In Vivo Antimicrobial Effects of Endodontic Treatment Procedures as Assessed by Molecular Microbiologic Techniques

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Abstract

Introduction: This culture-independent molecular microbiology study evaluated the antimicrobial effects of chemomechanical preparation supplemented by intracanal medication during treatment of teeth with apical periodontitis. **Methods:** Samples were taken from 24 necrotic root canals at the baseline (S1), after chemomechanical preparation by using 2.5% NaOCl as the irrigant (S2), and after a 7-day interappointment medication with calcium hydroxide paste in either glycerin (CHG) or camphorated paramonochlorophenol/glycerin (CHPG) (S3). Bacterial, archaeal, and fungal presence was evaluated by polymerase chain reaction (PCR), and bacterial identifications were performed by a closed-ended reverse-capture checkerboard approach targeting 28 candidate endodontic pathogens. **Results:** All S1 samples were positive for bacteria but negative for both archaea and fungi. Treatment procedures were highly effective in reducing the bacterial levels and number of taxa. Overall, 46% of S2 samples and 62.5% of S3 samples were PCR-negative for bacteria. Specifically, S2 and S3 samples yielded negative PCR results in 50% and 58% of the canals in the CHG group and in 42% and 67% of the canals in the CHPG group, respectively. Except for comparisons with S1 samples, no other statistically significant differences were observed for intragroup and intergroup comparisons involving S2 and S3. Several taxa were still found in S2 and S3 samples, and the most prevalent were *Propionibacterium acnes* and *Streptococcus* species. **Conclusions:** Bacterial levels and number of taxa were substantially reduced after chemomechanical preparation and intracanal medication. However, presence of detectable levels of persisting bacteria in many cases indicates that the search for more effective antimicrobial treatment strategies should be stimulated. *(J Endod 2011;37:304–310)*

Key Words

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

The infected root canal system acts as a reservoir of microbial cells, virulence products, and antigens, which collectively evoke and maintain apical periodontitis (1). Microbial organizations in the root canal system very often give rise to biofilm communities adhered to the root canal walls, isthmuses, and ramifications (2). Because apical periodontitis is recognizably an infectious disease, optimum treatment outcome can only be achieved when the endodontic infection is properly eradicated or controlled (1, 3, 4).

Essentially, endodontic infections are treated by chemomechanical preparation supplemented or not by an interappointment intracanal medication. Although a substantial reduction in intracanal microbial communities is usually reached after chemomechanical procedures with antimicrobial irrigants such as NaOCl, it has been shown that predictable disinfection in most cases can only be achieved after an interappointment intracanal medication (5–7). Calcium hydroxide is arguably the most used substance between treatment sessions, but studies have shown inconsistent results as to its efficacy in significantly enhancing disinfection (5, 6, 8, 9). Some authors have proposed adding other medicaments to calcium hydroxide, such as camphorated paramonochlorophenol (CPMC) or chlorhexidine, so as to circumvent its limitations and maximize bacterial elimination (10, 11). Although many *in vitro* studies have supported the advantages of combining calcium hydroxide with other antimicrobial substances (11, 12), there is only limited information from clinical studies comparing different calcium hydroxide pastes (13–15).

The great majority of clinical studies evaluating the antibacterial effects of endodontic treatment procedures have been based on culture techniques. Nonetheless, it is well-known that culture has important limitations, including low sensitivity, misidentification of cultivable strains with ambiguous phenotype, difficulties in detecting culture-difficult species, and inability to grow many oral species under laboratory artificial conditions (16). Although culture-independent molecular microbiology techniques can overcome many of the limitations of culture, there are not many studies using these techniques to investigate the antimicrobial efficacy of treatment procedures. Also, most studies have focused on bacteria, which are the main microorganisms found in endodontic infections (16). However, because there are some reports of the presence of archaea (17) and fungi (18) in primary endodontic infections, it seems interesting to evaluate the effects of endodontic procedures against these microorganims, in case they are present at all.

