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The Antibacterial Effect of QMix, a Novel Root Canal Irrigant (Ex vivo Study)

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الخلاصة

الأهداف: تهدف الدراسة إلى تقييم الفعالية المضادة للجراثيم لمحلول جديد لقناة الجذر من خلال تقييم تأثيرها على البكتيريا المكورة البرازية. **المواد وطرائق العمل:** التركيز المثبط الأصغري ، التركيز الأصغري المميت و إختبار التعرض المباشر كانت الإختبارات التي استخدمت لتقييم الفعالية المضادة للجراثيم. في اختبار التركيز المثبط الأصغري والتركيز الأصغري المميت، تم تخفيف المحاليل التالية (QMix, 17% EDTA, 2% CHX) بمستنبت نقيع الدماغ والقلب بشكل متسلسل وقد تم إضافة (٠.٢) مل من المحاليل المايكروبية. تم الحصول على النتائج من خلال ملاحظة عكرة المحاليل ونمو الجراثيم على صفائح الاغار. في إختبار التعرض المباشر،تم تعرض البكتيريا المكورة المعوية البرازية إلى المحاليل (QMix, 17% EDTA, 2% CHX) لمدة خمسة ثوان، ثلاثون ثانية وثلاث دقائق. بعد التعرض، تم آخذ العينات والقيام بتخفيفها بشكل متسلسل ثم وضعها في الحاضنة بعد زرعها على آغار البكتيريا الكروية المعوية البرازية لمدة ٢٤ ساعة لقياس عدد الخلايا البكتيرية التي تم قتلها. لغرض التحليل الإحصائي، تم إستخدام اختبار انوفا ذي الإتجاه الأحادي وكذلك إختبار دنكن. **النتائج:** بالنسبة لإختبارات التركيز المثبط الأصغري والتركيز الأصغري المميت، كان (CHX) اكثر فعالية من باقي المحاليل ضد البكتيريا المكورة المعوية البرازية، حيث كان تركيز (CHX) هو الأقل لمنع نمو البكتيريا. محلول (EDTA) كان الأقل فعالية. في إختبارالتعرض المباشر، كان (QMix) اكثر فعالية من (CHX و EDTA) حيث كان الوحيد القادر على قتل كافة خلايا البكتيريا، بينما كان هناك عدد قليل من خلايا البكتيريا بعد التعرض ل(EDTA و CHX) لمدة ثلاث دقائق. كانت هناك فروق معنوية بين QMix و كل من EDTA و CHX من حيث فعالية قتل البكتيريا عند التعرض لمدة خمس ثوان. لم يكن هنالك فروق معنوي بين المحاليل الثلاثة عند التعرض لمدة ثلاث دقائق. **الإستنتاجات:** أظهرت الأستنتاجات أنه في اختباري التركيز المثبط الأصغري والتركيز الأصغري المميت، كان (CHX) اكثر فعالية من بقية المحاليل. في إختبار التعرض المباشر، كان (QMix) الأكثر فعالية كونها المحلول الوحيد الذي قام بقتل جميع خلايا البكتيريا، فضلا عن قتل أكثر من ٩٥% من الخلايا البكتيرية خلال خمس ثوان فقط.

ABSTRACT

Aims: To evaluate the antibacterial effect of a novel root canal irrigant, QMix, by evaluating its effect against *E. faecalis* and comparing it to 17% EDTA and 2% Chlorhexidine digluconate. **Materials and Methods:** Minimum Inhibitory Concentration (MIC), Minimum bactericidal concentration (MBC) and direct exposure test were the techniques used. In the MIC and MBC technique, the irrigants (QMix, 17% EDTA, 2% CHX) were serially diluted in BHI broth and 0.2 mL of the tested bacterial suspensions was added. Results were obtained on the basis of turbidity and growth on agar plates. In the direct exposure test, *Enterococcus faecalis* were exposed to QMix, 2% Chlorhexidinedigluconate and 17% EDTA for 5 seconds, 30 seconds and 3 minutes. Following exposure, samples were taken and serially diluted and incubated anaerobically on *E. faecalis* selective media for 24 hours to count the resistance of the bacteria. **Results:** In the MIC and MBC technique, CHX showed to be more effective against *E. faecalis* than both QMix and EDTA, as lower dilutions were required to inhibit growth of both bacteria. Ethylene diamine tetra acid (EDTA) was the least effective. In the direct exposure test, QMix was more effective than CHX and EDTA as it was the only solution to be able to kill all bacteria. Few *E. faecalis* cells remained even after exposure of bacteria to 3 minutes of EDTA and CHX. There was significant difference between QMix and both EDTA and CHX in killing of *E. feacalis* at 5 seconds exposure ($p<0.05$). QMix killed more than 95% of bacteria, whereas CHX and EDTA killed fewer than 20% ($p<0.05$). There was no statistical significance between the irrigant solutions at 3 minutes of exposure. **Conclusions:** In the MIC

