Infection Control in Retreatment Cases: *In Vivo* Antibacterial Effects of 2 Instrumentation Systems

Renata C.V. Rodrigues, MSc, Henrique S. Antunes, MSc,*† Mônica A.S. Neves, PhD,* José F. Siqueira Jr, PhD,* and Isabela N. Rôças, PhD**

Abstract

Introduction: This *in vivo* study compared the antibacterial effects of 2 instrumentation systems in root canal–treated teeth with apical periodontitis. **Methods:** Forty-eight teeth with a single root and a single canal showing post-treatment apical periodontitis were selected for this study. For retreatment, teeth were randomly divided into 2 groups according to the instrumentation system used: Self-Adjusting File (SAF; ReDent-Nova, Ra'anana, Israel) and Twisted File Adaptive (TFA; SybronEndo, Orange, CA). In both groups, 2.5% sodium hypochlorite was the irrigant. Bacteriological samples were taken before (S1) and after chemomechanical preparation (S2). In the TFA group, passive ultrasonic irrigation (PUI) was performed after instrumentation, and samples were also taken after this supplementary step (S2b). DNA was extracted from the clinical samples and subjected to quantitative real-time polymerase chain reaction to evaluate the levels of total bacteria, streptococci, and *Enterococcus faecalis*. Statistical analyses from quantitative real-time polymerase chain reaction data were performed within groups using the Wilcoxon matched pairs test and between groups using the Mann-Whitney *U* test and the Fisher exact test with the significance level set at $P < .05$. **Results:** Bacteria were detected in S1 samples from 43 teeth, which were then included in the antibacterial experiment. Both SAF and TFA instrumentation protocols showed a highly significant intracanal bacterial reduction ($P < .001$). Intergroup quantitative comparisons disclosed no significant differences between TFA with or without PUI and SAF ($P > .05$). PUI did not result in significant improvement in disinfection ($P > .05$). **Conclusions:** Both instrumentation systems/treatment protocols were highly effective in significantly reducing the intracanal bacterial counts. No significant difference was observed between the 2 systems in disinfecting the canals of teeth with post-treatment apical periodontitis. *(J Endod 2015;41:1600–1605)*

Key Words

Chemomechanical preparation, endodontic retreatment, passive ultrasonic irrigation, post-treatment apical periodontitis, Self-Adjusting File, Twisted File Adaptive

Endodontic treatment failure usually occurs when an intraradicular infection is not properly controlled by treatment procedures (1). Infection is present in virtually all cases of post-treatment apical periodontitis (2–4). Studies have reported that the treatment outcome is negatively affected by bacterial persistence in the root canal at the time of filling (5, 6). Therefore, the main microbiological goal of the endodontic treatment and retreatment of teeth with apical periodontitis is to eradicate bacterial infection (1).

Given the lower success rates of retreatment when compared with the initial treatment in teeth with apical periodontitis (7), one may expect that proper disinfection is not easy to achieve in previously treated canals. Many studies have evaluated the efficacy of clinical procedures in reducing bacterial populations in teeth with primary intraradicular infection (8). However, only a few have investigated the antibacterial effects of chemomechanical procedures in retreatment cases (9–12). None of these previous studies have used contemporary rotary instrumentation techniques, and the microbiologic evaluation method consisted of culture (9–11, 13) or nonquantitative end-point polymerase chain reaction (PCR) (11, 12).

To improve the performance of instruments in cleaning and shaping root canals, new instruments are available. The self-adjusting file (SAF) (ReDent-Nova, Ra'anana, Israel) has been developed with a totally different concept of root canal instrumentation (14). This instrument consists of a hollow cylindric file with flexibility that allows it to adapt to the cross section of the root canal (15). The SAF has an abrasive surface that enlarges the canal while still preserving its original shape. The SAF design permits a continuous delivery and flow of irrigants through its hollow body. Studies have shown that the SAF system can enhance cleaning (16, 17) and disinfection of root canals when compared with conventional instruments (18, 19).

Conventional nickel-titanium (NiTi) rotary systems have continuously evolved, especially in terms of design and improvements in the NiTi alloy. The Twisted File Adaptive (TFA) instrument developed by SybronEndo (Orange, CA) is proposed for use in combined continuous rotation and reciprocating motions. The instrument uses continuous rotation when it is exposed to a minimal or no applied load and changes to reciprocating motion when it engages dentin and some load is applied. This adaptive technology and the twisted file design that uses R-phase treatment is claimed to reduce the risk of instrument failure and increase flexibility and canal centering ability (20, 21).

