

Comparison of the *In Vivo* Antimicrobial Effectiveness of Sodium Hypochlorite and Chlorhexidine Used as Root Canal Irrigants: A Molecular Microbiology Study

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Abstract

Introduction: The purpose of this clinical study was to compare the antimicrobial effects of 2.5% sodium hypochlorite (NaOCl) and 0.12% chlorhexidine digluconate (CHX) when used as irrigants during treatment of teeth with apical periodontitis. **Methods:** Forty-seven single-rooted single-canal teeth with necrotic pulps and asymptomatic apical periodontitis were selected for this study according to stringent inclusion/exclusion criteria. Bacterial samples were taken at the baseline (S1) and after (S2) chemomechanical preparation using 2.5% NaOCl ($n = 30$) or 0.12% CHX ($n = 17$) as the irrigant. Bacterial, archaeal, and fungal presence was evaluated by broad-range polymerase chain reaction (PCR), whereas bacterial identifications were performed by a closed-ended reverse-capture checkerboard approach targeting 28 candidate endodontic pathogens. **Results:** All S1 samples were PCR positive for bacterial presence but negative for both archaea and fungi. Both NaOCl- and CHX-based protocols were significantly effective in reducing the bacterial levels and number of taxa. No significant differences were observed between them in all tested parameters including the incidence of negative PCR results in S2 (40% for NaOCl vs 47% for CHX, $p = 0.8$), reduction in the number of taxa per canal ($p = 0.3$), and reduction in the bacterial levels ($p = 0.07$). The most prevalent taxa in S2 samples from the NaOCl group were *Propionibacterium acnes*, *Streptococcus* species, *Porphyromonas endodontalis*, and *Selenomonas sputigena*. In the CHX group, the most prevalent taxa in S2 were *Dialister invisus*, *Actinomyces israelii*, *Prevotella baroniae*, *Propionibacterium acidifaciens*, and *Streptococcus* species. **Conclusions:** Treatment protocols using irrigation with either NaOCl or CHX succeeded in significantly reducing the number of bacterial taxa and their levels in infected root canals, with no significant difference between these substances. (*J Endod* 2011;37:143–150)

Key Words

Apical periodontitis, checkerboard DNA-DNA hybridization, chlorhexidine, endodontic treatment, polymerase chain reaction, sodium hypochlorite

Apical periodontitis is an infectious disease caused by intraradicular microbial biofilms (1). Consequently, the outcome of the endodontic treatment depends on successful microbial elimination from the infected root canal system so as to achieve a host manageable bioburden (2). During treatment, chemomechanical preparation plays a critical role in disinfection by causing a drastic reduction in the bacterial populations located in the main root canal. In addition to the mechanical effects of instrumentation and irrigation procedures, the use of an antimicrobial substance for irrigation is indicated because it significantly enhances bacterial elimination (3–5).

Although many substances have been suggested for root canal irrigation, sodium hypochlorite (NaOCl) remains the most widely used irrigant solution because of its pronounced antimicrobial activity and the ability to dissolve organic matter (6). Chlorhexidine (CHX) has been proposed as a potential substitute for NaOCl given its optimum effects against endodontic bacteria (7, 8). Studies comparing the antimicrobial effectiveness of NaOCl and CHX have generated conflicting results. Some studies found that NaOCl is more effective (9, 10), others reported that CHX is more effective (11, 12), and others observed no significant difference between them (13–15). As for lipopolysaccharide (LPS) elimination from the root canal, a study reported that neither 2.5% NaOCl nor 2% CHX gel totally eliminated this virulence factor of gram-negative bacteria in any of the teeth evaluated, suggesting a low detoxifying activity for both substances (16).

Even though several *in vivo* studies have investigated the antibacterial effects of endodontic procedures, only a few have identified the bacterial taxa enduring treatment procedures (2). Most of these studies have been based on culturing techniques, which are largely known to have relevant limitations, most notably the low sensitivity and the inability to detect viable as-yet-uncultivated bacteria (17). Both limitations can underestimate the bacterial taxa occurring in endodontic infections and persisting after treatment. Culture-independent molecular microbiology methods can sidestep these shortcomings of culture methods because they exhibit increased sensitivity and specificity as well as the ability to reliably identify culture-difficult and even as-yet-uncultivated bacteria (17). Thus far, no molecular study has been used to compare the bacterial taxa identifications after chemomechanical procedures using either NaOCl or CHX as the irrigant.

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Although bacteria are the main microorganisms found in primary endodontic infections (17), there are some reports of the presence of archaea (18) and fungi (19) in primarily infected root canals. To the best of our knowledge, no study has consistently investigated the effects of intracanal procedures against these microorganisms using sensitive molecular techniques.

The purpose of this clinical study was to compare the antimicrobial efficacy of 2.5% NaOCl and 0.12% CHX when used as irrigants during the chemomechanical preparation of infected root canals associated with apical periodontitis lesions. Bacterial, archaeal, and fungal presence was evaluated by broad-range polymerase chain reaction (PCR), whereas bacterial identifications were performed by a closed-ended reverse-capture checkerboard DNA-DNA hybridization approach targeting 28 candidate endodontic pathogens.