and MBC techniques, Chlorhexidinedigluconate was found superior to both QMix and EDTA. In the Direct Exposure Test, QMix showed the best performance as it was the only irrigant solution to kill all *E. faecalis* cells, as well as killing more than 95% of all bacteria at 5 seconds exposure.

Keywords: Anibacterial effect, QMix, EDTA, CHX.

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INTRODUCTION

Bacteria are the main cause of periapical disease.[1] The main goal of root canal treatment is elimination of bacteria from the root canal and prevention of recontamination after treatment,[2] as well as the achievement and maintenance of a tight seal, chemical and/or mechanical, along the root canal system,[3] It has been reported that success rate of root canal treatment was higher when teeth were free of bacteria after chemomechanical instrumentation.[4, 5]

Mechanical preparation is the main mechanism to reduce the bacterial load in canals, which is enhanced by intracanal irrigants. In spite of these procedures, some bacteria might persist within the canals. *Enterococcus faecalis* is the most common and dominant bacterial species isolated from failed endodontic treatment cases and teeth with persistent periodontitis.[6] Consequently, current in vitro studies have placed great emphasis on the effectiveness of irrigants against *E. faecalis.*[7]

One of the most important requirements of an ideal endodontic irrigant is to possess antibacterial effect. Other desirable characteristics are ability to dissolve organic and inorganic tissue and to have lubricant as well as flushing effect. In addition, it should not be toxic for the surrounding tissue and not weaken the tooth structure.[8]

Sodium hypochlorite (NaOCl) in concentrations from 0.5% to 6% is the most commonly recommended irrigating solution. It has strong antibacterial and tissue dissolving effects.[9] However, it is toxic to periapical tissue[10] and has been suggested to degrade micromechanical characteristics of dentine.[11] Furthermore, it has no effect on the inorganic part of the smear layer.[12] EDTA, used usually in concentration of 17%, dissolves the inorganic portion of dentine and smear layer by chelation and is recommended for use after NaOCl to complete smear layer removal. Recently, Qian *et al*.,[13] showed that if NaOCl is used again after EDTA or citric acid as the final antibacterial rinse, it causes marked erosion of the root canal wall dentine.

Chlorhexidine has been shown to have a vast antimicrobial potential and has been recommended as an endodontic irrigant,[14, 15] particularly because of the fact that its antibacterial effect would increase with time when it remains for several days within the canals.[16,17] However, data suggest that CHX is highly cytotoxic in vitro and caution should be exercised with the use of this antiseptic in the oral surgical procedures.[18, 19]

An experimental antimicrobial root canal irrigant (QMix; Dentsply Tulsa Dental, USA) and its modifications containing a mixture of a bisbiguanide antimicrobial agent, a polyaminocarboxylic acid calcium-chelating agent, saline, and a surfactant have been found to be more effective than MTAD against bacterial biofilms.[13] Ma *et al.*[35] found that QMix is as effective as 6% NaOCL.

The aim of this study is to evaluate the antimicrobial efficacy of QMix against *E.faecalis* and compare it to EDTA and CHX.

MATERIALS AND METHODS

The solutions tested for their antimicrobial activity were as follows: QMix (Dentsply Tulsa Dental, OK, USA), EDTA 17% (Tg, UK), and CHX 2% (BasicPharma Life, India).

Enterococcus faecalis was isolated from tooth associated with apical periodontitis. Cultures of the *E. faecalis* strain were grown overnight at 37°C on *E. Faecalis* selective agar (Fluka, Switzerland).

