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Antimicrobial efficacy of 5.25% sodium hypochlorite, 0.12% chlorhexidine, and two commercial irrigation solutions (Qmix® and MTAD®): an in vitro study

Eficacia antimicrobiana del hipoclorito de sodio al 5,25 %, clorhexidina al 0,12 % y dos soluciones comerciales de irrigación (Qmix® y MTAD®): un estudio in vitro

Eficácia antimicrobiana de hipoclorito de sódio 5,25%, clorexidina 0,12% e duas soluções comerciais de irrigação (Qmix® e MTAD®): um estudo in vitro

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Abstract

Endodontic treatment includes mechanical therapy combined with irrigation and disinfection techniques to remove vital pulp, non-vital pulp, and infected or necrotic pulp aiming to reduce intracanal pathogens to a level compatible with endodontic health. Infections in the root canal system are polymicrobial in which diverse microorganisms aggregated within extracellular polysaccharide matrix biofilms, therefore endodontic irrigation and mechanical debridement must need to disrupt this established microbial community and its products. Brand new clinical irrigation systems are promoted by their properties against diverse pathogenic endodontic microbiota, but their antimicrobial effectiveness is a matter of discussion. This study aimed to determine the anti-microbial properties of four common endodontic irrigation products against five ATCC strains in an in vitro test.

Objective: Determine the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) of 5.25% NaOCl, 0.12% Chlorhexidine, MTAD® and Qmix® against reference strains including *Aggregatibacter actinomycetemcomitans* – ATCC 29522*, Porphyromonas gingivalis* – ATCC 33277*, Candida albicans* – ATCC 44858*, Enteroccocus faecalis* - ATCC 29212 and *Enterobacter cloacae* 13047.

Methods and Materials: A microdilution broth test was carried out in triplicate to determine the minimum inhibitory concentration and the minimum bactericidal concentration of endodontic irrigation against specific microbial strains. Microbial viability was determined using resazurin, and microbial subcultures according to the adapted Norms of Clinical and Laboratory Standards (NCCL) - 2015.

Results: C. albicans, E. faecalis and *E. cloacae* were the microorganisms more resistant against the microbicide action of Qmix® and *C. albicans,* and *E. faecalis* the more resistant microorganisms against BioPure MTAD®. The BioPure MTAD® had a MIC of (1:8) against *E. faecalis* and *C. albicans* and the more susceptible bacteria were *A. actinomycetemcomitans, P. gingivalis* and *E. cloacae,* with MIC (1:8192; 1: 2048; and 1: 1024, respectively). The most resistant against QMIX® (MIC) were *E. faecalis*, (1:4), followed by *E. cloacae* and *C. albicans* (1:8), and the more susceptible were *P. gingivalis* (1:1024), and *A. actinomycetemcomitans* (1:512), respectively. On the other hand, the less susceptible to Chlorhexidine was *P. gingivalis* (MIC, 75 µg/mL), followed by *A. actinomycetemcomitans* (MIC, 38 µg /mL), while the more susceptible were *C. albicans, MIC* 19 µg /mL, *E. cloacae* MIC 9 µg/ mL and *E. faecalis* MIC 4.7 µg /mL. For NaOCl, *C. albicans* (1,563 µg /mL) was the more susceptible, followed by *E. cloacae, P. gingivalis* and *E. faecalis* (781 µg/mL) and the less susceptible was *A. actinomycetemcomitans* (391 µg/mL).

Conclusions: C. albicans, E. cloacae and *E. faecalis* were the more resistant strains against the endodontic irrigation products tested. The (CIM) of BioPure MTAD® performed slightly better than QMix®. Chlorhexidine (0.12%) was more effective than NaOCl (5.25%) in inhibiting most microbial strains used. However, *P. gingivalis* was the less susceptible to Chlorhexidine, requiring 75 µg/mL for CIM. Overall, Chlorhexidine showed the best antimicrobial property as endodontic irrigation product in this *in vitro* test, therefore, this product efficacy might be proved against the complexity of biofilms in other *in vitro* test and also at the clinical setting.

