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ORIGINAL ARTICLE

# Evaluation of Antimicrobial Efficacy of Root Canal Sealers against Endodontic Pathogens: An *in vitro* Study

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## ABSTRACT

**Introduction:** The aim of the study is to evaluate the antimicrobial activity of AH Plus, Metaseal, Realseal SE, and EZ Fill root canal sealers against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

**Materials and methods:** Microbiological assays were carried in a laminar flow chamber using a standard ATCC strain. The microorganisms were cultivated at 37°C for 24 hours. Bacterial suspension was prepared to match the turbidity equivalent to 1.0 McFarland standard tube. Ten replica plates containing brain heart infusion (BHI) agar, Sabouraud's agar, and 20 plates containing Mueller Hinton agar were prepared. Four groups were made: Group I (*E. faecalis*), group II (*S. aureus*), group III (*P. aeruginosa*), and group IV (*C. albicans*). In each petri dish, five wells were made – AH Plus, Metaseal, Realseal SE, EZ Fill, and Control. The agar diffusion test was used for determining the zone of inhibition.

**Results:** In our study, control showed the maximum zone of inhibition against all microorganisms except *P. aeruginosa*. When the intragroup comparison of inhibition zones of all sealers in groups I, II, III, and IV were made, Metaseal showed larger zones of inhibition than EZ Fill, AH Plus, and Realseal SE.

**Conclusion:** All the tested groups of sealers except Realseal SE have shown some amount of zone of inhibition against all the tested microorganisms. After 24 hours, the maximum zone of inhibition was shown by Metaseal followed by EZ Fill and AH Plus, and the difference was statistically significant.


**Keywords:** Agar diffusion test, AH plus, Antimocrobial activity, E. Feacalis, Metaseal.

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**Conflict of interest:** None

## INTRODUCTION

Microorganisms and their products are the main etiological factors in dentinal, pulpal, and periapical pathogenesis. Therefore, the ultimate aim of endodontic treatment is absolute eradication of pathogenic organisms and their toxic products from the root canal space.

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Root canal instrumentation, irrigation, and intracanal medicaments significantly reduce the population of microorganisms inside the infected root canal. However, it is impossible to completely eliminate the microbes from the root canal system in all cases.

Consequently, the utilization of antibacterial root canal filling material is considered beneficial in elimination of microorganisms and in further reduction of infection. The traditional method of root canal filling uses a core material in combination with a root canal sealer. Root canal sealers with good sealing ability and antimicrobial properties can have a large impact on the overall success of endodontic treatment. This property will thus enable them to deal with residual infection and bacteria reentering from the oral cavity. Therefore, the aim of the study was to evaluate the antimicrobial activity of AH Plus, Metaseal, Realseal SE, and EZ Fill root canal sealers against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Candida albicans.*

## MATERIALS AND METHODS

The microbiological assays were carried out under aseptic conditions in a laminar flow chamber (Quimis Diadema, SP, Brazil). The antibacterial activity was evaluated using a standard strain of *E. faecalis* (ATCC 29212), *C. albicans* (ATCC 10231), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923).

The microorganisms were cultivated in brain heart infusion(BHI) broth (Merck, Darmstadt, Germany) for *E. faecalis*, Sabouraud's agar for *C. albicans*, and Mueller Hinton agar for *P. aeruginosa* and *S. aureus* at 37°C for 24 hours.

Then, a bacterial suspension was prepared with 0.85% of BHI broth for *E. faecalis* and *S. aureus* and in peptone water for *C. albicans* and *P. aeruginosa* to match the turbidity equivalent to a 1.0 McFarland standard tube, corresponding to $3 \times 10^8$ colony-forming units per mL.

Ten replica plates containing BHI agar, Sabouraud's agar, and 20 plates containing Mueller Hinton agar were spread with the bacterial suspension using a sterile swab.

These plates were then divided into four groups, i.e., group I (*E. faecalis*), group II (*S. aureus*), group III (*P. aeruginosa*), and group IV (*C. albicans*). In each petri dish, five wells were prepared: (A) AH Plus, (B) Metaseal, (C) Realseal SE, (D) EZ Fill, and (E) control.