From the *Molecular Microbiology Laboratory, Department of Endodontics, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro; and †Department of Endodontics, Faculty of Dentistry, Grande Rio University, Duque de Caxias, Rio de Janeiro, Brazil.

Address requests for reprints to Dr Renata Costa Val Rodrigues, Faculty of Dentistry, Estácio de Sá University, Av Alfredo Baltazar da Silveira, 580/cobertura, Recreio Rio de Janeiro, RJ, Brazil 22790-710. E-mail address: recostaval@gmail.com

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In addition to new instruments, strategies have been developed to improve root canal disinfection (22). One of these strategies is passive ultrasonic irrigation (PUI), which consists of the ultrasonic activation of an irrigant (usually sodium hypochlorite [NaOCl]) after root canal preparation. Data from *ex vivo* and *in vivo* studies evaluating the antibacterial effectiveness of PUI with NaOCl as a supplementary step have been inconclusive. Some authors reported superiority of the PUI approach over syringe irrigation (23, 24), whereas others showed no significant differences (25–27). To the best of our knowledge, no study has evaluated the *in vivo* effects of PUI in retreatment cases.

The purpose of this clinical study was to compare the antibacterial effectiveness of the SAF and TFA systems during the chemomechanical preparation of root canal–treated teeth with apical periodontitis. Counts of total bacteria, *Streptococcus* species, and *Enterococcus faecalis* were evaluated before and after preparation by means of quantitative PCR (qPCR). The null hypothesis was that there is no significant difference in intracanal bacterial reduction promoted by the SAF system, the TFA system, and the TFA with PUI.

Materials and Methods

Case Selection

Forty-eight patients (35 females and 13 males; mean age = 43; range, 12–72 years) attending the endodontic clinic at the Department of Endodontics, Estácio de Sá University, Rio de Janeiro, RJ, Brazil, for retreatment of teeth with post-treatment apical periodontitis were included in this study. All teeth had a single root and a single canal and showed radiograph evidence of periapical bone destruction. Treatments were performed at least 2 years previously. Root canal fillings were no more than 4 mm short of the apex. Symptoms were absent. All teeth were coronally restored and with no evidence of direct exposure of the root canal filling material to the oral cavity. Exclusion criteria included teeth with periodontal pockets deeper than 4 mm, teeth that could not be easily isolated with a rubber dam, and teeth with large intraradicular posts. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Sample Taking and Treatment Procedures

Samples from root canals were taken using strict aseptic techniques. After an oral rinse with 0.12% chlorhexidine for 1 minute, supragingival plaque biofilms were removed by scaling and cleansing with pumice. Next, the tooth was isolated with a rubber dam, and the operative field (tooth, clamp, and surrounding dam) was cleaned by using 3% hydrogen peroxide and disinfected with 2.5% NaOCl. After completing the access preparation with sterile burs under sterile saline irrigation, the operative field, this time also including the pulp chamber, was once again cleaned and disinfected as described previously. Residual NaOCl was neutralized with 10% sodium thiosulfate, and sterility control samples were taken by scrubbing sterile paper points on the cavosurface angle of the access cavity. These paper points were transferred aseptically to a cryotube containing Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH = 7.6) and immediately frozen at −20°C.

Gutta-percha fillings were removed by using the DR1 (size 30/.10, at 1000 rpm) and DR2 (size 25/.04, at 600 rpm) instruments from the D-Race system (FKG Dentaire, La Chaux-de-Fonds, Switzerland). Sterile saline solution was used for irrigation, and no solvent was used. The working length (WL) was established 1 mm short of the apical foramen with an apex locator (Novapex; Forum Technologies, Rishon Le-Zion, Israel) and confirmed by radiographs. Next, the canal was left filled with saline, and a small hand instrument was placed at the WL and used to gently file the canal walls. An initial microbiologic sample (S1) was taken from the root canal with sterile paper points consecutively placed at the WL. Each paper point was left in the canal for about 1 minute. Paper points were transferred to cryotubes containing RNA*later* (Ambion, Austin, TX), stored at 4°C for 12 hours, and then frozen at −20°C.

