

EFFICACY OF SODIUM HYPOCHLORITE AND CHLORHEXIDINE AGAINST *Enterococcus faecalis* – A SYSTEMATIC REVIEW

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ABSTRACT

The efficacy of the sodium hypochlorite (NaOCl) and chlorhexidine (CHX) on *Enterococcus faecalis* was evaluated by systematic review and meta-analysis. The search strategies included search in electronic biomedical journal databases (MEDLINE, EMBASE, CENTRAL) and handsearching records, using different matches of keywords for NaOCl, CHX and *Enterococcus faecalis*. From 41 *in vivo* studies, 5 studies met the inclusion criteria. In a sample containing 159 teeth, *E. faecalis* was detected initially in 16 (10%) teeth by polymerase chain reaction (PCR) and 42 (26.4%) teeth by microbial culture techniques. After root canal disinfection, this species was observed in 11 (6.9%) teeth by PCR and 12 (7.5%) teeth by culture. Risk differences of included studies were combined as generic inverse variance data type (Review Manager Version 5.0 – Cochrane Collaboration, <http://www.cc-ims.net>, accessed 15 May 2008), taking into account the separate tracking of positive and negative cultures/PCR. The level of statistical significance was set at $p < 0.05$. In conclusion, NaOCl or CHX showed low ability to eliminate *E. faecalis* when evaluated by either PCR or culture techniques.

Key words: *E. faecalis*. Sodium hypochlorite. Chlorhexidine. Irrigating solutions. Systematic review.

INTRODUCTION

The pathogenicity of endodontic microorganisms responsible for stimulating apical periodontitis creates the need for finding effective antimicrobial medicaments¹⁶. Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most frequently widely studied and employed antimicrobial agents for treatment of root canal infection^{2,5}. These medicaments present chemical characteristics that are particularly responsible for their distinct results when compared^{1,6,20,23}. These variations occur probably due to differences in methodology, biological indicators, concentrations, exposure time, the potential for different anatomical and treatment differences between patients^{1,6,20,23,27}.

The contemporary literature contains numerous reports on the antimicrobial efficacy of NaOCl and CHX in several experimental models - infected human teeth *in vivo*^{7,16-18,29,30}, infected human teeth *ex vivo*⁴, infected dog's teeth *in vivo*^{5,21}, infected bovine teeth *ex vivo*¹⁰, biofilm model in membrane

filters^{22,25}, direct contact and agar diffusion test^{6,9}.

E. faecalis is an extensively evaluated biological indicator^{12,15,19,26,27}. Some factors can explain the concern with this pathogen in endodontic infections. Its high prevalence in cases with post-treatment disease associated with virulence factors (aggregation substance, enterococcal surface proteins (Esp), gelatinase, cytolysin toxin, extracellular superoxide production, capsular polysaccharides, antibiotic resistance determinant) can facilitate the adherence of host cells and extracellular matrix, tissue invasions, immunomodulation effect and cause toxin-mediated damage¹⁹.

Actual thinking has recommended the implementation of evidence-based dentistry, which valorizes studies involving the systematic review or meta-analysis. Systematic reviews use a strict methodological approach to search, select, evaluate, and analyze original data from primary sources. Good scientific evidence is mandatory to elaborate clinical decisions, yet few systematic reviews or meta-analysis have been developed in Endodontics^{14,21,28}.

Torabinejad and Babjri²⁸ reported that the American Dental Association has adopted the concept of evidence-based healthcare, which requires the judicious integration of systematic assessments of clinically relevant scientific evidence.

Previous studies using *in vitro* experimental models have confirmed the antimicrobial efficacy of NaOCl and CHX against *E. faecalis*^{6,23}, while others using different study designs have not found same results^{4,5,7,16-18,29,30}. Relevant clinical questions based on evidence regarding the resistance of *E. faecalis* to NaOCl or CHX should be further discussed. Therefore, the aim of this study was to review findings on the antibacterial efficacy of NaOCl or CHX against *E. faecalis* in endodontic infection using systematic review and meta-analysis.

MATERIALS AND METHODS

Literature Review

This study was designed using an analysis of longitudinal studies from a quantitative systematic review. Prospective studies were selected towards the efficacy of NaOCl and CHX against *E. faecalis* identified in endodontic infections before and after root canal preparation. English-language articles were retrieved from electronic biomedical journal databases and handsearching records.