Amoxiclav was used as a control in groups I, II, and III, while fluconazole was used as a control in group IV.

### Agar Diffusion Test

After the preparation of the samples, five wells of 6 mm in diameter were made with a punch by a sterile pipette removing the agar at equidistant points. Amoxiclav and fluconazole discs were used as a control and placed at the center of the petri dishes in groups I, II, III, and IV respectively; all the sealers were mixed according to the manufacturer's instruction on a mixing pad with an agate spatula and were placed in the petri dishes.

All plates were maintained at room temperature for 2 hours for prediffusion of the materials and then incubated at 37°C for 24 hours. The inhibition zones around each one of the wells were then measured in millimeters. The data obtained were tabulated and subjected to statistical analysis.

### Statistical Analysis

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 15.0 statistical analysis software. The Kolmogorov–Smirnov test, Kruskal–Wallis H test, and Mann–Whitney U test were used. The values were represented in number (%) and mean ± standard deviation. The results were recorded and tabulated.

## RESULTS

For different test and control materials, the zones of inhibition ranged from 0 to 39 mm, with a mean value of 14.22 ± 12.04 mm and a median value of 12.00 mm.

The mean zone of inhibition was found to be the maximum in control (amoxiclav) (36.00 ± 2.00 mm; median 35.5 mm), whereas among the test materials, it was the maximum in Metaseal (13.20 ± 1.32 mm) and the minimum in Realseal SE (0 ± 0). The median value was also found to be the minimum in Realseal SE (0) and the maximum in Metaseal (13.50).

On comparing the antimicrobial efficacy of control and different test materials, the test material amoxiclav was seen to have significantly higher inhibition as compared with all the test materials. Among test materials, Metaseal and EZ Fill had significantly higher inhibitory values as compared with AH Plus and Realseal SE, whereas the difference between Metaseal and EZ Fill was not significant. Realseal SE was the least effective material. On the basis of the above evaluation, the following order of antimicrobial efficacy was noted in different test materials and controls:

### Outcome: Amoxiclav > Metaseal ~ EZ Fill > AH Plus > Realseal SE

Zone of inhibition of tested sealers against *E. faecalis* after 24 hours

- AH plus
- Metaseal
- Realseal SE
- EZ fill

Zones of inhibition of tested sealers against *S. aureus* after 24 hours

- AH Plus
- Metaseal
- Realseal SE
- EZ Fill

Zones of inhibition of tested sealers against *P. aeruginosa* after 24 hours

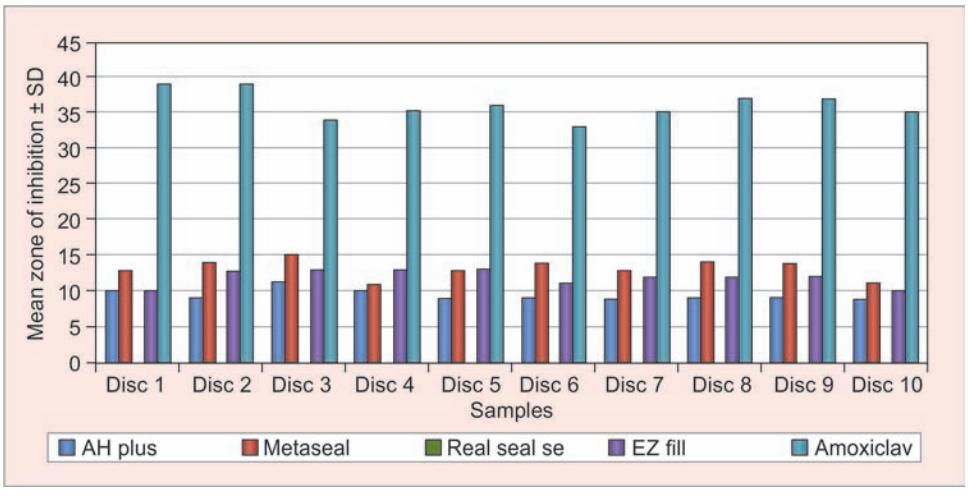
- AH Plus
- Metaseal
- Realseal SE
- EZ Fill

