Bacterial Reduction in Infected Root Canals Treated With 2.5% NaOCl as an Irrigant and Calcium Hydroxide/Camphorated Paramonochlorophenol Paste as an Intracanal Dressing

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Abstract

This clinical study investigated the bacterial reduction after instrumentation using 2.5% sodium hypochlorite (NaOCl) as an irrigant and further interappointment dressing with a calcium hydroxide (Ca(OH)₂)/camphorated paramonochlorophenol (CPMC) paste. Eleven teeth with primary intraradicular infections and chronic apical periodontitis selected according to stringent inclusion/exclusion criteria followed in the study. Bacterial samples were taken before treatment (S1), after chemomechanical preparation using hand NiTi files and 2.5% NaOCl (S2), and following a 7-day medication with a Ca(OH)₂ paste in CPMC (S3). Cultivable bacteria recovered from infected root canals at the three stages were counted and identified by means of 16S rRNA gene sequencing analysis. At S1, all cases harbored bacteria, with a mean number of 2.8 taxa per canal (range, 1–6). At S2, 6 of 11 (54.5%) of the cases yielded positive cultures, with one to three species per canal. At S3, only one case (9.1%) was positive for the presence of bacteria, with Propionibacterium acnes as the only taxon isolated. A significantly high reduction in bacterial counts was observed between S1 and S2, and S1 and S3. Significant differences were also observed for comparisons involving S2 and S3 samples with regard to both quantitative bacterial reduction (p = 0.029) and number of culture-negative cases (p = 0.03). It was concluded that chemomechanical preparation with 2.5% NaOCl as an irrigant significantly reduced the number of bacteria in the canal but failed to render the canal free of cultivable bacteria in more than one-half of the cases. A 7-day intracanal dressing with Ca(OH)₂/CPMC paste further significantly increased the number of culture-negative cases. (J Endod 2007;33:667–672)

Key Words

16S rRNA gene sequencing, antimicrobial treatment, apical periodontitis, endodontic microbiology

It has been shown that the endodontic treatment of teeth evincing apical periodontitis lesions has a lower success rate when compared with teeth with no apical periodontitis (1). Moreover, a better long-term prognosis is expected for teeth showing negative culture at the root canal–filling stage when compared with those presenting bacteria in the canals at the time of filling (2–5). Therefore, an optimal outcome of the endodontic treatment is dependent on eradication of the intraradicular infection before filling or at least reduction in bacterial counts to levels that cannot be detected by contemporary culturing techniques. Because routine bacterial monitoring of root canals during treatment using reliable anaerobic culturing techniques is not always feasible, clinicians should ideally adopt an antibacterial treatment protocol that has been shown to be effective in well-controlled studies so that a predictable outcome can be achieved.

Chemomechanical preparation using sodium hypochlorite (NaOCl) as an irrigant has been shown to be a critical step for reduction of bacterial populations in the root canal, but about 40% to 60% of the canals still yield positive cultures after instrumentation and irrigation with different NaOCl concentrations (2, 6–8). To supplement the antibacterial effects of chemomechanical procedures and eliminate persisting bacteria, the use of an interappointment medication has been recommended (8, 9). Calcium hydroxide (Ca(OH)₂) is arguably the most used intracanal medication. However, its effectiveness in significantly increasing the number of culture-negative canals after chemomechanical procedures has been somewhat inconsistent (8–10), indicating that this substance may have its own limitations when it comes to intracanal disinfection. In fact, Ca(OH)₂ is known to exert lethal effects on bacterial cells when in direct contact in solution (11). In such conditions, the concentration of hydroxyl ions is very high, reaching incompatible levels to bacterial survival. Clinically, this direct contact is not always possible. In addition, the low solubility and diffusibility of Ca(OH)₂ may make it difficult to achieve a rapid and significant increase in the pH to eliminate bacteria present in biofilms, dentinal tubules, tissue remnants, and anatomic variations. Likewise, the buffering ability of serum and dentin controls pH changes and thereby reduces calcium hydroxide antimicrobial effectiveness (12). Also, resistance to Ca(OH)₂ has been reported for some microbial species (11, 13, 14). In an attempt to sidestep these limitations, association of Ca(OH)₂ with other antibacterial substances, such as camphorated paramonochlorophenol (CPMC), has been proposed (14, 15). In vitro studies have shown that Ca(OH)₂ paste in CPMC has a broader antimicrobial spectrum (eliminating microorganisms that are resistant to Ca(OH)₂), a larger radius of antimicrobial action (eliminating microorganisms located in regions more distant from the vicinity...
where the paste was applied), and kills microorganisms faster than mixtures of Ca(OH)$_2$ with inert vehicles (water, saline, and glycerin) (14–18). To the best of our knowledge, no clinical study has investigated the antibacterial effects of Ca(OH)$_2$/CPMC paste.

