Antibacterial Effectiveness of 2 Root Canal Irrigants in Root-filled Teeth with Infection: A Randomized Clinical Trial

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Abstract

Introduction: This study compared the antibacterial effects of 1% sodium hypochlorite (NaOCl) and 2% chlorhexidine digluconate (CHX) during retreatment of teeth with apical periodontitis. **Methods:** Root canal–treated teeth with apical periodontitis were randomly distributed into 2 groups. Bacteriological samples were taken from the canals before (S1) and after (S2) preparation using either NaOCl or CHX irrigation and after calcium hydroxide medication (S3); 16S ribosomal RNA gene-based real-time quantitative polymerase chain reaction was performed to quantify total bacteria, streptococci, and *Enterococcus faecalis*. **Results:** Forty-nine teeth were available for analysis (NaOCl, *n* = 20; CHX, *n* = 29). Bacterial DNA occurred in all S1 samples, streptococci in 57% and *E. faecalis* in 6%. The total bacterial counts decreased from S1 to S2 in both groups ($P < .01$) but were higher in S3 than S2 ($P < .01$). Thirty-five percent of the teeth in the NaOCl group were positive in S2, decreasing to 20% in S3. In the CHX group, 41% were positive in S2, decreasing to 31% in S3. The bacterial load in S1 influenced the incidence of bacteria in S2 ($P < .01$). Streptococci were significantly reduced in both groups, and *E. faecalis* was found in only 1 S2 sample and not in S3. No significant difference between NaOCl and CHX was found. **Conclusions:** NaOCl and CHX both reduced bacterial counts and the number of infected canals. Intracanal medication with calcium hydroxide reduced the number of canals with persistent infection but resulted in overall larger bacterial counts in the cases positive for bacteria. The effectiveness of antimicrobial treatment can be influenced by the initial bacterial load. (*J Endod 2016;42:1307–1313*)

Key Words

16S ribosomal RNA gene, calcium hydroxide, chlorhexidine, endodontic retreatment, post-treatment apical periodontitis, quantitative real-time polymerase chain reaction, sodium hypochlorite

Post-treatment apical periodontitis is an infectious disease in root canal–treated teeth caused mainly by persistent intraradicular infection [1]. Persistent infections are more common in teeth with inadequate treatments [2], but even some adequately treated teeth fail. The main cause of failure in these cases is bacterial persistence in anatomically challenging areas, such as lateral canals, isthmi, apical ramifications, and dentinal tubules [3, 4]. The lower success rate for retreatment when compared with the initial treatment of teeth with apical periodontitis [5] indicates that achieving proper root canal disinfection during retreatment may be more difficult. Because the treatment outcome is negatively influenced by the presence of bacteria at the time of root filling [6, 7], the ultimate goal during root canal treatment or retreatment is to eradicate bacterial infection.

Mechanical instrumentation needs to be accompanied by a root canal irrigant with antimicrobial properties to reduce the intracanal bacterial populations [8–10]. Sodium hypochlorite (NaOCl) in concentrations ranging from 0.5%–5.25% has been widely used as a root canal irrigant. It has pronounced antimicrobial activity and the ability to dissolve organic matter [11]. However, NaOCl has an adverse effect on vital tissues, and it is toxic to periradicular tissues [12]. Chlorhexidine digluconate (CHX) exhibits broad-spectrum antimicrobial activity against endodontic bacteria [13] and substantivity to dentin [14, 15], but it lacks a tissue-dissolving ability [11]. CHX may be less irritant to vital tissues [16]. Most of the *in vitro* studies have indicated that increasing the concentration of CHX from 0.12%–2% improves the antimicrobial efficacy [17, 18]. NaOCl and CHX may differ in their effects on the various members of the endodontic microbiota [13, 17, 19]. This may have clinical significance because there are significant differences among the bacterial communities found in retreatment cases compared with primary endodontic infections [20].