For inclusion of the tooth in the study, sterility control samples had to be negative for end-point PCR using universal 16S ribosomal RNA gene bacterial primers, and S1 samples had to be positive for bacterial presence in the qPCR assay (see later). Accordingly, 5 teeth were excluded from the study because of negative PCR results in S1 samples after 2 separate qPCR runs in triplicates. Thus, 43 patients were included in the study. According to the treatment protocol, teeth were randomly distributed into 2 groups. Chemomechanical preparation was completed at the same appointment in all cases.

SAF Group

Twenty-one teeth had their root canals prepared using the SAF system. Initially, the canal was instrumented at the WL with the DR2 instrument. Irrigation was performed with 3 mL 2.5% NaOCl. Root canal preparation was then completed using the SAF 2-mm instrument operated for 4 minutes at the WL under continuous irrigation with 2.5% NaOCl. The irrigant solution was continuously delivered by a special irrigation device (VATEA, ReDent-Nova) at a flow rate of 5 mL/min (total of 20 mL per canal). The SAF system was used with the instrument operated by an in-and-out vibrating handpiece (GENTLEpower; KaVo, Bieberach a.d. Riß, Germany) combined with an RDT3 head 2 (ReDent-Nova) at 5000 rpm and an amplitude of 0.4 mm. Each root canal was instrumented with a single SAF, and each instrument was used to prepare only 1 canal. After preparation with the SAF, patency of the apical foramen was checked with small hand files, and a size 50/.02 NiTi hand instrument was used at the WL to finish apical preparation. The canal was then irrigated with 3 mL 2.5% NaOCl (Fig. 1).

The smear layer was removed by rinsing the canal with 1 mL 17% EDTA and then leaving the canal filled with this solution for 3 minutes. Next, the canal was irrigated with 5 mL 2.5% NaOCl. The total volume of NaOCl used to prepare the root canal with the SAF was 31 mL (Fig. 1). Each canal was flushed with 1 mL 10% sodium thiosulfate for 1 minute to inactivate any residual NaOCl. A postinstrumentation sample (S2) was taken from the root canal as outlined earlier.

TFA Group

Twenty-two teeth had their root canals prepared using the TFA system. Initial instrumentation with the DR2 instrument was performed at the WL, and the canal was rinsed with 5 mL 2.5% NaOCl. TFA instruments of the ML kit (sizes 25/.08, 35/.06, and 50/.04) were used up to the WL. After each instrument size, the root canal was rinsed with 6 mL 2.5% NaOCl. After apical preparation, the canal was dried by using sterile paper points and then flushed with 1 mL 10% sodium thiosulfate for 1 minute to inactivate NaOCl. Next, a sample (S2) was taken from the canals as described for S1. The smear layer was removed by rinsing the canal with 1 mL 17% EDTA and 3 mL 2.5% NaOCl. PUI was performed for 1 minute by using an Irrisonic E1 insert (Helse, Santa Rosa de Viterbo, SP, Brazil) coupled to an ultrasonic device (GVDentus, São José dos Campos, SP, Brazil) and placed to the WL. Then, the canal was rinsed with 5 mL 2.5% NaOCl, dried with sterile paper points, and then flushed with 1 mL 10% sodium thiosulfate for 1 minute. Another microbiological sample (S2b) was taken from the canal. Irrigation was performed using the total volume of 23 mL 2.5% NaOCl up to S2 and 31 mL up to S2b (Fig. 1). Irrigant was delivered by disposable syringes and NaviTip needles (Ultradent, South Jordan, UT) inserted up to 4 mm short of the WL.

