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Comparison of Antibacterial Effect of Four Irrigation Solutions in Primary Root Canal Infections: A Clinical Study

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ABSTRACT

Introduction: Reducing the bacterial count from the root canal system is one of the main stages in root canal treatment. The aim of the present clinical study was to compare the antibacterial effect of four intracanal irrigants in primary endodontic infections using both microbiological culture and quantitative Real-time Polymerase Chain Reaction (qRT-PCR) technique. **Methods and Materials**: Forty patients with primarily infected single rooted premolars were selected and then randomly divided into 4 groups according to the intra canal irrigant used: 5.25% Sodium hypochlorite (NaOCl), Hypoclean (Ogna Laboratori Farmaceutici, Muggiò, Italy), 2% chlorhexidine glouconate (CHX) and CHX-Plus (Vista Dental Products, Racine, WI, USA). Samples were collected before and after chemomechanical preparation and were evaluated by bacterial culture and RT-PCR technique for *Enterococcus faecalis* and *Fusobacterium nucleatum*. Data analyzed by repeated measured ANOVA. The significance level was set at 0.05. **Results**: Four irrigation solutions significantly reduced the total numbers of cultivable bacteria ($P<0.05$). No statistically differences were found among the antibacterial effects of 5.25% NaOCl (99.93%), Hypoclean (99.94%), 2% CHX (99.77%) and CHX-Plus (99.83%) in reducing cultivable bacteria. *Enterococcus faecalis* and *Fusobacterium nucleatum* were no longer detected after preparation using four irrigants (100% reduction). **Conclusions**: All tested irrigants including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus significantly reduced the number of bacterial colonies in primary endodontic infections.

Keywords: CHX-Plus; Endodontic Infection; *Enterococcus faecalis*; *Fusobacterium nucleatum*; Hypoclean

Introduction

The presence of infection in the apical periodontitis lesions is an important criterion of endodontic treatment failure [1]. To achieve a successful endodontic treatment, bacterial eradication from the root canal system is very important. There are some reports that show the influence of the residual bacterial in the root canal after endodontic treatment procedure on the post treatment outcome [2, 3]. Therefore, choosing an effective antibacterial endodontic irrigant during root canal therapy should be the main microbiological goal of the endodontic treatment to eradicate bacterial infection [4].

Sodium hypochlorite (NaOCl) has been used as an endodontic irrigant for many years and has paramount importance in root canal disinfection as an antimicrobial agent and an excellent tissue solvent [5-8] but its toxicity for periapical tissues is overlooked [9-11]. Also NaOCl has high surface tension that limits its penetration into dentinal tubules and irregularities of the root canal system [12]. Hypoclean is a NaOCl-based irrigant, composed of 5.25% NaOCl and two detergents (cetrimide and polypropylen glycol) [13].

Chlorhexidine glouconate (CHX) is another effective antimicrobial agent [14] that in recent decades has been used as an endodontic irrigant. This agent is suggested as an alternative to NaOCl with the same antibacterial effect and less toxicity [15, 16]. CHX-Plus is a new product from CHX which is composed of %2 CHX and a surfactant. Manufacturer states that this irrigant has not unpleasant smell and is able to kill the bacteria 2 times faster than CHX by reducing the viscosity of surface [17].

There is no clinical study on antibacterial effectiveness of Hypoclean and CHX-Plus in root canal treatment. Researchers reported that more than 500 bacterial species are present in the oral cavity, but interestingly, there are few species in the root canal that indicates the selective environment of the root canal so that some bacteria survive whereas others cannot [18]. *Enterococcus (E.) faecalis* is one of these bacteria in root canal system, that is present in primary endodontic infection and can persist in root canal system even after root canal therapy [19]. The high incidence of *E. faecalis* in persistent apical periodontitis might be related to its unique virulence factors including its ability to compete with other microorganisms, invade dentinal tubules, and resistance in nutritional deprivation. This species could survive in 500 μm depth of dentin for 3 weeks [20, 21]. The other bacterium with high prevalence in root canal infection is *Fusobacterium (F.) nucleatum* [22-24].