Keywords: Antimicrobianos, hipoclorito de sodio, clorhexidina, soluciones irrigantes

Resumen

El tratamiento endodóntico incluye la terapia mecánica combinada con técnicas de irrigación y desinfección para eliminar la pulpa vital, la pulpa no vital y la pulpa infectada o necrótica con el objetivo de reducir los patógenos intracanales a un nivel compatible con la salud endodóntica. Las infecciones en el sistema de conductos radiculares son polimicrobianas en las que diversos microorganismos se agregan dentro de biopelículas de

matriz de polisacáridos extracelulares, por lo tanto, la irrigación endodóntica y el desbridamiento mecánico deben interrumpir esta comunidad microbiana establecida y sus productos. Los nuevos sistemas de irrigación clínica se promueven en virtud de sus propiedades contra la variada microbiota endodóntica patógena, pero su eficacia antimicrobiana es un tema de discusión. Este estudio tuvo como objetivo determinar las propiedades antimicrobianas de cuatro productos de irrigación endodónticos comunes frente a cinco cepas de ATCC en una prueba in vitro.

Objetivo: determinar la concentración mínima bactericida (CMB) y la concentración mínima inhibitoria (CMI) de NaOCl al 5,25 %, clorhexidina al 0,12 %, MTAD y Qmix frente a cepas de referencia que incluyen Aggregatibacter actinomycetemcomitans (ATCC 29522), Porphyromonas gingivalis (ATCC 33277), Candida albicans (ATCC 44858), Enteroccocus faecalis (ATCC 29212) y Enterobacter cloacae 13047.

Métodos y materiales: se realizó una prueba de caldo de microdilución por triplicado para determinar la concentración mínima inhibitoria y la concentración mínima bactericida de la irrigación endodóntica contra cepas microbianas específicas. La viabilidad microbiana se determinó utilizando resazurina y subcultivos microbianos de acuerdo con las Normas de Estándares Clínicos y de Laboratorio adaptadas (NCCL, 2015).

Resultados: C. albicans, E. faecalis y E. cloacae fueron los microorganismos más resistentes a la acción microbicida de Qmix y C. albicans, y E. faecalis los microorganismos más resistentes a BioPure MTAD. El BioPure MTAD tuvo una MIC de (1:8) contra E. faecalis y C. albicans y las bacterias más susceptibles fueron A. actinomycetemcomitans, P. gingivalis y E. cloacae, con MIC (1:8192; 1:2048; y 1: 1024, respectivamente). Las más resistentes a QMIX (MIC) fueron E. faecalis, (1:4), seguida de E. cloacae y C. albicans (1:8), y las más susceptibles fueron P. gingivalis (1:1024), y A. actinomycetemcomitans (1:512), respectivamente. Por otro lado, la menos susceptible a Clorhexidina fue P. gingivalis (MIC, 75 µg/mL), seguida de A. actinomycetemcomitans (MIC, 38 µg/mL), mientras que la más susceptible fue C. albicans, MIC 19 µg/mL, E. cloacae MIC 9 µg/mL y E. faecalis MIC 4,7 µg/mL. Para NaOCl, C. albicans (1.563 µg/mL) fue la más susceptible, seguida de E. cloacae, P. gingivalis y E. faecalis (781 µg/mL) y la menos susceptible fue A. actinomycetemcomitans (391 µg/mL) .

Conclusiones: C. albicans, E. cloacae y E. faecalis fueron las cepas más resistentes a los productos de irrigación endodóntica probados. El CMI de BioPure MTAD se desempeñó ligeramente mejor que QMix. La clorhexidina (0,12 %) fue más eficaz que el NaOCl (5,25 %) para inhibir la mayoría de las cepas microbianas utilizadas. Sin embargo, P. gingivalis fue el menos susceptible a la clorhexidina, requiriendo 75 µg/mL para CMI. En general, la clorhexidina mostró la mejor propiedad antimicrobiana como producto de irrigación endodóntica en esta prueba in vitro, por lo tanto, la eficacia de este producto podría probarse frente a la complejidad de las biopelículas en otras pruebas in vitro y, también, en el ámbito clínico.