Although many species found in infected root canals can be overlooked by the inherent limitations of culturing methods (19), the greatest advantages of culture refer to the method’s ability to provide quantitative broad-range data and detect viable cells, which is particularly relevant when the immediate effects of antimicrobial treatment are being evaluated. Most studies of the antibacterial effectiveness of intracanal procedures have been based on traditional culture-dependent methods (2, 6–10, 20). Of these studies, only a few have identified the bacterial taxa present after chemomechanical preparation and/or intracanal medication (2, 7, 10, 20). These studies used phenotype-based methods to characterize the isolates, but a precise bacterial identification cannot always be achieved with phenotypic approaches, particularly for isolates that are biochemically inert, species with atypical biochemical patterns, rare isolates, or poorly described bacteria (19). The 16S ribosomal RNA (rRNA) gene-sequencing method has emerged as a valuable tool for accurate bacterial identification and can even lead to recognition of novel species and previously uncultivated bacteria (19, 21). The 16S rRNA gene is present in all bacteria and contains alternating regions of sequence conservation and heterogeneity. Polymerase chain reaction (PCR) primers complementary to conserved regions can amplify the 16S rRNA gene from virtually all bacteria, whereas the information in areas of sequence heterogeneity can be used to characterize isolates to the genus or species level. 16S rRNA gene sequencing allows identification of novel species and previously uncultivated bacteria. PCR primers to the 16S rRNA gene-sequencing method has emerged as a valuable tool for accurate bacterial identification and can even lead to recognition of novel species and previously uncultivated bacteria (19, 21).

The present study intended to investigate the bacterial reduction in infected root canals of teeth with apical periodontitis after chemomechanical preparation using 2.5% NaOCl as an irrigant and the additive antibacterial effect of interappointment medication with a Ca(OH)$_2$/CPMC paste. Cultivable bacteria recovered from infected root canals at three treatment stages were identified by means of 16S rRNA gene-sequencing analysis.

### Materials and Methods

#### Clinical Material

Patients presenting to the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, Brazil, for evaluation and treatment of apical periodontitis were recruited for this study. Twelve single-rooted teeth (six maxillary central incisors, two maxillary lateral incisors, one maxillary canine, one mandibular incisor, one mandibular canine, and one mandibular premolar) from 11 patients (8 females and 3 males, aged 12–60 years, mean 35 years) were selected for this study based on stringent inclusion/exclusion criteria. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative pulp vitality test, and cases showing periodontal pockets over 4 mm deep were excluded from the study. Before initiation, the experimental project was approved by the Ethics Committee of the Estácio de Sá University, and informed consent was obtained from each patient.

#### Endodontic Treatment and Sampling Procedures

Rubber dam and an aseptic technique were used throughout the endodontic treatment. Before isolation with 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 and 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 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 cleaned and disinfected once again the same way as described earlier. NaOCl 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.

Chemomechanical preparation was completed at the same appointment in all cases. Canals were instrumented by the alternated rotation motion technique (22). 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 using a back-and-forth alternating rotation motion. Master apical files ranged from #50 to #60. After each file size used in apical preparation, small files (#15 or #20 NitiFlex files) were placed in the patency length to keep the apical foramen cleaned and patent. Preparation was completed by using step-back of 1-mm increments with Flexofile instruments (Dentsply-Maillefer). NaOCl (2.5%) was used as an irrigant during the preparation. Two milliliters of this solution were 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 by using sterile paper points and then flushed with 5 mL of 5% sodium thiosulfate to inactivate the NaOCl. Subsequently, the root canal walls were gently filed and a postinstrumentation sample (S2) was taken from the canal as described earlier.

