



## Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis

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**Objective.** This clinical study was undertaken to compare the effectiveness of 2.5% sodium hypochlorite (NaOCl) and 0.12% chlorhexidine digluconate as irrigants in reducing the cultivable bacterial populations in infected root canals of teeth with apical periodontitis.

**Study design.** According to stringent inclusion/exclusion criteria, 32 teeth with primary intraradicular infections and chronic apical periodontitis were selected and followed in the study. Bacterial samples were taken at the baseline (S1) and after chemomechanical preparation using either NaOCl (n = 16) or chlorhexidine (n = 16) as irrigants (S2). Cultivable bacteria recovered from infected root canals at the 2 stages were counted. Isolates from S2 samples were identified by means of 16S rRNA gene sequencing analysis.

**Results.** At S1, all canals were positive for bacteria, and the median number of bacteria per canal was  $7.32 \times 10^5$  for the NaOCl group and  $8.5 \times 10^5$  for the chlorhexidine group. At S2, the median number of bacteria in canals irrigated with NaOCl and chlorhexidine was  $2.35 \times 10^3$  and  $2 \times 10^2$ , respectively. Six of 16 (37.5%) canals from the NaOCl group and 8 of 16 (50%) canals from the chlorhexidine group yielded negative cultures. Chemomechanical preparation using either solution substantially reduced the number of cultivable bacteria in the canals. No significant difference was observed between the NaOCl and chlorhexidine groups with regard to the number of cases yielding negative cultures ( $P = .72$ ) or quantitative bacterial reduction ( $P = .609$ ). The groups irrigated with NaOCl or chlorhexidine showed a mean number of 1.3 and 1.9 cultivable species per canal, respectively. The great majority of isolates in S2 were from gram-positive bacteria, with streptococci as the most prevalent taxa.

**Conclusions.** The present findings revealed no significant difference when comparing the antibacterial effects of 2.5% NaOCl and 0.12% chlorhexidine used as irrigants during the treatment of infected canals. (**Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:122-30**)

Apical periodontitis is an infectious disease caused by microorganisms colonizing the necrotic root canal system.<sup>1</sup> For an optimal outcome of the endodontic treatment to be achieved, bacterial populations within the root canal should be ideally eliminated—or at least significantly reduced to levels that are compatible with

periradicular tissue healing. Of the treatment steps involved with infection control, the chemomechanical preparation assumes a pivotal role in root canal disinfection, because instruments and irrigants act primarily on the main canal, which is the most voluminous area of the system and consequently harbors the largest

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number of bacterial cells. In addition to the mechanical effects exerted by instruments and the flow and back-flow of the irrigant solution during preparation, the use of an antimicrobial substance for irrigation has been shown to be necessary to enhance bacterial elimination from infected canals.<sup>2-4</sup>

Sodium hypochlorite (NaOCl) remains the most used irrigant due to its potent antimicrobial activity and lubricant and tissue-dissolving ability.<sup>5</sup> More recently, chlorhexidine has emerged as a potential irrigant,<sup>6</sup> given its broad-spectrum efficacy against oral bacteria.<sup>7,8</sup> In vivo and ex vivo studies comparing the antimicrobial effectiveness of chlorhexidine and NaOCl when used as irrigants during chemomechanical preparation have demonstrated conflicting results. Some studies have revealed that chlorhexidine is more effective,<sup>9-11</sup> others have reported that NaOCl is more effective,<sup>12,13</sup> whereas others have shown no significant difference between these 2 substances.<sup>14,15</sup> In vitro studies have also been inconsistent in their findings, with NaOCl more effective,<sup>16-18</sup> chlorhexidine more effective,<sup>8</sup> or no significant difference between the two.<sup>19-24</sup> In vitro effects of NaOCl—particularly in high concentrations—on single-species or multispecies bacterial biofilms have been shown to be superior to chlorhexidine.<sup>17,25-27</sup> However, a study revealed that chlorhexidine was more effective than NaOCl against fungi in both pure and mixed cultures as well as in biofilms.<sup>28</sup>

The present clinical study was undertaken to compare the antibacterial efficacy of 2.5% NaOCl and 0.12% chlorhexidine when used as irrigants during the chemomechanical preparation of infected root canals associated with apical periodontitis lesions. Cultivable bacteria recovered from the canals were counted, and identification of the taxa persisting after chemomechanical procedures was performed by means of 16S rRNA gene-sequencing analysis.