Zones of inhibition of tested sealers against *C. albicans* after 24 hours

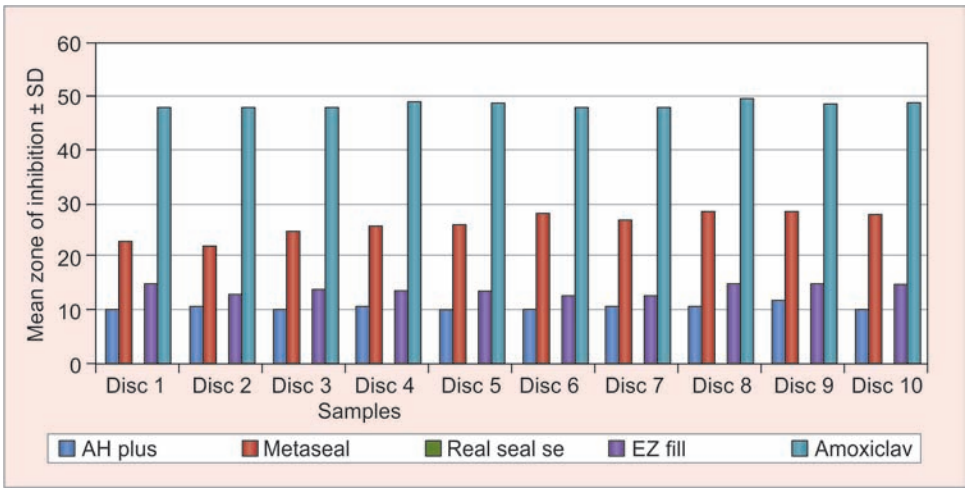
- AH Plus
- Metaseal
- Realseal SE
- EZ Fill

## DISCUSSION

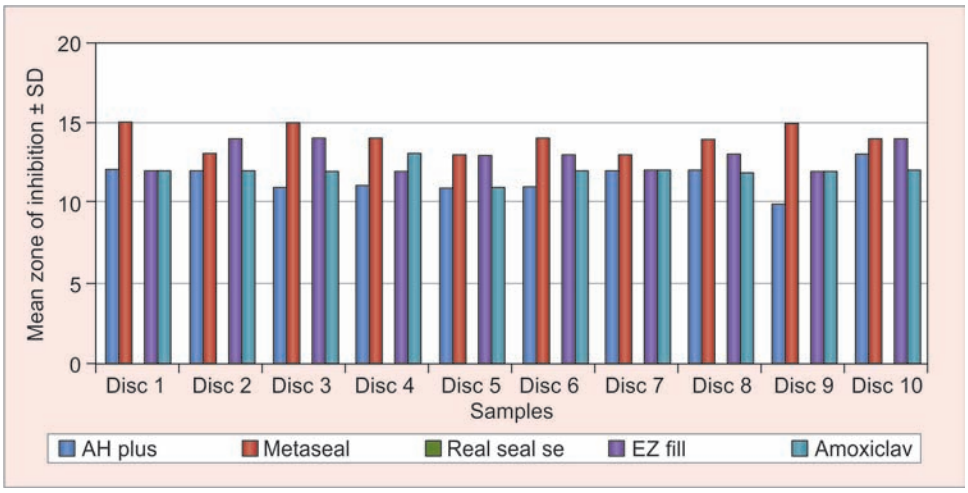
The pertinent aim of root canal treatment is to do away with the microbial entity and any future predilection of