Materials and Methods

Subjects

Fifty patients attending 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 included in this study. Teeth were selected based on stringent inclusion/exclusion criteria. Each patient contributed a single-rooted single-canal tooth. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of asymptomatic apical periodontitis lesions were included. The size of the apical periodontitis lesions ranged from 2×3 mm to 12×15 mm, and attempts were made to evenly distribute teeth with different lesion sizes between the two experimental groups. Exclusion criteria included 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, symptomatic teeth, and cases showing periodontal pockets deeper than 4 mm. Patients included in the study reported no significant systemic condition. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Treatment and Sampling Procedures

An aseptic technique was used throughout the endodontic treatment. Before rubber dam isolation, each tooth had supragingival biofilms 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 were cleaned with 3% hydrogen peroxide until no further bubbling of the peroxide occurred. All surfaces were then disinfected by vigorous swabbing with 2.5% NaOCl. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was once again cleaned and disinfected the same way as described previously. NaOCl was neutralized with 5% sodium thiosulfate, and 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 after PCR with universal primers 8f and 1492r. Based on this criterion, three teeth from the CHX group had to be excluded from the study.

The first root canal sample (S1) was taken as follows. The canal was filled with sterile saline solution with care to not overflow, and a sterile #15 K-file was introduced to a level approximately 1-mm short of the root apex, based on diagnostic radiographs, and a gentle filing motion was applied. Three sterile paper points were consecutively placed in the canal to the same level 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 transferred aseptically to cryotubes containing Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH = 7.6) and immediately frozen at -20°C .

Chemomechanical preparation was completed at the same appointment in all cases. The alternated rotation motion technique was used to prepare all canals (4, 20). Briefly, the coronal two thirds of the root canals were enlarged with Gates-Glidden burs. The working length was established 1-mm short of the root apex, and the patency length coincided with the radiographic root edge. This was established with an electronic apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel) and confirmed by radiographs. Apical preparation was completed to the working length with hand nickel-titanium files (Nitiflex; Dentsply-Maillefer, Ballaigues, Switzerland) in a back-and-forth alternating rotation motion. Master apical files ranged from #50 to #70, depending on both root anatomy and initial diameter of the root canal. Whenever instruments larger than #60 were required, stainless steel Flexofile instruments (Dentsply-Maillefer) were used. Apical patency was confirmed with a small file (#15 or #20 NitiFlex) throughout the procedures after each larger file size. Preparation was completed using stepback of 1-mm increments. In 30 root canals, the irrigant used was 2.5% NaOCl solution, whereas a 0.12% CHX solution was used in the other 20 canals (three were excluded later because of contamination of the sterility controls). A 27-G needle was used to deliver 2 mL of the test solutions after each instrument size.

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 CHX, respectively. Subsequently, the root canal walls were gently filed, and a postinstrumentation sample (S2) was taken from the canal using sterile paper points as described previously. Afterward, the smear layer was removed, the canals were medicated with a calcium hydroxide paste for 1 week, and then they were filled by the lateral compaction technique.

DNA Extraction

Clinical samples were brought to room temperature, and then DNA was extracted by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the protocol recommended by the manufacturer. DNA from a panel of several oral bacterial species was also prepared to serve as controls (21).

Broad-range PCR for Bacteria, Archaea, and Fungi

Aliquots of extracted DNA were used in 16S rRNA gene-based PCR protocols using universal primers for members of the domains bacteria (22) or archaea (23, 24) and in a 18S rRNA gene-based PCR assay with universal primers for fungi (domain eukarya) (25) (Table 1). PCR reactions were performed in 50 μL of reaction mixture containing 1 $\mu\text{mol/L}$ concentrations of each primer, 5 μL of $10\times$ PCR buffer (Fermentas, Ontario, Canada), 3 mmol/L MgCl_2 , 1.25 U *Taq* DNA polymerase (Fermentas), and 0.2 mmol/L each deoxyribonucleoside triphosphate (Biotools, Madrid, Spain). Positive and negative controls were included in each batch of samples analyzed. Positive controls consisted of DNA extracted from *Porphyromonas gingivalis* (ATCC 33277), *Methanobrevibacter arboriphilus* (DSMZ 744), and *Candida albicans* (ATCC 10231). Negative controls consisted of sterile ultrapure water instead of sample.