## MATERIAL AND METHODS

### Patient selection

Patients presenting to the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil, for evaluation and treatment of apical periodontitis were considered for this study. Thirty-four single-rooted teeth, each one with a single canal, were selected based on stringent inclusion/exclusion criteria. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of chronic apical periodontitis lesions were included in this study. The size of the apical periodontitis lesions ranged from 2 × 3 mm to 12 × 15 mm. Teeth with different lesion sizes were evenly distributed be-

tween the 2 experimental groups. Teeth from patients who received antibiotic therapy within the previous 3 months, teeth with gross carious lesions, teeth with fractures of the root or crown, teeth that had received previous endodontic treatment, and cases showing periodontal pockets more than 4-mm deep were excluded from the study. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University. The study and associated risks were explained to the patients and informed consent was obtained.

### Endodontic treatment and sampling procedures

Rubber dam and an aseptic technique were used throughout the endodontic treatment. Before isolation with the rubber dam, each tooth had supragingival plaque removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. After rubber dam application, dental floss was securely tied around the neck of the tooth. The operative field, including the tooth, clamp, and surroundings, was cleaned with 3% hydrogen peroxide until no further bubbling of the peroxide occurred. All surfaces were then disinfected by vigorous swabbing with a 2.5% NaOCl solution. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was then cleaned and disinfected once again in the same manner. Sodium hypochlorite was neutralized with 5% sodium thiosulfate, and then sterility control samples were taken from the tooth surface with sterile paper points. For inclusion of the tooth in the study, these control samples had to be uniformly negative.

The first root canal sample (S1) was taken as follows: Three sterile paper points were consecutively placed in the canal to a level approximately 1 mm short of the root apex, based on diagnostic radiographs, and used to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute. Paper points were then transferred aseptically to tubes containing 500 µl of reduced transport fluid.<sup>29</sup>

Chemomechanical preparation was completed at the same appointment in all cases. The alternated rotation motion technique was used to prepare all canals.<sup>2,30</sup> Briefly, the coronal two thirds of the root canals were enlarged with Gates Glidden burs. Working length was established 1 mm short of the root apex, and the patency length coincided with the radiographic root edge. Apical preparation was completed to the working length with hand nickel-titanium files (Nitiflex, Dentsply-Maillefer, Ballaigues, Switzerland), always by using a back-and-forth alternated rotation motion. Master api-

cal files ranged from size 50 to 60, depending on both the root anatomy and the initial diameter of the root canal. Apical patency was confirmed with a small file (size 15 or 20, Dentsply-Maillefer), throughout the procedure, after each larger file size. Preparation was completed using step-back of 1-mm increments. In 17 root canals, the irrigant used was 2.5% NaOCl solution, whereas in the other 17 canals, a 0.12% chlorhexidine digluconate solution was used. Two milliliters of the test solution was used to rinse the canals after each instrument. Irrigant was delivered in the canals by means of a 5-mL disposable syringe with a 23-gauge needle.

Each canal was dried using sterile paper points and then flushed with 5 mL of either 5% sodium thiosulfate or a mixture of 0.07% lecithin, 0.5% Tween 80, and 5% sodium thiosulfate to neutralize any residual NaOCl or chlorhexidine, respectively. Subsequently, the root canal walls were gently filed and a postinstrumentation sample (S2) was taken from the canal as aforementioned.

After smear layer removal, the canals were medicated with a calcium hydroxide paste and filled 1 week later with gutta-percha points and Sealer 26 (Dentsply, Petrópolis, RJ, Brazil) by using the lateral compaction technique.