Palabras clave: Antimicrobianos, hipoclorito de sodio, clorhexidina, soluciones irrigantes

Resumo

O tratamento endodôntico inclui terapia mecânica combinada com técnicas de irrigação e desinfecção para remover polpa vital, polpa não vital e polpa infectada ou necrótica com o objetivo de reduzir os patógenos intracanais a um nível compatível com a saúde endodôntica. As infecções no sistema de canais radiculares são polimicrobianas em que vários microrganismos se agregam dentro de biofilmes de matriz polissacarídica extracelular, portanto, a irrigação endodôntica e o desbridamento mecânico devem romper essa comunidade microbiana estabelecida e seus produtos. Novos sistemas de irrigação clínica são promovidos em virtude de suas propriedades contra a microbiota endodôntica patogênica, mas sua eficácia antimicrobiana é uma questão de debate. Este estudo teve como objetivo determinar as propriedades antimicrobianas de quatro produtos comuns de irrigação endodôntica contra cinco cepas de ATCC em um teste in vitro.

Objetivo: determinar a concentração bactericida mínima (CBM) e a concentração inibitória mínima (CIM) de 5,25% NaOCl, 0,12% clorexidina, MTAD e Qmix contra cepas de referência, incluindo Aggregatibacter actinomycetemcomitans (ATCC 29522), Porphyromonas gingivalis (ATCC 33277), Candida albicans (ATCC 44858), Enteroccocus faecalis (ATCC 29212) e Enterobacter cloacae 13047.

Métodos e Materiais: Um teste de caldo de microdiluição em triplicata foi realizado para determinar a concentração inibitória mínima e a concentração bactericida mínima da irrigação endodôntica contra cepas microbianas específicas. A viabilidade microbiana foi determinada usando resazurina e subculturas microbianas de acordo com as Diretrizes de Padrões Clínicos e Laboratoriais adaptadas (NCCL, 2015).

Resultados: C. albicans, E. faecalis e E. cloacae foram os microrganismos mais resistentes à ação microbicida de Qmix e C. albicans, e E. faecalis os microrganismos mais resistentes ao BioPure MTAD. O BioPure MTAD apresentou CIM de (1:8) contra E. faecalis e C. albicans e as bactérias mais suscetíveis foram A. actinomycetemcomitans, P. gingivalis e E. cloacae, com CIM (1:8192; 1:2048; e 1:1024, respectivamente). Os mais resistentes ao QMIX (MIC) foram E. faecalis, (1:4), seguido por E. cloacae e C. albicans (1:8), e os mais suscetíveis foram P. gingivalis (1:1024), e A actinomycetemcomitans (1:512), respectivamente. Por outro lado, o menos suscetível à Clorexidina foi P. gingivalis (CIM, 75 µg/mL), seguido por A. actinomycetemcomitans (CIM, 38 µg/mL), enquanto o mais suscetível foi C. albicans, CIM 19 µg/ mL. mL, CIM de E. cloacae 9 µg/mL e CIM de E. faecalis 4,7 µg/mL. Para NaOCl, C. albicans (1563 µg/mL) foi o mais suscetível, seguido por E. cloacae, P. gingivalis e E. faecalis (781 µg/mL) e o menos suscetível foi A. actinomycetemcomitans (391 µg/mL) . .

Conclusões: C. albicans, E. cloacae e E. faecalis foram as linhagens mais resistentes aos produtos de irrigação endodôntica testados. O CMI do BioPure MTAD teve um desempenho ligeiramente melhor do que o QMix. A clorexidina (0,12%) foi mais eficaz que o NaOCl (5,25%) na inibição da maioria das cepas microbianas utilizadas. No entanto, P. gingivalis foi o menos suscetível à clorexidina, necessitando de 75 µg/mL para CIM. Em geral, a clorexidina apresentou a melhor propriedade antimicrobiana como produto de irrigação endodôntica neste teste in vitro, portanto, a eficácia deste produto pode ser testada contra biofilmes complexos em outros testes in vitro e também no ambiente clínico.

