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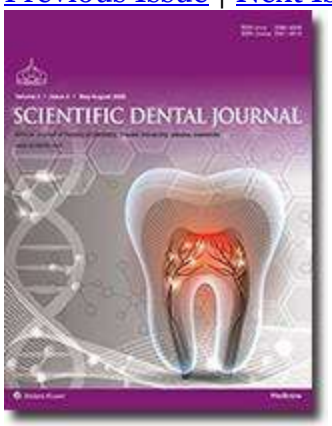

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Original Article

The Effectiveness of Mixtures of Tetracycline, Acid and Detergent, and Mixtures of Chlorhexidine and Ethylenediaminetetraacetic Acid in Preventing the Growth of *Enterococcus faecalis*: An *Ex vivo* Study

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Abstract

Background: Sodium hypochlorite (5.25% NaOCl) is the gold standard among irrigation solutions, but it is toxic to periapical tissue, decreases the micromechanical characteristics of dentin, has no effect on smear layer removal, and may not completely eradicate biofilms. Therefore, many new irrigation solutions, such as mixtures of tetracycline, citric acid, and detergent (MTAD) and a mixture of chlorhexidine (CHX) and ethylenediaminetetraacetic acid (EDTA) have been introduced as the alternatives to NaOCl. **Objectives:** The objectives of this study are to analyze the differences in the effects of MTAD and mixtures of CHX and EDTA on the growth of *Enterococcus faecalis ex vivo*. **Methods:** This study used 28 lower premolars, divided into seven groups. Group I received MTAD. Group II received MTAD with CHX. Group III received a mixture of CHX and EDTA. Group IV received a 2% CHX solution. Group V received 17% EDTA. Group VI received a 5.25% NaOCl solution, which served as the positive control, and Group VII received sterile distilled water, which was the negative control. The effectiveness of various irrigation solutions in preventing the growth of *E. faecalis* was measured by the zone of growth inhibition and colony counts. **Results:** A one-way analysis of variance revealed a significant difference among the tested irrigation solutions, both in terms of zone inhibition of *E. faecalis* and *E. faecalis* colonies counting ($P < 0.05$). **Conclusion:** There was a different antibacterial effect between MTAD and the mixture of CHX and EDTA. MTAD were more effective as irrigation solution compared to the mixture of CHX and EDTA. However, as irrigation solutions, both were less effective than 5.25% NaOCl.

Keywords: *Chlorhexidine, mixtures of tetracycline, citric acid and detergent, colony count, ethylenediaminetetraacetic acid, Enterococcus faecalis, growth inhibition, sodium hypochlorite*

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Background

Chemical-based irrigation solutions are used to clean and disinfect the complex root canals system.[1] Nearly 5.25% sodium hypochlorite (NaOCl) solutions as the gold standard have both antimicrobial and tissue-dissolving properties. However, this solution is toxic to the periapical tissue and may not completely eradicate biofilms.[2] Due to these limitations, many irrigation solutions have been introduced as alternatives. Torabinejad reported that mixtures of tetracycline, citric acid, and detergent (MTAD) had the strongest antimicrobial activity.[3] Other irrigation solutions-containing chlorhexidine (CHX) and ethylenediaminetetraacetic acid (EDTA) were introduced to remove the smear layer and kill bacteria.[4] This

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study will investigate the effectiveness of MTAD and mixtures of CHX and EDTA in preventing the growth of *Enterococcus faecalis*.

Materials and Methods

Zone of inhibition test

E. faecalis strain ATCC 29212 was grown in a tube-containing brain heart infusion (BHI) broth (Sigma-Aldrich, Germany), incubated at 37°C for 24 h under anaerobic conditions. Six agar plates were prepared, and 4 wells were made on each agar plate. A 100 µl of bacterial dilution (1×10^7 CFU/mL) were plated on the seven BHI agar plates. Treatment group were: MTAD (Group I), MTAD with CHX (Group II), mixture of CHX and EDTA (Group III), 2% CHX (Group IV), and 17% EDTA (Group V). Group VI was a 5.25% NaOCl solution, which served as the positive control, and Group VII was sterile distilled water, which was the negative control. Incubation was kept for 24 h at 37°C, and then, the plates were examined for evidences of inhibition zones, which appeared as a clear area around the well. The zone of inhibition that formed was measured in millimeters using the calipers.