The following databases were searched on January 2nd, 2007: MEDLINE (without filter, from 1966 to January 2nd, 2007), EMBASE (without filter, from 1980 to January 2nd, 2007), Cochrane Oral Health Group Trials Register and Cochrane Central Register of Controlled Trials (CENTRAL). For the electronic search strategy, the following terms were used as keywords in several combinations: 1. *faecalis* and sodium hypochlorite OR, 2. *faecalis* and chlorhexidine OR, 3. *faecalis* and root canal infections OR, 4. *faecalis* and endodontics infections OR, 5. *faecalis* and root canal irrigants OR, 6. *faecalis* and irrigating solution OR, 7. *faecalis* and endodontics irrigants

OR, 8. *faecalis* and intracanal irrigants.

A systematic review was conducted according to the guidelines of the Cochrane Collaboration, which recommend the search for the largest possible number of articles. Handsearching was conducted by the review of the reference lists of the eligible clinical trials and the review of author's personal databases of trial reports in an attempt to identify any other relevant studies.

Inclusion and Exclusion Criteria

The selected articles were identified from titles and abstracts by two independent reviewers, considering the tabulated inclusion and exclusion criteria. The inclusion criteria were studies in humans, studies related to the efficacy of NaOCl or CHX against *E. faecalis*, nonsurgical root canal treatment performed during the study, subjects with a noncontributory medical history, microbiological samples collected before and after root canal preparation, English-language articles. The exclusion criteria were *in vitro* and animal studies, studies related to the efficacy of intracanal irrigants and medications other than NaOCl or CHX, studies without collection of microbiological samples before or immediately after root canal preparation, non-English-language articles, studies abstract only or no abstract, literature reviews, studies involving primary teeth, case reports, studies related only to microbial identification or studies involving microorganisms other than *E. faecalis*.

Full-text reprints were obtained from all relevant and potentially relevant studies, which seemed to meet the inclusion criteria and from those that had insufficient data in the title and abstract to make a clear decision.

Meta-Analysis

The design of this meta-analysis was based on the guidelines of the Cochrane Collaboration and on the methodology of previous study²¹. Chi-square test was used to analyze the differences between the studies. The essential analysis of antibacterial efficacy was risk difference (difference in the ratio of positive bacterial identification

TABLE 1- Studies included related to the efficacy of the NaOCl and CHX against *E. faecalis*

Studies (ref.)	n	IET	Baseline infection types	<i>E. faecalis</i> before RCP		Irrigants	<i>E. faecalis</i> after RCP	
				PCR	Culture		PCR	Culture
Willian, et al.(29)	29	>5 years	primary (n=15)	2 (13.3%)	1 (6.6%)	1% NaOCl	3 (20%)	0 (0%)
			secondary (n=14)	6 (42.8%)	2 (14.2%)		8 (57.1%)	1 (7.1%)
Zerella, et al.(30)	40	-	secondary	8 (20%)	-	1% NaOCl	-	0 (0%)
Ferrari, et al.(7)	25	-	primary	-	4 (16%)	0.5% NaOCl + Endo PTC	-	0 (immediately) (0%) 11 (after 7 days) (44%)
Peciulienė, et al.(17)	40	5-10 years	secondary	-	21 (52.5%)	2.5% NaOCl	-	6 (15%)
Peciulienė, et al.(18)	25	-	secondary	-	14 (56%)	2.5% NaOCl	-	5 (20%)
Total	159	-	-	16 (10%)	42 (26.4%)	-	11 (6.9%)	12 (7.5%)

(n - number of samples, IET- initial endodontic treatment, RCP – root canal preparation)

obtained by PCR of culture techniques between pre- and post-disinfection). Risk differences of included studies were combined as generic inverse variance data type (Review Manager Version 5.0 – Cochrane Collaboration, <http://www.cc-ims.net>, accessed 15 May 2008), taking into account the separate tracking of positive and negative cultures/PCR. The level of statistical significance was set at $p < 0.05$.

RESULTS

The search retrieved 229 related articles, being 6 literature reviews, 39 articles related to *in vivo* studies (27 in humans and 12 in animals), and 189 related to *in vitro* studies. From the 39 *in vivo* studies, 5 met the inclusion criteria. In these 5 papers, from a total of 159 teeth with primary or secondary endodontic infections, *E. faecalis* was initially detected in 16 (10%) teeth by PCR and 42 (26.4%) teeth by culture. After disinfection (effect of root canal enlargement associated with the action of chemical irrigants), *E. faecalis* was identified in 11 (6.9%) teeth by PCR and 12 (7.5%) teeth by culture techniques. No longitudinal studies in humans investigating the efficacy of NaOCl and CHX against *E. faecalis* from endodontic infections were found (Table 1).