Palavras-chave: Antimicrobianos, hipoclorito de sódio, clorexidina, soluções irrigadoras

1. Introduction

The success of endodontic treatment depends on the good efficacy of root canal disinfection, mechanical preparation and obturation to prevent reinfection. Biomechanical preparation, which involves the use of irrigants, aims to remove inflamed/necrotic tissues, microbial biofilms, and virulence factors from the root-canal space (1).

Bacteria and their products are the main etiological factors of pulp and periapical diseases (2, 3). Apical periodontitis is essentially an inflammatory disease of polymicrobial etiology, which is mainly due to an infection of the root canal system (4-6). Although the presence of fungi, archaea, and viruses has being detected in endodontic infections (7-11); bacteria constitute the main microorganisms involved in the pathogenesis of apical periodontitis, and in the advanced phases of the endodontic

infectious process, as biofilms adhering to the walls of the root canal system has been observed (12-15).

Approximately 79 bacterial species have been reported in endodontic infections (16, 17). Intra-radicular infections can lead to the development of primary, secondary, or persistent apical periodontitis, and the latter has been associated with extra-radicular infection, where Gram-negative anaerobic bacteria predominate. Different species have been identified in infected root canals and periapical abscesses such as *Prevotella, Porphyromonas, Fusobacterium, Peptostreptococcus, Enterococcus faecalis (E. faecalis), Lactobacillus, Enterococcus, Actinomyces, Propionibacterium,* and Candida, among others (18).

The goal of endodontic treatment is to achieve complete disinfection, prevent reinfection of the root canal system and periapical tissue, prevent apical periodontitis to develop or, when the disease is already present, create the appropriate conditions for healing (18). Therefore, irrigation is an essential element during endodontic therapy and sodium hypochlorite and chlorhexidine are the gold standard. However; other products such as BioPure MTAD® a powder–liquid system that´s contains 4.25% citric acid and 0.5% polysorbate 80 detergent, and Qmix® is compound solution containing a bisbiguanide antimicrobial agent (2% CHX) and a polyaminocarboxylic acid calcium-chelating agent (17% EDTA), are recommended by their biocompatibility (19).

Although various irrigation protocols have been proposed, the most accepted it is sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 6% during biomechanical preparation, accompanied by sonic or ultrasonic activation (20, 21) that have the limitation to an efficient removal of the smear layer. To overcome this limitation, the use of EDTA 17%, citric acid (CA) and BioPure MTAD® has been recommended within a 30 second activation (22 ,23). Similarly, it has been suggested to carry out a final irrigation with NaOCl or chlorhexidine (CHX) to improve the disinfection process.

Newberry et al. (24) evaluated the antimicrobial effect of MTAD as final irrigant againts eight strains of *E. faecalis*. They measured (MIC) and (MBC), demonstrating that this irrigant effectively eliminated the growth of seven strains. On the other hand, Davis et al. (25) determined the antimicrobial action of BioPure MTAD® (Dentsply Tulsa Dental, Johnson City, TN), 2% Chlorhexidine (CHX; Ultradent, West Jordan, UT), and 5.25% sodium hypochlorite (NaOCl) against *E. faecalis* and found that BioPure MTAD showed significantly higher microbial inhibition zones than NaOCl 5.25% and that CHX 2%. Arslan et al. (26) measured the MIC and CBM of Propolis, BioPure MTAD, NaOCl 5%, and CHX 2% against *E. faecalis* and *Candida albicans*, finding that Propolis and other irrigants were effective against both microorganisms, but Propolis and

NaOCl were more effective in lower concentrations for *C. albicans* than for *E. faecalis*, and CHX and MTAD were more effective in lower concentrations for E. *faecalis* than for C. albicans.