PCR amplifications were performed in a DNA thermocycler (Mastercycler Personal; Eppendorf, Hamburg, Germany). Cycling conditions were as follows: for archaea, initial denaturation at $94^{\circ}\text{C}/2$ min, 36 cycles at $94^{\circ}\text{C}/30$ s, $58^{\circ}\text{C}/30$ s, and $72^{\circ}\text{C}/1$ min, and final extension

TABLE 1. Probes and Primers Used in This Study

Taxa	Sequence (5' – 3')	Base position *
<i>Actinomyces israelii</i>	TTTTTTTTTTTTTTTTTTGGCACAGCCAGAACAC	179
<i>Atopobium rimae</i>	TTTTTTTTTTTTTTTTTTACGGGAAGACGTATTCTCC	837
<i>Bacteroidetes</i> clone X083	TTTTTTTTTTTTTTTTTTACTTGAGTGGAGGGTAGG	132
<i>Campylobacter rectus</i>	TTTTTTTTTTTTTTTTTTGCTTATGCTTAGAAGGAGTATAGA	160
<i>Dialister invisus</i>	TTTTTTTTTTTTTTTTTTGCTCTTTCCGCTTTTTCG	67
<i>Dialister pneumosintes</i>	TTTTTTTTTTTTTTTTTTTACCATGCGATGCTTAGAA	181
<i>Eikenella corrodens</i>	TTTTTTTTTTTTTTTTTT GAATAACGCGAGGTCTTG	202
<i>Enterococcus faecalis</i>	TTTTTTTTTTTTTTTTTTGTCAGGGGACGTTCCAG	470
<i>Filifactor alocis</i>	TTTTTTTTTTTTTTTTTT GGCTCATCTTTGTCCACT	224
<i>Fusobacterium nucleatum</i>	TTTTTTTTTTTTTTTTTTTTCATAATTCTAGGATGCCCTATAATCAT	184
<i>Olsenella genomospecies C1</i>	TTTTTTTTTTTTTTTTTTTGACGGAAGTAAAGCCTC	835
<i>Olsenella uli</i>	TTTTTTTTTTTTTTTTTT GCTTCACCTGGATGCAA	959
<i>Pseudomonas aeruginosa</i>	TTTTTTTTTTTTTTTTTT CATCCAAGTGTGAACC	573
<i>Parvimonas micra</i>	TTTTTTTTTTTTTTTTTTTCCATTCCGGAAGAATTC	80
<i>Peptostreptococcus stomatis</i>	TTTTTTTTTTTTTTTTTTTAGTCTTAGAATACTCAATACTGAGC	69
<i>Porphyromonas endodontalis</i>	TTTTTTTTTTTTTTTTTTCAAACCTCATCTGCCATCATCTAAA	997
<i>Porphyromonas gingivalis</i>	TTTTTTTTTTTTTTTTTTACTGTTAGCAACTACCGATGT	1115
<i>Prevotella baroniae</i>	TTTTTTTTTTTTTTTTTTTGTATACCGAGTCGCG	138
<i>Propionibacterium acnes</i>	TTTTTTTTTTTTTTTTTTTCTGAGCACTCCCGAT	1000
<i>Propionibacterium acidifaciens</i>	TTTTTTTTTTTTTTTTTTTGAGCGCTTCCAGTACATG	995
<i>Pseudoramibacter alactolyticus</i>	TTTTTTTTTTTTTTTTTTTCCGGCACTGACTTATTCG	837
<i>Pyramidobacter piscolens</i>	TTTTTTTTTTTTTTTTTTGTGCTCAGCTTTATCTGGTA	1129
<i>Selenomonas sputigena</i>	TTTTTTTTTTTTTTTTTTTGAAGGGAGGAAGACTCTCT	1012
<i>Solobacterium moorei</i>	TTTTTTTTTTTTTTTTTTATTTAACCCTACTTAAACAGGC	588
<i>Streptococcus</i> species	TTTTTTTTTTTTTTTTTTTAGCCGTCCTCTTCTGGT	491
<i>Tannerella forsythia</i>	TTTTTTTTTTTTTTTTTTTGACAGGTATTGCTACCACCG	73
<i>Treponema denticola</i>	TTTTTTTTTTTTTTTTTTTCTGAAGCGGAGCCGTAG	207
<i>Treponema socranskii</i>	TTTTTTTTTTTTTTTTTTTCTACCTCCGTATACAGTGAT	182
Bacteria (universal) probe 1089	TTTTTTTTTTTTTTTTTTTCTCGTTGCGGGACTTAAC	1089
Bacteria (universal) probe 341	TTTTTTTTTTTTTTTTTTTCTGCTGCCTCCCGTAGG	341
Bacteria (universal) forward PCR primer 8f	AGA GTT TGA TYM TGG C	8
Bacteria (universal) reverse PCR primer 1492r	GYT ACC TTG TTA CGA CTT	1492
Archaea (universal) forward PCR primer 333f	TCC AGG CCC TAC GGG	333
Archaea (universal) reverse PCR primer 934r	GTG CTC CCC CGC CAA TTC CT	934
Fungi (universal) forward PCR primer B2f	ACT TTC GAT GGT AGG ATA G	209
Fungi (universal) reverse PCR primer B4r	TGA TCR TCT TCG ATC CCC TA	862

*Base positions for bacterial and archaeal primers and probes were relative to *Escherichia coli* 16S rRNA gene (GenBank accession no. J01695). Positions for fungi were relative to *Candida albicans* 18S rRNA gene (GenBank accession no. AF114470).

at 72°C/10 min; for bacteria, initial denaturation step at 95°C for 2 minutes, followed by 36 cycles at 95°C/30 s, 60°C/1 min, and 72°C/1 min, and final extension at 72°C/10 min; and for fungi, initial denaturation step at 95°C/30 s, followed by 40 cycles at 95°C/30 s, 55°C/1 min, 72°C/2 min, and a final step at 72°C/10 min.