This clinical study was undertaken to evaluate the antimicrobial effects of chemomechanical preparation with 2.5% NaOCl as the irrigant and the additive antibacterial effect of interappointment medication with either calcium hydroxide/glycerin (CHG) or calcium hydroxide/CPMC/glycerin (CHPG) paste during treatment of primarily infected root canals of teeth with apical periodontitis. Bacterial, archaeal, and fungal presence was evaluated by broad-range polymerase chain reaction (PCR), and bacterial

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identifications were performed by a closed-ended reverse-capture checkerboard DNA-DNA hybridization approach. In previous studies (7, 9) we have used culture methods to evaluate these 2 protocols separately. It is our intention in the present study to refine and expand our previous observations on these 2 antimicrobial protocols by using molecular microbiology analyses, also including now a direct comparison betweeen them.

Materials and Methods

Subjects and Case Selection

This study included 27 patients attending the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro for evaluation and treatment of apical periodontitis. Each patient contributed 1 tooth, and selection followed stringent inclusion/exclusion criteria. For inclusion, teeth had to be single-rooted and single-canalled and present with intact pulp chamber walls, necrotic pulps confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of asymptomatic apical periodontitis. All teeth had apical bone radiolucencies ranging in size from 2 × 3 mm to 12 × 15 mm. Exclusion criteria involved teeth from patients who received antibiotic therapy within the previous 3 months, teeth with gross carious lesions, teeth with root or crown fracture, teeth subjected to previous endodontic treatment, symptomatic teeth, and patients with marginal periodontitis exhibiting pockets deeper than 4 mm. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Treatment and Sampling Procedures

Before rubber dam application, supragingival biofilms were removed from each tooth by scaling and cleansing with pumice. Caries and/or defective coronal restorations were then removed by using sterile high-speed and low-speed burs. After rubber dam application, the operative field was cleaned and disinfected with 3% hydrogen peroxide, followed by 2.5% NaOCl. After completing the access preparation with another sterile bur under sterile saline irrigation, the operative field, now including the pulp chamber, was once again cleaned and disinfected as above. 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 bacterial primers. On the basis of this criterion, 3 teeth had to be excluded from the study.

A microbiologic sample was taken from the root canal immediately before preparation (S1 sample). For sample taking, sterile saline solution was placed in the pulp chamber without overflowing, and a small instrument was used to carry the solution into the canal. The root canal walls were gently filed with the small instrument so as to suspend the canal contents in saline. Three sterile paper points were consecutively placed in the canal to a level approximately 1 mm short of the root apex and used to soak up the fluid in the canal. Each paper point was left in the canal for about 1 minute and then transferred to cryotubes containing Tris–ethylenediaminetetraacetic acid (EDTA) (TE) 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 alternating rotation motion (ARM) technique was used to prepare all canals (1). 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 apical foramen with an 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 alternated rotation motion. Master apical files ranged from #50–#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. Patency of the apical foramen was confirmed with a small file (#15 or #20 NitiFlex) throughout the procedures and after each file size. Preparation was completed by using step-back of 1-mm increments. The irrigant used was 2.5% NaOCl solution. A 27-gauge needle was used to deliver 2 mL of NaOCl after each instrument size.

Each canal was dried by using sterile paper points and then flushed with 5 mL of 5% sodium thiosulfate to inactivate any residual NaOCl. Subsequently, the root canal walls were gently filed, and a postinstrumentation sample (S2) was taken from the canal as outlined above.

Smear layer was removed by rinsing the canal with 3 mL of 17% EDTA and then leaving the canal filled with this solution for 3 minutes. After irrigation with 5 mL of 2.5% NaOCl, the canal was dried with sterile paper points and medicated with either CHG (n = 12) or CHPG (n = 12) paste. The paste was placed in the canals by means of lentulo spiral fillers and packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide paste in the canal. Access cavities were filled with at least 4-mm thickness of a temporary cement (Coltosol; Coltène/Whaledent Inc, Cuyahoga Falls, OH).

Seven days later, the tooth was isolated with a rubber dam, the operative field was cleaned and disinfected, and the NaOCl was neutralized, as outlined earlier. A sterility control sample of the operative field was obtained. The temporary filling was removed, and the calcium hydroxide paste was rinsed out of the canal by using sterile saline solution and the master apical file. The root canal walls were gently filed, and a postmedication sample (S3) was taken as above. Subsequently, the canals were filled with gutta-percha and sealer by the lateral compaction technique, and the tooth was temporized with glass ionomer cement.

DNA Extraction

Clinical samples were brought to room temperature, and 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 (19).