Several *in vivo* studies have compared the antimicrobial effectiveness of CHX and NaOCl with conflicting results [21–26]. Most of these studies investigated the antibacterial effects by culture-dependent methods. Culture-dependent studies have limitations related to low sensitivity and the inability to detect many difficult-to-grow or uncultivable bacteria [20]. Culture-independent molecular microbiology methods can overcome these shortcomings of culture-dependent techniques [27]. Few studies

Significance
This study evaluates the clinical efficacy of 2% chlorhexidine in comparison with 1% sodium hypochlorite used as irrigants in the treatment of infected root-filled teeth. We show that both irrigants are similarly efficient in bacterial reduction and removal.

have used molecular methods to compare the antibacterial effectiveness of NaOCl and CHX (23, 25, 26). Using the reverse-capture checkerboard assay, Rôças and Siqueira (23) found no significant difference between 2.5% NaOCl and 0.12% CHX in terms of the incidence of bacterial persistence after irrigation. When comparing 2.5% NaOCl and 2% CHX in a quantitative polymerase chain reaction (qPCR), Rôças et al (26) also found no significant differences between them. Another study used qPCR to evaluate total bacteria and showed that 2.5% NaOCl was significantly more effective than 2% CHX gel (25). To our knowledge, there are no studies comparing the antibacterial effectiveness of these root canal irrigants in root canal–treated teeth with apical periodontitis using culture-independent molecular approaches such as qPCR.

The aim of this clinical study was to compare the antibacterial efficacy of 1% NaOCl and 2% CHX used as root canal irrigants in teeth with post-treatment apical periodontitis as evaluated by a molecular microbiology approach. Counts of total bacteria, *Streptococcus* species, and *Enterococcus faecalis* were evaluated before and after chemomechanical preparation and also after calcium hydroxide intracanal medication by means of qPCR.

Materials and Methods

Patient Selection

Sixty-seven consecutive patients (39 men and 28 women; mean age = 50 years; range, 21–91 years) presenting to the endodontic clinic at the School of Dentistry, University of Oslo, Oslo, Norway, and in a private practice limited to endodontics were invited to participate in this study. All treatments were performed by 1 of the authors (H.Z.). All patients exhibited post-treatment apical periodontitis either in a single-rooted tooth or in 1 root with a single canal from a multirooted tooth. Teeth with gross carious lesions, fractures involving the periodontium, and/or periodontal pockets more than 4 mm deep were excluded from the study. For all included cases, the quality of the root fillings and coronal restorations were regarded as technically adequate. Patients were not included in the study if they had diabetes, human immunodeficiency virus infection, or other immunocompromising conditions or received antibiotic therapy within the previous 3 months. On admission, cases were randomly distributed into NaOCl and CHX groups by the flipping of a coin. This randomization process resulted in 29 teeth (43%) in the NaOCl group and 38 teeth (57%) in the CHX group. Approval for the study protocol was obtained from the Regional Ethics Committee of the University of Oslo. The study and associated risks were explained to the patients, and written informed consent was obtained.

Treatment and Sampling Procedures

A rubber dam and the aseptic technique were used throughout endodontic treatment. Before rubber dam isolation, supragingival plaque was 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, the operative field, including the tooth, clamp, and surroundings, were disinfected with 3% hydrogen peroxide followed by 2.5% NaOCl. After completing the access opening with sterile burs under aseptic conditions, the operative field, including the pulp chamber, was cleaned and disinfected once again. NaOCl was neutralized with 5% sodium thiosulfate (Sigma-Aldrich, St Louis, MO), and sterility control samples (SR1) were taken from the tooth surface with a sterile Omni Swab (Whatman FTA, Sigma-Aldrich) with an ejectable head. The swab was transferred to a cryotube containing Tris-EDTA buffer (10 mmol/L Tris-HCL, 1 mmol/L EDTA, pH = 7.6) (Sigma-Aldrich) and immediately placed in a Labtop cooler (−20°C Naglene Labtop cooler, Sigma-Aldrich) or directly to a freezer (−80°C). Samples in the Labtop cooler were later transferred to a freezer. For

the inclusion of a tooth in the study, sterility control samples had to be uniformly negative after polymerase chain reaction with universal primers 8f and 1492r (28, 29).