The outcomes of the 5 selected studies are shown in Tables 2 and 3. The analysis was made between pre- and post-disinfection of the same root canals. The 5 studies were heterogeneous (Test of Homogeneity Cochran), considering samples evaluated by culture technique ($\chi^2 = 45.85$, $df=4$, $p < 0.00001$) and PCR ($\chi^2 = 1.65$, $df=1$, $p=0.20$). Thus, NaOCl or CHX showed low ability to eliminate *E. faecalis* when evaluated by either culture or PCR techniques.

DISCUSSION

The success of endodontic treatment is closely associated to the control of endodontic microbiota. Several chemical irrigants have been suggested for use in the treatment of infected root canal^{1,2,4-7,9-11,16-18,20,22,23,25,28-30}.

Studies with similar results have been reported when NaOCl and CHX were compared^{9,30}. However, other investigations have shown that NaOCl presents better antimicrobial activity than CHX^{1,20,23}, or, conversely, that CHX has better antimicrobial activity than NaOCl²³. Recently, it was demonstrated in a model of *E. faecalis* biofilm in human root canals that ozonated water, 2.5% NaOCl, 2% CHX and the application of gaseous ozone for 20 min were not sufficient to inactivate *E. faecalis*⁴. These differences may have been caused by differences in the experimental methodology, concentration, type of irrigating solution, patient and anatomical differences in root canal anatomy or the period of time used in the analysis.

It is important to emphasize the methodology used in the present investigation. Studies based on scientific evidence have stood out in dentistry^{14,21}. Various advantages

of systematic review can be observed: explicit methods limit bias in identifying and rejecting studies; conclusions are more reliable and accurate; more information can be rapidly assimilated by health care providers, researchers and policymakers; delay among research discoveries, implementation of effective diagnostic and therapeutic strategies is potentially reduced; results of different studies can be formally compared to establish generalization of findings and consistency of results (lack of heterogeneity); reasons for heterogeneity (inconsistency in results across studies) can be identified and new hypotheses generated about particular subgroups; quantitative systematic reviews (meta-analyses) increase the accuracy of the overall result.

The investigation model adopted in the present essay involved 5 studies, characterized by the heterogeneity of the clinical protocols. The analysis was made between pre- and post-disinfection of the same root canals considering samples evaluated by culture or PCR techniques. Thus, the NaOCl or CHX showed low ability to eliminate *E. faecalis* when evaluate by both techniques. An identical problem occurs when calcium hydroxide [$\text{Ca}(\text{OH})_2$] is tested against *E. faecalis*^{14,21}. Depending on the methodology - direct contact, agar diffusion or contaminated dentin test - this intracanal medicament may either present efficacy or not. This fact was not confirmed in human studies^{7,17,18,29,30} (Table 1), but the limitations of the methodology employed in this study should be considered. The application and validation of the results of longitudinal studies, as far as evidence-based view is concerned, are essential to determine the scientific value of the selected studies. Knowledge of the strategies to be applied for study selection is also important. Therefore, planning and development of this study model should be done with great caution⁸.

The first aspect to be considered is related to the bacteria location. If bacteria are located only on the root canal surface, where the intracanal medicaments can reach, NaOCl and CHX may present efficacy against *E. faecalis*. However, when bacteria are lodged within the dentinal tubules or in deep layers, *E. faecalis* can be more resistant to the antibacterial action of NaOCl and CHX.

Five studies met the inclusion criteria established for the present investigation^{7,17,18,29,30}. Peciuliene, et al.¹⁸ observed *E. faecalis* in 25 asymptomatic teeth with secondary infection. Avoiding contamination, microbiological samples were collected from the canals before and after preparation and irrigation with NaOCl and EDTA. *E. faecalis* was isolated from 14 of those 20 culture positive teeth, usually in pure culture or as a major component of the flora. Second samples taken after preparation revealed growth in 7 of the 20 teeth. Five of the 7 cases were *E. faecalis* in pure culture. Isolation of *E. faecalis* was not related to the use of any specific root filling material in the original root filling. Peciuliene, et al.²⁹ determined the occurrence and role of yeasts, enteric Gram-negative rods and *Enterococcus* species in root-filled teeth with chronic apical periodontitis. After collection of the first microbiological sample, the root canals were prepared to a size 40 file using 2.5% NaOCl and 17% EDTA as irrigating solutions. Microbes were isolated from

