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ORIGINAL ARTICLE

Evaluation of Bacterial Reduction at Various Stages of Endodontic Retreatment After Use of Different Disinfection Regimens: An *In Vivo* Study

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ABSTRACT

Objective: The present study was conducted to evaluate the presence of aerobic bacteria, anaerobic bacteria, *E. faecalis*, *F. nucleatum, Propionibacteria* sp., *Actinomyces* sp., and their reduction at various stages of endodontic retreatment with the use of conventional protocol (5.25 % Sodium Hypochlorite (NaOCl) as the irrigant along with Calcium Hydroxide ($Ca(OH)_2$) as intracanal medicament and advocated protocol (SmearOFF as the irrigant along with 2% Chlorhexidine (CHX) gel as intracanal medicament).

Methods: Twenty eight patients fulfilling the eligibility criteria were selected for root canal retreatment and randomly allocated into two groups. Group 1: Final irrigant as SmearOFF+Chlorhexidine 2% gelas intracanal medicament (n=14). Group 2: Final irrigant as 5.25% NaOCl+$Ca(OH)_2$ as intracanal medicament (n=14). With aseptic environment, access opening was performed followed by Gutta Percha (GP) removal and sample S1 was collected for bacterial analysis. The biomechanical preparation was done by using Reciproc system with additional finishing with XP-Endo Finisher R. Sample S2 was then collected for bacterial analysis after the final irrigation protocol in the respective groups. Intracanal medicaments were placed for one week and sample S3 was collected. All the samples were subjected to qualitative analysis using PCR and quantification was done by Colony Forming Unit (CFU) analysis.

Results: Aerobic [28/28], Anaerobic [28/28], Propionibacterium sp. [20/28] and *F. nucleatum* [24/28] were the most frequently isolated in S1 sample followed by *Actinomyces* sp. [16/28] and *E. faecalis* sp. [19/28]. Chemico-mechanical preparation followed by irrigation (S2 sample) resulted in significant reduction of all types of bacteria in both groups. Group-1 (SmearOFF as the final irrigant) had significantly superior efficacy against aerobic bacteria, *E. faecalis* and *F. nucleatum* ($P<0.05$) as compared to Group-2 (NaOCl). After medicament placement, significant differences between the groups were noted only for the *E. Faecalis* group. For the S3 samples, the mean bacterial reduction was significant in Aerobic and *F. nucleatum* in S3 samples for Group 1 and Group 2.

Conclusion: Chemico-mechanical preparation followed by irrigation resulted in significant reduction in bacterial load irrespective of the final irrigant. SmearOFF was significantly better than NaOCl in minimizing bacterial load of *E. faecalis* and *F. nucleatum*. 2% Chlorhexidine gel has superior antimicrobial efficacy against *E. faecalis* and may be recommended in secondary endodontic treatment.

Keywords: Chlorhexidine, endodontic microbiology, endodontic retreatment, SmearOFF

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HIGHLIGHTS

- Instrumentation with Reciproc followed by XP Endo Finisher R along with irrigant activation with PUI device results in significant reduction of bacterial load.
- Irrigation with SmearOFF lead to a significant reduction in *E. Faecalis* and *Fusobacterium* species and can be recommended in secondary endodontic therapy.
- 2% CHX gel may be advisable over $Ca(OH)_2$ in retreatment cases.

INTRODUCTION

An individual infected root canal system harbours an endodontic microbial community composed of many bacterial microcolonies whose interaction plays a crucial role in ecological balance and establishment of bacterial community (1). The persistence of these microorganisms causes intraradicular or extraradicular infections and lead to endodontic treatment failure (2).

Molecular technology methods have refined our knowledge on root canal biodiversity and have identified several other bacterial species to be majorly linked with post treatment apical periodontitis (3). Earlier studies reported *E.*

faecalis to be predominantly implicated in failure of root canal treatment (4, 5). However, recent studies have raised a question for *E. faecalis* being the main pathogen in secondary endodontic treatment. Several other species have been reported in various studies including *Actinomyces* sp, *Prevotella* sp and *F. nucleatum* (6).

Success of secondary root canal treatment depends on complete sealer and gutta percha removal (7). Supplementary instrumentation has been proposed to aid in the removal of the obturatigmaterial (8). Recently, XP-Endo Finisher R (FKG Dentaire) file, manufactured through an innovative Maxwire Technology, having a unique design has been introduced and proposed to improve the effectiveness in touching and displacing root canal filling materials which may be difficult to access with conventional instrumentation (9).