Broad-range PCR for Bacteria, Archaea, and Fungi

Aliquots of extracted DNA were used in 16S rRNA gene-based PCR protocols with universal primers for members of the domains Bacteria (8f: 5′ - AGA GTT TGA TYM TGG C – 3′ and 1492r: 5′ - GYT ACC TTG TTA CGA CTT – 3′) (20) or Archaea (333f: 5′ - TCC AGG CCC TAC GGG - 3′ and 934r: 5′ - GTG CTC CCC CGC CAA TTC CT - 3′) (21, 22) and in a 18S rRNA gene-based PCR assay with universal primers for fungi (domain Eukarya) (B2f: 5′ - ACT TTC GAT GGT AGG ATA G - 3′ and B4r: 5′ - TGA TCR TCT TCG ATC CCC TA - 3′) (23). PCR reactions were performed in 50 μL of reaction mixture containing 1 μmol/L concentrations of each primer, 5 μL of 10× PCR buffer (Fermentas, Ontario, Canada), 3 mmol/L $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Fermentas), and 0.2 mmol/L of 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. All reactions were run in triplicate.

TABLE 1. Incidence of Positive PCR Results for Broad-range Bacterial Detection after Chemomechanical Preparation with 2.5% NaOCl as the Irrigant and 2 Intracanal Medications

	Sample		
Medication	Initial (S1)	Postinstrumentation (S2)	Postmedication (S3)
CHG paste	12/12 (100)*	6/12 (50)	5/12 (42)
CHPG paste	12/12 (100)	7/12 (58)	4/12 (33)

CHG, calcium hydroxide paste in glycerin; CHPG, camphorated paramonochlorophenol/glycerin.
*No. of positive samples/no. of samples analyzed (percent).

PCR amplifications were performed in a DNA thermocycler (Mastercycler personal; Eppendorff, Hamburg, Germany). Cycling conditions were as follows. For bacteria, it included initial denaturation step at 95°C for 2 minutes, followed by 36 cycles at 95°C/30 seconds, 60°C/1 minute, and 72°C/1 minute, and final extension at 72°C/10 minutes. For archaea, it included initial denaturation at 94°C/2 minutes, 36 cycles at 94°C/30 seconds, 58°C/30 seconds, and 72°C/1 minute, and final extension at 72°C/10 minutes. For fungi, it included initial denaturation step at 95°C/30 seconds, followed by 40 cycles at 95°C/30 seconds, 55°C/1 minute, 72°C/2 minutes, and a final step at 72°C/10 minutes.

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 as positive result. A 100 base pair 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 by using universal bacterial primers 8f and 1492r, with the forward primer labeled at the 5′ end with digoxigenin. PCR amplifications were performed as described above for bacteria.

The reverse-capture checkerboard assay was conducted to determine the presence and levels of 28 bacterial taxa as described previously (20, 24, 25). Probes were based on 16S rRNA gene sequences of the target bacteria and were described and validated elsewhere (20, 24, 26, 27).

Data Analysis

Prevalence of the target taxa was recorded as the percentage of cases examined. A semiquantitative analysis of the checkerboard findings was conducted as follows. The obtained chemiluminescent signals were evaluated by 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 0–5, where 0 indicated no signal, 1 indicated a signal weaker than the intensity of the signal generated by the 10^5 standard, 2 indicated a signal equal to the 10^5 standard, 3 indicated a signal stronger than the 10^5 standard but weaker than the 10^6 standard, 4 indicated a signal equal to the 10^6 standard, and 5 indicated a signal stronger than the 10^6 standard. No signal (score 0) meant absence of the target taxon or presence in numbers below the method's detection threshold, which was approximately 10^3.

Data were statistically analyzed, taking into consideration either all of the 24 cases, regardless of the specific interappointment medication, so as to evaluate the overall effects of irrigation and interappointment medication, or the 12 cases medicated with either CHG or CHPG separately to evaluate the intragroup effects of each specific medication and compare their efficacies through intergroup analyses. The Fisher exact test was used to compare the number of cases yielding negative PCR results after S2 and S3 (intragroup) and in S3 for the 2 groups (intergroup). The Mann-Whitney test was used to evaluate the reduction in the number of target bacterial taxa from S1 to S2, S1 to S3, and S2 to S3 (intragroup analysis) and to compare the number of taxa persisting at S3 after medication with either CHG or CHPG (intergroup analysis). 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 non-targeted taxa could have been present. Scores for bacterial levels were averaged across the subjects in S1, S2, and S3 samples, and the ability of each procedure to reduce the levels of the target taxa was assessed for intragroup and intergroup differences by the Mann-Whitney test. Intragroup analysis took into account the reduction from S1 to S2, S1 to S3, and S2 to S3. Intergroup analysis used the difference values from S1 to S3 (bacterial reduction data) to compare the 2 medicationś ability to reduce the overall bacterial load. The significance level for all tests was set at 5% ($P < .05$).

