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# Antimicrobial activity of three different endodontic sealers on the enterococcus faecalis and lactobacillus (in vitro)

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## Abstract

**Introduction:** Growth and proliferation of the remaining microorganisms within the root canals may destroy the surrounding tissue of the root and leads to periapical lesion. Consequently, the complete elimination of microorganisms from the root canal is an important goal of endodontic therapy. Endodontic sealers do not provide complete seal in root canal system, and micro spaces have always remained between the material and canal walls that lead to penetration of these spaces, so, an antibacterial activity is essential for sealers. The aim of the present study was the in vitro evaluation of antimicrobial activity of the three endodontic sealers on two microorganisms.

**Methods:** To study the effect of each sealer; AH26, MTA Fillapex and ADseal on Enterococcus Faecalis and Lactobacillus bacteria 10 samples were considered. In this experimental study, 60 plates were exposed to bacteria and 10 plates were considered for control group. Sealer antibacterial effect on bacterial growth was studied after 48 hours. Firstly, the freshly prepared sealers were poured inside the micro tube and diffused in the wall of the micro tube. Then solution of nutrient broth was poured into a micro tube and the determined volume of solution of bacterial suspension was added into a microtube and was kept 24 hours in the incubator to grow the bacteria. Then, it was poured in the plates of blood agar and cultured after 24 hours and then the colonies grown on the plates were counted in sufficient light. The data were analyzed with MANOVA statistical test and SPSS Version 18.

**Results:** Most bacteria grew in the plates of ADseal sealer and MTA fillapex sealer with means of 5113.00CFU and 3077.00CFU respectively, while the lowest number of bacteria grew in the plates of AH26 sealer with a mean of 1345.15CFU.

**Conclusions:** Most antibacterial activities of each enterococcus faecalis and lactobacillus bacteria sample was for AH26 sealer and MTA fillapex sealer. The lowest antibacterial activity was for ADseal sealer.

**Keywords:** Endodontics sealers, Antibacterial activity, Microorganisms

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# ارزیابی فعالیت ضد میکروبی سه نوع سیلر مختلف اندودنتیک علیه انتروکوک فکالیس و لاکتوباسیل به روش آزمایشگاهی

## چکیده

**مقدمه:** رشد و تکثیر میکروارگانیسم های باقیمانده درون کانال ریشه، ممکن است بافتهای اطراف ریشه را تخریب کرده و باعث ایجاد ضایعه پری اپیکال گردد. بنابراین حذف کامل میکروارگانسم ها ازکانال ریشه همواره ازاهداف مهم درمان اندودنتیک بوده است، هیچکدام ازمواد دندانپزشکی سیل کامل با دیواره های حفره فراهم نمی کند وهمیشه فضاهای میکرونی درفاصله بین ماده ودیواره حفره باقی می ماند که میکروارگانیسم ازاین فضاها می تواند نفوذ کند که این خود تاکید یبیشتر برضرورت داشتن خاصیت ضدباکتریایی این مواد می باشد. بنابراین هدف ازمطالعه حاضر ارزیابی آزمایشگاهی فعالیت ضدمیکروبی سیلـرهای AH26، MTA Fillapex وADseal است.

**مواد و روش ها:** برای مطالعه اثر هر سیلر AH26، MTA Fillapex وADseal بر هر یک ازباکتری های انتروکوکفکالیس و لاکتوباسیل تعداد ۱۰ نمونه درنظرگرفته شد. دراین تحقیق مجموعاً ۶۰ پلیت مورد مطالعه قرار گرفتند و جهت گروه کنترل ۱۰ پلیت درنظرگرفته شد. ابتدا مقدارمعینی سیلر تازه تهیه شده درداخل میکروتیوب ها ریخته ودردیواره آنها پخش شد، سپس محیط کشت به داخل میکروتیوب ریخته و بعد حجم معینی از سوسپانسیون باکتریایی به داخل آن اضافه شد و میکروتیوب ها برای مدت ۲۴ ساعت در داخل انکوباتور نگهداری شدند تا باکتریها رشد کنند، سپس حجم معینی ازمحلول داخل میکروتیوب را برداشته و بر روی پلیت حاوی بلاد آگار ریخته وبه مدت ۲۴ ساعت کشت داده شد سپس تعداد کلنیهای رشد کرده در هر پلیت مورد شمارش قرار گرفت و بعد داده ها با استفاده از تست های آماری MANOVA–Tukey Test و بوسیله نرم افزارآماری SPSS Version 18 آنالیز گردیدند.

**یافته ها:** بیشترین تعداد باکتری رشد کرده درمجموع هر دو باکتری انتروکوک فکالیس ولاکتوباسیل، در پلیت های سیلر Adseal با میانگینCFU 5113.00 وسپس در پلیت های سیلر MTA Fillapex با میانگین 3077.00 CFUو کمترین تعداد باکتری رشد کرده با میانگین 1345.15 CFU در پلیت های سیلر AH26 مشاهده شد.