Preparation of the teeth

The selection criteria for the teeth used in this study were lower premolars with completely developed roots, without root caries or previous endodontic treatment. All the root canals were instrumented using a rotary ProTaper Universal NiTi according to the manufacturer's instructions at a rotational speed of 300 rpm. The coronal two-thirds of the canals were prepared using SX and S1 shaping files. Instrumentation was accomplished using S1, S2, F1, F2, and F3 to the working length. Each canal was irrigated with 1 ml of 2.5% NaOCl between each instrumentation using a disposable 2 ml syringe and 30 G needle. After the instrumentation, the canals were filled with 1 ml of 15% EDTA for 2 min, followed by a final rinse with 1 ml of 2.5% NaOCl, and 1 ml of saline solution. Finally, the canals were dried with sterile F3 paper points. The apical sections were covered with composite resin, and two layers of the cuticle were then applied to the entire root surface. The teeth were then sterilized in an autoclave for 20 min at 121°C.

Cultivation of *Enterococcus faecalis* and root canal inoculation

E. faecalis strain ATCC 29212 from the laboratory stock was grown in a tube-containing BHI broth (Sigma-Aldrich, Germany), incubated at 37°C for 24 h under anaerobic conditions. Subsequently, the 28 teeth (four were used as the negative control group) were each inoculated with 50 µl of *E. faecalis* and then incubated at 37°C for 48 h in a vertical position in the microtube.

Colony counting

After 48 h of incubation, each tooth was rinsed with 5 ml of phosphate-buffered saline (PBS) to eliminate the inoculation broth. The teeth were then randomly divided into seven experimental groups, with four teeth in each group. The composition of the treatment groups was as described above. Then, 5 ml of solution from each group was irrigated into the root canal of each tooth using a 30 G side-vented needle. The irrigation solution was remained in the root canal for 5 min, and the teeth were then dried with sterile paper points. Subsequently, the teeth were washed with 5 m of PBS solution. The bacteria were then cultured, and incubation was then carried out for 24 h at 37°C under anaerobic condition. Subsequently, the number of *E. faecalis* colonies on agar plates was counted to calculate the number of bacteria remaining after the application of the various irrigation solutions.

Statistical analysis

The Shapiro–Wilk test was used to analyze whether the data are normally distributed. One-way analysis of variance (ANOVA) test was applied to analyze the significant differences among the tested irrigation solutions, both in terms of zone of inhibition of *E. faecalis* and *E. faecalis* colonies counting. Differences were considered statistically significant if $P < 0.05$. Statistical calculations were performed with MiniTab 18 statistical software (Minitab, LLC software. Pennsylvania, USA).

Results

Zone of inhibition

From agar-well diffusion method, the zone of inhibition formed by MTAD (Group I) after incubation for 24 h at 37°C was the most extensive, followed by MTAD with CHX (Group II) as compared to the other irrigation solution tested [Figures 1 and 2]. The results of the one-way ANOVA confirmed that the differences were statistically significant in all irrigation solutions to exhibit good antibacterial properties against *E. faecalis* compared to the negative control ($P < 0.05$).

Colony counting

From the plate count method, the *E. faecalis* colonies were significantly decreased after irrigation with 5.25% NaOCl and 2% CHX. This showed that the most effective agents against *E. faecalis* were 5.25% NaOCl and 2% CHX [Figures 3 and 4]. The results of the one-way ANOVA confirmed that the differences were statistically significant in all irrigation solutions to exhibit good antibacterial properties against *E. faecalis* compared to the negative control ($P < 0.05$).