Results

All S1 samples were positive for bacteria as determined by broad-range PCR. Overall, 11 of 24 (46%) S2 samples and 15 of 24 (62.5%) S3 samples yielded negative PCR results for bacteria. Intragroup evaluations demonstrated that the protocol with CHG resulted in 6 of 12 (50%) S2 samples and 7 of 12 (58%) S3 samples exhibiting negative PCR results for bacteria, whereas respective figures for the CHPG group were 5 of 12 (42%) S2 samples and 8 of 12 (67%) S3 samples. All these results were confirmed in the checkerboard assay and are depicted in Table 1. No significant difference was observed when comparing the incidence of negative PCR results in S2 and S3 samples ($P > .05$). No significant difference was observed when comparing the incidence of negative PCR results after CHG or CHPG medication ($P = .5$). No case was positive for the presence of archaeal and fungal DNA. Positive and negative PCR controls showed the predicted results.

Overall, 26 of the 28 taxon-specific checkerboard probes were positive for at least 1 of the 24 S1 samples. The most prevalent taxa in S1 were *Propionibacterium acnes* (75%), *Bacteroidetes* oral clone X083 (63%), *Selenomonas sputigena* (63%), *Porphyromonas endodontalis* (58%), and *Propionibacterium acidifaciens* (54%). After chemomechanical preparation with 2.5% NaOCl as the irrigant (S2 samples), 17 taxa were still detected in at least 1 canal, and the most prevalent were *P. acnes* (38%), *P. endodontalis* (21%), and *Streptococcus* species (17%).

Specifically in the CHG group (n = 12), 24 of the 28 taxon-specific checkerboard probes were positive for at least 1 S1 sample. The most prevalent taxa in S1 were *S. sputigena* (83%), *P. acidifaciens* (75%), *P. endodontalis* (75%), and *Actinomyces israelii* (75%) (Fig. 1). Of