PCR products were subjected to electrophoresis in a 1.5% agarose gel–Tris–borate–EDTA buffer. The gel was stained with GelRed (Biotium, Hayward, CA) and visualized under ultraviolet illumination. The presence of amplicons of the expected size for each primer pair was considered a positive result. A 100-bp DNA ladder (Biotools) was used as a parameter for amplicon size.

Reverse-capture Checkerboard Assay

For bacterial identification in the checkerboard assay, a practically full-length 16S rRNA gene fragment was amplified using universal primers 8f and 1492r, with the forward primer labeled at the 5' end with digoxigenin. PCR amplifications were performed as described previously.

The reverse-capture checkerboard assay was performed as described previously (22, 26, 27). Labeled PCR products (40 µL) were used in a reverse-capture checkerboard assay to determine the presence and levels of 28 bacterial taxa. Probes were based on 16S rRNA gene sequences of the target bacteria and were described and validated previously (22, 26, 28, 29). In addition to the 28 taxon-specific

probes, two universal probes were included in the assay to serve as controls. Two lanes in the membrane contained standards at the concentration of 10⁵ and 10⁶ cells, which were treated the same way as the clinical samples.

The reverse-capture checkerboard assay was performed using the Minislot-30 and Miniblotter-45 system (Immunetics, Cambridge, MA). First, 100 pmol of probe in Tris–EDTA buffer (10 mmol/L Tris HCl, 1 mmol/L EDTA, pH = 8.0) were introduced into the horizontal wells of the Minislot apparatus and crosslinked to the Hybond-N+ nylon membrane (AmershamPharmacia Biotech, Buckinghamshire, England) by ultraviolet irradiation using a Stratalinker 1800 (Stratagene, La Jolla, CA) on autocrosslink position. The polythymidine tails of the probes are preferentially crosslinked to the nylon, which leaves the specific probe available for hybridization. The membrane was then prehybridized at 55°C for 1 hour. Subsequently, 40 µL of the labeled PCR products with 100 µL of 55°C preheated hybridization solution was denatured at 95°C for 5 minutes and loaded on the membrane using the Miniblotter apparatus. Hybridization was performed at 54°C for 2 hours.

After hybridization, the membrane was washed and blocked in a buffer with casein. The membrane was sequentially incubated in anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany) and ultrasensitive chemiluminescent substrate CDP Star (Roche Molecular Biochemicals). Finally, a square of x-ray film was exposed to the membrane in a cassette for 10 minutes in order to detect the hybrids.