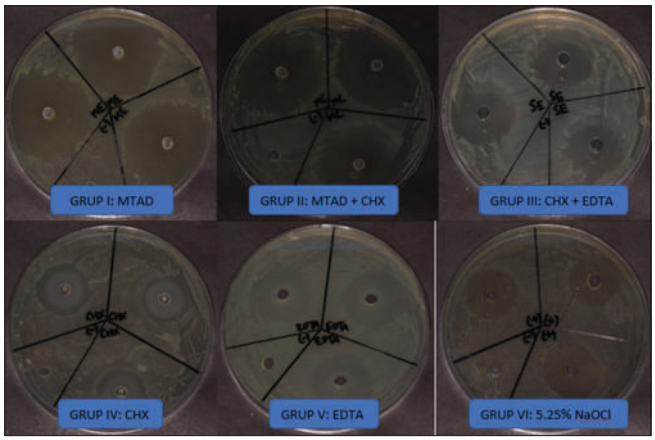

Figure 1: Zone of inhibition formed by the different irrigation solutions after incubation for 24 h at 37°C, using 5.25% sodium hypochlorite as a positive control and sterile distilled water as a negative control showed that Group I (MTAD) formed the most extensive zone of inhibition. MTAD: Mixture of tetracycline, citric acid, and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

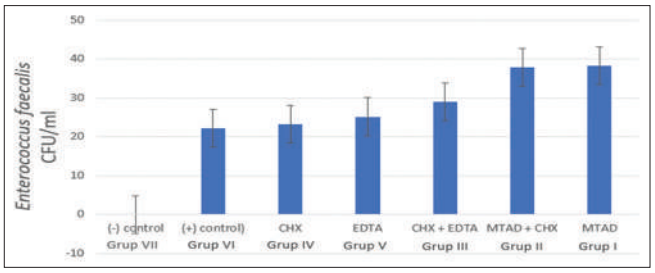

Figure 2: Zone of inhibition formed by the different irrigant after incubation for 24 h at 37°C, using 5.25% sodium hypochlorite as a positive control and sterile distilled water as a negative control. MTAD: Mixtures of tetracycline, citric acid and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

Discussion

This study revealed that there were opposite antibacterial effects of the irrigations solutions when tested with the zone of inhibition method and colony count method. The results of the inhibition zone test against *E. faecalis* showed that MTAD had the most extensive antibacterial effect (38.28 mm ± 1.10). In terms of the antibacterial effect of the irrigation solutions (i.e., elimination of *E. faecalis* biofilms) on the number of *E. faecalis* colonies, the results showed that the effectiveness of MTAD (567.50 CFU/ml ± 254.74) was lower than that of 5.25% NaOCl and 2% CHX (0.00 CFU/ml). These findings were in accordance with those of earlier research by Pappen *et al*., who showed that MTAD was more effective and faster in killing planktonic bacteria compare to MTAD with modifications. However, in the same study, when the effect of MTAD on biofilms was examined, its ability to kill microbes was not as rapid as that Tetraclean irrigation solutions.[5] Similar results were reported by Davis *et al*., who found that MTAD exhibited the most extensive zone of growth inhibition when compared with that of 2% CHX and 5.25% NaOCl.[6] Based on a literature review, the exposure time appeared to affect the effectiveness of a MTAD and CHX. Murad *et al*. concluded that 2.5% and 5.25% NaOCl were the most effective solutions in eliminating *E. faecalis* biofilms, although the time of exposure was different (1 min, 5 min, 15 min, and 30 min), whereas MTAD was effective only with an exposure time after 5 min. In the same study, based on the exposure time and concentration of the irrigation solutions, MTAD and CHX were less effective in terms of biofilm elimination as compared with that of 2.5% or 5.25% NaOCl.[7] Darrag demonstrated that CHX showed extensive antimicrobial activity against various bacteria, including *E. faecalis*, because the irrigation solution has the ability to be absorbed and released gradually from the hydroxyapatite surfaces.[8] Stojici *et al*. showed that biofilms under 2 weeks old were more sensitive to CHX than biofilms more than 3 weeks old.[9] The findings of our study were similar to those of Murad *et al*.; MTAD treatment with 5 min exposure time in the root canal not eliminating *E. faecalis* completely when compared with that of 5.25% NaOCl with 5 min exposure time.[7]