The coronal two thirds of the root filling was mechanically removed with Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), PreRaCe burs (FKG Dentaire, La Chaux/de/Fonds, Switzerland), and/or hand files. The canal was filled with sterile saline solution with care to not overflow; a sterile #15 K-file (Dentsply Maillefer) was placed to a level approximately 1 mm short of the apical foramen, based on diagnostic radiographs and with the aid of the Root ZX electronic apex locator (J Morita Corp, Tokyo, Japan); and a gentle filing motion was applied. A larger endodontic file was used to engage the root canal filling material. On withdrawal from the canal, the instrument was cut with a presterilized wire cutter, and the fragment with attached root filling material was put in a cryotube. In addition, the root canal walls were filed with sterile saline without suction, and the entire canal content was absorbed onto 3 sterile paper points and transferred to Tris-EDTA buffer (S1). Each paper point was inserted to the full length of the instrumented canal and left for about 1 minute. Apical preparation was completed to the working length with hand nickel-titanium files (NitiFlex, Dentsply Maillefer) in a back-and-forth alternating rotation motion. Master apical files ranged from #40 to #60 depending on both the root anatomy and the initial diameter of the root canal. The irrigants used were 1% NaOCl solution in 29 cases and 2% CHX solution in 38 cases. In each group, the total volume of irrigant was 10 mL delivered by a 30-G needle (Max-i-Probe, Dentsply Maillefer). Each canal was dried using sterile paper points, and then 5 mL 5% sodium thiosulfate or a mixture of 0.07% lecithin, 0.5% Tween 80, and 5% sodium thiosulfate solutions (Sigma-Aldrich) was used to neutralize any residual NaOCl or CHX, respectively (23, 26, 30). Subsequently, the root canal walls were filed, and a postinstrumentation sample (S2) was taken from the canal using sterile paper points as described earlier. Calcium hydroxide paste mixed with sterile saline was placed with engine-driven Lentulo spiral fillers (Dentsply Maillefer) in the entire root canal extent and packed with paper points. A 2-mm plug of Cavit-G (3M ESPE, St Paul, MN) was placed in the coronal portion of the canal orifice. On top of that, a thick layer of IRM (Denstply, York, PA) was used as a temporary filling. The dressing was left in place for an average of 25 days (median = 18 days).

At the second visit, the tooth was isolated with a rubber dam, and disinfection of the operative field was performed as mentioned earlier. The temporary restoration was removed, and the operative field, including the pulp chamber, was cleaned and disinfected once again. Sterility control samples were taken (SR2). The intracanal dressing was removed with sterile saline and with gentle filing using an endodontic instrument under magnification in a microscope. The canal was dried with sterile paper points, and the canal walls were gently filed with a Hedstrøm instrument. Sterile saline was placed in the canal, and a postmedication sample was taken using 3 sterile paper points (S3). The root canal was then irrigated with 10 mL either 1% NaOCl or 2% CHX, dried, and obturated with gutta-percha and AH Plus (Denstply) sealer using the cold lateral compaction technique. The tooth was sealed with Cavit and IRM, and a final radiograph was taken.

DNA Extraction and qPCR Analysis

DNA from clinical samples was extracted by using the MasterPure DNA isolation kit from Epicenter (MCD85201; Epicenter Illumina, Cambridge, UK). To quantify the levels of total bacteria, *Streptococcus* species and *E. faecalis* before and after treatment procedures, 16S ribosomal RNA gene target qPCR was performed with Power SYBR Green PCR Master MIX (Applied Biosystems, Foster City, CA) on an ABI 7500

real-time PCR instrument (Applied Biosystems) in a total reaction volume of 20 μL. Primers, qPCR conditions, and data analysis were as described previously (31, 32). Negative controls consisted of the reaction mix with sterile water instead of the sample. Sensitivity of the qPCR assays was set at 10^2 bacterial cell equivalents. All measurements were taken in triplicate for samples, standards, and controls.