**نتیجه گیری:** بیشترین اثر ضد باکتریایی برروی هر دو باکتری انتروکوک فکالیس ولاکتوباسیل مربوط به سیلر AH26 وپس ازآن سیلر MTA Fiilapex و کمترین اثر ضدباکتریایی مربوط به سیلر ADseal می باشد.

**واژگان کلیدی:** سیلرهای اندودنتیک، اثر آنتی باکتریال، میکروارگانیسمها

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## Introduction

One of the major aims of endodontic treatment is sealing the root canal system, which is directly related to the omission of microorganisms and their products by means of cleansing, mechanical shaping, irrigating with antibacterial solutions, filling the root canal and using the anti-bacterial dressing in sessions of treatments if necessary (calcium hydroxide) (1-3).

This process does not completely sterilize root canals (4). Proliferation of the remaining microorganisms may damage the surrounding tissues of the root and cause periapical lesions (5). The presence of bacteria and infection may cause apical periodontitis (6). Thus the root canal filling materials must be anti-bacterial or anti-microbial (7).

Adding anti-bacterial agents to the endodontic sealers is a method which leads to antimicrobial activity of sealers (1). Nowadays, the different sealers with specific formula such as resin, calcium hydroxide and MTA (Mineral Trioxide Aggregate) based sealers are manufactured. Resin based sealers like AH26 (Dentsply, Detrey, Konstanz Germany) are applied commonly and are useful for posterior and anterior teeth. ADseal (Meta, Michigan, United States) is a newly developed resin based sealer which a limited data about its anti-microbial features is available (8). MTA fillapex is a MTA base sealer which has useful features like insolubility in wet environment, lack of allergic reactions after treatment and dimensional stability and appropriate setting time (9-10).

al-Khatib et al. were the first promoters for the investigation of anti-bacterial endodontic sealers in 1990 (11). From then on, some researchers used a similar model to investigate the anti-microbial features of sealers, while the different microorganisms sensitivity to antimicrobial agents following contact test is different (12-13).

In this study, Enterococcus faecalis and lactobacilli were used. With regard to the significance of the study and lack of relevant studies, we aimed to investigate the anti-bacterial features of the different types of sealers to improve endodontic treatment outcome choosing the proper sealer in clinics, and prevent from further problems.

## Methods

The present study was an experimental study and the endodontic sealers such as ADseal (Meta, United States), MTA fillapex (Angelus, Brazil) and AH26 (Dentsply, Detrey, Germany) were investigated and compared.

The microorganisms of enterococcus faecalis (1394 PTCC) and lactobacilli (1643 PTCC) were prepared from the samples in standard species of Asre-Enghelab Corporation, Tehran, Iran. This study was conducted in the microbiology laboratory of the Faculty of Medicine of Babol, Iran. To study the effect of each sealer on specific bacteria, 10 samples of each case were prepared.

In this study, 60 plates were measured and after 48 hours, the effect of sealers on the bacterial growth was investigated and 10 plates were selected for the control group. Firstly, the microtubes were placed in autoclave and sterilized.

Then, the sealers were prepared based on the manufacturer's instruction and immediately, 0.1 cc of each sealer was added to the micro tube through a syringe and distributed homogeneously on the wall of the micro tube. 1.49 cc of nutrient broth was added to the micro tube through a sampler and then 0.01 cc of bacterial suspension solution containing 1500000 bacteria was added to the micro tube.

Finally micro tubes contained 1.50cc solution containing 1500000 bacteria. The micro tube lid was closed and kept in autoclave at 37ºC for 24 hours. With respect to the anaerobic feature of lactobacilli, the micro tubes and plates were placed in an anaerobic jar.

**Culturing the Microorganisms on the Blood Agar Medium:**

24 hours after the incubation of the microtubes, their lids were opened and 0.01cc of the solution was added to the plate containing blood agar through the sampler.

After sterilizing the metal loop, it was used to distribute the entire solution on the plate. Then, all the petteries were incubated at 37ºC for 24 hours, the number of microorganisms cultured was counted based on colony count.

**Bacterial Counting:**

The number of colonies on each plate was counted. Any decrease in the number of bacteria on each plate indicated the effect of anti-bacterial activity of sealer.

**Analysis:**

The mean of log 10 CFU (Colony Forming Unit)/ml and Standard Deviation (SD) of bacteria was calculated and the mean, standard deviation,

distribution and data were analyzed by MANOVA and the comparison of intergroup data by TUKEY TEST using SPSS Version 18. The data from counting CFU in each group were compared and a p-value of 0.05 was determined for identifying the significance of the result.