The results of this study are supported by those of Afzal *et al*., who used the same method, agar disc diffusion and culture of the number of *E. faecalis* colonies, as applied in the present study. In the study by Afzal *et al*., based on an assessment of growth inhibition zones, the antibacterial abilities of MTAD and CHX were better than the antibacterial ability of 5.25% NaOCl. This may be due to the fact that in case of agar well-diffusion method, *E. faecalis* is in a planktonic forms.[10] According to Luddin and Ahmed, there are many factors that might affect the reliability of the agar disc-diffusion method beside the antibacterial activity of the tested solution such as the chemical agent's formulation, solubility, and diffusion ability of the irrigation solutions through the agar medium, agar viscosity, storage conditions of the agar plates, and incubation time.[11] Therefore, in agar well method, the bacteria are more sensitive to antibacterial solutions such as MTAD, whereas 5.25% NaOCl due to the chlorine could be evaporated and then reducing the antibacterial property. However, the zone of inhibition does not represent clinical conditions, and as such, do not reflect the ability of an irrigation solution to eliminate the biofilms.[10]

Therefore, in this study, we also assessed the antibacterial effects of irrigation solutions against of *E. faecalis* biofilms on lower premolars to represent clinical conditions. The results of Afzal *et al*. revealed significant differences in the number of bacteria on teeth treated with 5.25% NaOCl and 2% CHX as

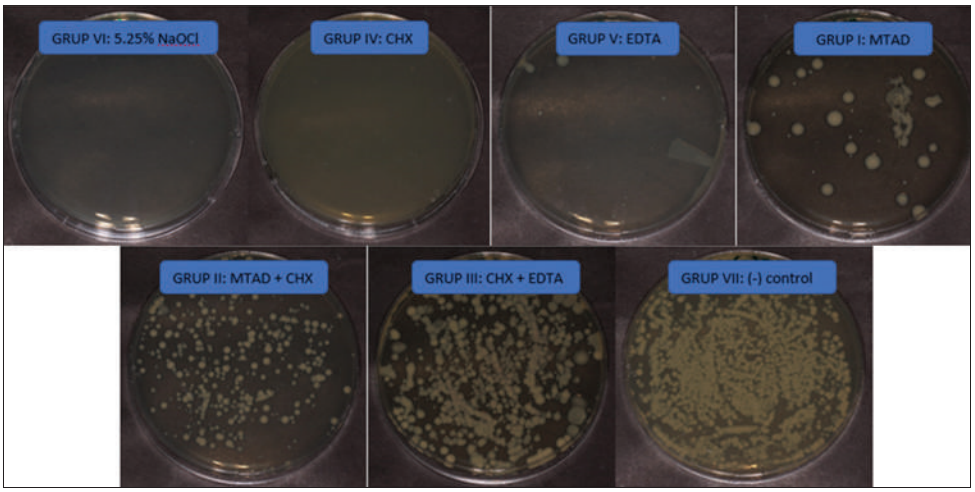

Figure 3: *Enterococcus faecalis* colonies after treatment with the different irrigation solutions in BHI agar. 5.25% sodium hypochlorite was used as a positive control, and sterile distilled water was used as a negative control. This showed that the most effective agents against *Enterococcus faecalis* were 5.25% sodium hypochlorite and 2% CHX. MTAD: Mixtures of tetracycline, citric acid, and detergent. CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

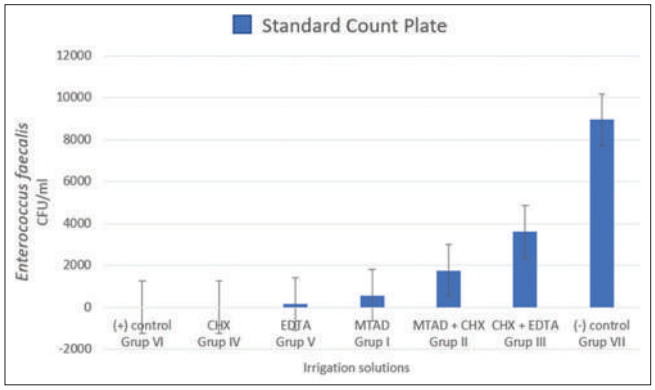

Figure 4: *Enterococcus faecalis* number (CFU/ml) after treatment with various irrigation solutions and the standard method plate count method were carried out. As shown, 5.25% sodium hypochlorite (positive control) and CHX 2% completely eliminated bacteria. MTAD: Mixtures of tetracycline, citric acid, and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

compared with those in MTAD-treated teeth. In their study, the 5.25% NaOCl irrigation solution was the most effective, with no colonies detected. The findings of their study were similar to those in our present study, where the number of *E. faecalis* colonies was zero. This may due to the efficiency of irrigation solutions is tested against *E. faecalis* biofilm. Furthermore, when testing as irrigation solution, we use freshly prepared solutions of NaOCl, which is most active.[10]