For smear layer removal, 17% ethylenediaminetetraacetic acid (EDTA) was left in the canal for 3 minutes followed by irrigation with 5 mL of 2.5% NaOCl. The canal was dried with paper points and medicated with a Ca(OH)$_2$/CPMC/glycerin paste. The paste was prepared by initially mixing equal volumes of CPMC and glycerin, which was added to dilute CPMC and facilitate paste manipulation and further removal from the canal. Calcium hydroxide powder was then added until a creamy consistency was achieved. 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 paste in the canal. The access cavities were filled with at least 4 mm thickness of a temporary cement (Coltosol; Coliène/Whaledent Inc., Gualahoga Falls, OH).

The second appointment was scheduled for 1 week thereafter. At this time, the tooth was isolated with a rubber dam, the operative field disinfected, and the NaOCl neutralized, as outlined previously. A sterility control sample of the operative field was obtained. The temporary filling
was removed and the Ca(OH)$_2$ paste rinsed out of the canal by using sterile saline solution and the master apical file. The root canal walls were lightly filed, and a postmedication sample (S3) was taken from the canals. Subsequently, the canals were filled with gutta-percha and Sealer 26 (Dentsply, Petrópolis, Brazil) using cold lateral compaction. The tooth was temporized with glass ionomer cement and a permanent restoration planned. All clinical procedures were conducted by one experienced endodontist (KMM).

**Microbiologic Analysis**

Samples in reduced transport fluid vials were transported to the laboratory within 15 minutes for microbiologic processing. Samples were dispersed with a vortex for 30 seconds and 10-fold serial dilutions to $10^{-3}$ (for S1 samples) or $10^{-2}$ (for S2 and S3 samples) were made in prereduced anaerobically sterilized buffered salt solution. Aliquots of 100 µL 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 incubated anaerobically within anaerobic jars (GasPak System, BBL Microbiology Systems) at 37°C for 14 days. After incubation, the total colony-forming units (CFUs) were counted, and actual counts were calculated based on the known dilution factors. One or two colonies of each different colony type were isolated, and each one was individually placed in cryovials containing TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, and pH 8). Cryovials were then stored at −20°C until further bacterial identification by 16S rRNA gene sequencing.

**16S rRNA Gene Identification**

Genomic DNA was extracted from each colony by heating the suspension for 10 minutes at 97°C with a thermocycler. The vials were then stored for 5 minutes on ice and centrifuged, and 5-µL aliquots of the supernatant were used as template in the 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 786-808 and 1,369-1,387, respectively, and spanning the variable regions V5-V8 of the *Escherichia coli* 16S rRNA gene. PCR amplification was performed in a reaction volume of 50 µL, consisting of 0.8 µmol/L concentration of each primer, 5 µL of 10X PCR buffer, 2 mmol/L MgCl$_2$, 1.25 U of *Tth* DNA polymerase, and 0.2 mmol/L concentration of each deoxyribonucleoside triphosphate (all reagents from Biotools, Madrid, Spain). Cycling parameters included an initial denaturation step at 95°C for 2 minutes, followed by 36 cycles of a denaturation step at 95°C for 30 seconds, a primer annealing step at 60°C for 1 minute, an extension step at 72°C for 1 minute, and a final step of 72°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.

Polymerase chain reaction products were purified by using a PCR purification system (Wizard PCR Prep; Promega, Madison, WI) and then sequenced directly on the ABI 377 automated DNA sequencer using dye terminator chemistry (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Sequence data and electropherograms were then sequenced directly on the ABI 377 automated DNA sequencer.

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**Statistical Analysis**

Effectiveness of each treatment step in rendering root canals free of cultivable bacteria was recorded as percentage of cases yielding negative cultures. In this regard, a one-tailed Fisher exact test was used to compare S2 and S3 samples. Percent reduction in the number of CFUs after each treatment step was calculated based on quantitative data obtained from samples S1, S2, and S3. Quantitative data were statistically analyzed for differences by using the Mann-Whitney test comparing pairs of groups. Significance level was always set at 5% (p < 0.05).