## Culture

Samples were transported to the laboratory within 15 minutes for microbiological processing. Samples in reduced transport fluid (RTF) vials were dispersed with a vortex for 30 seconds and 10-fold serial dilutions to  $10^{-3}$  (for S1 samples) or  $10^{-2}$  (for S2 samples) were made in prerduced anaerobically sterilized buffered salt solution.<sup>31</sup> Aliquots of 100  $\mu$ L from the undiluted suspension and the highest dilution were each spread onto brucella agar plates (BBL Microbiology Systems, Cockeysville, MD) supplemented with 5% defibrinated sheep blood, hemin (5 mg/L) and menadione (1 mg/L), and mitis salivarius agar plates (Difco, Detroit, MI). Plates were immediately placed in anaerobic jars (GasPak system, BBL Microbiology Systems) and incubated anaerobically at 37°C for 14 days.<sup>32,33</sup> Following incubation, the total colony forming units (CFUs) were counted, and actual counts were calculated based on the known dilution factors.

## 16S rRNA gene identification

Bacterial isolates recovered from S2 samples were identified by 16S rRNA gene sequencing. One or 2 colonies of each different colony type were isolated, and each one was individually placed in cryogenic

vials containing TE buffer (10-mM TRIS-HCl, 1-mM EDTA, pH 8). Cryogenic vials were stored at  $-20^{\circ}\text{C}$  until further bacterial identification by 16S rRNA gene sequencing.

Genomic DNA was extracted from each colony by heating the suspension for 10 minutes at  $97^{\circ}\text{C}$  with a thermocycler. The vials were then stored for 5 minutes on ice and centrifuged, and 5- $\mu$ L aliquots of the supernatant were further used as template in the polymerase chain reaction (PCR) assay.

Polymerase chain reaction amplification of 16S rRNA genes was used for bacterial identification. The pair of universal 16S rRNA gene primers used was 5'-GAT TAG ATA CCC TGG TAG TCC AC-3' and 5'-CCC GGG AAC GTA TTC ACC G-3', corresponding to base positions 786 to 808 and 1369 to 1387, respectively, of the *Escherichia coli* 16S rRNA gene sequence (accession No. J01695). This universal primer pair flanks the variable regions V5, V6, V7, and V8 of the 16S rRNA gene. Polymerase chain reaction amplification was performed in a reaction volume of 50  $\mu$ L, consisting of 0.8- $\mu$ M concentration of each primer, 5  $\mu$ L of  $10 \times$  PCR buffer, 2-mM  $\text{MgCl}_2$ , 1.25 U of *Tth* DNA polymerase, and 0.2-mM concentration of each deoxyribonucleoside triphosphate (all reagents from Biotoools, Madrid, Spain). Cycling parameters included an initial denaturation step at  $95^{\circ}\text{C}$  for 2 minutes, followed by 36 cycles of a denaturation step at  $95^{\circ}\text{C}$  for 30 seconds, a primer annealing step at  $60^{\circ}\text{C}$  for 1 minute, an extension step at  $72^{\circ}\text{C}$  for 1 minute, and a final step of  $72^{\circ}\text{C}$  for 2 minutes. The results of PCR amplification were examined by electrophoresis in 1.5% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength UV light.

Amplicons were purified using a PCR purification system (Wizard PCR Preps, Promega, Madison, WI) and then sequenced directly on the ABI 377 automated DNA sequencer by using dye terminator chemistry (Amersham Biosciences, Little Chalfont, Buckinghamshire, England). Sequence data and electropherograms were inspected and corrected when obvious sequencing-software errors were observed. Sequences generated were compared with the GenBank database to identify the closest relatives by using the basic local alignment search tool algorithm.<sup>34</sup> A  $\geq 99\%$  identity in 16S rRNA gene sequence was the criterion used to identify an isolate to the species level. A 97% to 99% identity in 16S rRNA gene sequence was the criterion used to identify an isolate at the genus level, whereas  $< 97\%$  identity in 16S rRNA gene sequence was the criterion used to define a potentially new bacterial species.<sup>35</sup>