According to Jorgensen and Ferraro, the diameter of an inhibition zone depends on the susceptibility of the isolated bacteria and the rate of diffusion of the drug used to penetrate the agar medium.[12] Ballal *et al.* reported that a mixture of CHX and EDTA provided clinical benefits because of the presence of the CHX content, thus providing antibacterial ability after endodontic treatment.[13] Research on the mixture of CHX and EDTA solution against bacterial growth inhibition zones and the number of *E. faecalis* colonies has not yet reported.

In this study, the tested solution was an experimental solution, with an adjusted composition. The MTAD is more effective as an irrigation solution compared to the mixture of CHX and EDTA, but both of these solutions were less optimal when compared with 5.25% NaOCl. Thus, a mixture of CHX and EDTA with NaOCl appears to be needed for an effective irrigation solution. MTAD and a mixture of CHX and EDTA have not been proven to be more effective than CHX. Thus, limitation of this present study was that the test solution was an experimental solution not the commercial MTAD. Therefore, to assess the effectiveness of this experimental solution, we need to do the stability testing.

Conclusion

The results of this study indicate that there was a different antibacterial effect between MTAD and the mixture of CHX and EDTA. MTAD was more effective as irrigation solution compared to the mixture of CHX and EDTA. However, as irrigation solutions, both were less effective than 5.25% NaOCl. Further study is needed to explore this result and analyze the mechanism of irrigation solutions in inhibiting the biofilms.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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The Effectiveness of Mixtures of Tetracycline, Acid and Detergent, and Mixtures of Chlorhexidine and Ethylenediaminetetraacetic Acid in Preventing the Growth of Enterococcus faecalis

by Tien Suwartini

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Original Article

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Tien Suwartini, Elfira Anggraini[1], Meiny Faudah Amin, Boedi Oetomo Roeslan[2]

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E. faecalis strain ATCC 29212 was grown in a tube-containing brain heart infusion (BHI) broth (Sigma-Aldrich, Germany), incubated at 37°C for 24 h under anaerobic conditions. Six agar plates were prepared, and 4 wells were made on each agar plate. A 100 µl of bacterial dilution (1×10^7 CFU/mL) were plated on the seven BHI agar plates. Treatment group were: MTAD (Group I), MTAD with CHX (Group II), mixture of CHX and EDTA (Group III), 2% CHX (Group IV), and 17% EDTA (Group V). Group VI was a 5.25% NaOCl solution, which served as the positive control, and Group VII was sterile distilled water, which was the negative control. Incubation was kept for 24 h at 37°C, and then, the plates were examined for evidences of inhibition zones, which appeared as a clear area around the well. The zone of inhibition that formed was measured in millimeters using the calipers.

Preparation of the teeth

The selection criteria for the teeth used in this study were lower premolars with completely developed roots, without root caries or previous endodontic treatment. All the root canals were instrumented using a rotary ProTaper Universal NiTi according to the manufacturer's instructions at a rotational speed of 300 rpm. The coronal two-thirds of the canals were prepared using SX and S1 shaping files. Instrumentation was accomplished using S1, S2, F1, F2, and F3 to the working length. Each canal was irrigated with 1 ml of 2.5% NaOCl between each instrumentation using a disposable 2 ml syringe and 30 G needle. After the instrumentation, the canals were filled with 1 ml of 15% EDTA for 2 min, followed by a final rinse with 1 ml of 2.5% NaOCl, and 1 ml of saline solution. Finally, the canals were dried with sterile F3 paper points. The apical sections were covered with composite resin, and two layers of the cuticle were then applied to the entire root surface. The teeth were then sterilized in an autoclave for 20 min at 121°C.