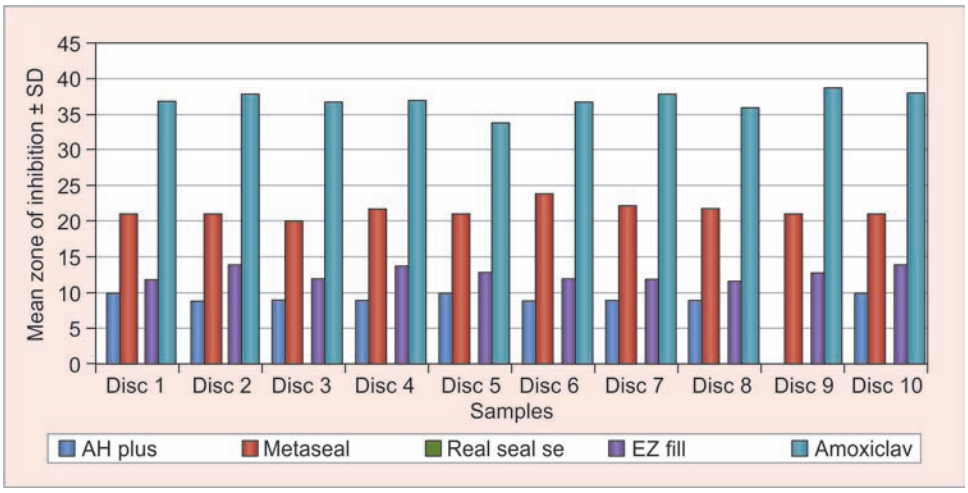

**Graph 1:** Zones of inhibition of tested sealer against *E. faecalis*




**Graph 2:** Zones of inhibition of tested sealers against *S. aureus*




**Graph 3:** Zones of inhibition of tested sealer against *P. aeruginosa*




**Graph 4:** Zones of inhibition of tested sealers against *Candida*

reinfection. Cleaned and shaped root canals must be 3D filled, eliminating the empty space, which has the potential to be infected or reinfected.[1]

Most root canal filling techniques use core materials associated with endodontic sealers. Several properties are required for an ideal endodontic sealer. Among them sealing ability, biocompatibility, antimicrobial activity, adhesiveness, dimensional stability, insolubility to oral and tissue fluids, and adequate flow rate are the properties that will probably influence the root canal treatment.[1,2]

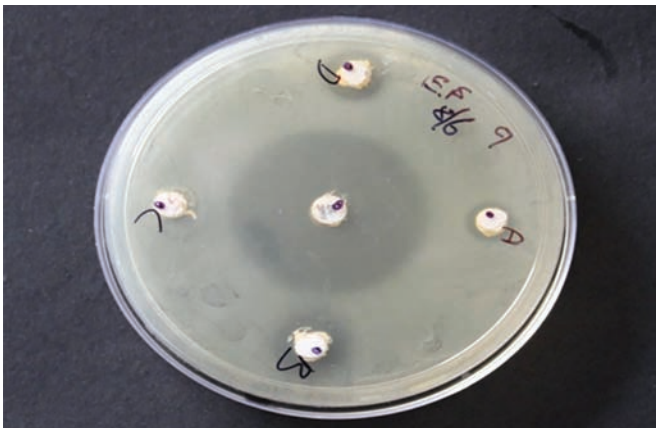


**Fig. 1:** Zone of inhibition of tested sealers against E. faecalis after 24hrs, AH plus, Metaseal, Realseal SE, EZ fill

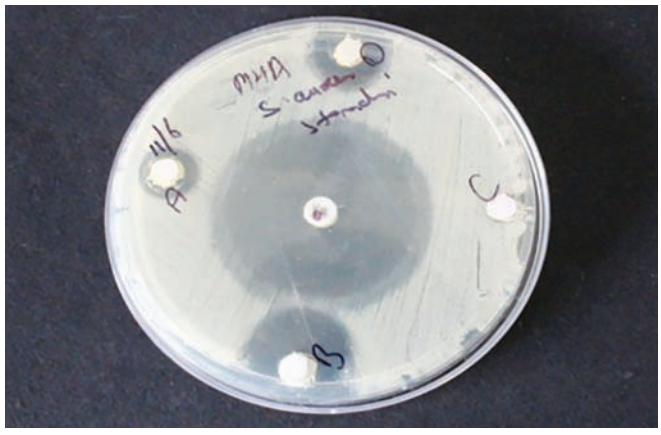


**Fig. 2:** Zones of inhibition of tested sealers against S.aureus after 24hrs, AH Plus, Metaseal, Realseal SE, EZ fill

**Fig. 3:** Zones of inhibition of tested sealers against, P. aeruginosa after 24hrs, AH Plus, Metaseal, Realseal SE, EZ fill