**Results**

Of the 12 teeth sampled, 1 showed bacterial growth for the sterility control of the working field and was excluded from the study. Bacteria were found in all initial samples (S1) from the other 11 root canals. The median value of the number of CFUs in the initial samples was $3.02 \times 10^3$, ranging from $1.68 \times 10^4$ to $3.3 \times 10^7$.

After chemomechanical preparation using 2.5% NaOCl as an irrigant, 5 of the 11 canals (45.5%) showed negative culture results. The median number of CFUs in postinstrumentation samples was $1.5 \times 10^2$, ranging from 0 to $1.3 \times 10^4$. When compared with initial samples, chemomechanical preparation promoted reduction in bacterial counts ranging from 84.56% to 100%.

After 7 days of dressing with Ca(OH)$_2$/CPMC paste, 10 of the 11 canals (90.9%) yielded no cultivable bacteria. The only case positive for bacteria in S3 harbored $4 \times 10^2$ CFUs. Percent reduction in this case was 99.87%. All the other cases showed 100% reduction in the number of cultivable bacteria.

Analysis of the quantitative data revealed that the number of CFUs in S2 and S3 was significantly reduced in comparison to S1 (p < 0.001 for both comparisons). Significant differences were also observed for comparisons involving S2 and S3 samples with regard to the number of cases yielding negative cultures (p = 0.03, Fisher exact test) or quantitative bacterial reduction (p = 0.029, Mann-Whitney test). Quantitative data and percent reductions are depicted in Table 1 and shown in Figure 1.

Thirty isolates belonging to 24 bacterial taxa were identified in initial samples. One additional isolate was not identified because of sequences with low-scoring homologies (<97% similarity) to sequences deposited in the GenBank. The mean number of bacterial taxa per canal was 2.8, ranging from 1 to 6. The most prevalent taxa were *Micrononas microbi* (3 cases), *Streptococcus constellatus/intermedius* (3 cases), *Pseudoramibacter alactolyticus* (2 cases), and *Streptococcus oralis* (2 cases) (Table 2).

Eleven isolates belonging to 10 distinct taxa were recovered from S2 samples (mean, 1.8 taxon per case; range, 1–3 taxa). *Streptococci* were the most frequent species in S2 (5 isolates in 4 cases). Of the 6 cases showing culture-positive results, two cases harbored one taxon each (*Streptococcus parasanguinis* and *Delftia sp*), three cases showed two taxa each (*S constellatus/intermedius* and *P alactolyticus*, *S oralis* and *Staphylococcus aureus*, and *Propionibacterium acnes* and *Cellulomonas parabominis*), and another case harbored 3 taxa (*S oralis*, *Streptococcus anginosus*, and *M micros*). All these bacteria were also found in S1, except for *C parabominis*, which was exclusively found in S2. This species may have been in S1 but in numbers below the detection levels of culture, or it may have been a contaminant introduced in the canal during intervention or during handling of samples. This sample was not eliminated from the study because the origin
In a previous study using similar methodology (26), we found that 45.5% of the canals sampled bacteria after chemomechanical preparation using 2.5% NaOCl as an irrigant and after a 7-day intracanal dressing with a calcium hydroxide/CPMC paste.

**Discussion**

In this study, bacterial elimination in infected root canals promoted by a specific treatment protocol was assessed by means of anaerobic-culturing procedures and 16S rRNA gene sequencing for identification of isolates. Even though several cultivable bacterial species were disclosed by these techniques, it is salient to point out that the endodontic microbiota is often underrepresented by this sort of analysis. About 40% to 55% of the bacteria found in endodontic infections have not yet been cultivated by standard culture techniques (24, 25). Furthermore, the low sensitivity of culture-dependent approaches may have made some taxa (even cultivable ones) pass unnoticed in this study. As a consequence of these limitations, it is entirely possible that the culture-positive cases had more taxa than revealed, and those showing negative cultures in fact harbored undetected bacteria. Likewise, bacterial counts cannot be considered as an accurate representation of the bacterial community in the canal. Even considering these limitations, culture was used because of its ability to detect exclusively viable bacteria and because of the correlation shown by previous studies between negative cultures and favorable treatment outcomes (2, 4). 16S rRNA gene sequencing analysis was used in order to achieve a more precise identification of the isolates recovered from the canals.