Adequate disinfection is crucial for success in root canal therapy. Till date, no single solution alone has completely removed the organic and inorganic parts of the smear layer. Incomplete eradication of microbiota from the root canals was observed using Sodium hypochlorite (NaOCl) irrigant (10). SmearOFF is a novel irrigant composed of Ethyelene Diamine Tetra acetic acid (EDTA) and chlorhexidine along with a detergent. According to the manufacturers, EDTA present in SmearOFF is in lower concentrations, which is less aggressive on the dentine and the irrigant is claimed to have antibacterial properties (11). As SmearOFF is a recent irrigating solution, not much of literature is currently available regarding its *in vivo* antibacterial efficacy and hence was evaluated in the present study.

The use of intracanal medicament has been advocated to enhance the outcome of root canal treatment. Some studies have reported that use of calcium hydroxide (Ca $(OH)_2$) may deteriorate the mechanical properties of the root dentine (12). Also its antibacterial efficacy against *E. faecalis* is in comparison to Chlorhexidine (CHX) (13).

To our knowledge, very few *in vivo* studies (Rodriques et al. (14) (2015), Zandi et al. (15) (2016), Rodriques et al. (16) (2017), Zandi et al. (17) (2019)) have evaluated the bacterial load reduction at various retreatment stages. Also, it is important to find an effective protocol that minimizes the bacterial load in the root filled canal. Therefore, the present double blinded *in vivo* study aimed to evaluate the presence of aerobic bacteria, anaerobic bacteria, *E. faecalis, F. nucleatum, Propionibacteria* sp., *Actinomyces* sp., in retreatment cases with the use of conventional protocol (5.25 % sodium hypochlorite ((NaOCl)+Calcium hydroxide (Ca $(OH)_2$) and advocated protocol (2% Chlorhexidine (CHX) gel). Furthermore, the study evaluated the individual bacterial reduction as well as the total overall reduction at various stages of endodontic retreatment with the above mentioned regimens.

MATERIALS AND METHODS

The study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with Centre for Advanced Research. The study design was approved by the internal and external ethical review board under the protocol number ITS CDSR/IIEC/2018-20/CONS/03. A written informed consent was obtained from all participants before the start of the study.

Sample Size (n) was calculated using the formula:

n=(Zα/2+Zβ)2 *2*σ2/d2,

where:

Zα/2 is the critical value of the Normal distribution at α/2 (e.g. for a confidence level of 95%

α is 0.05 and the critical value is 1.96)

Zβ is the critical value of the Normal distribution at β (e.g. for a power of 80%

β is 0.2 and the critical value is 0.84)

σ2 is the population variance

d is the difference to be detected

Sample Size was calculated as 28.

Criteria for selection of patients:

1. ***a. Inclusion criteria:***

- Single root filled teeth indicated for root canal retreatment as assessed with clinical and radiological signs.(PAI Score 1-4) (14).
- Root canal treatment completed for more than 2 years.

b. Exclusion criteria:

- Subjects with history of antibiotic treatment usage in the last 3 months.
- Patients reporting with systemic disease starting with ASA Grade 3.
- Teeth in which rubber dam application is not feasible (15).
- Teeth with pocket depth greater than 3 mm (15).

Randomization of teeth with Final Irrigation Regimens

The twenty eight infected single-rooted teeth diagnosed as post-treatment apical periodontitis were randomly divided into two treatment Groups (14 each) according to the various irrigation and intracanal medicament regimens. Randomization was achieved using sealed envelope into the following groups:

a. Group 1 (n=14)- Final irrigant as SmearOFF (Vista Dental Products, USA)+CHX (2% Gel, Cerkamed Medical Co. Poland) as intracanal medicament.

b. Group 2 (n=14)- Final irrigant as 5.25% NaOCl (Coltene, Switzerland) followed by Calcium Hydroxide (Apexcal, IvoclarVivadent, Schaan, Liechtenstein) as intracanal medicament.

Endodontic clinical procedures and sample collection

- Rubber dam isolation was done following which the crown and adjoining structures were disinfected thoroughly with 30% (v/v) hydrogen peroxide for 30 seconds followed by 2.5% NaOCl and then further inactivated with 5% sodium thiosulfate (8). Disinfection of the external crown surfaces was checked by incubating the crown swab sample on blood agar plate.