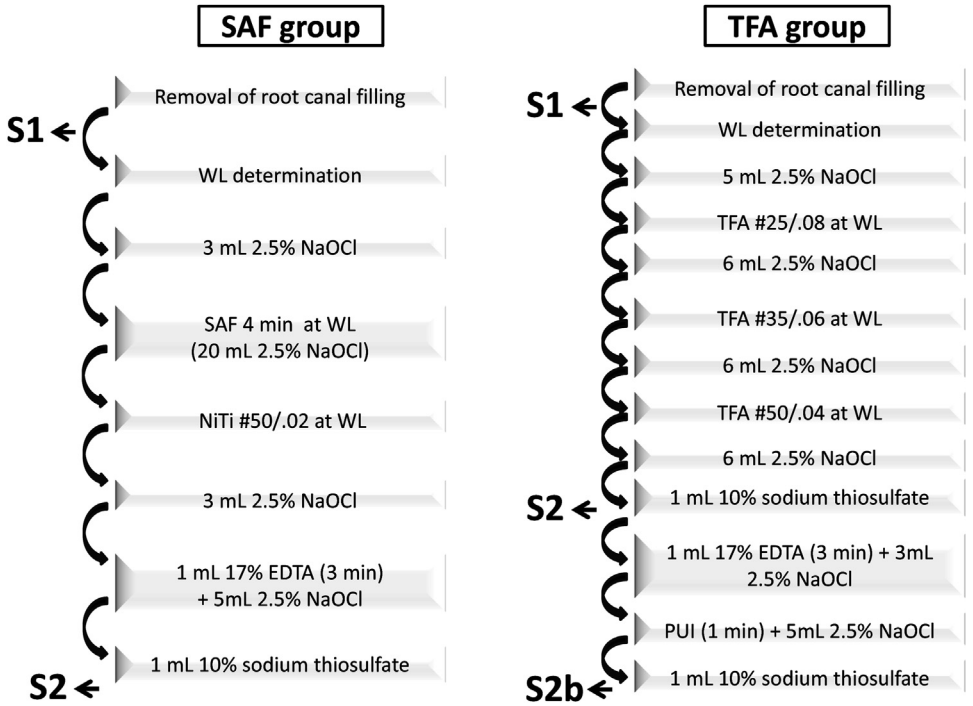

Figure 1. Flowchart of the clinical procedures. PUI, passive ultrasonic irrigation; SAF, Self-Adjusting File; TFA, Twisted File Adaptive; WL, working length.

After preparation in both groups, the canal was medicated with a calcium hydroxide paste; 1 week later, it was filled with gutta-percha and sealer, and the tooth was coronally restored.

DNA Extraction and Quantitative Real-time PCR Analysis

Clinical samples were thawed to room temperature, and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the protocol recommended by the manufacturer. To quantify the total bacterial load and levels of *E. faecalis* and *Streptococcus* species before and after treatment procedures, 16S ribosomal RNA gene–targeted 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 analyses were as described previously (2, 28). All measurements were taken in triplicate for samples and standards.

Statistical Analysis

Sample size calculation revealed that 21 specimens per group would be sufficient to show a 5% difference in S2 or S2b/S1 proportions with a power of 90%. The Wilcoxon matched pairs test was used to compare S1 and S2/S2b samples for intragroup bacterial reduction. The nonparametric Mann-Whitney *U* test was used to compare S1 samples from both groups. Because there was no statistically significant difference between S1 for both groups ($P > .05$), the absolute values in S2/S2b could be used for intergroup comparisons using the Mann-Whitney *U* test. S2 samples from the SAF group were compared with S2 (before PUI) and S2b (after PUI) from the TFA group. For intergroup analysis of the presence/absence (qualitative) data, the Fisher exact test was used. The significance level for all tests was set at $P < .05$.

Results

In the SAF group, a mean number of 1.78×10^4 bacterial cell equivalents were found in S1 samples and decreased substantially in S2 to a mean of 9.43×10^2 cells ($P < .001$). This comprised an 83.7% reduction in total bacterial counts. In the TFA group, the initial mean number of 8.24×10^4 bacterial cell equivalents was significantly reduced in S2 to a mean of 7.71×10^1 cells ($P < .001$) with a 94.8% reduction. After PUI (S2b), the mean number of total bacteria still decreased to 5.35×10^1 with a 96.9% reduction in relation to S1. However, the reduction from S2 to S2b was not statistically significant ($P = .3$). Table 1 depicts the mean, median, and range of bacterial counts (quantitative data) observed for the test groups.

No significant difference was observed when comparing quantitative S2 data between the groups or S2 data from the SAF group with S2b data from the TFA group ($P > .05$). Likewise, qualitative analysis of the same data showed no significant difference for the incidence of negative PCR results ($P > .05$). Table 2 displays the incidence of positive results in qPCR before (S1) and after chemomechanical preparation (S2 or S2b) in the test groups.