All initial samples were positive for the presence of bacteria and the number of CFUs per canal ranged from $10^4$ to $10^7$. Substantial bacterial reduction was observed after chemomechanical preparation with NaOCl as an irrigant, which is in consonance with other studies (7, 8, 22). Except for one case (7TK), percent reduction of the bacterial counts was always more than 96%. These findings confirm the important role played by chemomechanical preparation in bacterial elimination from infected root canals.

Although a significant reduction in bacterial counts occurred after chemomechanical procedures, about one half of the root canals still cultured bacteria in S2. Other studies have reported similar figures (2, 6–8, 26). Apart from a *Delftia* sp, all the other taxa found in S2 samples were gram-positive bacteria. Streptococci were found in 4 of 6 of these culture-positive cases. Other studies have also reported gram-positive species, particularly streptococci, as the most commonly found taxa enduring chemomechanical procedures (7, 27, 28).

Virtually all of the taxa found in S2 samples were eliminated after a 7-day intracanal medication with Ca(OH)$_2$/CPMC. The only exception was *P. acnes*, which was found in all three samples taken from case 12TK. The reason why this gram-positive anaerobic species persisted after every treatment step remains unknown, but it may be related to its ability to form biofilms and resistance to antimicrobial agents (29) or presence in regions inaccessible to intracanal disinfection procedures. Although *P. acnes* has not been considered as a candidate endodontic pathogen involved with persistent disease, this species has been previously isolated from samples taken after chemomechanical preparation using NaOCl (2, 10) and Ca(OH)$_2$ medication (10), as well as from root canal–treated teeth with persistent apical periodontitis (30, 31).

In a previous study using similar methodology (26), we found that...
Ca(OH)$_2$ with other disinfectants, such as CPMC (32). Although CPMC is not known to have strong cytotoxic activity, a favorable periradicular tissue response to Ca(OH)$_2$/CPMC mixture has been reported (33). This association probably owes its biocompatibility to the slow release of PMC from the paste; the denaturing effect of calcium hydroxide on connective tissue, which may prevent further tissue penetration by PMC, reducing its toxicity; and the fact that the effect on periradicular tissues is probably associated with the antimicrobial effect of the paste, which allows natural healing to occur without persistent infectious irritation (32). If the wound area is free of bacteria when the transitory and mild chemical injury occurs, there is no reason to believe that tissue repair would not take place as the initial chemical irritant is removed at the time of filling.

Analyses of the absolute bacterial counts and the number of culture-negative cases revealed statistically significant differences when comparing S2 and S3 samples. These findings confirm the importance of applying an interappointment dressing to supplement the antibacterial effects of the chemomechanical preparation. Although the impact of intracanal medication on the treatment outcome still remains debatable (34), mostly because of lack of well-controlled clinical studies with adequate sample size, it seems logical to adopt a treatment protocol that more reliably renders root canals free of cultivable bacteria before filling. Because bacteria are the major etiologic agents of apical periodontitis, the microbiologic goal of the endodontic treatment must be the complete eradication or at least the maximum reduction of the bacterial population that can be achieved. Although contrary evidence is not available, it does not seem reasonable to dismiss the significant additive antimicrobial effects of an interappointment medication in favor of saving one visit.

A culture-negative case does not imply sterility because it can be a result of limitations of the experimental protocol. One disadvantage of the experimental design used in this and other clinical studies is that samples are obtained from the main canal only. Thus, other regions in the root canal system that also harbor bacteria cannot be reached by sampling procedures. In addition, bacteria may be in the canal in levels below the sensitivity of the culture method or they can be unable to grow under artificial laboratory conditions (as-yet-uncultivated bacteria). Even so, studies have shown that the chance for a better prognosis of the endodontic treatment significantly increases when the root canal does not culture bacteria at the root canal-filling stage (2, 4). In fact, a culture-negative canal usually means that bacterial reduction has reached levels that are below the detection limits of culture procedures, and these levels may be compatible with healing of the periradicular tissues. Therefore, it is fair to assume that the routine use of intracanal medication with certain substances, such as the Ca(OH)$_2$/CPMC paste used herein, may be necessary to obtain a high incidence of negative cultures before filling and then improve the outcome of the endodontic treatment.