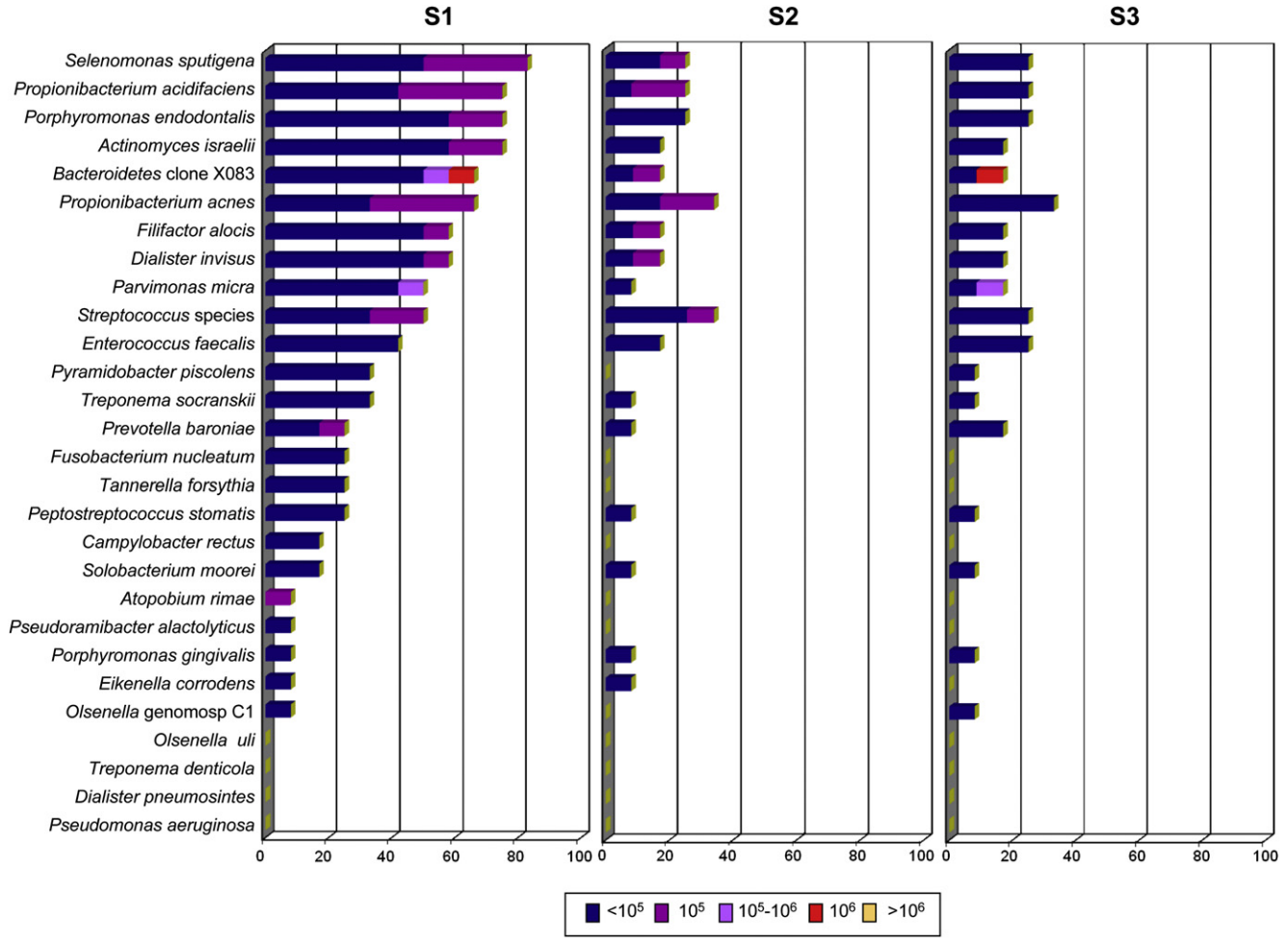

Figure 1. CHG group. Stacked bar chart of frequency of detection and levels of bacterial taxa in root canal samples of teeth with asymptomatic apical periodontitis. Total length of each bar stack indicates percentage of positive samples. Different colors within each bar indicate percentage of samples containing different levels of the taxon.

the 17 taxa still detected in S2, the most prevalent were *P. acnes* (33%) and *Streptococcus* species (33%) (Fig. 1). Of the 18 taxa detected after 7-day medication with CHG (S3), the most prevalent was *P. acnes* (33%). Other 5 taxa were found in 25% of the S3 samples (Fig. 1).

Specifically in the CHPG group (n = 12), 21 of the 28 taxon-specific checkerboard probes were positive for at least 1 S1 sample. The most prevalent taxa in S1 were *P. acnes* (83%), *Bacteroidetes* oral clone X083 (58%), and *P. acidifaciens* (50%) (Fig. 2). Only 5 taxa were found in S2 samples, and the most prevalent was *P. acnes* (25%) (Fig. 2). After 7-day medication with CHPG (S3), 3 taxa were detected, with *P. acnes* still prevailing (25%) (Fig. 2).

In the CHG group, the mean number of target bacterial taxa per canal in S1 was 9.4 (range, 3–19), in S2 it was 2.8 (range, 0–14), and in S3 it was 3.2 (range, 0–14). Intragroup analysis revealed high significance for the differences in the number of taxa per canal from S1 to S2 ($P = .003$) and from S1 to S3 ($P = .007$), but not from S2 to S3 ($P = .9$). In the CHPG group, the mean number of target bacterial taxa per canal in S1 was 6.8 (range, 1–15), in S2 it was 1 (range, 0–5), and in S3 it was 0.4 (range, 0–2). Intragroup analysis demonstrated results similar to the CHG group, with highly significant reduction from S1 to S2 and S1 to S3 ($P < .001$ for both), but not from S2 to S3 ($P = .2$). Intergroup comparison demonstrated no significant difference in the number of taxa persisting in S3 samples from canals medicated with either CHG or CHPG ($P = .3$).

Data about bacterial levels are shown in Figures 3 and 4. When the levels of target taxa were averaged across the 24 subjects, data revealed that the bacterial taxa found in the highest levels in S1 were *Bacteroidetes* clone X083, followed by *S. sputigena, P. endodontalis,* and *P. acidifaciens*; in S2 they were *P. acnes* and *Streptococcus* species; and in S3 they were *P. acnes* and *S. sputigena*. Overall analysis of the 24 samples, not distinguishing the 2 interappointment medications, also revealed significant differences between S1 and S2 and S1 and S3 ($P < .01$ for both), but not between S2 and S3 ($P = .8$).