Streptococci occurred in 17 of 21 (81%) S1 samples from the SAF group in mean counts of 8.50×10^3 cell equivalents. After SAF instrumentation, streptococci were still detected in 9 samples in mean levels of 1.06×10^3 cells. In the TFA group, streptococci were present in 19 of 22 (86%) S1 samples with a mean number of 9.08×10^4 cells. After TFA instrumentation, streptococci were still present in 6 samples in a mean count of 6.83×10^1 cells. After PUI, streptococci still remained in the 6 cases, slightly decreasing in numbers to 6.57×10^1 cells (Table 3). Results for streptococci were the same as those for total bacteria in terms of statistical differences.

E. faecalis was detected in 5 of 21 (24%) S1 samples from the SAF group in mean levels of 4.47×10^4 cell equivalents. After SAF instrumentation, this species was no longer detected (100% reduction). In the TFA group, *E. faecalis* was found in 7 of 22 (32%) S1 samples

TABLE 1. Incidence of Positive Results in Quantitative Polymerase Chain Reaction before (S1) and after Chemomechanical Preparation Using Different Instrumentation Systems (S2) in Teeth with Post-treatment Apical Periodontitis

Groups	Total bacteria		Streptococci		*Enterococcus faecalis*	
	S1	S2	S1	S2	S1	S2
Self-Adjusting File	21/24 (87.5)	10/21 (48)*	17/21 (81)	9/21 (43)	5/21 (24)	0/21 (0)
Twisted-File Adaptive	22/24 (92)	7/22 (32)	19/22 (86)	6/22 (27)	7/22 (32)	0/22 (0)
Twisted-File Adaptive + passive ultrasonic irrigation†	22/24 (92)	6/22 (27)	19/22 (86)	6/22 (27)	7/22 (32)	0/22 (0)

*Number of cases with positive result/number of positive cases for total bacteria in S1 (%).

†In the Twisted File Adaptive group, a final step of passive ultrasonic activation was included. Therefore, S1 results from both groups are the same.

with a mean number of 8.63×10^3 cells. After instrumentation and before or after PUI, this species was not detected either (Table 4).

Discussion

This clinical study compared the antibacterial effects of 2 instrumentation systems during retreatment of teeth with post-treatment apical periodontitis. Both systems were associated with irrigation with 2.5% NaOCl and showed a highly significant reduction of the intracanal bacterial populations. This is in agreement with the previous studies that evaluated the effects of retreatment on bacterial elimination from the root canal (9–13).

The mean total bacterial reduction was 83.7% for SAF, 94.8% for TFA, and 96.9% for TFA + PUI. No significant difference was observed between the groups. Attempts were made to reduce the influence of other variables on the results by standardizing the apical size of instrumentation (size 50) and the volume of NaOCl used, which was the same for the comparison between SAF and TFA + PUI. Time of NaOCl permanence in the canal is another factor that may potentially influence the results, and *in vitro* studies have shown that a significantly higher bacterial reduction was observed after longer exposures to NaOCl (29, 30). However, this was difficult to standardize in the present *in vivo* study because of the differences between the 2 systems; one used a single instrument with concomitant irrigation (SAF), and the other used multiple instruments alternated with syringe irrigation (TFA). How the exposure time to NaOCl influences the *in vivo* antibacterial results is worth further research.

The SAF system is a single-instrument approach whose main advantages are that the instrument is designed to adapt to the root canal morphology in cross section and the irrigant solution is concomitantly and continuously delivered during the instrument action. *Ex vivo* studies have shown that the SAF ability of cleaning, shaping, and disinfecting canals that are curved and/or irregular in cross section is superior to conventional instruments (16, 17, 19, 31, 32). In terms of antibacterial effects of the SAF, the only previous clinical study published so far revealed that the SAF performed significantly better than a hand instrumentation technique in untreated teeth with primary infection (18). However, the results of the present study in root canal–treated teeth showed no superior results for the SAF when compared with conventional rotary instruments. There are some potential explanations for the lack of improved effects for the SAF system in the present study. One is that the teeth included in this study did not have significant anatomic variations, and under these conditions the SAF system may not perform differently from conventional rotary instruments. Moreover, remnants of the previous filling may have made it difficult or even precluded the SAF from properly reaching and affecting bacteria located in root canal irregularities.