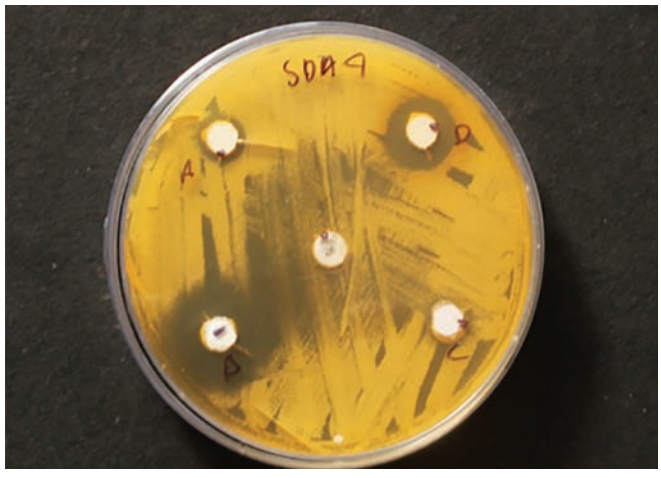


**Fig. 4:** Zones of inhibition of tested sealers against C.albicans after 24hrs, AH Plus, Metaseal, Realseal SE, EZ fill

Sealers should have microbicidal activity or, at a minimum, they should not encourage microbial growth. Studies have reported that several endodontic sealers have antimicrobial effects.[3-6] Sealers having antimicrobial effects may help to eliminate residual microorganisms unaffected by the effects of both chemomechanical preparation and intracanal medication. In addition, they may limit the ingress of microorganisms from saliva, impeding or at least retarding the complete recontamination of the root canal after saliva challenge.

In the present study, the antimicrobial activity of sealers AH Plus, EZ Fill, Realseal SE, and Metaseal were evaluated against *E. faecalis*, *C. albicans*, *P. Aeruginosa*, and *S. aureus* using agar diffusion test. The mean zones of inhibition were calculated and compared. The results were subjected to statistical analysis.

Kruskal–Wallis test was applied for means, and the means were later compared with Mann–Whitney U test. This test was used to check the multiple comparisons, i.e., between all possible combinations of two groups.

In our study, control showed the maximum zone of inhibition against all microorganisms except *P. aeruginosa*. This might be due to the fact that control (amoxiclav) is not the first line of drug of choice against *P. aeruginosa*.

The mean zone of inhibition against *E. faecalis* was 9.40 mm for AH Plus, 13.20 mm for Metaseal, 12.50 mm for EZ Fill, 0 for Realseal SE, and 39 mm for control group (amoxiclav).

### Amoxiclav > Metaseal ~ EZ Fill > AH Plus > Realseal SE

The mean zone of inhibition against *S. aureus* was found to be the maximum in the control (amoxiclav) (48.30 ± 1.16 mm), whereas among the test materials, it was the maximum in Metaseal (26.30 ± 2.41 mm; median 26.50 mm) followed by EZ Fill (14.10 ± 0.88 mm; median 14 mm), AH Plus (10.60 ± 0.70 mm; median 10.50 mm) and the minimum in Realseal (0 ± 0).

### Amoxiclov > Metaseal > EZ Fill > AH Plus > Realseal SE

The mean zone of inhibition against *P. aeruginosa* was found to be the maximum in test material Metaseal

(14.0 ± 0.82 mm; median 14 mm) followed by EZ Fill (12.9 ± 0.82 mm; median 13 mm), control (12.0 ± 0.47 mm; median 12), and AH Plus (11.5 ± 0.85 mm; median 11.5 mm). Realseal SE did not show any inhibitory activity.