Intragroup analysis revealed that both protocols performed equally well in reducing the overall levels of the targeted taxa ($P < .01$ for both S1-S2 and S1-S3 differences in the 2 groups). No significant difference between S2 and S3 was observed for either CHG or CHPG ($P = .6$ for both). Intergroup analysis by using the S1-to-S3 reduction values showed no significance difference between CHG and CHPG in reducing the overall levels of target bacteria ($P = .8$).

Discussion

The present culture-independent molecular microbiology study evaluated the antimicrobial effects of chemomechanical preparation with NaOCl as the irrigant, supplemented by a 7-day intracanal medication with either CHG or CHPG paste during root canal treatment of teeth with apical periodontitis. The parameters examined included bacterial,

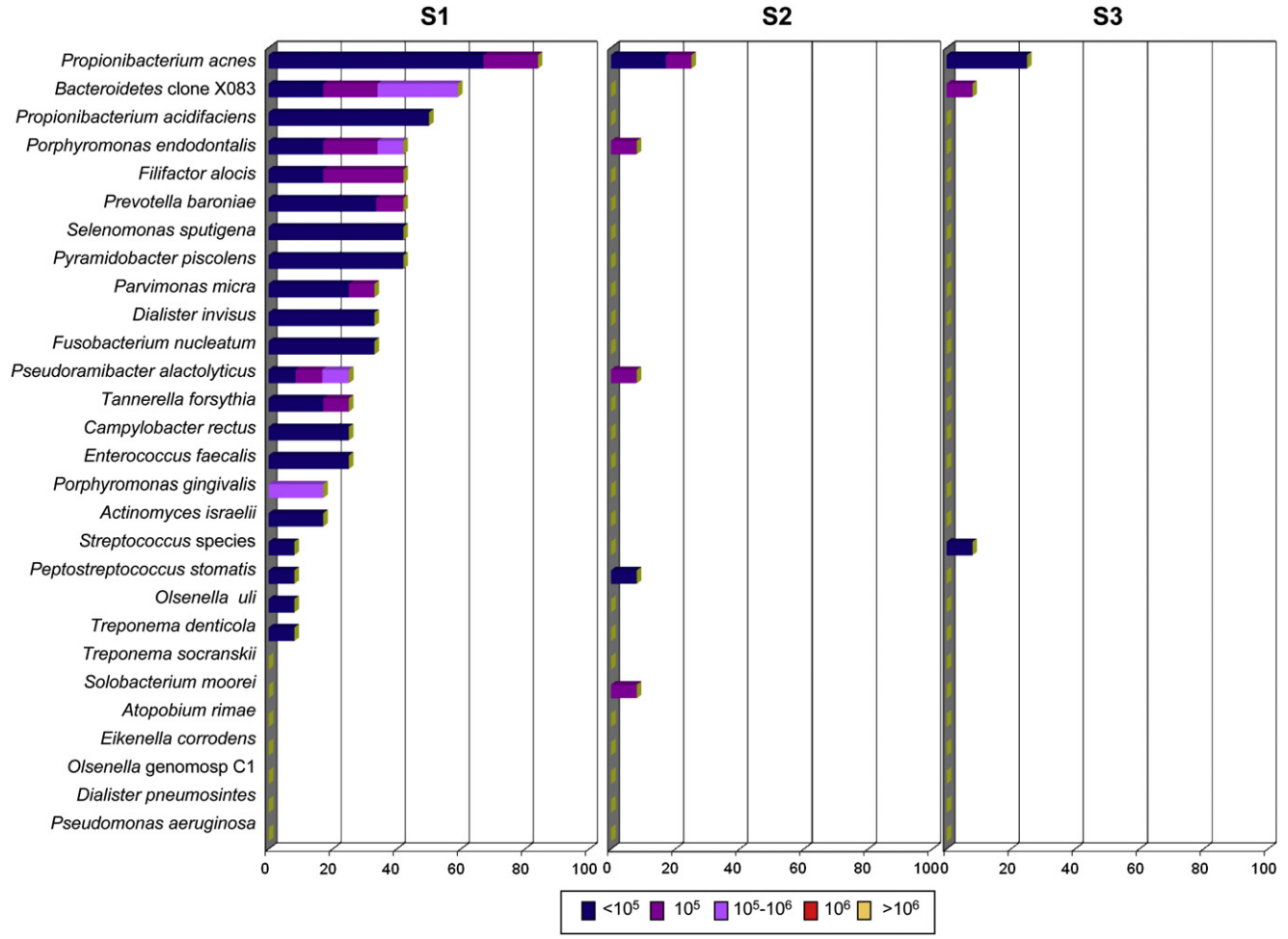

Figure 2. CHPG group. Stacked bar chart of frequency of detection and levels of bacterial taxa in root canal samples of teeth with asymptomatic apical periodontitis. Total length of each bar stack indicates percentage of positive samples. Different colors within each bar indicate percentage of samples containing different levels of the taxon.

fungal, and archaeal elimination or reduction to undetectable levels after treatment as evaluated by broad-range PCR. Because neither archaea nor fungi were detected in any samples, the analyses were limited to bacteria. The effects of treatment on the number of bacterial taxa and their levels were then evaluated by the checkerboard approach targeting 28 candidate endodontic pathogens.

Bacterial levels and number of taxa were substantially reduced after chemomechanical preparation with 2.5% NaOCl irrigation. This corroborates several other studies (9, 28, 29). Even so, 54% of the cases were still positive for the presence of bacteria as detected by broad-range PCR. This figure is within the range reported by several other studies (9, 28–31) and indicates the need for additional or alternative antimicrobial strategies. After intracanal medication (with no distinction of