Statistical Analysis

Sample size calculation revealed that 22 patients per group would be sufficient to show a 25% difference with a power of 90%. For each patient, counts of bacteria were obtained at 3 different time points corresponding to S1, S2, and S3. Such clustered data, in this case at the patient level, are usually correlated or highly interdependent. The Poisson regression model is the basic model for modeling such counts of bacteria. The model assumes that the observations are independent and that the mean of the distribution is equal to the variance, a relationship called equidispersion. However, because of clustering of bacteria data, the response variance was greater than the mean (ie, overdispersion). The problem of overdispersion was solved by introducing patient random effects. Intergroup comparison of bacterial counts in S2 and S3 was performed by adjusting for bacterial counts in S1. For evaluation of associations showing the presence/absence of bacteria load in S1 and the incidence of positive/negative in S2 and S3, the chi-square test was used. Both the chi-square and Fisher exact tests were then used to evaluate qualitative data showing the presence/absence of bacteria between the groups. All statistical analyses were performed using StataSE 13 (StataCorp LP, College Station, TX), and the significance level was set at $P < .05$.

Results

Table 1 summarizes the distribution of age, sex, diagnosis, and tooth type from 49 patients after exclusions. Reasons for exclusion from the study were the development of a flare-up and pain postoperatively with the need for antibiotic therapy; a positive sterility control test in SR1 or SR2; S1 samples yielding negative results for bacteria; evidence of coronal leakage at the second appointment; and vertical root fracture. Forty-nine root canal–treated teeth with apical periodontitis remained in the study: 20 from the 1% NaOCl group and 29 teeth from the 2% CHX group. A flowchart of the trial is provided in Supplemental Figure S1 (available online at www.jendodon.com).

Total Bacterial Counts

Table 1 shows the mean bacterial counts in S1. The quantitative and qualitative data are summarized in Table 2. In the NaOCl group, a mean number of 7.96×10^4 bacterial cell equivalents was found in S1 samples, decreasing significantly in S2 to a mean of 2.95×10^2 cell equivalents ($P < .01$) (ie, a 99.6% reduction in total bacterial counts). A mean number of 3.51×10^2 bacterial cell equivalents was detected in S3 with a significant reduction from S1 (99.5% reduction). However, this represented 19% more bacterial cells than detected in S2. In the CHX group, a mean number of 5.37×10^5 bacterial cell equivalents was found in S1 samples; this decreased significantly in S2 to a mean of 1.10×10^3 cell equivalents ($P < .01$) (99.8% reduction). A mean number of 1.95×10^3 cell equivalents was detected in S3 with a significant reduction from S1 (99.6% reduction). S3 counts in the CHX group represented 77% more bacterial cells than S2. A highly significant decrease of bacterial counts was found when comparing S2 with S1, whereas the bacterial counts increased significantly from S2 to S3 ($P < .01$), with no differences between the 2 irrigants ($P = .62$). No significant difference was observed when comparing quantitative S2 or S3 data between the NaOCl and CHX groups ($P > .01$).

In the NaOCl group, 13 of 20 canals were negative for bacteria in S2, increasing to 16 of 20 in S3. Of the 7 cases still positive for bacteria in S2, 4 were converted to negative after intracanal medication. Of the 13 bacteria-negative teeth, 12 remained negative, whereas 1 tooth became positive in S3. In the CHX group, 17 of 29 were negative for bacteria in S2, increasing to 20 of 29 in S3. Of the 12 positive cases in S2, 6 became negative in S3. Of the 17 bacteria-negative cases, 14 remained negative, and 3 reverted to positive in S3. Qualitative analysis of the same data showed no significant difference in achieving bacteria-free root canals in S2 ($P = .65$) and S3 ($P = .62$). Figure 1A and B shows the quantitative and qualitative data in both test groups.