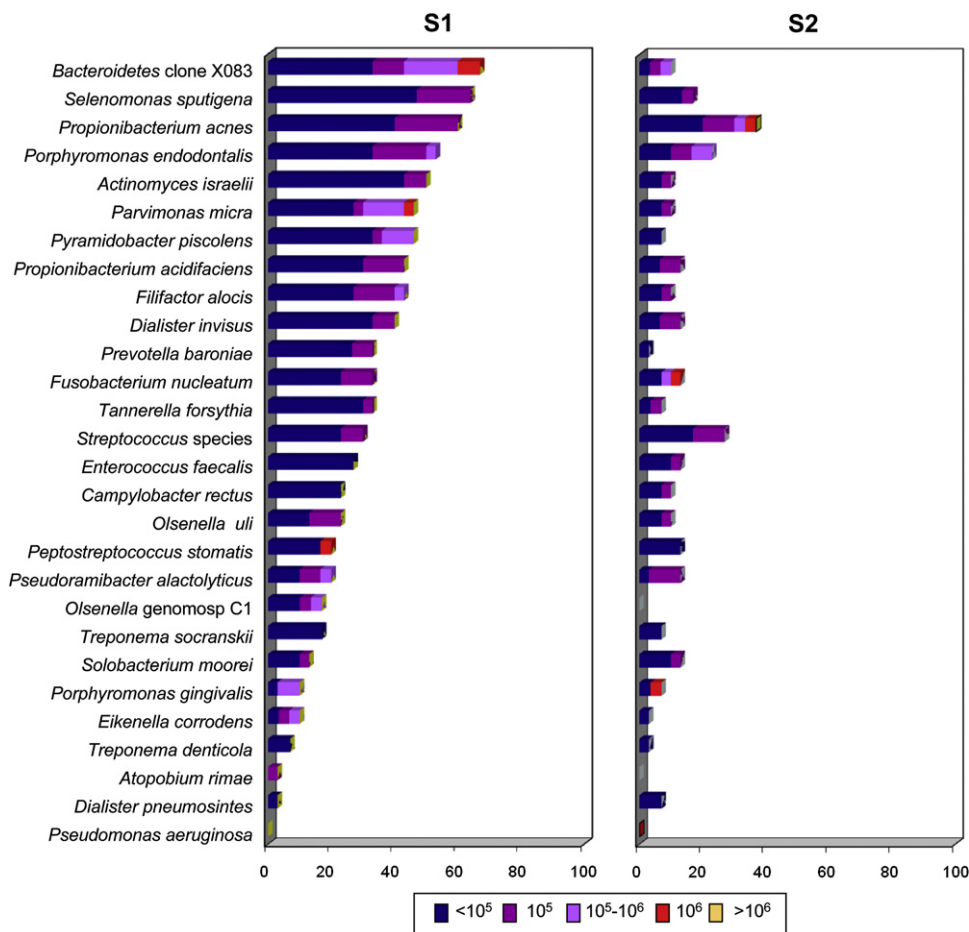


Figure 1. The NaOCl group: a stacked bar chart of frequency of detection and levels of bacterial taxa in root canal samples of teeth with asymptomatic apical periodontitis. The total length of each bar stack indicates percentage of positive samples. Different colors within each bar indicate the percentage of samples containing different levels of the taxon. S1, initial samples; S2, postinstrumentation samples of canals irrigated with 2.5% NaOCl.

Data Analysis

Prevalence of the target taxa was recorded as the percentage of cases examined. A semiquantitative analysis of the checkerboard findings was performed as follows. The obtained chemiluminescent signals were evaluated using ImageJ (W. Rasband, <http://rsb.info.nih.gov/ij/>) and converted into counts by comparison with standards at known concentrations run on each membrane. Because of the recognized difficulties in inferring absolute counts for PCR-amplified samples and because estimates had to be made for counting as-yet-uncultivated phylotypes or culture-difficult species, counts were transformed into semiquantitative data and categorized into a scale of 0 to 5, where 0 indicated no signal, 1 indicated a signal weaker than the intensity of the signal generated by the 10^5 standard, 2 a signal equal to the 10^5 standard, 3 a signal stronger than the 10^5 standard but weaker than the 10^6 standard, 4 a signal equal to the 10^6 standard, and 5 a signal stronger than the 10^6 standard. No signal (score 0) means absence of the target taxon or presence in numbers below the method’s detection threshold, which was approximately 10^3 .

The two-tailed Fisher exact test was used to compare the number of cases yielding negative PCR results after treatment with either NaOCl or CHX. The Mann-Whitney *U* test served to evaluate the reduction in the number of target bacterial taxa from S1 to S2 (intragroup analysis) and to compare the number of taxa persisting at S2 in the two groups (intergroup analysis). For statistical purposes, cases showing positive results

only for universal checkerboard probes and negative results for all the 28 target taxon-specific probes were considered as harboring one species, even though it is entirely possible that many more nontargeted taxa could have been present.

Scores for bacterial levels were averaged across the subjects in S1 and S2 samples, and the ability of each irrigant to reduce the levels of the target taxa was assessed for intragroup and intergroup differences by the Mann-Whitney *U* test. Intragroup analysis took into account the reduction from S1 to S2 within each group. Intergroup analysis used the difference values from S1 and S2 (bacterial reduction data) per taxon to compare the ability of NaOCl and CHX to reduce the overall bacterial load. The significance level for all tests was set at 5% ($p < 0.05$).

Results

Initial (S1) samples from all teeth yielded positive PCR results for bacteria. In the 2.5% NaOCl group, 12 of 30 (40%) S2 samples were PCR negative for bacterial presence. In the CHX group, 8 of 17 (47%) cases exhibited negative PCR results for bacteria in S2. All these results were confirmed in the checkerboard assay. No significant difference was observed when comparing the incidence of negative PCR results in S2 samples from NaOCl and CHX groups ($p = 0.8$). No case was positive for the presence of archaeal and fungal DNA. Positive and negative PCR controls showed the predicted results.