the medication used), the number of positive PCR results was further decreased to 37.5% of the cases. This reduction in the number of PCR-positive cases after intracanal medication is in agreement with other studies using culture. However, this 16.5% difference was not found to be statistically significant with the sample size used, which was recognizedly small, given the difficulties posed by the rigid inclusion/exclusion criteria set for this study.

When distinction was made between the intracanal medications, the results revealed that a 7-day medication with CHG decreased the number of PCR-positive cases from 50% after preparation to 42%, an 8% decrease. Intracanal medication for the same period with CHPG reduced the number of PCR-positive cases from 58% to 33%, a 25% decrease. No significant differences were observed for intragroup and intergroup comparisons, but this is also very likely to have been influenced by sample size.

Analyses of reductions in both the number of taxa per canal and levels of each taxa demonstrated that chemomechanical preparation with NaOCl as the irrigant was highly effective. Although these parameters were still reduced after intracanal medication, the results failed to reach statistical significance when compared with chemomechanical procedures. No significance was observed when comparing CHG and CHPG either.

Accurate analysis of the antimicrobial effects of treatment by means of DNA-based molecular microbiologic methods might be hampered by the risks of detecting DNA from microbial cells that died very recently. There are, however, technical strategies that can be successfully used for molecular detection of viable bacteria. Examples include the use of propidium monoazide before DNA extraction (32), reverse transcriptase–PCR assays (33), or PCR primers that generate large amplicons (34). The latter approach was used in this study, and our overall results are in agreement with most previous studies with either culture (7, 9, 14, 31) or RNA-based molecular microbiology analyses (33). It is possible that DNA from moribund