33 of 40 teeth in the initial sampling. Yeasts were isolated from 6 teeth, 3 of them together with *E. faecalis*. *E. faecalis* was isolated from 21 of the 33 culture positive teeth, 11 in pure culture. Growth was detected in 10 teeth of the second samples. Six of the 10 cases were *E. faecalis*, with five being a pure culture. Ferrari et al.⁷ detected enterococci, enteric bacteria and yeast species from 25 root canals with primary endodontic infections before and after canal preparation and to test the antibiotic susceptibility of enterococcal strains isolated. The canals were instrumented using a simple stepback technique with Endo PTC cream associated 0.5% NaOCl and EDTA. Microorganisms were isolated from 92% of the samples following intracoronary access, 22% were enterococci, enteric bacteria or yeast species. After biomechanical preparation, these species were no longer detected. After 7 days without intracanal dressing, 100% of the canals contained microorganisms, 52% of which were target species. *E. faecalis* and *E. faecium* were resistant to removal by root canal preparation followed by intracanal dressing. Zerella, et al.¹⁵ compared the effect of a slurry of Ca(OH)₂ mixed in aqueous 2% CHX versus aqueous Ca(OH)₂ slurry alone on the disinfection of the pulp space of failed root-filled teeth during endodontic retreatment in 40 teeth. The root canal was then cleaned and shaped with endodontic files using conventional endodontic technique. A copious amount of 1.0% NaOCl solution was used for irrigation. The results of this analysis were previously reported. The teeth were nonsurgically retreated and medicated over 3 treatment visits with 7-10-day intervals with either Ca(OH)₂ in water or Ca(OH)₂ in 2% aqueous CHX. Of the total sample population, 12 of 40 (30%) were positive for bacteria before root filling. The control medication disinfected 12 of 20 (60%) teeth including 2 of 4 teeth originally diagnosed with enterococci. The experimental medication resulted in 16 disinfected teeth out of 20 (80%) at the beginning of the third appointment. None of the teeth originally containing enterococci showed persistent bacterial growth. Canal dressing with a mixture of 2% CHX and Ca(OH)₂ slurry is as efficacious as aqueous Ca(OH)₂ on the disinfection of failed root-filled teeth. Williams, et al.²⁹ compared real-time quantitative PCR (qPCR) assay to cultivation for *E. faecalis* detection and quantification during endodontic treatment. Final shaping and mechanical root canal debridement was achieved using nickel-titanium files in a rotary crown-down technique. Teeth were irrigated with 1.05% NaOCl between files and after the final file. In primary infections, *E. faecalis* was present in Sample 1 in 7% (1/15) of cases by cultivation and 13% (2/15) by qPCR. No tooth was positive for the bacterium in either Sample 2 or Sample 3 by cultivation, indicating the removal of culturable *E. faecalis* by the instrumentation - irrigation protocol. Using qPCR, 3 teeth (the two teeth identified at Sample 1 and another tooth) harbored the bacterium at both Sample 2 and Sample 3. As observed with the primary infections, there was a nonsignificant trend for the number of *E. faecalis* positive cases detected by qPCR to increase to 57% (8/14) in Sample 2 and 50% (7/14) in Sample 3. qPCR detected up to three times more *E. faecalis*

in Sample 1 than cultivation, but the difference was not statistically significant. At collection times in Samples 2 and 3, qPCR identified more *E. faecalis* infections in refractory lesions than cultivation (Table 1).

Based on the meta-analysis results, in the selected 5 studies⁹⁻¹³, from a total of 159 teeth with endodontic infections, *E. faecalis* was detected initially in 16 (10%) teeth by PCR and 42 (26.4%) teeth by microbial culture techniques. Immediately after root canal preparation using 0.5% to 2.5% NaOCl, it was possible to identify *E. faecalis* in 11 teeth (6.9%) by PCR and in 12 teeth (7.5%) by culture. No longitudinal studies in humans investigating the efficacy of NaOCl and CHX against *E. faecalis* from endodontic infections were found

The difficulty in comparing the studies retrieved in the present search is due to differences on the methodological design of each investigation: standardization of the limit of preparation, choice of the preparation technique, standardization of tooth type and sample size, time of the initial endodontic treatment in cases of secondary infection, quality control of the chemical irrigants and variation in their concentration, criteria for the detection of the periapical lesion etc, in addition to other important data that were not mentioned in these studies (Table 1).

The selection of endodontic irrigants that aggregate the largest possible number of ideal properties was a major point in the present study. There was a concern in this systematic review¹⁰ regarding to the process of making clinical decisions in the control of microorganisms in endodontic infections.