PUI has been recommended as an additional step after chemomechanical preparation for supplementary disinfection. The potential benefits of ultrasonic activation of NaOCl are related to acoustic streaming, cavitation, and/or warming of the irrigating substance (33, 34). In the present study, PUI led to a mean percent reduction of 30.6% when compared with samples taken immediately after chemomechanical preparation. This reduction in bacterial counts was not statistically significant and is in consonance with other studies (25–27). PUI is expected to enhance bacterial elimination in recesses of oval and flattened canals, ramifications, or areas of isthmus. However, the sampling method with paper points only provides information about the bacteriologic conditions of the main root canal. Thus, our findings along with others (25–27) allow us to conclude that PUI does not significantly improve disinfection of the main canal. The *in vivo* effects of PUI in other areas of the root canal system require further investigation in a modified experimental protocol.

Despite the substantial bacterial reduction after chemomechanical procedures, 48% of the teeth in the SAF group, 32% in the TFA group, and 27% after PUI in the TFA group were still positive for bacteria. Except for 1 study, which found 100% of the cases had no bacteria after chemomechanical procedures (11), the present data are in agreement

TABLE 2. Total Bacterial Load in Root Canal Samples of Teeth with Post-treatment Apical Periodontitis Taken before (S1) and after Chemomechanical Preparation Using 2 Instrumentation Systems (S2)

Groups	*N**	S1			S2			Mean % S1 to S2 reduction
		Mean	Median	Range	Mean	Median	Range	
Self-Adjusting File	21	1.78 E + 04	1.13 E + 03	1.00 E + 02–2.22 E+05	9.43 E + 02	0	0–1.10 E + 04	83.7
Twisted-File Adaptive	22	8.24 E + 04	1.00 E + 03	1.06 E + 02–7.80 E + 05	7.71 E + 01	0	0–3.71 E + 02	94.8
Twisted-File Adaptive + passive ultrasonic irrigation†	22	8.24 E + 04	1.00 E + 03	1.06 E + 02–7.80 E + 05	5.35 E + 01	0	0–3.19 E + 02	96.9

Data from quantitative polymerase chain reaction analysis.

*Number of cases positive for total bacteria in S1.

†In the Twisted File Adaptive group, a final step of passive ultrasonic activation was included. Therefore, S1 results from both groups are the same.

TABLE 3. Levels of Streptococci in Root Canal Samples of Teeth with Post-treatment Apical Periodontitis Taken before (S1) and after Chemomechanical Preparation Using 2 Instrumentation Systems (S2)

Groups	N*	S1 Mean	S1 Median	S1 Range	S2 Mean	S2 Median	S2 Range	Mean % S1 to S2 reduction
Self-Adjusting File	17	8.50 E + 03	8.44 E + 02	1.01 E + 02–5.14 E + 04	1.06 E + 03	1.09 E + 02	0–1.00 E + 04	85
Twisted-File Adaptive	19	9.08 E + 04	6.38 E + 02	1.02 E + 02–7.66 E + 05	6.83 E + 01	0	0–3.66 E + 02	95.4
Twisted-File Adaptive + passive ultrasonic irrigation†	19	9.08 E + 04	6.38 E + 02	1.02 E + 02–7.66 E+05	6.57 E + 01	0	0–3.02 E + 02	96.9

Data from quantitative polymerase chain reaction analysis.

*Number of cases positive for streptococci in S1.

†In the Twisted File Adaptive group, a final step of passive ultrasonic activation was included. Therefore, S1 results from both groups are the same.

with previous culture studies on retreatment, which reported bacterial persistence ranging from 23%–67% of cases (9, 10, 13). A study using nonquantitative end-point PCR found 29% of the cases were still positive for bacteria after chemomechanical preparation in retreatment cases (12). Because bacterial persistence is a risk factor for an unfavorable treatment outcome (5, 6), developing improved methods for intracanal disinfection during retreatment should be encouraged. In addition to these presence/absence data, this study also quantified bacteria after preparation. Most cases that were positive for bacteria presented counts in the order of 10^2 to 10^3 bacterial cells; 1 case from the SAF group had 10^4 cells. Quantification of bacteria may be a more important piece of information than mere presence, but the association of residual bacterial counts with treatment outcome still remains to be established.