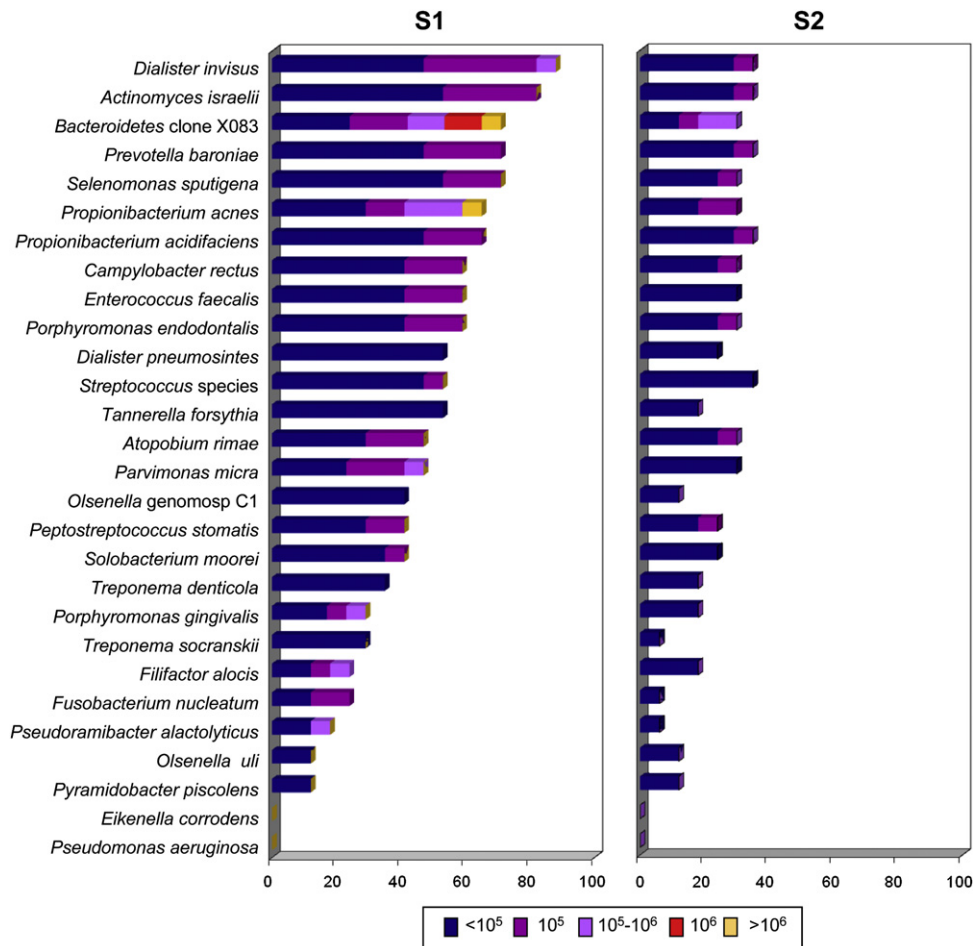


Figure 2. The chlorhexidine group: a stacked bar chart of frequency of detection and levels of bacterial taxa in root canal samples of teeth with asymptomatic apical periodontitis. The total length of each bar stack indicates percentage of positive samples. Different colors within each bar indicate percentage of samples containing different levels of the taxon. S1, initial samples; S2, postinstrumentation samples of canals irrigated with 0.12% chlorhexidine digluconate.

In the NaOCl group, 27 of the 28 taxon-specific checkerboard probes were positive for at least one S1 sample. The most prevalent taxa in S1 were *Bacteroidetes* oral clone X083 (20/30, 67%), *Selenomonas sputigena* (19/30, 63%), *Propionibacterium acnes* (18/30, 60%), *Porphyromonas endodontalis* (16/30, 53%), and *Actinomyces israelii* (15/30, 50%) (Fig. 1). After chemomechanical preparation using irrigation with 2.5% NaOCl, 25 taxa were detected, and the most prevalent were *P. acnes* (11/30, 37%), *Streptococcus* species (8/30, 27%), *P. endodontalis* (7/30, 23%), and *S. sputigena* (5/30, 17%) (Fig. 1). Only the following 5 taxa were found at levels above 10^5 in S2 samples: *P. acnes* (7% of the cases), *P. endodontalis* (7%), *F. nucleatum* (7%), *Bacteroidetes* clone X083 (3%), and *P. gingivalis* (3%).

In the CHX group, 26 of the 28 taxon-specific checkerboard probes were positive for at least one S1 sample. The most prevalent taxa in S1 were *Dialister invisus* (15/17, 88%), *A. israelii* (14/17, 82%), *Bacteroidetes* clone X083 (12/17, 71%), *Prevotella baroniae* (12/17, 71%), and *S. sputigena* (12/17, 71%) (Fig. 2). After chemomechanical preparation using irrigation with 0.12% CHX, the same 26 taxa found in S1 were again detected but with overall reduced prevalence and levels. The most prevalent taxa in S2 samples were *D. invisus*, *A. israelii*, *P. baroniae*, *Propionibacterium acidifaciens*, and *Streptococcus* species, all of them found in 6 of 17 (35%) samples (Fig. 2). The only taxon found at levels above 10^5 in S2 samples was *Bacteroidetes* clone X083 (12%).

In the NaOCl group, the mean number of target bacterial taxa per canal in S1 was 9 (range, 3-19) and in S2 it was 3 (range, 0-14). Intragroup analysis revealed that this reduction in the number of taxa per canal was highly significant ($p < 0.01$). In the CHX group, the mean number of target bacterial taxa per canal in S1 was 12 (range, 4-22) and in S2 it was 7 (range, 0-17). This reduction was also statistically significant ($p = 0.04$). The intergroup comparison showed no significant difference in the number of taxa persisting in S2 samples from canals irrigated with either NaOCl or CHX ($p = 0.3$).