**Table I.** Bacterial counts and reduction percentage determined for root canal samples

Sodium hypochlorite				Chlorhexidine			
Case number	S1 samples*	S2 samples†	Reduction (%)	Case number	S1 samples*	S2 samples†	Reduction (%)
1	$2.2 \times 10^6$	$1.1 \times 10^4$	99.5	1	$1 \times 10^6$	$3.5 \times 10^3$	99.65
2	$7.65 \times 10^5$	0	100	2	$1.0 \times 10^4$	0	100
3	$5.25 \times 10^5$	$2.3 \times 10^3$	99.56	3	$7.2 \times 10^6$	$4 \times 10^2$	99.99
4	$5.49 \times 10^5$	$2.77 \times 10^5$	49.54	4	$2.8 \times 10^7$	$2.48 \times 10^5$	99.11
5	$2.39 \times 10^4$	0	100	5	$1.1 \times 10^6$	$5.12 \times 10^5$	53.45
6	$2 \times 10^8$	$1 \times 10^7$	95	6	$2.2 \times 10^6$	0	100
7	$2 \times 10^5$	$5.1 \times 10^3$	97.45	7	$5.21 \times 10^5$	0	100
8	$1.08 \times 10^7$	$9.2 \times 10^3$	99.91	8	$2.12 \times 10^7$	0	100
9	$3.24 \times 10^4$	0	100	9	$1.63 \times 10^7$	$2 \times 10^5$	98.77
10	$6 \times 10^6$	$2.4 \times 10^3$	99.96	10	$6.08 \times 10^4$	$4.4 \times 10^3$	92.76
11	$7 \times 10^5$	$1.3 \times 10^4$	98.14	11	$1.83 \times 10^4$	$5 \times 10^3$	72.68
12	$7.3 \times 10^6$	0	100	12	$5.3 \times 10^3$	0	100
13	$2.92 \times 10^4$	$1.5 \times 10^2$	99.49	13	$2.8 \times 10^3$	0	100
14	$3.3 \times 10^7$	0	100	14	$1 \times 10^8$	0	100
15	$6.41 \times 10^4$	$9.9 \times 10^3$	84.56	15	$7 \times 10^5$	0	100
16	$5.72 \times 10^6$	0	100	16	$3.18 \times 10^5$	$2.2 \times 10^5$	30.82
Median	$7.32 \times 10^5$	$2.35 \times 10^3$		Median	$8.5 \times 10^5$	$2 \times 10^2$	

Samples of teeth with apical periodontitis lesions were taken before treatment and after instrumentation, with either 2.5% sodium hypochlorite or 0.12% chlorhexidine as an irrigant.

\*S1 samples were taken before chemomechanical preparation.

†S2 samples were taken after chemomechanical preparation.

**Statistical analysis**

Data concerning bacterial counts were checked for normality by using the Kolmogorov-Smirnov test. In spite of presenting normal distribution but taking into account the great variability and sample size, data were analyzed by means of nonparametric statistics. Therefore, bacterial counts in the initial (S1) samples from both experimental groups were tested for differences by using the Mann-Whitney *U* test. The same test was used to compare the bacterial counts achieved after instrumentation using each irrigant solution. For intra-group evaluation of the bacterial reduction after the antimicrobial treatment, data from S1 and S2 samples were compared by the Wilcoxon test. The 2-tailed Fisher exact test was used to compare the number of cases yielding negative cultures after treatment with either NaOCl or chlorhexidine. The significance level for every test used was established at 5% ( $P < .05$ ).