CONCLUSION

In summary, the disinfection of the root canal system produced by emptying, enlargement and action of NaOCl reduces the remaining endodontic microbiota, which optimizes the efficacy of the intracanal dressing and favors the achievement of a higher level of success of the endodontic treatment.

REFERENCES

- 1 - Ayhan H, Sultan N, Cirak M, Ruhi MZ, Bodur H. Antimicrobial effects of various endodontic irrigants on selected microorganisms. *Int Endod J.* 1999;32:99-102.
- 2 - Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J.* 1985;18:35-40.
- 3 - Estrela C, Estrela CRA, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. *Braz Dent J.* 2002;2:113-7.
- 4 - Estrela C, Estrela CRA, Decurcio DA, Hollanda ACB, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. *Int Endod J.* 2007;40:85-93.

- 5 - Estrela C, Holland R, Bernabé PFE, Souza V, Estrela CRA. Antimicrobial potential of medicaments used in healing process in dog's teeth with apical periodontitis. *Braz Dent J.* 2004;15:181-5.
- 6 - Estrela C, Ribeiro RG, Estrela CRA, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. *Braz Dent J.* 2003;14:58-62.
- 7 - Ferrari PH, Cai S, Bombana AC. Effect of endodontic procedures on enterococci, enteric bacteria and yeasts in primary endodontic infections. *Int Endod J.* 2005;38:372-80.
- 8 - Glenny AM, Esposito M, Coulthard P, Worthington HV. The assessment of systematic reviews in dentistry. *Eur J Oral Sci.* 2003;111:85-92.
- 9 - Gomes BPFA, Ferraz CCR, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J.* 2001;34:424-8.
- 10 - Haapasalo M, Ørstavik D. *In vitro* infection and disinfection of dentinal tubules. *J Dent Res.* 1987;66:1375-9.
- 11 - Holland R, Otoboni-Filho JA, Souza V, Nery MJ, Bernabé PFE, Dezan E Jr. A comparison of one versus two appointment endodontic therapy in dogs' teeth with apical periodontitis. *J Endod.* 2003;29:121-5.
- 12 - Jeansonne MJ, White RR. A comparison of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigant. *J Endod.* 1994;20:276-8.
- 13 - Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. *J Clin Periodontol.* 1988;15:415-24.
- 14 - Law A, Messer H. An evidence-based analysis of the antibacterial efficacy of intracanal medicaments. *J Endod.* 2004;30:689-94.
- 15 - Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J.* 1998;31:1-7.
- 16 - Nair PNR, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after one-visit-endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;99:231-52.
- 17 - Peciuliene V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root-filled canals in a Lithuanian population. *J Endod.* 2000;26:593-5.
- 18 - Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J.* 2001;34:429-34.
- 19 - Portenier I, Waltimo TMT, Haapasalo M. *Enterococcus faecalis*: the root canal survivor and 'star' in post-treatment disease. *Endod Top.* 2003;6:135-9.
- 20 - Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. *J Endod.* 1982;8:200-4.
- 21 - Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. *Int Endod J.* 2007;40:2-10.
- 22 - Siqueira JF Jr, Batista MMD, Fraga RC, Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented Gram-negative anaerobes and facultative bacteria. *J Endod.* 1998;24:414-6.
- 23 - Siqueira JF Jr, Rôças IN, Santos SRL, Lima KC, Magalhães FAC, Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. *J Endod.* 2002;28:181-4.
- 24 - Siwek J, Gourlay ML, Slawson DC, Shaughnessy AF. How to write an evidence-based clinical review article. *Am Fam Physician.* 2002;65:251-8.
- 25 - Spratt DA, Pratten J, Wilson M, Gulabivala K. An *in vitro* evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *Int Endod J.* 2001;34:300-7.
- 26 - Sundqvist G, Figdor D. Life as an endodontic pathogen. Ecological differences between the untreated and root-filled root canals. *Endod Top.* 2003;6:3-28.
- 27 - 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;85:86-93.
- 28 - Torabinejad M, Babjri K. Essential elements of evidenced-based endodontics: steps involved in conducting clinical research. *J Endod.* 2005;31:563-9.
- 29 - Williams JM, Trope M, Caplan DJ, Shugars DC. Detection and quantitation of *Enterococcus faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. *J Endod.* 2006;32:715-21.
- 30 - Zerella JA, Fouad AF, Spangberg LS. Efficacy of a calcium hydroxide and chlorhexidine digluconate mixture as disinfectant during retreatment of failed endodontic cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;100:756-61.