Cultivation of *Enterococcus faecalis* and root canal inoculation

E. faecalis strain ATCC 29212 from the laboratory stock was grown in a tube-containing BHI broth (Sigma-Aldrich, Germany), incubated at 37°C for 24 h under anaerobic conditions. Subsequently, the 28 teeth (four were used as the negative control group) were each inoculated with 50 µl of *E. faecalis* and then incubated at 37°C for 48 h in a vertical position in the microtube.

Colony counting

After 48 h of incubation, each tooth was rinsed with 5 ml of phosphate-buffered saline (PBS) to eliminate the inoculation broth. The teeth were then randomly divided into seven experimental groups, with four teeth in each group. The composition of the treatment groups was as described above. Then, 5 ml of solution from each group was irrigated into the root canal of each tooth using a 30 G side-vented needle. The irrigation solution was remained in the root canal for 5 min, and the teeth were then dried with sterile paper points. Subsequently, the teeth were washed with 5 m of PBS solution. The bacteria were then cultured, and incubation was then carried out for 24 h at 37°C under anaerobic condition. Subsequently, the number of *E. faecalis* colonies on agar plates was counted to calculate the number of bacteria remaining after the application of the various irrigation solutions.

Statistical analysis

The Shapiro–Wilk test was used to analyze whether the data are normally distributed. One-way analysis of variance (ANOVA) test was applied to analyze the significant differences among the tested irrigation solutions, both in terms of zone of inhibition of *E. faecalis* and *E. faecalis* colonies counting. Differences were considered statistically significant if $P < 0.05$. Statistical calculations were performed with MiniTab 18 statistical software (Minitab, LLC software. Pennsylvania, USA).

Results

Zone of inhibition

From agar-well diffusion method, the zone of inhibition formed by MTAD (Group I) after incubation for 24 h at 37°C was the most extensive, followed by MTAD with CHX (Group II) as compared to the other irrigation solution tested [Figures 1 and 2]. The results of the one-way ANOVA confirmed that the differences were statistically significant in all irrigation solutions to exhibit good antibacterial properties against *E. faecalis* compared to the negative control ($P < 0.05$).

Colony counting

From the plate count method, the *E. faecalis* colonies were significantly decreased after irrigation with 5.25% NaOCl and 2% CHX. This showed that the most effective agents against *E. faecalis* were 5.25% NaOCl and 2% CHX [Figures 3 and 4]. The results of the one-way ANOVA confirmed that the differences were statistically significant in all irrigation solutions to exhibit good antibacterial properties against *E. faecalis* compared to the negative control ($P < 0.05$).

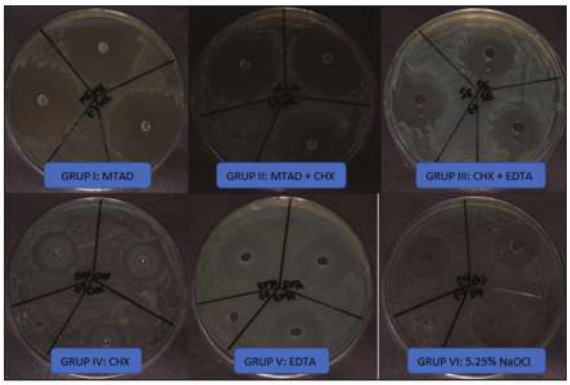

Figure 1: Zone of inhibition formed by the different irrigation solutions after incubation for 24 h at 37°C, using 5.25% sodium hypochlorite as a positive control and sterile distilled water as a negative control showed that Group I (MTAD) formed the most extensive zone of inhibition. MTAD: Mixture of tetracycline, citric acid, and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

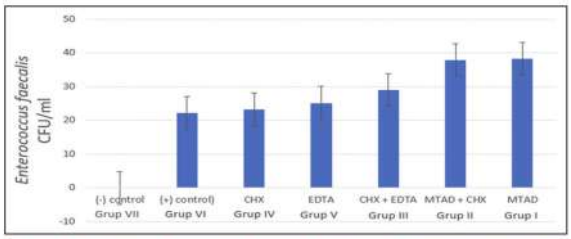

Figure 2: Zone of inhibition formed by the different irrigant after incubation for 24 h at 37°C, using 5.25% sodium hypochlorite as a positive control and sterile distilled water as a negative control. MTAD: Mixtures of tetracycline, citric acid and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

