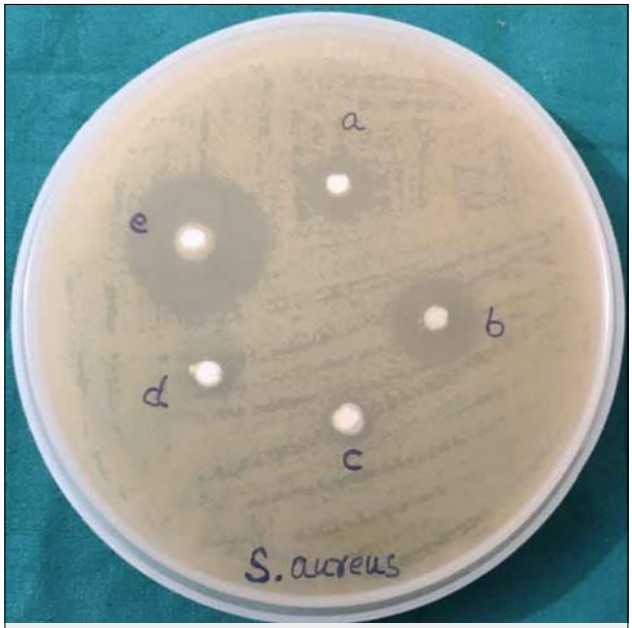

Figure 3. Zones of inhibition representing antibacterial activity of test materials; a, b, c, d, e, against *s.aureus*

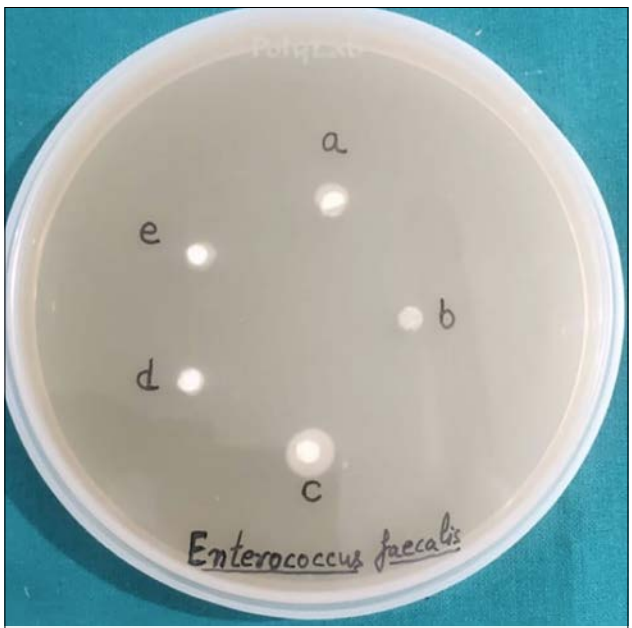

Figure 4. Zone of inhibition representing antimicrobial efficacy of test materials; a, b, c, d, e, against *e. faecalis*

canal space often remains untouched during cleaning and shaping regardless of the technique used.[5,6] Bystrom et al[12] postulated that if the microorganism prevails in the root canal after obturation there is a higher risk that treatment will fail. Hence for a successful endodontic treatment the role of quality obturation, in which a sealer has a part to play cannot be overlooked. Antimicrobial agents are incorporated into the root canal sealers to enhance their antimicrobial activity so that the space which remains untouched, is filled by the sealer and thus eliminates microorganisms.[9] *E. faecalis, S. aureus, P. aeruginosa*, and *E. coli* have been reported to be found in root canal treatment failures.[13] Their ability to form biofilm renders them to prevail in the root canal even under unrelenting conditions,[14] and hence these organisms were chosen for the present study. A variety of laboratory methods can be used to evaluate the *invitro* antibacterial activity of various agents but the most commonly known and basic method is the Agar Diffusion Test which was used in our study. The Agar Diffusion test proposed by Gomes et al[15], allows a direct comparison of the root canal sealers against the microorganisms to be tested and the visual indication of which sealer has the potential to eradicate microorganisms in the local microenvironment of the root canal system. This process is relatively inexpensive and can be performed rapidly and easily and the test also serves as a bioassay to measure the diffusion of the antibacterial agents into its surroundings.[16]

The highest zone of inhibition formed after 24 hours was by Zinc oxide + Thyme oil followed by AH 26>MTA Fillapex> Apexit Plus> Zinc Oxide Eugenol. Antimicrobial activity of Thyme oil is due to the presence of various agents like carvacol, B-cymene, Pinene, terpiene acid, menthone and cineole.[13] As there are very few studies available that have compared zinc oxide thyme oil with commercially available sealers like Apexit Plus; AH 26; MTA Fillapex, therefore the present study was carried out to assess the same. The second highest antimicrobial efficiency was exhibited by AH26 with a statistically significant difference in the zone of inhibition followed by MTA Fillapex, Apexit Plus, and ZOE respectively, being the least. These obtained results are in correlation with the study conducted by Jafari et al[17] and Nejadshamsi et al[18], Saha et al[19], and Shantiaee et al[20], who also concluded for AH 26 having higher antimicrobial effectiveness in comparison to MTA Fillapex and Apexit Plus and ZOE respectively. The antimicrobial activity of AH 26 is attributed to the release of formaldehyde.[19] The antimicrobial activity of MTA Fillapex was statistically significant against Apexit Plus and ZOE and these results are in correlation with the similar study conducted by Kumar et al[21], Reyhai et al[22] and Mangat et al[23] who concluded that MTA Fillapex exhibited higher antimicrobial activity against Apexit. MTA Fillapex contains calcium silicate which on hydration from dentine forms calcium silicate hydrogel and CaOH, which is eventually attributed to alkaline pH and its antimicrobial activity.[24] In the Studies conducted by Kumar et al[21] and Saha et al[19],

Apexit Plus demonstrated very little antimicrobial activity. The results of these studies are in correlation with the present study. Apexit Plus contains $Ca(OH)_2$ as the main constituent and it's due to the property of calcium hydroxide to dissociate into Ca^{++} and OH^{+}, increasing the pH, which reversibly or irreversibly inactivates the cellular membrane of microorganisms resulting in loss of biological activity of cytoplasm and thus providing some antimicrobial effect.[9] Zinc oxide eugenol demonstrated the least antimicrobial activity, the result of the present study coincides with the studies conducted by Singh et al[25] and Leonardo et al[26].

Conclusion

Within the limitation of this study, it is concluded that zinc oxide with thyme oil exhibited a significantly higher value of zone of inhibition and hence highest antimicrobial effectiveness, this might be due to agents like carvacrol, B-cymene, Pinene, terpenes acid, Menthone, and Cineole however, an elaborative experimental study should be done to assess tissue biocompatibility and, flowability, strength, and toxicity properties, and thus its use in dentistry.

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