**Controlling the Positive Group:**

(They are involved in the study for approving the bacteria purity and ensuring the bacteria growth during testing): 0.01 cc of enterococcus faecalis and lactobacilli bacteria grown was poured by a sampler on the seprate blood agar culture medium.

**Controlling the negative group:**

(For ensuring the disinfection of tested sealers): 0.1 cc of AH26, MTA Fillapex and ADseal sealer was poured by Syringe on the separate blood agar culture medium. All of the 70 plates were placed in the incubator at 37 ºC for 24 hours and the number of CFU colonies in plates was counted by colony count and the data were analyzed using SPSS Version 18.

## Results

The analysis of the data showed that for enterococcus faecalis bacteria, AH26 sealer with mean growth (1482/40CFU) in each plate had the most anti-bacterial effect and ADseal (5352/00CFU) had the least anti-bacterial effect ($p \leq 0.001$) (table1) (figure 1). Also, with regard to lactobacilli, the most anti-bacterial effect was related to the AH26 sealer (1207/90 CFU) and the least anti-bacterial effect was related to the ADseal (4874/00CFU) ($p \leq 0.001$) (table1) (figure2). In each bacterium, the sealers were significantly different based on the p-value count (table1).

In the positive control group, the bacteria grew completely on the plate and this rejected the presence of growth restricting infection while in the negative control group, no bacteria grew on the plate, and this rejected the possibility of infection from the sealers or plates.

On the average, the greatest number of bacterial loss in each plate (8454/85CFU) was observed for AH26 sealer and MTA Fillapex (6923/00CFU) and the least number of bacterial loss belonged to ADseal (4887/00CFU).

The ANOVA test determined the significant difference between the studied sealers regarding the anti-bacterial effect ($p \leq 0.001$) (figure 2). The most amount of bacterial growth in ADseal plates was 5113/00CFU and the least amount of bacterial growth in AH26 sealer plates was 1345/15CFU. ($p \leq 0.001$) (table 1).

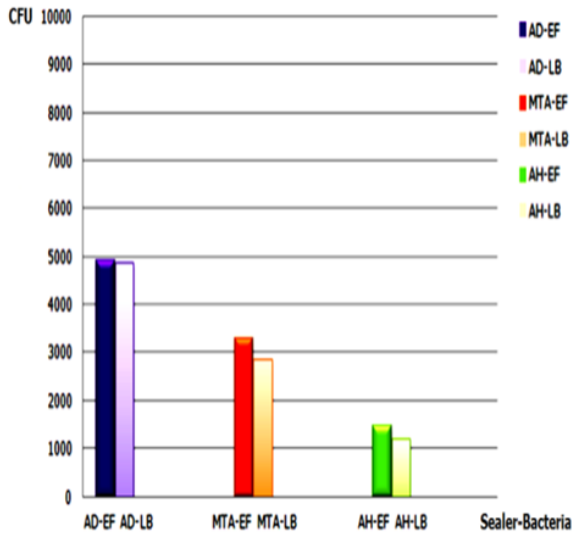


**Figure 1. Mean number of lost bacteria on all plates of Enterococcus Faecalis and Lactobacillus with regard to the type of sealer**

*AH=AH26, MTA=MTA Fillapex, AD=ADseal, EF=Enterococcus Faecalis, LB=Lactobacillus

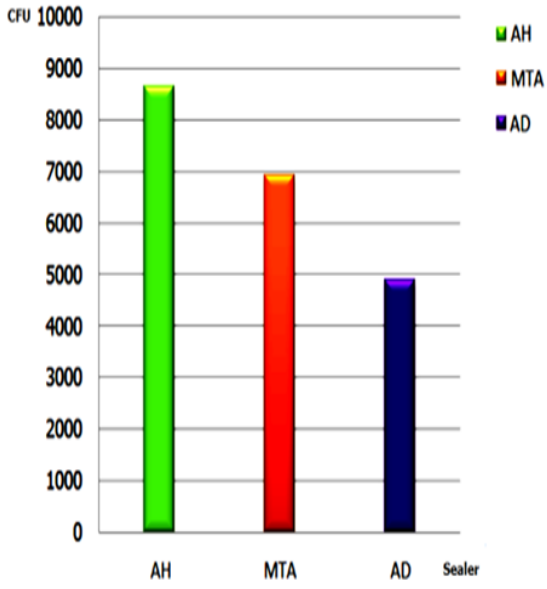


**Figure 2. Mean number of grown bacteria in each plate with regard to the type of sealer and bacteria**

AH=AH26, MTA=MTA Fillapex, AD= ADseal


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**Table 1. Mean amount of grown bacteria in each plate with regard to the type of sealer and bacteria**

| sealer / bacteria | AH26 Mean±SD | MTA Fillapex Mean±SD | ADseal Mean±SD | P-value | Total Mean±SD |
|---|---|---|---|---|---|
| Enterococcus Faecalis | 1482.40±532.553 | 3282±354.520 | 5352±321.310 | <0.001 | 3372.13±1656.791 |
| Lactobacillus | 1207.90±311.223 | 2872±368.504 | 4874±489.403 | <0.001 | 2984.63±1571.747 |
| P-value | 0.176 | 0.02 | 0.02 | | 0.357 |
| Total | 1345.15±447.273 | 3077±409.995 | 5113±471.683 | <0.001 | |