Minimum Inhibitory Concentration (MIC)

An overnight culture of *E. faecalis* was harvested in brain-heart infusion (BHI) broth and the concentration was adjusted to optical density of 0.11 at 570 nm using spectrophotometer.[7] To determine the MIC of the irrigant solutions (QMix, 2% CHX, 17% EDTA), the serial microdilution assay was used. Thirty test tubes were prepared for the test. Ten sterile test tubes were used for each irrigant. 2 mL of brain-heart infusion (BHI) broth was poured into each test tube using a sterile pipette, capped tightly using foil and then autoclaved. The tubes were then divided into three groups of 10 test tubes each. 2 mL of the selected irrigant was placed in the first test tube of each group and shaken thoroughly. Subsequently, 2 ml of the content of the first test tube was added to the next test tube using a sterile pipette and thoroughly mixed. This process was performed serially to test tube number ten. Finally, 200 µL of bacterial suspensions of *E. faecalis* were added to test tubes 1 to 10. The irrigant was gradually diluted from 1:2 to 1:1024 in the last test tube. The test tubes were incubated at 37°C for 24 hrs. Then, the bacterial growth was determined by the presence or absence of turbidity. The highest dilution for each respective irrigant that had no turbidity represents the minimum inhibitory concentration (MIC).[25]

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration was determined by subculturing MIC tubes of the previous test on *E. facealis* plates to examine bacterial growth. The plates were incubated at 37°C for 48 hours. Those plates whose MIC concentration revealed no bacterial growth was referred to as Minimum Bactericidal Concentration (MBC).[25]

Direct Exposure Test

An overnight culture of *E. faecalis* was harvested in brain-heart infusion (BHI) broth and the concentration was adjusted to optical density of 0.11 at 570 nm using spectrophotometer.[7] 50-µL samples of *E. faecalis* suspension were obtained and mixed with 0.45 mL of the disinfecting solutions (QMix, 2% CHX, 17% EDTA) for experimental times of 5 seconds, 30 seconds, and 3 minutes. After indicated times of exposure, 100-µL samples were obtained and mixed with sterile 900-µL brain heart infusion broth (BHI) contained in tightly foil-capped sterile test tubes and serially ten-fold diluted, so that 30 test tubes were used for this part, in addition to 10 test tubes that were used for counting the number of bacterial cells from the initial inoculum, in which 100-µL of bacterial suspension was obtained and mixed with sterilie900-µL of brain heart infusion broth and serially ten-fold diluted. A vortex mixer (DragonLab) was used throughout the experiment to ensure homogenous mixing of test tubes contents. Finally, droplets of 20-µL from the dilution tubes were cultured on *E. faecalis* selective media at 37°C for 48 hours. The results were expressed as the percentage of surviving bacteria from the initial inoculum. All experiments were performed in triplicate under strict aseptic conditions.[21]

Data Analysis

Each solutions and each time of exposure were considered as experimental groups. The results from killing tests were analyzed using One way analysis of Variance (ANVOA) and Duncan's multiple range test (SPSS version 11.5, Chicago, IL, USA) at level of significance $p < 0.05$.

RESULTS

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) is defined as the minimum concentration of the irrigant required to inhibit growth of *E. faecalis*. Results of the (MIC) test, shown in table (1), revealed that all irrigant solutions used had an inhibitory effect against *E. faecalis* after 24 hours of incubation. However, 2% Chlorhexidinedigluconate was the most effective among irrigants. It required the lowest concentration, where its ninth dilution showed no turbidity after 24 hours of incubation, whereas 17% EDTA was the least effective having shown its inhibitory effect against *E. faecalis* down to its fifth dilution. QMix was less effective than 2% CHX, but more effective than 17% EDTA.

Table (1): Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) test of (QMix, EDTA, CHX) against *E. faecalis*

Entercoccousfaecalis	**QMix**	**17% EDTA**	**2% CHX**
MIC	1:128	1:64	1:512
MBC	1:32	1:16	1:128

Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) is that concentration of irrigants that showed no growth of *E. faecalis*. Results, shown in table (1), revealed that all irrigation solutions were bactericidal after 24 hours of incubation. Chlorhexidinedigluconate at 2% was the most effective among all irrigants where the lowest concentration (seventh dilution) was needed to kill all bacteria, whereas 17% EDTA was the least effective.

Direct Exposure Test

QMix was the only irrigant solution to kill all planktonic *E. faecalis* cells at 3 minutes of exposure. Although there was no significant difference between QMix and both EDTA and CHX at 3 minutes, the latter were unable to completely eliminate *E. faecalis* strains, as shown in table (7). At 5 seconds of exposure, QMix was significantly different from both EDTA and CHX in that it killed more than 98% of the *E. Faecalis* cells, compared to EDTA that was ineffective and CHX that killed as little as 12.5% during that period, as shown in tables (2 and 3).