Primary apical periodontitis have a polymicrobial etiology, and the bacterial community profiles significantly vary from one individual to another (25), with differences being even more pronounced when samples are taken from different geographical locations (35). Because of

### Table 2: Cultivable bacterial taxa detected during treatment of 11 infected root canals associated with apical periodontitis lesions

<table>
<thead>
<tr>
<th>Bacteria Only in Initial Samples (S1)*</th>
<th>Persisting Bacteria</th>
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<tbody>
<tr>
<td></td>
<td>Postinstrumentation (S2)†</td>
</tr>
<tr>
<td><strong>Streptococcus constellatus/intermedius (2)</strong></td>
<td><strong>Propionibacterium acnes (1)</strong></td>
</tr>
<tr>
<td><strong>Porphyromonas gingivalis (1)</strong></td>
<td><strong>Micromonas micros (1)</strong></td>
</tr>
<tr>
<td><strong>Fusobacterium nucleatum (1)</strong></td>
<td><strong>Streptococcus anginosus (1)</strong></td>
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<tr>
<td><strong>Propionibacterium propionicum (1)</strong></td>
<td><strong>Streptococcus constellatus/intermedius (1)</strong></td>
</tr>
<tr>
<td><strong>Fusobacterium oral clone B5019/Fusobacterium periodonticum (1)</strong></td>
<td><strong>Streptococcus parasanguinis (1)</strong></td>
</tr>
<tr>
<td><strong>Capnocytophaga sp. (1)</strong></td>
<td><strong>Propionibacterium acnes (1)</strong></td>
</tr>
<tr>
<td><strong>Actinomyces naeslundii (1)</strong></td>
<td><strong>Staphylococcus aureus (1)</strong></td>
</tr>
<tr>
<td><strong>Dietzia sp. E9_2 E1 oral isolate (1)</strong></td>
<td><strong>Deltia sp. (1)</strong></td>
</tr>
<tr>
<td><strong>Bifidobacterium dentium (1)</strong></td>
<td><strong>Cellulomonas parahominis (1)</strong>§</td>
</tr>
<tr>
<td><strong>Eubacterium yurii (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus sanguinis (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus oralis/mitis/sanguinis (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Brachybacterium nesterenkovii (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uncultured Staphylococcus sp. clone EarCan063 (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus circulans (1)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Rhodococcus rhodochrous (1)</strong></td>
<td></td>
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<tr>
<td><strong>Unidentified (1)</strong></td>
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</tbody>
</table>

Data are based on 16S rRNA gene sequencing identification of isolates.

* Bacteria eliminated after treatment and not found in S2 or S3.

† Bacteria found in both S1 and S2, except where indicated.

‡ Bacteria found in S1, S2, and S3.

§ Isolate not found in S1.
these characteristics, primary intraradicular infections should be ideally treated by using a broad-spectrum antimicrobial strategy, which has the potential to reach most possible members of the endodontic bacterial communities. The present study lends support to the assertion that negative cultures are consistently and predictably obtained after adequate instrumentation and irrigation with NaOCl, smear layer removal, and application of an intracanal antimicrobial dressing. Chemomechanical preparation using NiTi files and irrigation with 2.5% NaOCl was highly effective in reducing the bacterial population within the root canal, but about one half of the samples still showed positive results for cultivable bacteria. A 7-day intracanal medication with Ca(OH)₂/CPMC further significantly decreased the number of canals with detectable bacteria. The excellent results regarding the effectiveness of antibacterial interappointment dressing were similar to those reported in previous studies (9, 11). Our findings confirm the excellent results from in vitro studies (14–18) regarding the antibacterial effects of Ca(OH)₂/CPMC paste and reinforce the need to use an interappointment medication after chemomechanical procedures to predictably eliminate or reduce bacteria from the canals to levels below the sensitivity of culture.

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