Bacterial culture is the most commonly employed technique for bacterial identification and pathogen detection. However, molecular techniques are more sensitive and effective than culture techniques and biochemical methods [25]. The quantitative Real-time Polymerase Chain Reaction (qRT-PCR) method provides detection and quantitative results about the exact bacterial counts in the root canal samples before and after chemomechanical root canal preparation [26, 27].

The aim of this clinical study was to compare the antibacterial effect of four irrigation solution including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus in teeth with primary endodontic infection using both microbiological culture and RT-PCR technique.

Materials and Methods

Patient selection

Forty patients referred to endodontic department of Tehran University of Medical Sciences for root canal treatment of single rooted premolars with one canal participated in the present study. This study protocol was approved by the ethics committee of Tehran University of Medical Sciences in Iran (IR.TUMS.VCR.REC.1395.1232). All patients signed the informed consent form. The teeth had necrotic pulps which was confirmed with absence of response to cold test and evidence of primary endodontic infection in radiography. Patients with systemic disease and patients who had received antibiotics in past 3 months were excluded. Teeth that could not be isolated with a rubber dam or teeth with periodontal pockets deeper than 4 mm were excluded as well. All patients were treated in the postgraduate clinic of the Endodontic Department of Tehran Dental School.

All patients who agreed to participate in the study were randomly divided into 4 groups of 10 patients each. To randomize the patients, each patient was asked to choose one of 4 sealed envelopes. Based on the number of the sealed envelope, the patients received one of the irrigants. The irrigants include 5.25% NaOCl (Noradiska Dental, Tartu, Estonia), Hypoclean (OgnaLaboratoriFarmaceutici, Muggiò, Italy), %2 CHX (Cerkamed, Stalowa, Poland) and CHX-Plus (Vista Dental Products, Racine, WI, USA).

Instruments and all materials used in this study were sterile throughout the endodontic treatment and all samples were collected under strictly aseptic conditions. The teeth were isolated with a rubber dam. At first the crown and surrounding operation area were cleaned with 30% hydrogen peroxide and 5.25% NaOCl. Then 5% sodium thiosulfate was used to inactive 5.25% NaOCl. The access cavity was prepared with high-speed diamond under manual irrigation with sterile saline solution. Procedures were performed aseptically.

For sample collection, 3 sterile paper points were used successively in full length of the canal that was determined by apex locator (I-Root Apex Locator, META, Chungcheongbuk-do, South Korea) and confirmed by using periapical radiography. Every paper point remained in the root canal for 1 min. The samples which were collected before and after irrigation were transferred into 1 mL thioglycollate broth (Quelab, Quebec, Canada) and immediately were cultured on Crystal-Violet Erythromycin (CVE) agar and brain-heart infusion (BHI, Merck, Darmstadt, Germany) in the Microbiology Department of Tehran University of Medical Sciences.

All groups were prepared in 20 min by using Mtwo files (VDW, Munchen, Germany) according to the manufacturer's instructions. Then 2 mL of each irrigant was used to rinse the canals after each instrument. At the end of preparation and before the second sampling, 5 mL 0.5% sodium thiosulfate was used in NaOCl and Hypoclean groups for 60 sec to inactive the irrigants. CHX and CHX Plus were inactivated by Tween 80 and alpha-lecithin. The second sampling was taken from the canal as mentioned earlier. Then all teeth were obturated with gutta-

percha (Aryadent, Tehran, Iran) and AH-26 silver free sealer (Dentsply, DeTrey, Konstanz, Germany) by lateral compaction technique. At the end of procedure, the access cavities were sealed with Zonalin™ (Kemedent, UK) and the patients were referred to the Operative Dentistry Department of Tehran University of Medical Sciences for coronal restoration.