E. faecalis is an important endodontic pathogen on which irrigants must be tested due to the fact that this bacterial specie is frequently detected in secondary endodontic failures and besides it can survive during long time due to its ability to create biofilms, to compete with other endodontic microorganisms, invade dentinal tubules, and resist nutritional deprivation (27). There are few reports of *in vitro* studies Elakanti et al, 2015 (27) comparing the MIC and CBM of NaOCl, CHX, MTAD and Qmix against various endodontic pathogens. The aim of this study was to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of four irrigation products such as NaOCl at 5.25%, Chlorhexidine at 0.12%, MTAD® and Qmix® against ATCC® strains in vitro. It is important to state that Candida is a yeast and it is not a bacterium; however, MBC is extrapolated to this particular specie along the manuscript just for practical reasons.

2. Materials and methods

This experimental study was carried out in the Oral Microbiology Laboratory. The MIC and the MBC was determined on *Aggregatibacter actinomycetemcomitans* ATCC 29522™, *Porphyromonas gingivalis* ATCC 33277™, *Candida albicans* ATCC 44858 ™, Enteroccocus *faecalis* ATCC 29212™ and on *Enterobacter cloacae* ATCC 13047™. Challenged against Chlorhexidine 0.12%, 5.25% sodium hypochlorite, Qmix® from Dentsply, Tulsa Dental, Tulsa, OK, USA and BioPure MTAD® from Dentsply, Tulsa Dental, Tulsa, OK, USA. Those reference strains were suspended in Brain Heart Infusion Broth (BHI) (Sharlau®), incubated at 37°C for 48 hours. Subsequently, *P. gingivalis* was sub-cultured on Brucellla agar (Difco, BD®) supplemented with sheep blood, Hemin (Sigma Aldrich®) and Menadione (Sigma Aldrich®), incubated at 37°C in anaerobiosis for 72 hours. While *C. albicans, E faecalis* and *E. cloacae* were cultured in tripticase soy (Merck®) at 37°C for 24 hours. Finally, *A. actinomycetemcomitans* was culture on tripticase soy supplemented with horse serum (Gibco®), Bacitracin (Sigma Aldrich®) and Vancomycin (Sigma Aldrich®) at 37°C with CO_2 at 5 %. To determine the MIC and MBC, a microdilution test was carried out in broth following the recommendations and guidelines of the Clinical and Laboratory Standards (CLSI, 2015)(28) and microbial viability was determined using resazurin and microbial viability by microbial subcultures.

To evaluate bacterial susceptibility of the reference strains against each irrigant, a microdilution test was performed in BHI with double serial dilutions to determine the minimum inhibitory concentration MIC and the minimal bactericidal concentration MBC. Sterile 96-well plates were used to set those serial dilutions of biocides by triplicate (Table 1). Because the exact concentration of antimicrobials in the BioPure MTAD® and the Qmix® were unknown since these data is not reported from commercial companies, the MIC and MBI values were expressed in terms of dilution in a range from 1: 2 (biocide diluted 2 times) to 1: 16,384 (biocide diluted 16,384 times) for these 2 products. Regarding the chlorhexidine gluconate (0.12%) and the sodium hypochlorite (5.25%), the percentage units were transformed into µg/mL. The dilution range was evaluated 1.200 µg / mL - 2.3 µg/mL for chlorhexidine and 25,000 µg/mL - 97.7 µg/mL for sodium hypochlorite as depicts Table 2. A bacterial inoculum was prepared for each test, which consisted of standardizing a concentration of $1.5x10^8$ bacteria/mL by using a turbidimeter (Ultrospec10®, Biochrom) to 0.08 absorbance. This inoculum of each microorganism was deposit at the 96 wells plate to perform the MIC and MBI assays.

Cell viability

Non-viable bacterial cells are those than lose their metabolic capacity to continue its bacterial growth due to adverse conditions or having exposed to structural damage to their structure that constrain replication. A colorimetric test with rezarzurin (blue color) was performed, where the cells that are viable conserved their ability to reduce this compound to resofurin (pink color) as an indicator of cell viability. A cell titer-blue-Promega® kit was used to carry out these experiments. The procedure consisted of adding the individual reagent directly to the strains after being expose to the different biocides in 100 µl of BHI broth in a 96-well plate. It was incubated for 3 hours with rezarzurin and later, the color change was measure with a plate reader and using positive and negative controls to determine at which biocide concentration, the respective microbial strains were no longer viable and it was considered (MBC).