Discussion

This study revealed that there were opposite antibacterial effects of the irrigations solutions when tested with the zone of inhibition method and colony count method. The results of the inhibition zone test against *E. faecalis* showed that MTAD had the most extensive antibacterial effect (38.28 mm ± 1.10). In terms of the antibacterial effect of the irrigation solutions (i.e., elimination of *E. faecalis* biofilms) on the number of *E. faecalis* colonies, the results showed that the effectiveness of MTAD (567.50 CFU/ml ± 254.74) was lower than that of 5.25% NaOCl and 2% CHX (0.00 CFU/ml). These findings were in accordance with those of earlier research by Pappen *et al.*, who showed that MTAD was more effective and faster in killing planktonic bacteria compare to MTAD with modifications. However, in the same study, when the effect of MTAD on biofilms was examined, its ability to kill microbes was not as rapid as that Tetraclean irrigation solutions.[5] Similar results were reported by Davis *et al.*, who found that MTAD exhibited the most extensive zone of growth inhibition when compared with that of 2% CHX and 5.25% NaOCl.[6] Based on a literature review, the exposure time appeared to affect the effectiveness of a MTAD and CHX. Murad *et al.* concluded that 2.5% and 5.25% NaOCl were the most effective solutions in eliminating *E. faecalis* biofilms, although the time of exposure was different (1 min, 5 min, 15 min, and 30 min), whereas MTAD was effective only with an exposure time after 5 min. In the same study, based on the exposure time and concentration of the irrigation solutions, MTAD and CHX were less effective in terms of biofilm elimination as compared with that of 2.5% or 5.25% NaOCl.[7] Darrag demonstrated that CHX showed extensive antimicrobial activity against various bacteria, including *E. faecalis*, because the irrigation solution has the ability to be absorbed and released gradually from the hydroxyapatite surfaces.[8] Stojici *et al.* showed that biofilms under 2 weeks old were more sensitive to CHX than biofilms more than 3 weeks old.[9] The findings of our study were similar to those of Murad *et al.*; MTAD treatment with 5 min exposure time in the root canal not eliminating *E. faecalis* completely when compared with that of 5.25% NaOCl with 5 min exposure time.[7]

The results of this study are supported by those of Afzal *et al.*, who used the same method, agar disc diffusion and culture of the number of *E. faecalis* colonies, as applied in the present study. In the study by Afzal *et al.*, based on an assessment of growth inhibition zones, the antibacterial abilities of MTAD and CHX were better than the antibacterial ability of 5.25% NaOCl. This may be due to the fact that in case of agar well-diffusion method, *E. faecalis* is in a planktonic forms.[10] According to Luddin and Ahmed, there are many factors that might affect the reliability of the agar disc-diffusion method beside the antibacterial activity of the tested solution such as the chemical agent's formulation, solubility, and diffusion ability of the irrigation solutions through the agar medium, agar viscosity, storage conditions of the agar plates, and incubation time.[11] Therefore, in agar well method, the bacteria are more sensitive to antibacterial solutions such as MTAD, whereas 5.25% NaOCl due to the chlorine could be evaporated and then reducing the antibacterial property. However, the zone of inhibition does not represent clinical conditions, and as such, do not reflect the ability of an irrigation solution to eliminate the biofilms.[10]

Therefore, in this study, we also assessed the antibacterial effects of irrigation solutions against of *E. faecalis* biofilms on lower premolars to represent clinical conditions. The results of Afzal *et al.* revealed significant differences in the number of bacteria on teeth treated with 5.25% NaOCl and 2% CHX as