Bacterial culture procedure

The transport medium containing the samples was shaken for 1 min. Fifty microliters of each sample were plated onto BHI medium and CVE agar using sterile spreaders to culture nonselective aerobes and anaerobes; respectively. The plates were incubated at 37ºC in both aerobic and anaerobic atmosphere for up to 7 days. After this period, colony-forming units (CFUs) were visually quantified for each plate. Biochemical differential diagnosis tests were used to distinguish various bacterial colonies [28].

Real Time-PCR analysis

The total DNA of the samples was isolated using YTA Genomic DNA Extraction Min Kit (Yektatajhiz, Tehran, Iran) according to the manufacturer's protocol. Before DNA extraction, DNAse was added to all samples to eliminate free DNAs. The primers and probes used in Real-Time PCR were selected to detect and quantify *E. faecalis* (Genesig Standard kit, Genesig, UK) and *F. nucleatum* (FusNucdtec-qPCR kit, GPS, Alicante, Spain). Real-time polymerase chain reaction was performed based on the Step One Plus Real-Time PCR System (ABI, California, USA) with amplification conditions for *F. nucleatum* as follows: 95°C/15 sec for denaturation followed by 60°C/60 sec for 40 cycles. Amplification conditions for *E. Faecalis* was: 95°C/10 sec for denaturation followed by 60°C/60 sec for 50 cycles. Then the quantification of each bacterium was calculated.

Statistical analysis

The results were analyzed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). After checking the normal distribution of the results, comparison between the experimental groups was performed by applying Repeated measure ANOVA of General Linear Model. Mean of data was used for analysis. Between subject factor was experimental group (NaOCl, Hypoclean, CHX, CHX-Plus) and repeated factor was the colony count before and after using irrigation solutions. The significance level for all tests was set at 0.05.

Table 1. Mean of total bacterial load (CFUs/mL) in samples taken before and after chemomechanical preparation in 4 irrigation groups (n=10)

Groups	Before treatment	After treatment
NaOCl	5.5×10^5	70
Hypoclean	4.8×10^5	60
CHX	5.2×10^5	80
CHX-Plus	6.4×10^5	90

Table 2. Number of *Enterococcus faecalis* in samples of teeth with primary endodontic infection taken before and after chemomechanical preparation using 4 irrigation solutions

	Before treatment			After treatment		
Groups (N)	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
NaOCl (3)	0	1864174.59	9641568	0	0	0
Hypoclean (2)	0	83.19	9.1760	0	0	0
CHX (1)	0	121.56	12.15	0	0	0
CHX-Plus (3)	0	54.28	5.4280	0	0	0

*Data from quantitative polymerase chain reaction analysis; *Number of samples that were positive before chemomechanical preparation*

Table 3. Number of *Fusobacterium nucleatum* in samples of teeth with primary endodontic infection taken before and after chemomechanical preparation using 4 irrigation solutions

	Before treatment			After treatment		
ups (N)	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
NaOCl (5)	0	9943698.00	995168.74	0	0	0
Hypoclean (4)	0	1108.68	141.25	0	0	0
CHX (3)	0	1967.33	261.63	0	0	0
CHX-Plus (4)	0	9718.42	1658.65	0	0	0

*Data from quantitative polymerase chain reaction analysis; *Number of samples that were positive before chemomechanical preparation*

Results

Bacterial culture

Bacterial cultures showed presence of bacteria in all root canal samples before preparation. After preparation using 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus, the cultivable bacteria decreased significantly ($P<0.004$) (Table 1). All irrigation solutions reduced the cultivable bacteria more than 99%. There was no significant difference among 5.25% NaOCl (99.93%), Hypoclean (99.94%), 2% CHX (99.77%) and CHX-Plus (99.83%) in reducing colony number of the bacteria.