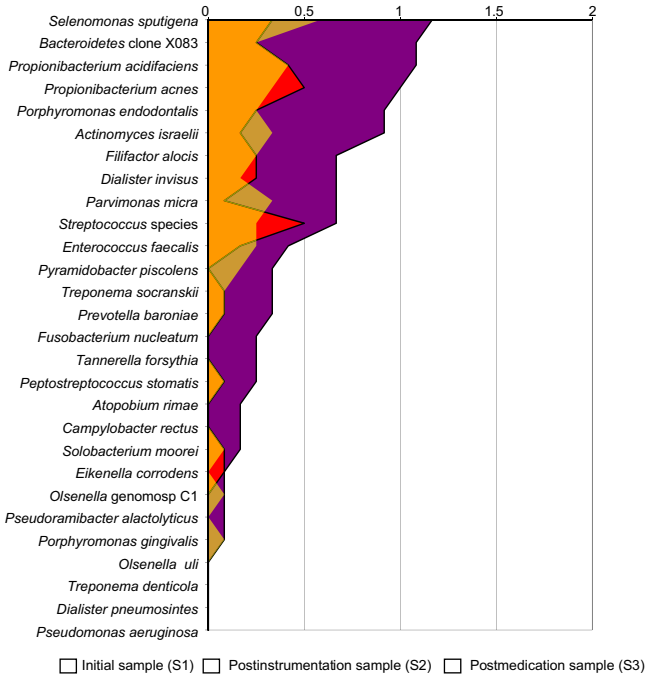

Figure 3. Reduction in bacterial levels (per taxon) after chemomechanical preparation with 2.5% NaOCl as irrigant and 7-day medication with CHG paste. Scores of the counts for each taxon were averaged across subjects and presented in descending order of the mean levels in S1 samples.

or dead cells might be destroyed by the effects of substances, such as NaOCl and calcium hydroxide, used during root canal treatment (35). The present results reinforce the conclusion of previous studies that DNA-based molecular microbiology assays with special care and optimized protocols can also be used for detection and identification of endodontic bacteria after treatment (33, 35).

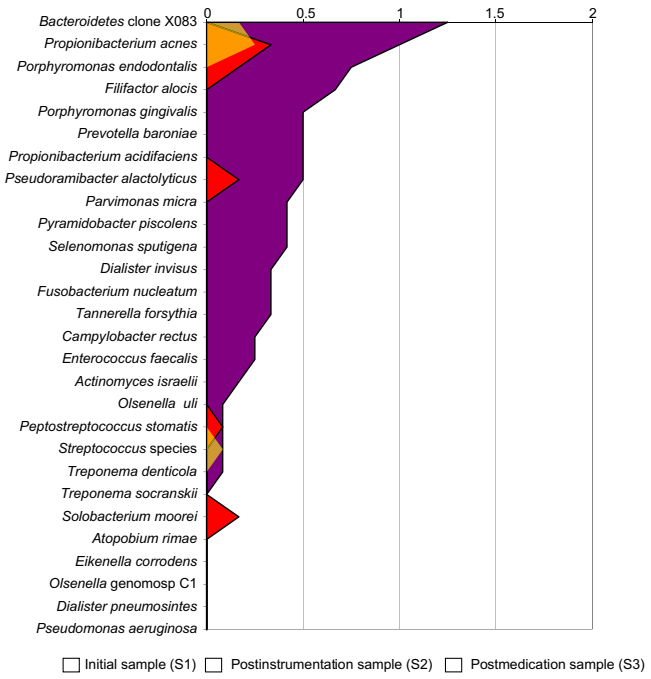

Figure 4. Reduction in bacterial levels (per taxon) after chemomechanical preparation with 2.5% NaOCl as irrigant and 7-day medication with CHPG paste. Scores of the counts for each taxon were averaged across subjects and presented in descending order of the mean levels in S1 samples.

Although no particular taxon was found to be associated with post-treatment samples, *P. acnes* and *Streptococcus* species were the most prevalent. These bacteria have already been previously found to endure endodontic treatment procedures (7–9, 33, 35, 36). This finding is in line with studies showing that gram-positive bacteria might be more resistant to treatment procedures (37). However, the finding that several other species were found in S2 and S3 samples might also indicate that bacterial persistence can be related to factors other than the intrinsic resistance to treatment procedures and substances by a specific taxon. For instance, bacteria organized in intraradicular biofilm communities can be collectively more resistant to antimicrobial agents, and those present in anatomical irregularities can evade the effects of instruments, irrigants, and even medications. Moreover, bacterial taxa found in the canal initially in high populational densities might also have theoretically more chances to survive treatment. This was somewhat supported by our present findings (Figs. 3 and 4).

In conclusion, bacterial counts and number of taxa were clearly reduced after chemomechanical preparation and then after the supplementary effects of the intracanal medication. Most taxa were completely eradicated, or at least reduced in levels, in the huge majority of cases. However, detectable levels of bacteria were still observed after chemomechanical preparation by using NaOCl and a 7-day intracanal medication with either of 2 calcium hydroxide pastes. Because persisting bacteria might put the treatment outcome at risk, the search for more effective antimicrobial treatment strategies and substances should be stimulated. Accurate detection and identification of bacteria persisting after treatment by molecular microbiology methods have the potential to set the standards for determining the antimicrobial efficacy of therapeutic procedures.

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The authors deny any conflicts of interest related to this study.

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