**RESULTS**

Of the 34 teeth sampled, 2 (1 from each group) showed bacterial growth for the sterility control of the working field and had to be excluded from the study. Bacteria were found in all initial samples from the other 32 root canals. The median value of the number of CFUs in the initial samples was  $7.32 \times 10^5$  (range,  $2.39 \times 10^4$  to  $2 \times 10^8$ ) for the group treated with 2.5% NaOCl and  $8.5 \times 10^5$  (range,  $2.8 \times 10^3$  to  $1 \times 10^8$ ) for the group treated with 0.12% chlorhexidine. After ch-

emomechanical preparation, the median number of CFUs present in samples from canals irrigated with NaOCl was  $2.35 \times 10^3$  (range,  $0-1 \times 10^7$ ) and  $2 \times 10^2$  (range,  $0-5.12 \times 10^5$ ) for canals irrigated with chlorhexidine. Six of 16 (37.5%) canals treated with NaOCl and 8 of 16 (50%) canals treated with chlorhexidine showed negative culture results. When compared with S1 samples, S2 samples revealed reduction in the number of bacteria ranging from 49.54% to 100% for canals treated with NaOCl and from 30.82% to 100% for canals treated with chlorhexidine. Quantitative data and percentage reductions are depicted in Table I and illustrated in Fig. 1.

Intergroup comparison between S1 samples showed no differences ( $P = .678$ ), indicating a similar level of infection for the 2 groups before treatment. Intragroup comparison between S1 and S2 samples revealed that both NaOCl and chlorhexidine were significantly effective in reducing bacterial populations within the canals ( $P < .001$  for both groups). Intergroup analysis of quantitative data from S2 samples showed no significant difference between NaOCl and chlorhexidine ( $P = .609$ ; Mann-Whitney *U* test). Comparison of the number of cases yielding negative cultures in the 2 groups did not reveal a significant difference either ( $P = .72$ ; Fisher exact test, 2-tailed).

Thirteen isolates belonging to 12 bacterial taxa were identified in the 10 cases showing positive culture after irrigation with NaOCl (mean, 1.3 taxon per canal). Each culture-positive canal harbored 1 or 2 taxa in S2.

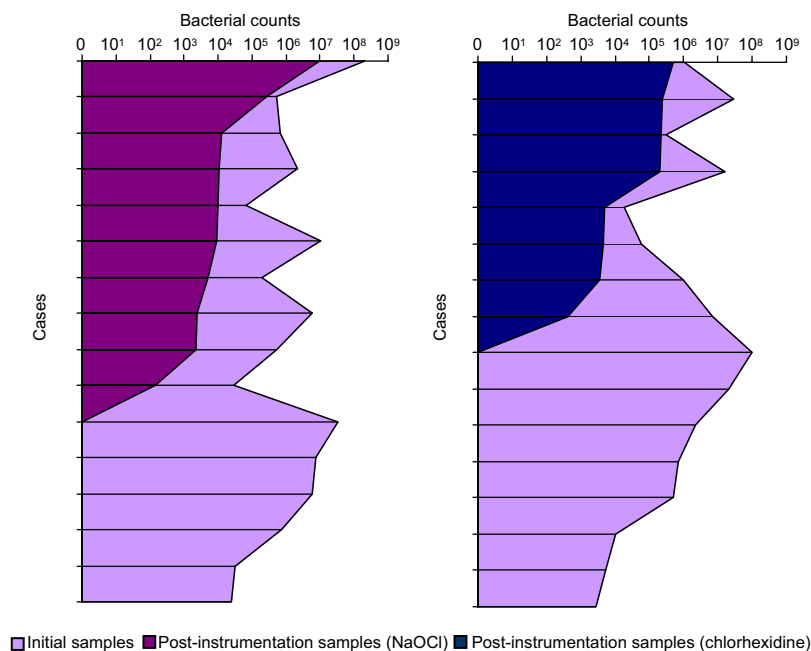


Fig. 1. Bacterial reduction per case after chemomechanical preparation using either 2.5% sodium hypochlorite (NaOCl) or 0.12% chlorhexidine solution as irrigants. Cases are ordered according to the bacterial counts after preparation.

In the chlorhexidine group, 13 isolates from 11 taxa and 2 other unidentified isolates were recovered from the 8 cases showing positive culture (mean, 1.9 taxon per canal). Each culture-positive canal harbored 1 to 4 taxa in S2. Unidentified isolates showed sequences with low-scoring homologies (<97% similarity) to sequences deposited in the GenBank and may represent novel species. Although a few gram-negative taxa were isolated from S2, the great majority of isolates were from gram-positive bacteria. The most prevalent taxa were *Streptococcus* species, corresponding to 6 of 13 isolates found in the NaOCl group and 5 of 15 isolates found in the chlorhexidine group. Table II depicts all the cultivable taxa isolated from S2 samples.