Data about bacterial levels are shown in Figs. 3 and 4. Intragroup analysis revealed that both groups performed equally well in reducing the overall levels of the targeted taxa ($p < 0.001$ for both groups). No significant difference between NaOCl and CHX was observed after intergroup analysis of the S1 to S2 bacterial reduction data ($p = 0.07$).

Discussion

The present culture-independent molecular microbiology study evaluated the antimicrobial effects of chemomechanical preparation using either NaOCl or CHX as the irrigant in root canals of teeth with apical periodontitis. The parameters examined included bacterial, fungal, and archaeal elimination or reduction to undetectable levels after treatment as evaluated by broad-range PCR. The effects of treatment on the number of bacterial taxa and their levels were evaluated

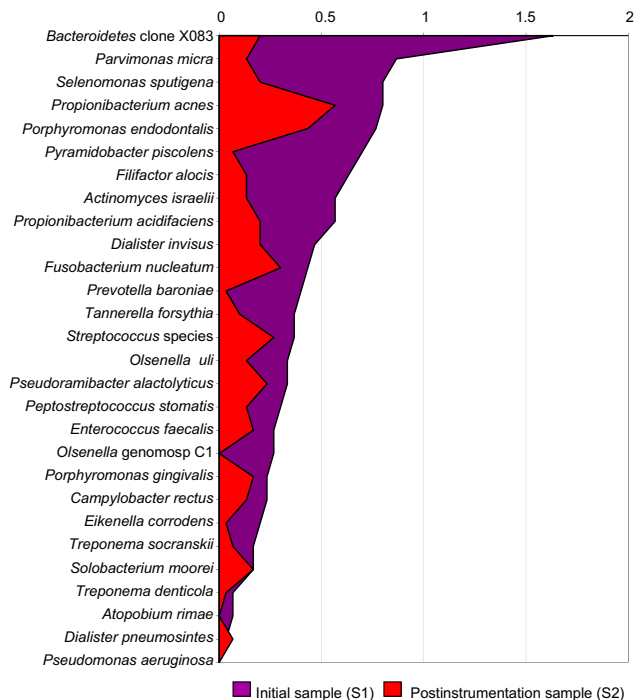


Figure 3. Reduction in bacterial levels (per taxon) after chemomechanical preparation using 2.5% NaOCl as the irrigant. The scores of the counts for each taxon were averaged across subjects and presented in descending order of the mean levels in S1 samples.

by the checkerboard approach targeting 28 putative endodontic pathogens.

A substantial reduction in the bacterial levels and number of taxa was observed after chemomechanical preparation using either irrigants. This finding is in consonance with many other studies (9, 20, 30), confirming the essential role of chemomechanical procedures in eliminating intraradicular bacteria. These effects are promoted by the mechanical debriding action of instruments and irrigant hydrodynamics and substantially enhanced by the antimicrobial ability of the irrigant solution (3–5).

No significant differences were observed for chemomechanical preparation protocols using either NaOCl or CHX with regard to the several parameters evaluated including incidence of negative PCR results, reduction in the number of taxa per canal, and reduction in the bacterial levels. This lack of significant difference between the antibacterial effects of irrigation with NaOCl or CHX is in line with other *in vivo* or *ex vivo* studies (13–15).

Both substances are known to possess excellent antimicrobial activities. CHX is a cationic bis-biguanide with good efficacy against several gram-positive and gram-negative bacteria found in endodontic infections (7, 8). Its antibacterial effects are likely to be related to the induction of damage to the bacterial cytoplasmic membrane and precipitation of intracellular constituents (31). Although some may claim for higher concentrations of CHX, *in vitro* antibacterial studies suggest that even lower concentrations may perform equally well (8); 0.12% CHX is widely used as a mouthrinse and has good tissue compatibility (32). Further studies are required to evaluate whether a higher concentrated CHX solution may offer better clinical performance.

NaOCl has a broad-spectrum antimicrobial activity, rapidly killing vegetative and spore-forming bacteria, fungi, protozoa, and viruses (33). Candidate endodontic pathogens are highly susceptible to NaOCl

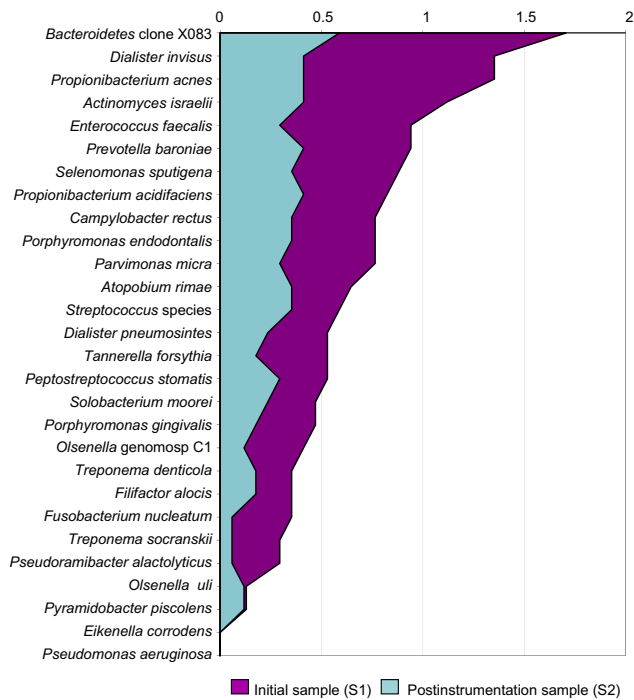


Figure 4. Reduction in bacterial levels per taxon after chemomechanical preparation using 0.12% chlorhexidine digluconate as the irrigant. The scores of the counts for each taxon were averaged across subjects and presented in descending order of the mean levels in S1 samples.