Streptococcus Species

Analysis by qPCR revealed streptococci in 28 of 49 (57%) S1 samples, with a mean count of 4×10^3 cell equivalents (Table 3). In the samples positive for streptococci, this bacterial group comprised 0.02%–100% of the total bacterial counts in S1 (median = 4.86%, mean = 27.5%). In 17 cases, *Streptococcus* species corresponded to less than 10% of the population; in 3 cases, they comprised

TABLE 1. Data Sorted by Categories, Age, Sex, Diagnosis, and Tooth Type: Distribution in the Treatment Groups and Mean Total Bacterial Counts

Group	NaOCl* (*n* = 20)	CHX* (*n* = 29)	Mean total bacterial counts in NaOCl and CHX (*n* = 49)	Mean total bacterial counts NaOCl (*n* = 20)	Mean total bacterial counts CHX (*n* = 29)
Age[†]					
≥50	9	16	7.84×10^4	7.60×10^4	7.98×10^4
<50	11	13	6.34×10^5	8.25×10^4	1.10×10^6
Sex					
Male	11	18	5.48×10^5	7.09×10^4	8.39×10^5
Female	9	11	6.39×10^4	9.02×10^4	4.24×10^4
Diagnosis					
CAP with symptoms	9	6	1.08×10^5	1.59×10^5	3.11×10^4
CAP without symptoms	11	23	4.57×10^5	1.49×10^4	6.69×10^5
Tooth type					
Anterior and premolar	8	16	3.16×10^5	1.53×10^4	4.66×10^5
Molar	12	13	3.83×10^5	1.22×10^5	6.24×10^5

CAP, chronic apical periodontitis; CHX, chlorhexidine digluconate; NaOCl, sodium hypochlorite.

*Mean age = 49.69 years.

[†]The 2 treatment groups showed a similar distribution in the different categories (*P* values from .07–.62).

TABLE 2. Total Bacterial Counts in Root Canal Samples of Teeth with Post-treatment Apical Periodontitis Taken before S1* and after S2† and S3‡ Using Sodium Hypochlorite (NaOCl) and Chlorhexidine Digluconate (CHX) as Irrigation Solutions

	S1	S2	S3	% reduction S1 to S2	Total bacteria-free root canals (S2), n (%)	% increase S2 to S3	Total bacteria-free root canals (S3), n (%)	% reduction S1 to S3
Total								
Mean	3.50×10^5	7.73×10^2	1.30×10^3	99.7	30 (61.2)	68	36 (73.5)	99.6
Median	1.86×10^4	0	0					
Range	1.02×10^2–7.43×10^6	0–8.49×10^3	0–4.03×10^4					
N	49	49	49	49	49	19	49	49
NaOCl								
Mean	7.96×10^4	2.95×10^2	3.51×10^2	99.6	13 (65)	19.2	16 (80)	99.5
Median	1.20×10^4	0	0					
Range	1.02×10^2–7.07×10^5	0–2.8×10^3	0–4.71×10^3					
n	20	20	20	20	20	7	20	20
CHX								
Mean	5.37×10^5	1.10×10^3	1.95×10^3	99.8	17 (58.6)	77.3	20 (69)	99.6
Median	2.42×10^4	0	0					
Range	1.07×10^2–7.43×10^6	0.0–8.49×10^3	0.0–4.03×10^4					
n	29	29	29	29	29	12	29	29

*S1 samples were taken before chemomechanical preparation.

†S2 samples were taken after chemomechanical preparation.

‡S3 samples were taken after intracanal medication.

10%–50% of the community; and in 8 cases, they comprised more than 50% of the total community.

In the NaOCl group, *Streptococcus* species were detected in 10 S1 samples, with a mean count of 7.38×10^3 cell equivalents. Five of these cases were still positive in S2, with a mean count of 8.25×10^2 cell equivalents (88.8% reduction), and 3 teeth remained positive in S3, with a mean count of 2.14×10^3 cell equivalents. In the CHX group, *Streptococcus* species were detected in 18 cases, with a mean count of 2.12×10^3 bacterial cell equivalents. Seven cases were still positive for streptococci in S2, with a mean count of 3.45×10^2 cell equivalents (83.7% reduction), and only 2 teeth remained positive in S3, with a mean count of 1.73×10^3 cell equivalents. Of the teeth negative for *Streptococcus* species in S1, 2 from the CHX group became positive in S3, with a mean count of 3.39×10^2 cell equivalents.