## DISCUSSION

A culture-dependent approach was used in the present study because it is one of the most reliable methods of detecting viable bacteria, particularly when samples are taken immediately after antimicrobial treatment where viability may not be ascertained by most culture-independent methods.<sup>36</sup> Furthermore, studies using cultures have shown a correlation between negative cultures and favorable treatment outcome.<sup>37,38</sup> However, it is worth pointing out that the bacterial diversity and bacterial counts are usually underrepresented by culturing analysis.<sup>36</sup> Also, because of limitations of sampling procedures, low sensitivity of the culturing techniques, and presence of as yet unculti-

vated bacteria, a negative culture does not imply sterility. Indeed, a negative culture usually means that cultivable bacterial populations were reduced to levels below the detection ability of culture-dependent methods, and that these levels can be compatible to periradicular tissue healing in most cases.

Identification of the isolates from S2 samples was performed by 16S rRNA gene sequencing, as this method has been demonstrated to provide a more reliable identification of bacteria that are difficult or even impossible to identify by conventional methods, including isolates that are unreactive in biochemical tests, strains with atypical phenotypes, rare isolates, or poorly described bacteria.<sup>35,36,39</sup> The technique can also lead to recognition of novel species and as yet uncultivated bacteria.<sup>40</sup>

All root canals included in this investigation harbored bacteria before treatment, confirming the strong correlation between bacteria and apical periodontitis. The initial number of bacteria in the infected canals ranged from  $10^3$  to  $10^8$ , which is concurrent with previous studies.<sup>4,41</sup> Irrespective of the irrigant used, substantial bacterial reduction was observed after chemomechanical preparation, which also parallels other findings from the literature.<sup>2,3,12,42,43</sup> Except for 2 cases in the NaOCl group and 4 cases in the chlorhexidine group, percentage reduction of the bacterial counts was always more than 95%. These findings confirm the important role played by instrumentation and irrigation

**Table II.** Cultivable bacterial taxa detected after chemomechanical preparation

<i>Sodium hypochlorite</i>			<i>Chlorhexidine</i>		
Taxa	No. of isolates	Case No.	Taxa	No. of isolates	Case No.
<i>Streptococcus oralis</i>	2	7, 13	<i>Streptococcus mitis</i> biovar 2	3	3, 4, 16
<i>Fusobacterium nucleatum</i>	1	4	<i>Streptococcus oralis</i>	1	5
<i>Streptococcus mitis</i> biovar 2	1	4	<i>Streptococcus sanguinis</i>	1	5
<i>Pseudoramibacter alactolyticus</i>	1	11	<i>Pseudoramibacter alactolyticus</i>	1	10
<i>Streptococcus constellatus</i> / <i>Streptococcus intermedius</i>	1	11	<i>Prevotella</i> oral clone FM005	1	3
<i>Streptococcus gordonii</i>	1	10	<i>Actinomyces odontolyticus</i>	1	3
<i>Streptococcus parasanguinis</i>	1	15	<i>Actinomyces urogenitalis</i>	1	11
<i>Propionibacterium acnes</i>	1	1	<i>Staphylococcus aureus</i>	1	9
<i>Neisseria sicca</i>	1	7	Beta proteobacterium clone FAC20	1	10
<i>Staphylococcus epidermidis</i>	1	8	<i>Delftia</i> sp	1	10
<i>Escherichia coli</i>	1	3	<i>Propionibacterium granulosum</i>	1	3
<i>Flavobacterium</i> sp.	1	6	Unidentified	2	1, 16

Cultivable bacterial taxa were detected after instrumentation with either 2.5% sodium hypochlorite or 0.12% chlorhexidine as an irrigant. Isolates were identified by a 16S rRNA gene sequencing approach.

with antimicrobial substances in reducing the bacterial populations in infected root canals.