A limitation of this and other *in vivo* studies is the sampling method using paper points, which can reveal the bacteriologic conditions only in the main root canal and the tissues in its immediate vicinity. Bacterial infection in teeth with post-treatment apical periodontitis can occur in lateral canals, isthmuses, dentinal tubules, and apical ramifications (4, 35, 36). Present in these locations, bacteria can pass unnoticed by the paper point sampling approach. Another limitation of this sampling procedure is that it is not possible to infer bacterial location by root canal segments (apical, middle, or coronal) because the paper points are placed in the entire extent of the main canal. Bacteria located in the apical canal are those directly involved with persistent disease (37).

Many studies have reported that *E. faecalis* is the most frequently detected species in canals of teeth with post-treatment apical periodontitis (3, 38, 39). However, its status as the most important pathogen associated with this condition has been questioned by many studies (2, 40, 41), including the present one. Overall, *E. faecalis* occurred in 12 of 43 samples (28%), 5 from the SAF group and 7 from the TFA group. This species has also been suggested to resist treatment procedures, but our findings revealed that *E. faecalis* was not detected in any sample taken after chemomechanical procedures in the test groups. This indicates that the treatment protocols adopted in this study were highly effective in eliminating *E. faecalis* from the root canals.

The presence and levels of streptococci before and after chemomechanical procedures were also evaluated. Streptococci were included in the analysis because this bacterial group is among the most prevalent bacterial taxa in postinstrumentation samples (12, 18, 42, 43) and retreatment cases (2, 3, 38, 39). Our findings confirmed that streptococci were highly frequent in S1 samples (overall 36/43 [84%] samples, 17 from the SAF group and 19 from the TFA group), and they were still found after instrumentation in 5 and 7 of the samples from the SAF and TFA groups, respectively.

The previous studies evaluating the antibacterial effects of treatment procedures in retreatment cases used culture (9–11, 13) or nonquantitative end-point PCR (11, 12). The qPCR approach used in this study has higher sensitivity and can detect difficult-to-culture and even as-yet-uncultivated bacteria. This permits for a more accurate evaluation of the treatment effects. The method used can detect DNA from dead cells, and this may represent both an advantage and a disadvantage. DNA from cells that recently died as a result of antimicrobial treatment can still be detected, and this may underestimate the effects of treatment (12). However, an *ex vivo* study using extracted human teeth contaminated with *E. faecalis* reported no significant differences in bacterial counts after chemomechanical preparation with NaOCl irrigation analyzed by culture or DNA-based qPCR. The possibility exists that free DNA is degraded by NaOCl and thereby made undetectable. This can also be inferred from the present results showing that most cases that were positive for bacterial DNA in S1 were negative in S2 or S2b. The present findings are in line with previous clinical studies that also

TABLE 4. Levels of *Enterococcus faecalis* in Root Canal Samples of Teeth with Post-treatment Apical Periodontitis Taken before (S1) and after Chemomechanical Preparation Using 2 Instrumentation Systems (S2)

Groups	N*	S1 Mean	S1 Median	S1 Range	S2 Mean	S2 Median	S2 Range	Mean % S1 to S2 reduction
Self-Adjusting File	5	4.47 E + 04	2.66 E + 03	6.65 E + 02–2.12 E + 05	0	0	0	100
Twisted-File Adaptive	7	8.63 E + 03	6.30 E + 03	3.43 E + 02–3.05 E + 04	0	0	0	100
Twisted-File Adaptive + passive ultrasonic irrigation†	7	8.63 E + 03	6.30 E + 03	3.43 E + 02–3.05 E + 04	0	0	0	100

Data from quantitative polymerase chain reaction analysis.

*Number of cases positive for *Enterococcus faecalis* in S1.

†In the Twisted File Adaptive group, a final step of passive ultrasonic activation was included. Therefore, S1 results from both groups are the same.

used qPCR to analyze the antibacterial effects of chemomechanical preparation with NaOCl irrigation (18, 44).

In conclusion, the test treatment protocols using the SAF system or TFA instruments with or without PUI were highly effective and statistically similar in reducing the bacterial populations during endodontic retreatment of teeth with apical periodontitis. PUI did not succeed in significantly enhancing disinfection. Some cases still harbored residual bacteria in counts that are as yet unknown to be sufficient to cause persistent infection and disease. If the goal of endodontic retreatment is to attain complete bacterial eradication from the root canal, development of strategies and substances to improve disinfection should be encouraged.

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

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