Biochemical tests identified *Streptococci, Micrococcus, Staphylococcus* and *Fusobacterium* species in root canals with primary endodontic infection before preparation. *E. faecalis* was not detected in bacterial cultures and *Fusobacterium* species were detected in 4 of 40 samples. After chemomechanical preparation using four tested irrigants only *Streptococci* species were detected in the specimens.

Real-time PCR

The Real-time PCR analysis of the root canal samples before root canal preparation using irrigation solution revealed the presence of *E. faecalis* and *F. nucleatum* in 9 and 16 specimens; respectively. After root canal preparation, *F. nucleatum* and *E. faecalis* species were no longer detected in four groups. There was no difference among four irrigants in terms of eradicating these two species. The number of *E. faecalis* and *F. nucleatum* in the positive samples before and after using irrigation solutions are shown in Tables 2 and 3.

Discussion

The present clinical study compared the antibacterial effect of 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus during chemomechanical preparation in teeth with primary endodontic infection. Before preparation, all root canal samples were positive for the presence of many cultivable bacteria including aerobes and anaerobes. This finding supported the polymicrobial feature of primary endodontic infections reported by other investigations [24, 29].

Root canal preparation using four irrigation solutions showed an extremely significant reduction of the cultivable bacteria and *F. nucleatum* and *E. faecalis* species in all the root canal samples. These results confirmed the essential role of instrumentation by using antimicrobial irrigation in reducing the bacterial populations in infected root canals and are in agreement with the previous studies [16, 30-32].

Bacterial culture is a routine technique for bacteria detection in microbiology. However, an important concern of such methods is the fact that about one half of the endodontic bacteria could not grow and have not been detected by standard culture techniques [33]. Furthermore, culture technique is not suitable for detection of bacteria in low number and culture-difficult species like anaerobic bacteria. To overcome to these problems, molecular techniques were developed to detect uncultivated bacteria, culture-difficult species and minimum number of bacteria.

Polymerase chain reaction (PCR) assay is used for assessment of clinical samples and detection and identification of bacteria at the DNA level. An established method for precise detection and quantification of bacterial DNA is RT-PCR. This technique allows exact determination of nucleic acid levels of known microbial species by monitoring the fluorescent signals per cycle [34]. Studies have shown that the sensitivity of RT-PCR is 41 fold more than bacteria detected using bacterial culture and colony counting techniques especially for anaerobic species [25, 35].

We used DNA-based RT-PCR screening in the present study to detect *E. faecalis* and *F. nucleatum.* These bacteria are cultivable but don't survive or grow well in culture. *E. faecalis* has been commonly reported as recovered species from root canals whenever the endodontic treatment is not successful. Sundqvist *et al.* [21] found that 38% of the recovered bacteria after root canal treatment was *E. faecalis*. On the other hand *F. nucleatum* has the unique properties like the ability to survive even under difficult stresses and resistance to medications applied in the endodontic procedures [36]. Also, this species has been recovered from infected root canals with high prevalence and is one of the most common species in post-instrumentation samples [37]. So these two species selected to study the ability of irrigants for eliminating the root canal infection.

There is no clinical study that compares the antibacterial effect of Hypoclean and CHX-Plus with other endodontic irrigants. Some *in vitro* studies reported Hypoclean has more antibacterial effect compared to NaOCl [38, 39]. According to the result of this study the antibacterial effect of Hypoclean was the same as NaOCl. In the study by Mohammadi *et al.* [40], 5.25% NaOCl and Hypoclean were more effective against the microorganisms tested compared to %2 CHX. Their *in vitro* study showed depending on the taxa of bacteria, that NaOCl and Hypoclean have different abilities to eliminate the bacteria. NaOCl was the most effective irrigant against *E. faecalis* and Hypoclean was the preferred irrigant against *C. albicans, P. aeroginosa,* and *L. casei.*

In the present study, %2 CHX and CHX-Plus had the same antibacterial effect against *E. faecalis*. This result corresponded with the *in vitro* study by Williamson, *et al.* [41] who showed the same effectiveness of %2 CHX and CHX-Plus for eradication of *E. faecalis*.