Chlorhexidine is probably the most widely used agent in antiseptic products, particularly in hand-washing and oral products, but also as a disinfectant and preservative.<sup>44</sup> This cationic bisbiguanide is highly efficacious against several gram-positive and gram-negative oral bacterial species as well as yeasts.<sup>7,8,28</sup> Chlorhexidine is bacteriostatic at low concentrations and bactericidal at high concentrations.<sup>45</sup> It can induce damage to the outer microbial cell layers, but this effect is usually insufficient to cause lysis or cell death. Chlorhexidine crosses the cell wall, presumably by passive diffusion, and subsequently attacks the cytoplasmic membrane. Damage to this delicate membrane is followed by leakage of intracellular constituents. At high concentrations, chlorhexidine causes precipitation of intracellular constituents, particularly phosphated entities such as adenosine triphosphate and nucleic acids.<sup>45</sup> As a consequence, the cytoplasm becomes congealed, with a consequent reduction in leakage.<sup>44</sup>

Sodium hypochlorite has a broad-spectrum antimicrobial activity, rapidly killing vegetative and spore-forming bacteria, fungi, protozoa, and viruses.<sup>46,47</sup> Most oral bacteria are killed after a short time of contact with NaOCl.<sup>8,21</sup> Sodium hypochlorite exerts its antibacterial effect by inducing the irreversible oxidation of sulfhydryl groups of essential bacterial enzymes, resulting in disulfide linkages, with consequent disruption of the metabolic functions of the bacterial cell.<sup>46,48</sup> Sodium hypochlorite can also have deleterious effects on bacterial DNA, which involve the formation of chlorinated derivatives of nucleotide bases. In addition, NaOCl has been reported to disrupt membrane-associated activities.<sup>44,49</sup>

Although bacterial reduction after chemomechanical preparation was significant in quantitative terms, 10 of 16 (62.5%) cases treated with NaOCl and 8 of 16 (50%) irrigated with chlorhexidine were still positive for the presence of cultivable bacteria. These figures are within the range reported by other studies (Table III).<sup>3,12,50-52</sup> Streptococci as a group were the most frequent taxa found in the canals that cultured bacteria in S2. Other studies have also reported streptococci as one of the most commonly found species in postinstrumentation samples positive for bacteria.<sup>3,43,57,58</sup> There are many possible reasons for bacterial persistence in the canals after instrumentation and irrigation with NaOCl or chlorhexidine: (a) persisting bacteria may be intrinsically resistant to the irrigant; (b) persisting bacteria may have been present in areas inaccessible to the effects of instruments and irrigants; (c) short contact time of the irrigant with bacteria; (d) persisting bacteria may have been embedded in tissue remnants or arranged in biofilm structures, being protected from the lethal effects of irrigants; and (e) inactivation or decreased activity of the irrigant induced by dentin constituents, inflammatory exudate seeping into the canal, bacterial products, and components of the necrotic tissue. The impact of persisting bacteria on treatment outcome is predictable. Although some cases of apical periodontitis lesions can even heal when bacteria are found in the canal at the root-canal filling stage,<sup>37,59</sup> it has been demonstrated that the outcome of the endodontic treatment is significantly influenced by the presence of bacteria in the canals at the time of filling.<sup>37,38,59</sup> The findings of this and the other studies listed in Table III with regard to the antimicrobial effectiveness of chemomechanical procedures reinforce the need for using additional or