3. Results

Turbidity in the medium quickly generated in the positive control samples was correlated with microbial growth. In the negative control samples, absence of turbidity was evident at 24 hours and no microbial growth was determined, demonstrating the correct functioning of positive and negative controls. The antimicrobial inhibition

(MIC) activity of the BioPure MTAD® and the Qmix® showed that *C. albicans* and *E. faecalis* were the microorganisms less susceptible to their microbicidal action as depicted Table 1. For BioPure MTAD® in relation to (MIC), the less susceptible microorganisms were *E. faecalis* and *C. albicans* (1: 8), while the more susceptible were *A. actinomycetemcomitans, P. gingivalis and E. cloacae*, (1: 8192; 1: 2048; and 1: 1024; respectively). For QMIX®, the less susceptible microorganism was again *E. faecalis*, (1: 4), followed by *E. cloacae* and *C. albicans* (1: 8) and the more susceptible were *P. gingivalis* and *A. actinomycetemcomitans*, (1: 1024 and 1: 512), respectively.

Table 1. Evaluation of the antimicrobial activity of different chemical agents against bacteria of clinical importance after 24-48 hours of exposure.

Microbial Strains	MTAD®		QMIX®		Chlorhexidine (µg/mL)		NaOCl (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis ATCC 29212	1:8	1:8	1:8	1:8	4,7	4,7	781	781
E. cloacae ATCC 13047	1:1024	1:1024	1:8	1:8	9	9	781	781
C. albicans ATCC 44858	1:8	1:8	1:8	1:8	19	19	1563	1563
P. gingivalis ATCC 33277	1:2048	1:2048	1:1024	1:1024	75	75	391	391
A. actinomycetemcomitans ATCC 29522	1:8192	1:8192	1:512	1:32	38	38	391	391

For MBC, the microorganism less susceptible to the BioPure MTAD® were *C. albicans* (1: 8) and *E. faecalis* (1: 8) and *E. cloacae* was susceptible (1: 1024), and the more susceptible were *P. gingivalis* and *A. actinomycetemcomitans*, (1: 2048 and 1: 8192), respectively. For the Qmix®, the less susceptible microorganisms were *E. faecalis, C. albicans, E. cloacae* and *A. actinomycetemcomitans* (1: 32), while the most susceptible was *P. gingivalis.*

Regarding the (MIC), the microorganism less susceptible to Chlorhexidine was *P. gingivalis* (75 µg / mL) followed by *A. actinomycetemcomitans* (38 µg / mL), while the more susceptible were *E. faecalis, E. cloacae and C. albicans* (4.7 µg / mL, 9 µg / mL and 19 µg / mL respectively). Compared to NaOCl, the more susceptible microorganism was *C. albicans* (1,563 µg / mL), followed by *E. cloacae, P. gingivalis* and *E. faecalis* (781 µg / mL) and the less susceptible was *A. actinomycetemcomitans* (391 µg / mL) (Table 1 and figure).

For MBC, the microorganism more susceptible to Chlorhexidine was *P. gingivalis* (75 µg / mL) followed by *A. actinomycetemcomitans* (38 µg / mL), while the less susceptible were *E. faecalis, E cloacae* and *C. albicans* (4.7 µg / mL, 9 µg / mL and 19 µg / mL, respectively. (Table 1 and figure).