In this study, four used irrigants showed comparable results regarding the bacterial elimination from infected root canals *in vivo*. This indicates that these agents can be assumed as equally effective antibacterial irrigants during chemomechanical preparation.

In the present study, only two culture-difficult species of bacteria detected by PCR method; further studies for detecting bacteria in low number and uncultivable bacteria of the primary infected root canal samples should be considered.

Conclusion

Four irrigation solutions including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus significantly reduced the intracanal bacteria. There was no difference in antibacterial effect of these irrigation solutions in single rooted premolars with primary endodontic infection.

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Conflict of Interest: 'None declared'.

References

1. Siqueira JF, Jr., Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-301 e3.
2. Fabricius L, Dahlen G, Sundqvist G, Happonen RP, Moller AJ. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. Eur J Oral Sci. 2006;114(4):278-85.
3. Waltimo T, Trope M, Haapasalo M, Orstavik D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. J Endod. 2005;31(12):863-6.
4. Siqueira JF, Jr. Endodontic infections: concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(3):281-93.
5. Siqueira Jr J, Machado A, Silveira R, Lopes H, De Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J. 1997;30(4):279-82.
6. Zand V, Lotfi M, Soroush MH, Abdollahi AA, Sadeghi M, Mojadadi A. Antibacterial efficacy of different concentrations of sodium hypochlorite gel and solution on enterococcus faecalis biofilm. Iran Endod J. 2016;11(4):315.
7. Samiei M, Shahi S, Abdollahi AA, Eskandarinezhad M, Negahdari R, Pakseresht Z. The Antibacterial Efficacy of Photo-Activated Disinfection, Chlorhexidine and Sodium Hypochlorite in Infected Root Canals: An in Vitro Study. Iran Endod J. 2016;11(3):179.
8. Nourzadeh M, Amini A, Fakoor F, Raoof M, Sharififar F. Comparative antimicrobial efficacy of Eucalyptus galbie and Myrtus communis L. extracts, chlorhexidine and sodium hypochlorite against Enterococcus faecalis. Iran Endod J. 2017;12(2):205.
9. Gernhardt C, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. Int Endod J. 2004;37(4):272-80.
10. Nabavizadeh M, Ghahramani Y, Abbaszadegan A, Jamshidzadeh A, Jenabi P, Makarempour A. In Vivo Biocompatibility of an Ionic Liquid-protected Silver Nanoparticle Solution as Root Canal Irrigant. Iran Endod J. 2018;13(3):293.
11. Abbaszadegan A, Gholami A, Abbaszadegan S, Aleyasin ZS, Ghahramani Y, Dorostkar S, Hemmateenejad B, Ghasemi Y, Sharghi H. The Effects of Different Ionic Liquid Coatings and the Length of Alkyl Chain on Antimicrobial and Cytotoxic Properties of Silver Nanoparticles. Iran Endod J. 2017;12(4):481.
12. Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L. Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. Int Endod J. 2012;45(2):129-35.
13. Mohammadi Z, Mombeinipour A, Giardino L, Shahriari S. Residual antibacterial activity of a new modified sodium hypochlorite-based endodontic irrigation solution. Med Oral Patol Oral Cir Bucal. 2011;16(4):e588-e92.
14. Samiei M, Torab A, Hosseini O, Abbasi T, Abdollahi AA, Divband B. Antibacterial Effect of Two Nano Zinc Oxide Gel Preparations Compared to Calcium Hydroxide and Chlorhexidine Mixture. Iran Endod J. 2018;13(3):305.
15. Ercan E, Özekinci T, Atakul F, Gül K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study. J Endod. 2004;30(2):84-7.
16. Siqueira Jr JF, Rôças IN, Paiva SS, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(1):122-30.
17. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. J Endod. 2012;38(7):948-53.
18. Sundqvist G, Figdor D. Life as an endodontic pathogen: Ecological differences between the untreated and root-filled root canals. Endod Topics. 2003;6(1):3-28.
19. Vidana R, Sullivan Å, Billström H, Ahlquist M, Lund B. Enterococcus faecalis infection in root canals–host-derived or exogenous source? Lett Appl Microbiol. 2011;52(2):109-15.

20. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. journal of endodontics. 2006;32(2):93-8.
21. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(1):86-93.
22. Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, Hazlett K, Radolf JD. PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol. 2002;40(9):3223-31.
23. Jung I-Y, Choi B-k, Kum K-Y, Roh B-D, Lee S-J, Lee C-Y, Park D-S. Molecular epidemiology and association of putative pathogens in root canal infection. J Endod. 2000;26(10):599-604.
24. Ferreira NS, Martinho FC, Cardoso FG, Nascimento GG, Carvalho CA, Valera MC. Microbiological profile resistant to different intracanal medications in primary endodontic infections. J Endod. 2015;41(6):824-30.
25. Martin FE, Nadkarni MA, Jacques NA, Hunter N. Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. J Clin Microbiol. 2002;40(5):1698-704.
26. Sedgley C, Nagel A, Dahlen G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. J Endod. 2006;32(3):173-7.
27. Sedigh-Shams M, Gholami A, Abbaszadegan A, Yazdanparast R, Nejad MS, Safari A, Nabavizadeh M, Ghasemi Y. Antimicrobial efficacy and cytocompatibility of calcium hypochlorite solution as a root canal irrigant: an in vitro investigation. Iran Endod J. 2016;11(3):169.
28. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology-E-Book: Elsevier Health Sciences; 2014.
29. Didilescu A, Rusu D, Anghel A, Nica L, Iliescu A, Greabu M, Bancescu G, Stratul S. Investigation of six selected bacterial species in endo-periodontal lesions. Int Endod J. 2012;45(3):282-93.
30. Iqbal A. Antimicrobial irrigants in the endodontic therapy. Int J Health Sci (Qassim). 2012;6(2):186.
31. Sakamoto M, Siqueira Jr J, Rôças I, Benno Y. Bacterial reduction and persistence after endodontic treatment procedures. Oral Microbiol Immunol. 2007;22(1):19-23.
32. Ramezanali F, Samimi S, Kharazifard M, Afkhami F. The in vitro antibacterial efficacy of persian green tea extract as an intracanal irrigant on enterococcus faecalis biofilm. Iran Endod J. 2016;11(4):304.
33. Sakamoto M, Rôças I, Siqueira Jr J, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. Oral Microbiol Immunol. 2006;21(2):112-22.
34. Morrison TB, Weis JJ, Wittwer CT. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. Biotechniques. 1998;24(6):954-8, 60, 62.
35. Verner C, Lemaitre P, Daniel A, Giumelli B, Lakhssassi N, Sixou M. Carpegen® real-time polymerase chain reaction vs. anaerobic culture for periodontal pathogen identification. Oral Microbiol Immunol. 2006;21(6):341-6.
36. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J. 1998;31(1):1-7.
37. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 1997;30(5):297-306.
38. Bolfoni MR, Ferla MdS, Sposito OdS, Giardino L, Jacinto RdC, Pappen FG. Effect of a surfactant on the antimicrobial activity of sodium hypochlorite solutions. Send to Braz Dent J. 2014;25(5):416-9.
39. Giardino L, Cavani F, Generali L. Sodium hypochlorite solution penetration into human dentine: a histochemical evaluation. Int Endod J. 2017;50(5):492-8.
40. Mohammadi Z, Shalavi S, Giardino L, Palazzi F, Mashouf R, Soltanian A. Antimicrobial effect of three new and two established root canal irrigation solutions. Gen Dent. 2012;60(6):534-7.
41. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of Enterococcus faecalis. J Endod. 2009;35(1):95-7.

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