**Table III.** Data from different clinical studies, showing the incidence of positive cultures

Study	Irrigant	Positive cultures†
Byström and Sundqvist (1983) <sup>53</sup>	0.5% sodium hypochlorite	13/15 (86.7%)*
Byström and Sundqvist (1985) <sup>3</sup>	0.5% sodium hypochlorite	12/20 (60%)**
	5% sodium hypochlorite	10/20 (50%)**
Sjögren et al. (1997) <sup>37</sup>	0.5% sodium hypochlorite	22/55 (40%)*
Leonardo et al. (1999) <sup>52</sup>	2% chlorhexidine	13/22 (59.1%)*
Shuping et al. (2000) <sup>42</sup>	1.25% sodium hypochlorite	15/41 (36.6%)*
Peciulienė et al. (2001) <sup>54,‡</sup>	2.5% sodium hypochlorite	10/33 (30.3%)*
Peters et al. (2002) <sup>55</sup>	2% sodium hypochlorite	10/42 (23.8%)*
Card et al. (2002) <sup>56</sup>	1% sodium hypochlorite	3/38 (7.9%)*
Ercan et al. (2004) <sup>14</sup>	2% chlorhexidine	3/15 (20%)*
Ercan et al. (2004) <sup>14</sup>	5.25% sodium hypochlorite	4/15 (26.7%)*
Kvist et al. (2004) <sup>51</sup>	0.5% sodium hypochlorite	58/94 (61.7%)*
McGurkin-Smith et al. (2005) <sup>50</sup>	5.25% sodium hypochlorite	16/29 (55.2%)*
Vianna et al. (2006) <sup>12</sup>	2.5% sodium hypochlorite	4/16 (25%)*
Vianna et al. (2006) <sup>12</sup>	2% chlorhexidine	8/16 (50%)*
This study	2.5% sodium hypochlorite	10/16 (62.5%)*
This study	0.12% chlorhexidine	8/16 (50%)*

Cultures were taken after chemomechanical preparation with either sodium hypochlorite or chlorhexidine as an irrigant.

\*Samples taken immediately after chemomechanical procedures.

\*\*Samples taken some days after chemomechanical procedures.

†Number of cases positive for bacteria in posttreatment samples/No. of cases positive for bacteria in initial samples.

‡Retreatment cases.

alternative antimicrobial strategies to predictably render root canals free of cultivable bacteria before filling.

Comparisons between chlorhexidine and NaOCl as irrigants should take into account several aspects. If the main goal of the endodontic treatment of teeth with apical periodontitis is bacterial elimination from the root canal, the most important property of an irrigant to be used during chemomechanical preparation is antibacterial efficacy. The present findings and those from several other clinical and laboratory studies have shown no significant differences between chlorhexidine and NaOCl with regard to antibacterial effectiveness.<sup>14,15,19-24</sup> Therefore, it seems that, from a microbiological point of view, it makes no difference whether NaOCl or chlorhexidine is used as an irrigant. Other properties are also important and should not be dismissed when one is weighing the pros and cons of a

substance to be used as an irrigant during root canal preparation. Both chlorhexidine and NaOCl have been demonstrated to have no significant effects on bacteria lipopolysaccharide.<sup>60</sup> Chlorhexidine has been shown to be less toxic than NaOCl,<sup>9,61</sup> though a comparative study using fluorescence assay on human periodontal ligament cells showed corresponding cytotoxicity with 0.4% NaOCl and 0.1% chlorhexidine.<sup>62</sup> Chlorhexidine exhibits substantivity to dentin and consequently may present residual antimicrobial effects for days to weeks and help prevent root canal reinfection.<sup>52,63-69</sup> This seems to be an important advantage over NaOCl. Nevertheless, one important disadvantage of chlorhexidine is that it lacks tissue-dissolving ability, which is one of the obvious benefits of NaOCl.<sup>70,71</sup> In fact, a study<sup>72</sup> demonstrated that root canal cleaning by chlorhexidine was inferior compared with the cleaning by NaOCl with and without EDTA.

In conclusion, both solutions used herein presented comparable results as to the bacterial elimination from infected root canals in vivo. This indicates that both can be used as irrigants and that perhaps the election of NaOCl or chlorhexidine as an irrigant should rely on the differences in their other properties (e.g., substantivity, tissue-dissolving ability, and toxicity). The impact of all these properties on the long-term outcome of the endodontic treatment will drive the option for the better substance. As of now, there is no such prospective study comparing the 2 substances. The findings that more than one half of the cases still harbored detectable levels of bacteria after chemomechanical preparation with either substance indicate that the search for more effective substances and protocols that can predictably render the canals free of bacteria in 1 appointment should be encouraged.

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