Table (2): One way ANOVA on the percentage of killed *E. faecalis* at 5 seconds of exposure using QMix, EDTA and CHX in the direct exposure test

	Sum of Squares	**Degree of Freedom**	**Mean square**	***F***	**Sig.**
Between Groups	174433.317	2	8716.659	55.438	.000
Within Groups	943.402	6	157.234		
Total	18376.719	8			

*Significance level = $p < 0.05$

Table (3): Duncan's Multiple Range Test on the percentage of killed *E. faecalis* at 5 seconds of exposure using QMix, EDTA and CHX in the direct exposure test

		Subset for Alpha = .05	
Irrigant	**N**	**A**	**B**
CHX	3	.0000	
EDTA	3	12.5000	
QMix	3		98.9833

*Different letters means significant difference.

At 30 seconds of exposure, both CHX and QMix were effective in killing *E. Faecalis* as opposed to EDTA, as shown in tables (4 and 5).

Table (4): One Way ANOVA on the percentage of killed *E. faecalis* at 30 seconds using QMix, EDTA and CHX in the direct exposure test

	Sum of Squares	**Degree of freedom**	**Mean square**	***F***	**Sig.**
Between Groups	12110.889	2	6055.444	18.307	.003
Within Groups	1984.667	6	330.778		
Total	14095.556	8			

*Significance level = $p < 0.05$

Table (5): Duncan's test on the percentage of killed *E. faecalis* at 30 seconds of exposure using QMix, EDTA and CHX in the direct exposure test

		Subset for Alpha = .05	
Irrigant	**N**	**A**	**B**
EDTA	3	16.6667	
CHX	3		89.0000
QMix	3		99.0000

*Different letters means significant difference.

The mean percentage (± S.D) of killed *E.Faecalis* using all irrigantsare shown together in table (7), and the mean percentage in a histogram shown in Figure (1).

Table (6): One Way analysis of Variance (ANOVA) of the irrigant solutions against *E. faecalis* at 3 minutes in the direct exposure test

	Sum of Squares	**Degree of freedom**	**Mean square**	**F**	**Sig.**
Between Groups	2.056	2	1.028	1.609	.276
Within Groups	3.883	6	.639		
Total	5.889	8			

*Significance level = $p < 0.05$

Table (7): Mean Percentage (± Standard Deviation) of killed *E. faecalis* of all experimental solutions in direct exposure test

Irrigant	**5 seconds**	**30 seconds**	**3 minutes**
QMix	98.98 ± 1.71	99.00 ± 1.73	100.00
CHX	.00	89.00 ± 12.49	99.33 ± .57
EDTA	12.5 ± 21.65	16.60 ± 28.86	98.83 ± 1.25

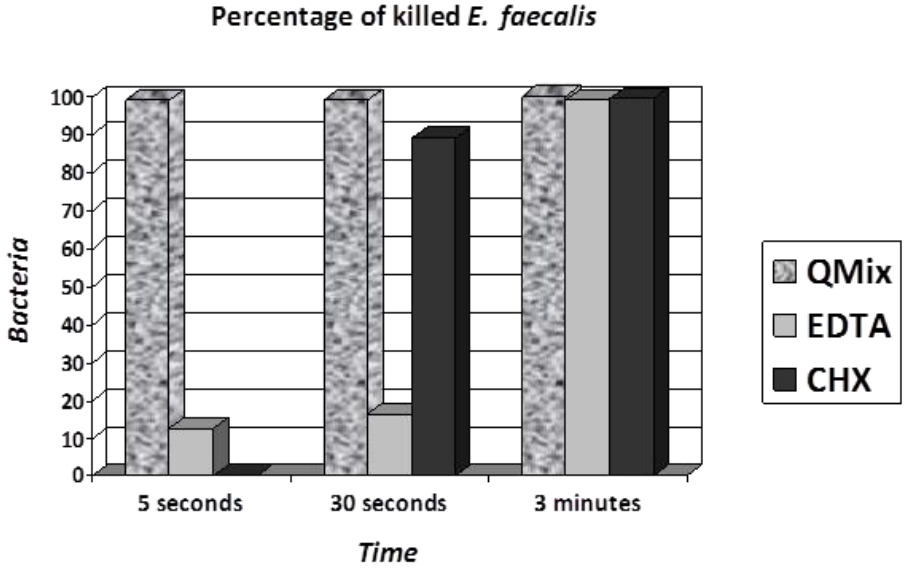

Figure (1): Mean percentage of killed *E.faecalis*.