**Metaseal > EZ Fill > Amoxiclav ~ AH Plus > Realseal SE**

The mean zone of inhibition against *C. albicans* was found to be the maximum in control (37.10 ± 1.37 mm; median 37 mm). Among test materials, Metaseal (21.50 ± 1.08 mm; median 21 mm) had the highest value followed by EZ Fill (12.8 ± 0.92 mm; median 12.5 mm) and AH Plus (9.4 ± 0.52 mm; median 9 mm). Realseal SE did not show any inhibitory activity.

**Fluconazole > Metaseal > EZ Fill > AH Plus > Realseal SE**

When the intragroup comparison of inhibition zones of all sealers in groups I, II, III, and IV were made, Metaseal showed larger zones of inhibition than EZ Fill, AH Plus, and Realseal SE.

It has been shown that sealers that have strong antibacterial effects are also toxic to the host; thus, the antimicrobial activity of Metaseal could be attributed to the cytotoxicity of its components 4-methacryloyloxyethyl trimellitate anhydride and hydroxyethylmethacrylate (HEMA).

Imazato et al[7] showed that cytotoxic effect of Metaseal is because of HEMA, which is known to be cytotoxic even at low concentrations.

Elgendy and Mahran[8] have reported that HEMA of Metaseal is of low molecular weight, has high hydrophilicity, and can thus easily diffuse and flow into the surrounding environment.

On comparison of zones of inhibition of EZ Fill and AH Plus in all groups, EZ Fill showed larger inhibition zones as compared with AH Plus. This could possibly be due to the higher amount of formaldehyde release (540 ppm) in EZ Fill as compared with AH Plus (3.9 ppm; Cohen et al) despite the composition of both the sealers being quite similar.[9]

Cobankara et al[9] reported that besides formaldehyde release, EZ Fill contains bisphenol A diglycidyl ether, which could be particularly responsible for its cytotoxic effect.

AH Plus also showed zones of inhibition against all the tested microorganisms. Its zones of inhibition were greater than those of Realseal SE, but less than those of Metaseal and EZ Fill. The antimicrobial effect of AH Plus may be related to bisphenol A diglycidyl ether. In addition, it has been reported that the material releases formaldehyde during polymerization,[10] which could also contribute to its antimicrobial effect.[11]

Yasuda et al[11] compared the antimicrobial activity of AH plus and other sealers against *S. aureus*, *C. albicans*, *S. mutans*, and *E. faecalis* and showed that AH Plus had the strongest antimicrobial activity among all the tested sealers, and the antimicrobial activity is due to bisphenol A diglycidyl ether and other components, such as epoxy resins and amines.

The finding contradictory to our study has been reported by Miyagak et al[12] and Mickel et al,[13] who have reported no antimicrobial activity of AH Plus against *E. faecalis*.

Cohen et al[14] studied that AH26 and endomethasone sealers release formaldehyde after setting. Only a minimum release was observed for AH Plus, followed by EZ Fill endodontic sealers, and AH26 yielded the greatest formaldehyde release.

In this study, Realseal SE has not shown any antibacterial activity despite being cytotoxic. This might be because of the nondiffusibility of the material across the medium. Studies have shown that in agar diffusion test, a material that diffuses more easily will probably provide larger zones of microbial growth inhibition.[15-16]

Barry and Thornsberry[17] have reported that the size of zones of inhibition not only depends on the toxicity of the material but also its diffusibility and rate of diffusibility.

The present study has compared only the antimicrobial activity of Metaseal, AH Plus, Realseal SE, and EZ Fill root canal sealers. Further laboratory studies and clinical trials are needed to evaluate the long-term efficacy of these sealers.

## CONCLUSION

Within the limitations of this study, all the tested groups of sealers except Realseal SE have shown some amount of zone of inhibition against all the tested microorganisms. After 24 hours, the maximum zone of inhibition was shown by Metaseal followed by EZ Fill and AH Plus, and the difference was statistically significant.

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