E. faecalis

E. faecalis was detected by qPCR in only 3 of 49 root canals, with a mean count of 1.67×10^3 cell equivalents. Two of the cases were positive for *E. faecalis* in the NaOCl group, with a mean count of 2.23×10^2 cell equivalents, and 1 was positive in the CHX group, with 4.56×10^3 cell equivalents. In the 2 cases in the NaOCl group

that were positive for this species, it was no longer found in S2. In the only case positive for this species in the CHX group, it was reduced in S2 and no longer detected in S3.

Association between Initial Bacterial Load and Bacterial Presence after Treatment

Table 4 illustrates the cases with total bacterial load categorized as 10^2–10^6 cells in S1 and the number of positive/negative root canals after irrigation (S2). By ranking all samples by their S2 levels, an arbitrary cutoff level of 2.7×10^4 bacterial cell equivalents in S1 was set for illustration of the effect of the initial bacterial load. Of 30 teeth with a bacterial load below 2.7×10^4 cells in S1, 25 teeth became bacteria free after irrigation. Of the 19 teeth with a bacterial load above this level, only 5 teeth became bacteria free in S2. This difference was statistically significant ($P < .01$). No such association between S1 and S3 values was found ($P = .1$).

Discussion

The present study evaluated the antibacterial effects of NaOCl and CHX during retreatment of teeth with post-treatment apical periodontitis. The counts of total bacteria, *Streptococcus* species, and *E. faecalis*
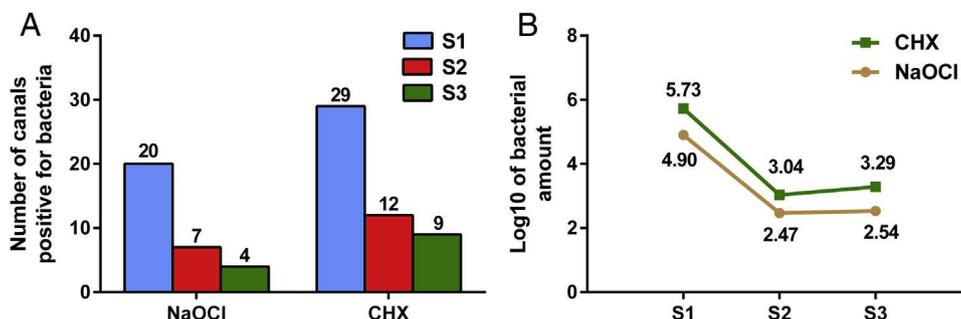
Figure 1. (*A*) The number of root canals positive for bacteria before (S1) and after (S2) chemomechanical preparation using NaOCl and CHX as irrigation solutions and after intracanal medication (S3). (*B*) Log10 of the mean total counts in S1, S2, and S3 for CHX (*n* = 29) and NaOCl (*n* = 20). A significant increase in the amount of bacteria from S2 to S3 was found (*P* < .01, Poisson regression), with no difference between NaOCl and CHX (*P* = .62).

TABLE 3. Estimate for Total Bacterial Load, *Enterococcus faecalis*, and *Streptococcus* Species in S1 Samples from Teeth with Post-treatment Apical Periodontitis

Statistic	Total bacteria	*E. faecalis*	*Streptococcus* spp.	% of total bacteria that were *E. faecalis*	% of total bacteria that were *Streptococcus* spp.
Mean	3.50×10^5	1.67×10^3	4.00×10^3	5.44	27.5
Median	1.86×10^4	3.44×10^2	1.8×10^3	3.5	4.86
Range	1.02×10^2–7.43×10^6	1.01×10^2–4.56×10^3	1.16×10^2–5.53×10^4	0.02–12.8	0.02–100
N	49	3	28	3	28

as determined by qPCR were variables examined before and after irrigation and also after intracanal medication with calcium hydroxide dressing.