DISCUSSION

Infections in root canals have a polymicrobial nature. Many microorganisms including Enterococci can be detected in the infected root canals.[22] *Enterococcus faecalis*, is frequently found in endodontic infections, especially in secondary and persistent root canal infections.[23] Bacterial elimination from the root canal is achieved by means of the mechanical action of instruments and irrigation as well as the antibacterial effects of the irrigating solutions. To further reduce the number of viable organisms, antimicrobial irrigating solutions are applied.[24]

Recently a new irrigant, QMix, has been introduced as a final irrigant to be used after NaOCl for disinfecting the root canal given that it has both antibacterial effects and has the ability to remove the smear layer.[21] This ex-vivo study was aimed to evaluate the antibacterial effect of QMix against *E. faecalis*.

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.[25] The microdilution method is the standard in vitro method for determining MIC and MBC for most of the bacterial strains.[26] A lower MIC is an indication of a better antimicrobial agent.[25] Results of the MIC test, shown in table (2) revealed that all irrigant solutions (QMix, 17% EDTA, 2%CHX) effectively inhibited the growth of *E. faecalis*. Chlorhexidinedigluconate, however, was the most effective against *E. faecalis*, whereas EDTA was the least.

Chlorhexidine inhibited the growth of *E. faecalis* in as high as a 1:512 dilution. This result comes in agreement with Saraf*i et al*.,[27] who studied the MIC of CHX against *E. faecalis* and showed that the MIC range was (0.0004-.004%). Furthermore, these findings corroborate the results reported by Bulacio*et al*.,[28] who evaluated the MIC of both CHX and EDTA and showed that both had an inhibitory effect against *E. faecalis*, the latter being the most effective requiring more dilutions. However, these results are in contrast with those reported by Estrela*et al*.,[29] who showed in their study on the control of microorganisms in vitro by endodontic irrigants that the MIC of 2% CHX was 0.02%. Similarly, Gorduysus*et al*.[30] showed in their study comparing the MIC of CHX to EDTA against *E. faecalis* that the bacterial strains were resistant to CHX rendering it ineffective. This discrepancy might be related to the different strains of *E. faecalis* that were examined in the different studies.

EDTA proved to have an inhibitory effect against *E. faecalis*, however, it required a higher concentration (1:64) compared to both QMix and CHX. This finding coincides with the finding of Gurduysus*et al*.,[30] who revealed that EDTA is effective in inhibiting growth of *E. faecalis*. However, he showed that dilutions as high as 1:512 were effective. In contrast, El-Sharif and Hussain,[31] showed that EDTA failed to have inhibitory activity against *E. faecalis*. These discrepancies among the results might be attributed to the experimental methods, biological indicators and exposure time.

Minimum bactericidal concentration (MBC) is the lowest concentration of antibiotic required to kill a particular bacterium.[32] Results of the MBC test, shown in Table (2) revealed all irrigant solutions used (QMix, EDTA and CHX) were bactericidal to *E. faecalis*. This comes in agreement with Fidalgo*et al*.,[33] who detected a high inhibitory effect on the metabolic activity of *E. faecalis* when the microorganisms were incubated with 17% EDTA and CHX. Chlorhexidinedigluconate was the most effective against *E. faecalis* requiring the least concentration (1:512) compared to EDTA and QMix.

The direct contact test, despite not being able to fully reproduce the clinical conditions observed in endodontic infections, provides some insights and allows comparison between the substances, without external factors that might interfere with their antimicrobial action.[34] QMix is a new root canal irrigant (Dentsply, USA) that contains a mixture of a bisbiguanide antimicrobial agent, a polyaminocarboxylic acid calcium-chelating agent, saline, and a surfactant. When the smear layer has been removed, it is rational to finalize the irrigation with another disinfecting rinse to attack the remaining bacteria. A combinational product provides that option without