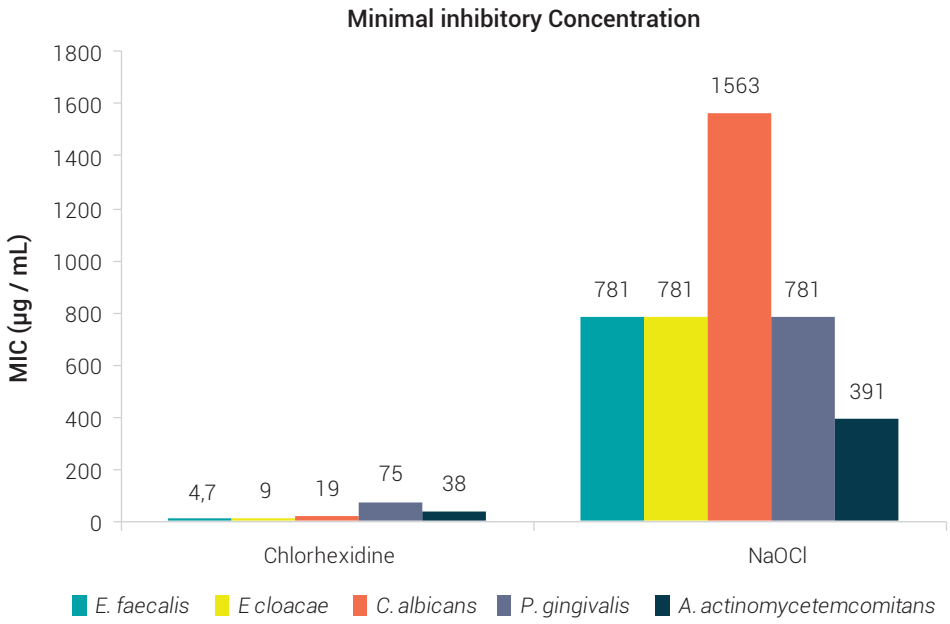

Figure depicts the minimal inhibitory concentration of chlorhexidine 0.12% and sodium hypochlorite 5.25% against diverse microbial strains. In general, chlorhexidine performed better than sodium hypochlorite as irrigation agent.

Source: own work

Overall, the ATCC strains of *C. albicans, E cloacae* and *E. faecalis* was more resistant than *P. gingivalis* and *A. actinomycetemcomitans* in terms of MIC determination against irrigants tested. The irrigant BioPure MTAD® performs slightly better than QMix® with respect to the (MIC). Chlorhexidine 0.12% was more effective than Hypochlorite 5.25% in eliminating most of microbial strains used in this study.

4. Discussion

The objective of this study was to determine the minimum bactericidal concentration and minimum inhibitory concentration of Sodium Hypochlorite 5.25%, Chlorhexidine 0.12%, MTAD® and Qmix® against ATCC strains of *A. actinomycetemcomitans, P. gingivalis, C. albicans, E. faecalis* and *E. cloacae* growing in vitro. Chlorhexidine 0.12% was more effective than Hypochlorite 5.25% to inhibit test microorganisms at lower concentrations (figure and Table 1). BioPure MTAD® exercised a better antimicrobial effect when compared to Qmix. Since it´s well known that, endodontic biofilms are organized in multi-species biofilms and these results are related to single microbial species, our results must be interpreted with some caution.

Shen Ya et al (29) in 2011 suggested that CHX 0.12% holds more bacteriostatic properties; and to achieve the bactericidal effect, their concentration should be greater than 2%, but higher concentration seems to disturb tissue healing and increase the risk of endodontic failure. In the present study, the investigators used 0.12% CHX and 5.25% NaOCl that were able to inhibit those microbial strains tested. Chlorhexidine to inhibit *P. gingivalis* requires a minimum inhibitory concentration of 75 µg /mL, being this bacterium the less susceptible to CHX. However, chlorhexidine is considered inferior to NaOCL because its inability to dissolve organic matter and lesser antimicrobial effect on established biofilm (30). Some attempts have been made to evaluate the activity of CHX to dissolve organic matter, and the actual facts are that CHX in aqueous solution or gel, could not dissolve pulp tissue as reported (Marley JT) (30). And pulp bleeding in the case of vital pulp only might stop when a complete removal of the pulp tissue occurs after full instrumentation and whole root canal extension. Therefore, when CHX is used as irrigant, emphasis should be given to full canal instrumentation to remove pulp tissue, as CHX does not promote superficial necrosis. (Gomes BP) (31).