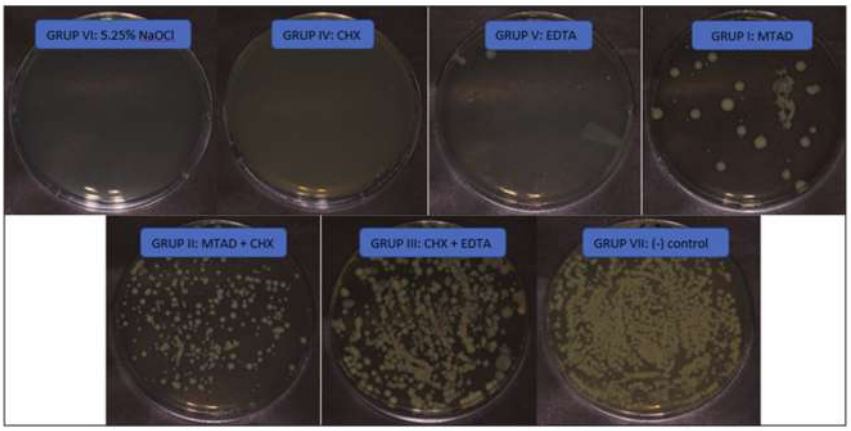

Figure 3: *Enterococcus faecalis* colonies after treatment with the different irrigation solutions in BHI agar. 5.25% sodium hypochlorite was used as a positive control, and sterile distilled water was used as a negative control. This showed that the most effective agents against *Enterococcus faecalis* were 5.25% sodium hypochlorite and 2% CHX. MTAD: Mixtures of tetracycline, citric acid, and detergent. CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

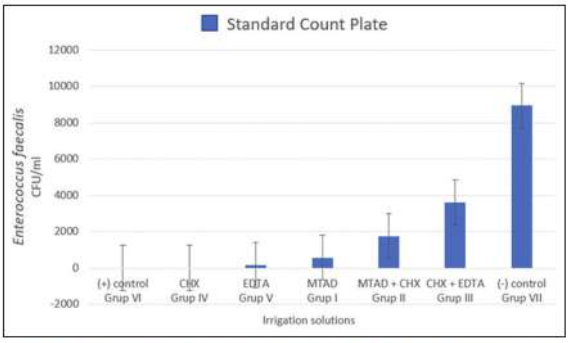

Figure 4: *Enterococcus faecalis* number (CFU/ml) after treatment with various irrigation solutions and the standard method plate count method were carried out. As shown, 5.25% sodium hypochlorite (positive control) and CHX 2% completely eliminated bacteria. MTAD: Mixtures of tetracycline, citric acid, and detergent, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid

compared with those in MTAD-treated teeth. In their study, the 5.25% NaOCl irrigation solution was the most effective, with no colonies detected. The findings of their study were similar to those in our present study, where the number of *E. faecalis* colonies was zero. This may due to the efficiency of irrigation solutions is tested against *E. faecalis* biofilm. Furthermore, when testing as irrigation solution, we use freshly prepared solutions of NaOCl, which is most active.[10]

According to Jorgensen and Ferraro, the diameter of an inhibition zone depends on the susceptibility of the isolated bacteria and the rate of diffusion of the drug used to penetrate the agar medium.[12] Ballal *et al.* reported that a mixture of CHX and EDTA provided clinical benefits because of the presence of the CHX content, thus providing antibacterial ability after endodontic treatment.[13] Research on the mixture of CHX and EDTA solution against bacterial growth inhibition zones and the number of *E. faecalis* colonies has not yet reported.

In this study, the tested solution was an experimental solution, with an adjusted composition. The MTAD is more effective as an irrigation solution compared to the mixture of CHX and EDTA, but both of these solutions were less optimal when compared with 5.25% NaOCl. Thus, a mixture of CHX and EDTA with NaOCl appears to be needed for an effective irrigation solution. MTAD and a mixture of CHX and EDTA have not been proven to be more effective than CHX. Thus, limitation of this present study was that the test solution was an experimental solution not the commercial MTAD. Therefore, to assess the effectiveness of this experimental solution, we need to do the stability testing.

Conclusion

The results of this study indicate that there was a different antibacterial effect between MTAD and the mixture of CHX and EDTA. MTAD was more effective as irrigation solution compared to the mixture of CHX and EDTA. However, as irrigation solutions, both were less effective than 5.25% NaOCl. Further study is needed to explore this result and analyze the mechanism of irrigation solutions in inhibiting the biofilms.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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