dentinal erosion was observed with sodium hypochlorite as the final rinse.[21] In the present study, QMix was found to be superior to CHX and EDTA in that it was the only solution to be able to completely eradicate *E. faecalis* cells at 3 minutes in planktonic culture. Furthermore, QMix proved highly effective for killing more than 97 % of the bacteria at only 5 seconds, whereas CHX and EDTA killed fewer than 13% of *E. facealis* cells at the same time interval. These results come in agreement with a recent study conducted by Stojicic *et al*.,[21] who used the same methodology in his study comparing QMix to CHX and NaOCL, and concluded that QMix killed all planktonic *E. faecalis* strains at 5 seconds, unlike CHX which killed fewer than 20% within the same time interval. A recent study conducted by Ma *et al*.,[35] showed QMix to be as effective as 6% sodium hypochlorite against *E. faecalis*, which corroborate the findings of our study. Surfactants are potent antimicrobial agents.[36] The addition of a surfactant to EDTA and CHX in the composition of QMix may have accounted for its potent antimicrobial efficacy. Arias-Moliz *et al*.,[36] showed that the association of a surfactant (cetrimide) to CHX provided better results than their application as single agents against *E. faecalis*. Furthermore, EDTA is regarded as a potentiator of the activity of other antimicrobial agents.[37] One of the recognized modes of action of EDTA is the disruption of the lipopolysaccharide structure in the outer membrane of bacteria. Through this disruption the membrane becomes more permeable to other agents, hence the potentiating action.[38] Therefore, adding EDTA and CHX together in the composition of QMix might have accounted for the significant antimicrobial potency of QMix given their synergism.

The use of CHX as an endodontic irrigant has also been indicated due to its broad spectrum of antimicrobial activity and substantivity.[39] In the present study, Chlorhexidine was able to eliminate 89% of planktonic *E. faeacalis* cells after 30 seconds of exposure. This result closely resembles the results of Guerreiro-Tanomaru *et al.*,[34] in their study evaluating the antibacterial effectiveness of different endodontic irrigants against *E. faecalis* at different exposure times, and showed that 2% CHX was able to eliminate *E. feacalis* at 30 seconds. Arias-Moliz*et al*.,[43] however, showed in their contact test evaluating CHX and other irrigants that 2% CHX was only able to eliminate *E. faecalis* biofilms after 5 minutes of exposure, whereas 1% CHX needed 10 minutes to eliminate *E. faecalis* biofilms. Stojicic*et al*.,[21] showed that CHX killed fewer than 30% of planktonic bacteria after 30 seconds of exposure. This comes in consistency with our study that showed CHX was unable to completely eradicate *E. faecalis* after 30 seconds of exposure.

EDTA inhibits the growth of microorganisms by the disruption of the lipopolysaccharide structure in the outer membrane of bacteria.[38] In the present study, it was the least effective among all irrigants at 5 seconds, and 30 seconds of exposure. However, it was almost able to completely inhibit *E. faeaclis* at 3 minutes of exposure. This result comes in agreement with Akcay *et al*.,[41] and Gorduysus *et al.*,[30] Akcay *et al.*[41] in their in vitro study comparing several types of root canal irrigants against *E. faecalis*, showed that EDTA was able to kill 99.9% of *E. faecalis* cells at 3 minutes exposure. Gorduysus *et al.*[30] also showed that EDTA had antimicrobial effects. In contrast, Arias-Moliz *et al.*,[45] showed in his contact test evaluating its bactericidal activity, that EDTA lacked inhibitory action against *E. faecalis*. Similary, Arias-Moliz *et al*.,[43] showed that 17% EDTA was not effective against *E. faecalis* biofilms at any time or concentration tested. They reported in their direct contact test evaluating both 17% EDTA and 2% CHX that, unlike CHX which eradicated *E. faecalis* biofilms after 5 minutes exposure, EDTA was ineffective. This discrepancy in the results may be attributed to the difference in methodology, as using bacteria in its planktonic phase versus biofilms. Organization of bacteria within biofilms confers a range of phenotypic properties that are not evident in their planktonic counterparts and amongst other characteristics, and confers a reduced susceptibility to antimicrobial agents.[43] EDTA has a high surface ten-

sion.[44] Therefore, the reduced effect of EDTA against *E. faecalis* biofilms in some studies maybe be due to the inability of EDTA to penetrate the dentinal tubules for having high surface tension.

CONCLUSIONS

Considering the methodology employed and the results obtained in this study, it may be concluded that all endodontic irrigants used in this study were effective against *E. faecalis.* QMix was superior to CHX and EDTA being the only irrigant able to completely eradicate *E. faecalis*. Furthermore, QMix was the most effective at 5 seconds of exposure.

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