## Discussion

In this study, we focused on the anti-bacterial activity of three different sealers: ADseal, MTA Fillapex and AH26 on enterococcus faecalis and lactobacillus were examined. In a study by Al-khatib et al. the anti-microbial effect of tubliseal, calciobiotic, sealapex, hypocal, nogenol, eucapercha and AH26 sealers on the streptococcus mutants, staphylococci aurous, bacteriodus endodontalis were investigated.

Various kinds of sealers and both anaerobic and aerobic bacteria and control groups were investigated. The result was similar to the result of the current study and showed that AH26 sealer had the most effect on both the aerobic and anaerobic microorganisms. And in contrast to our study, the cavity was created on the agar jelly for pouring the sealers and microbial suspension must have not been distributed on agar surface, it should have been mixed with culture medium. The number of samples and plates for each sealer and bacteria was not identified either.

In Pumarola et al. study, the anti-microbial effect of traitementspad, N2 universal, diaket, endomethasone, tublisealsealapex and AH26 on 120 species of staphylococci aurous was investigated. The results showed that diaket and traitment had the most anti-bacterial features (14). In our study, AH26 (like diaket has epoxy) had the most anti-bacterial effect. In the study by Chong et al. the anti-microbial effect of ZOE, glass ionomer cement and amalgam on the streptococcus miller and enterococcus faecalis was investigated. The result showed that glass ionomer cement had the most effect on both bacteria and ZOE placed the second, and Amalgam did not show anti-bacterial features (15).

According to our study, anaerobic bacteria were grown under anaerobic conditions in order to be matched with clinical conditions however, they did not use the control group. In Torabinejad et al. study, the anti-microbial effect of MTA and ZOE sealer and amalgam was investigated on 9 species of optional anaerobic bacterium and 7 species of obligatory anaerobic bacterium. The results showed that MTA affected on some optional anaerobic bacterium (16). In our study, MTA sealer had effect on anaerobic bacterium.

In a study by Abulkadar et al. the anti-microbial effect of Ketac-Endo tubliseal, sealapex, apexit, and roth on porphyromonas gingivalis, peptostreptocucus micros and capnocytophagaochracea was investigated. The result showed that roth´s antibacterial effect was more than the tubliseal and apexit on peptostreptocucus micros (4). Like our study, the use of the oral anaerobic bacteria was very important. But, the sample size was restricted to two plates while in the present study; the number of samples in each group was 10 plates that was adequate.

In the study by Heling et al. the anti-microbial effect of sealapex, Ketac-Endo, AH26 sealers on enterococcus faecalis was investigated. The result showed that AH26 had the most anti-bacterial effect (17). Similar to our study, they used different kinds of sealers with various bases but they did not use the control groups.

In the study by Gorduysus et al. the anti-microbial effect of Endo-Fill sealer on the staphylococcus aeureus, streptococcus pyogenes, E. Coli and pseudomonas aeruginosa was investigated. The result showed that Endo-Fill did not show any anti-bacterial features (18).

The anti-microbial feature of new sealers was investigated in their study while the number of samples was not identified, and Escherichia coli were not considered as the oral pathogens.

In Mickel et al. study, the anti-microbial effect of apexit, roth, CRCS and sealapex on the streptococcus miller was investigated. The result showed that roth had the most anti-bacterial effect and there was no significant difference between apexit and CRCS (12). The processes of study were illustrated in details and




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the positive and negative control groups were used in the study which was so significant.

Siqueira et al. studied the anti-microbial effect of Grossman's, EWT, sealer 26, AHplus, and sealerplus on 8 optional anaerobic bacteria and 2 obligatory anaerobic bacteria and showed that there was no significant difference between the sealers and most of the sealers had the anti-bacterial features (19). They investigated the wide spectrum of bacteria and various sealers described the processes of research in details similar to our research. However, they studied Escherichia coli bacterium which was not related to microbial floor of infected tooth root canal.

Tanomaru-Filho et al. compared the anti-bacterial effect of MTA and AH26 sealer and portland cement and concluded that AH26 had more anti-bacterial activity than MTA and portland cement and MTA and portland cement had similar anti-microbial features (20), while in our study, AH26 sealer had more anti-microbial activity than MTA sealer.

## Conclusions

With regard to enterococcus faecalis and lactobacillus bacteria, AH26 sealer had the most anti-bacterial effect and ADseal had the least anti-bacterial effect.

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