(7, 8). This substance purportedly exerts its antibacterial effect by inducing the irreversible oxidation of sulfhydryl groups of essential bacterial enzymes but may also have deleterious effects on bacterial DNA and membrane-associated activities (31). The choice for a 2.5% NaOCl solution was based on the fact that no significant differences in intracanal antibacterial effects have been observed when comparing it with higher NaOCl concentrations (4).

Antimicrobial effectiveness is surely the most important property required for an irrigant solution to be used during treatment of teeth with apical periodontitis. The results of the present clinical study show that, from a microbiological point of view, it makes no difference to use NaOCl or CHX. This is further reinforced by the findings that both NaOCl and CHX exhibit no significant effects on LPS (16). Therefore, other properties should then be considered when choosing one or the other as the irrigant. CHX but not NaOCl has substantivity to dentin, which provides CHX with residual antimicrobial effects for days to weeks (34, 35). However, NaOCl but not CHX possesses a soft-tissue-dissolving ability, which may help clean the root canal (6). To take advantage of the properties of both substances, some authors have recommended a combined use, with NaOCl being used throughout instrumentation followed by a final rinse with CHX, and good results have been reported in terms of negative bacterial cultures (36).

Although an overall significant reduction in bacterial levels occurred after chemomechanical preparation, 18 of 30 (60%) cases treated with NaOCl and 9 of 17 (53%) irrigated with chlorhexidine were still positive for the presence of bacteria as detected by broad-range PCR. These molecular findings are very similar to those of a previous culture study that reported positive cultures in 62.5% and 50% of the cases treated with NaOCl and CHX, respectively (15). The present results are also within the range reported by several other studies (9, 20, 30, 37) and highlight the need for using supplementary or

alternative antimicrobial strategies to predictably render root canals free of detectable bacteria before obturation is placed.

Allegedly, molecular methods targeting DNA may not be the best ones to detect bacteria immediately after treatment procedures because they can detect DNA from cells that recently died. Strategies for successful molecular detection of viable bacteria may be made necessary, such as using propidium monoazide before DNA extraction (38), targeting RNA (39), or using PCR with primers that amplify large products (40). The latter was used in this study. In addition to corroborating the results from previous culture studies, our present data for NaOCl are also comparable to a study using reverse transcriptase PCR (39) in which 60% of the cases were positive for bacterial presence after chemomechanical preparation. Although direct comparisons with culture results were not made in the present study, our findings suggest that broad-range PCR for DNA detection using primers that generate a large amplicon may be reliably used to detect bacteria-enduring treatment procedures.

No particular taxon was found to be associated with S2 samples. In the NaOCl group, the taxa found more frequently after chemomechanical preparation were *P. acnes*, *Streptococcus* species, *P. endodontalis*, and *S. sputigena*. In the CHX group, *D. invisus*, *A. israelii*, *P. baroniae*, *P. acidifaciens*, and *Streptococcus* species were the most prevalent in S2. These findings suggest that bacterial persistence after chemomechanical preparation may be more related to factors other than the intrinsic resistance to treatment procedures and substances by certain taxa. These factors may include the ability of involved bacteria to form and coexist in biofilm communities, spatial location of the biofilm and species distribution in the root canal system, and the levels of infection by each species in an individual case. Bacteria in biofilms are more resistant to treatment and may be located in areas unaffected by instruments and irrigants. Heavy infections (high bacterial density) may be more difficult to deal with, and the bacterial species occurring in high counts have theoretically more chances to persist. With some clear exceptions, this statement was generally supported by our findings (Figs. 3 and 4).

Because archaea and fungi were not detected in any sample, it was not possible to evaluate the effects of chemomechanical procedures on these microorganisms. Even so, the present results join others to confirm that both archaea and fungi are rarely, if ever, found in primary endodontic infections (17). These observations suggest they are not important pathogens in primary apical periodontitis, and, therefore, the antimicrobial therapy does not necessarily need to target them.

In conclusion, chemomechanical preparation using either NaOCl or CHX as the irrigant succeeded in significantly reducing the number of bacterial taxa and their levels in infected root canals, with no significant difference between these substances. The impact of protocols using either of these two irrigants on treatment outcome awaits further evaluation by clinical trials so that one, the other, or even none can be elected as the best. Because predictable infection eradication was not observed for any of the protocols, the search for more effective root canal disinfecting approaches should not be discontinued.

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