Bacterial reduction after chemomechanical preparation was substantial both in qualitative and quantitative terms, and there were no significant differences between the 2 irrigation protocols in reducing the bacterial counts or in promoting bacteria-free canals. These results for teeth with persistent/secondary endodontic infections are in accordance with those from several clinical studies of primary infections (21, 23, 24, 33).

NaOCl has long been used in endodontics, and studies evaluating the clinical antibacterial effects of different NaOCl concentrations have shown an incidence of negative cultures after irrigation ranging from 40%–60% (9, 24, 34–37). There seems to be no significant differences in the intracanal antimicrobial effects of different NaOCl concentrations (9). Because the toxic effects of NaOCl increase at higher concentrations (38) whereas the antibacterial effects within the canal remain the same, we decided to use 1% NaOCl in the present clinical study. Our results showed that 65% of the canals had no detectable bacteria after NaOCl irrigation. This figure is comparable with a previous study on persistent/secondary infections that found 68% of bacteria-free canals after using rotary nickel-titanium instrumentation (39).

So far, there seems to be no previous molecular study evaluating 2% CHX irrigation in retreatment cases. In the present study, the protocol using 2% CHX rendered 59% of canals bacteria free. One of the advantages of using CHX in the root canal is its substantivity to dentin, which may be an advantage in extending its antimicrobial effects for days or weeks and preventing root canal reinfection (15, 40). CHX is bacteriostatic in low concentrations and bactericidal in higher concentrations. The main disadvantage of CHX when compared with NaOCl is its inability to dissolve organic matter. Also, serum albumin and dentin matrix have inhibitory effects on CHX activity (41).

The main goal of using calcium hydroxide is to eliminate microorganisms that persist after chemomechanical procedures and inhibit the regrowth of these microorganisms in an empty root canal between appointments (42). Clinical studies have shown inconsistent results regarding the ability of calcium hydroxide dressings to promote negative cultures after chemomechanical procedures (37, 42, 43). Most studies showed a decrease in the number of positive cases from S2 to S3 (37, 42, 44, 45), which is in agreement with the present study. However, our findings showed that the samples that were positive for

bacteria at S3 had significantly higher counts than S2. Peters et al (43) also found similar results in their culture study in which the mean total colony-forming unit counts of positive samples increased from 1.8×10^3 cells in S2 to 9.3×10^3 cells in S3. One reason for the increase in bacterial counts from S2 to S3 may have been contamination or leakage through the temporary restoration between the first and second visits, even though this was not clinically evident. Another reason may have been regrowth of residual bacteria present in dentin tubules, isthmi, ramifications, or recesses, which may have passed unnoticed in S2.

There are some concerns regarding the reliability of using DNA-based molecular methods after treatment procedures because DNA from dead cells can also be detected. A study performed on rRNA detection, which is more likely to evaluate viable cells, still showed 60% of the cases positive for bacteria after chemomechanical preparation; this is similar to DNA-based studies (46). The high incidence of negative results in a very sensitive technique like qPCR suggests that DNA from dead cells may not have been a significant source of the results. DNA fragments from dead cells may have been degraded by NaOCl (47, 48) or washed away during irrigation. An *in vitro* study showed that even though qPCR revealed significantly higher bacterial counts in S1 than culture, counts in S2 did not significantly differ when using NaOCl as the irrigant (49). On the other hand, CHX has been shown not to significantly affect DNA detection by polymerase chain reaction under laboratory conditions (50), and how this may have influenced the results in this group during the clinical use remains to be addressed.