The antimicrobial effect of 2.5% NaOCl and 0.2% chlorhexidine used together is greater than if they are used each one separately, but the combination of both irrigants produces an acid-base reaction leading to the formation of a neutral salt Insoluble called para-chloroaniline (PCA) Basrani BR et al (32). PCA has been found to be mutagenic for microorganisms and cytotoxic to cells according to Prasad I et al (33). Some concern over possible carcinogenicity has also been expressed Gomes BP et al. (34)

Different scientific evidence related to the use of sodium hypochlorite has been reported by Byström et al (35), 1983 and Haapasalo (36) et al, 2010, as they considered NaOCL as "gold standard" for endodontic irrigation although some bacteria can remain viable. A Cochrane Review whose objective was to assess the effects of irrigants used in non-surgical root canal treatment of mature permanent teeth, one of the aspects analyzed was the proportion of participants or 'samples' with positive bacterial growth culture following the procedure and until 72 hours at follow-up. Four trials compared sodium hypochlorite versus chlorhexidine, however, no primary outcomes and only one secondary outcome, bacterial growth cultures, was reported for two of these trials (20% and 50% of teeth in the control group had positive bacterial culture). The meta-analysis indicated no strong evidence of a difference in the existence of bacterial growth between the interventions. (37)

Another In vitro study of Daniel et al, 2017 (38) demonstrated a high susceptibility of *E. faecalis* against BioPure MTAD, even when this irrigation solution was diluted 200 times, while NaOCl, lost its antibacterial activity against *E. faecalis* at dilution 32. Other microbial strains were susceptible up to dilution 8,192 regarding (MIC) and in

relationship with (MBC), BioPure MTAD inhibit *E. faecalis* at a dilution of up to 1,024. We observed that most strains of *E. faecalis* were inhibit by MTAD until 1: 512 dilution and more effective at higher concentrations (dilutions 1: 128 and 1: 256). Our results showed that BioPure MTAD effectively inhibits the growth of *E. faecalis* up to a dilution of 2.048. This finding might be clinically significant because lower level of antimicrobials can get inside dentinal tubules and inhibit endodontic microbes. Dioguardi M et al. (2019) (17) recently indicated a better bactericidal effect of MTAD associated with a carryover effect of doxycycline during preparation.

Jose J (39) et al in 2016 compared the antimicrobial efficacy of different irrigants such as Qmix, guava leaf extract, aloe-vera extract, 2.5% sodium hypochlorite and 2% chlorhexidine gluconate against *E. faecalis* and *C. albicans*. Qmix showed an inhibitory effect against *E. faecalis* and *C. albicans* followed by 2% chlorhexidine, 2.5% sodium hypochlorite, guava leaf extract and aloe-vera extract were compared to our results Qmix was effective at low concentrations against the *P. gingivalis* strain and a high concentration was required to inhibit the *E. faecalis* strain.

The clinical importance of these findings should be interpreted with caution as the same work with multispecies biofilms could generate different results. Taking into account the results obtained in this research, and in terms of cost-effectiveness, it is suggested to carry out other studies that better represent the clinical environment, where the various factors that can influence the antimicrobial effect of the substances used for irrigation during root canal treatment, and that meet the ideal properties of an irrigant for endodontic use, including the ability to dissolve organic tissue and remove smear layer.

5. Conclusion

Strains of *C. albicans* ATCC® 44858 ™, *E. cloacae* ATCC®13047 ™ and *E. faecalis* ATCC® 29212 ™ were more resistant than these of *P. gingivalis* ATCC® 33277 ™, and *A. actinomycetemcomitans* ATCC® 29522 ™ in terms of MIC to the four irrigation products tested. The irrigant BioPure MTAD® performed better than QMix® with respect to the (MIC) and Chlorhexidine 0.12% was more effective than Sodium Hypochlorite 5.25% in inhibiting and eliminating the tested strains.

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