Although it may be impossible to eradicate all bacteria in the root canal system, a more realistic therapeutic goal may be to reduce the bacterial counts to a level below that needed to uphold the disease (51). We found that there was a significantly higher chance of achieving bacteria-free canals after chemomechanical preparation and irrigation if the initial bacterial load was lower than 2.7×10^4 cell equivalents. Root canals with bacterial counts above this threshold value remained positive for bacteria after instrumentation and irrigation in 14 of 19 cases (74%). To our knowledge, this is the first molecular study showing an association between initial bacterial counts and achieving bacteria-free root canals after treatment.

The effects of treatment on the presence and levels of *Streptococcus* species and *E. faecalis* were also assessed. Streptococci as a group are among the most prevalent bacteria found in postinstrumentation samples (9, 46, 52) and root canal– treated teeth (32, 39, 53, 54). Streptococci occurred in 57% of the initial S1 samples, which is in the range of 37%–84% found in other molecular studies (32, 39, 55). When present, *Streptococcus* species comprised an average of 27.5% of the total bacterial community; in 8 cases, they represented more than 50% of the total community. After chemomechanical procedures, 25% of the cases in the NaOCl group and 24% of the cases in the CHX group were still positive for these bacteria. There were no significant differences between the 2 irrigation groups in reducing the streptococcal counts. Of the teeth negative for *Streptococcus* species in S1, 2 from the CHX group became positive in S3. This may be caused by coronal leakage unnoticed during the

TABLE 4. Association between Total Bacterial Load in S1 and the Number of Bacteria-positive or -negative Root Canals after Chemomechanical Preparation (S2)

	Total bacterial amount in S1					
	10^2	10^3	10^4	10^5	10^6	Total
Negative root canals in S2	6	7	17	0	0	30
Positive root canals in S2	0	4	7	6	2	19

clinical examination, but one cannot discard the possibility that these bacteria occurred in undetectable levels in S1 and S2 and found conditions to grow between appointments. Limitations of the paper point sampling approach may also have accounted for this finding.

E. faecalis was found in only 3 cases (6%), which is lower compared with most previous studies (56). Differences in these results may be related to the specific primers used, but even less sensitive polymerase chain reaction techniques using the same primers have found a higher prevalence (57). Whether this low prevalence was related to a possible geographic species variation or other factors remains elusive. The 2 cases positive for *E. faecalis* were negative after irrigation with NaOCl, whereas irrigation with CHX could not completely eliminate this species from the 1 case in which it was found, even though the counts were reduced. The mean *E. faecalis* counts initially were higher in the CHX case than in the 2 NaOCl cases, which may partially explain this result. There was no detectable *E. faecalis* after medication. It seems that both chemomechanical preparation and intracanal medication were effective in reducing and eliminating this species.

The Poisson regression model was used in the current study for 2 reasons. First, the Poisson model is the basic model for analyzing count data (58). Second, clustered observations from longitudinal studies are usually correlated. Therefore, by extending the Poisson regression model with the introduction of patient random effects, the confounding effects of correlated bacterial counts were controlled. Further calculations and results are provided in Supplemental Tables S1 and S2 (available online at www.jendodon.com). Incidence rate ratios (IRRs) from the random effect models represent the change in counts of bacteria in S2 and S3 relative to S1 and also the changes between S2 and S3 after adjusting for counts in S1. If the IRR is equal to 1, then there is no difference between NaOCl and CHX. However, if the IRR is significantly less than 1, then a reduction in counts of bacteria is observed relative to the reference treatment method.

In conclusion, irrigation protocols with either 1% NaOCl or 2% CHX effectively reduced both the number of positive root canals and the counts of total bacteria, streptococci, and *E. faecalis* in root canal–treated teeth with apical periodontitis with no significant differences between them. Although the amount of positive root canals decreased after intracanal medication with calcium hydroxide dressing, bacterial counts in the positive cases increased significantly. Finally, a significant association between the initial bacterial counts and achieving bacteria-free root canals after chemomechanical preparation was observed.

Supplementary Material

Supplementary material associated with this article can be found in the online version at www.jendodon.com (http://dx.doi.org/10.1016/j.joen.2016.06.006).

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