- Local anesthesia (2% lidocaine with 1:100,000 epinephrine) was administered and access cavity preparation was done with sterile high-speed diamond burs. This was followed by disinfection of access cavity as mentioned previously.

A sterile Headstrom file was used to engage the the Gutta Percha cone which was then removed with a sterile plier. The same was transferred to an Eppendorf tube containing PBS (Phosphate buffer solution) (sample S1) as a transport media. Root Dentine chips were obtained using a #25 Hedstroem files stroked circumferentially along the root canal wall (2 strokes against each wall). Thereafter, 4 sterile saline moistened paper points were consecutively pressed against the canal walls for 1 min and put into the same Eppendorf tubes containing gutta-percha (16). The samples were then transported to Advanced Research Lab within 15 minutes of the procedure to determine the bacterial load. The samples were preserved at -20°C for bacterial analysis.

Working length was estimated using apex locators and cross checked radiographically. The biomechanical preparation was done by using Reciproc R25 followed by R 40files (VDW, Munich, Germany) in 3 pecking motions and then the instrument was removed from the canal and cleaned. XP-Endo Finisher R (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) was introduced as a final step in improving root canal cleaning. The instrument was brushed gently against the walls of the canals at a speed of 800 rpm and torque of 1 Ncm for 60 seconds.

In Both Group 1 and 2, irrigation was done with 3% NaOCl for five minutes followed by normal saline. Smear layer removal was done using 17% EDTA for 60 seconds which was followed by saline irrigation.

The Final irrigation protocol was as follows:

Group-1: Final irrigation using 5ml of SmearOFF for 1 minute with Passive ultrasonic irrigation activation (PUI) activation.

Group-2: Final irrigation of 5ml of 5.25% NaOCl for 1 minute with PUI activation.

To neutralize CHX after the final rinse of SmearOFF, each canal was dried using sterile paper points and treated with 3% Tween 80 (3 ml) and 0.3% lecithin for 5 min followed by 5 ml of saline irrigation (17).

To neutralize NaOCl, the canals were dried and flushed with 5% sodium thiosulfate. S2 samples were taken by the same procedure from the root canal as taken previously in S1 sample. This was followed by placement of intracanal medicament.

In Group-1: CHX gel (Gluco-Chex 2% Gel, Cerkamed Medical Co. Poland) was placed along the root canal length using lentulo spiral #40.

In Group 2: Apexcal (IvoclarVivadent, Schaan, Liechtenstein) was placed over the entire length of the prepared root canal. The access cavity was then sealed with intermediate GIC Type II (G-Coat [GC]. All the Patients were recalled after 7 days.

After 7 days, permanent restoration was removed which was followed by neutralization of the medicament. For Group-1, to neutralize CHX, 3% Tween 80 (3 ml) and 0.3% a lecithin were used as shown previously followed by saline irrigation (18). For Group-2, $Ca(OH)_2$ was inactivated with 5 ml of 10% citric acid followed by saline (volume=5 ml/canal) (19). S3 samples were then obtained in the same manner as described previously.

Genomic analysis

DNA isolation

Following the standard protocol of DNA isolation, High molecular weight DNA isolation was done by using Genomics DNA Purification kit (HiPurA). The extracted genomic DNA was quantified and checked for purity using a UV Transilluminator (Spectro Ultraviolet-Visible Double Beam PC, UVD Model 2950; LABOMED, Inc., Culver City, CA, USA). Presence of DNA in the samples was evaluated by Ethidium bromide 1% agarose gel electrophoresis.

Qualitative analysis was done using a Conventional Polymerase chain Reaction

Polymerase chain reaction (PCR) amplification was performed for checking the presence of aerobic and anerobic bacteria using consensus degenerate primers. Hi-Chrome master mix was thawed at the room temperature. Master mix was vortexed and then spinned briefly in a microcentrifuge to collect the material at the bottom of the tube. Electrophoresis of PCR product was done on a 1% gel.

The following primer sequence were used for different bacteria (Table 1):

Microbiological analysis

Quantitative analysis of all the PCR positive samples was done by culturing the samples and calculating colony forming unit by using electronic colony counter.

Sterile plastic spreaders were used to plate fifty microlitres of serial dilutions into 5 % defibrinated sheep blood fastidious anaerobe agar (HI-Media, USA) to determine the total load of aerobic bacteria, anaerobic bacteria, *E. faecalis, F. nucleatum, Propionibacterium* and *Actinomyces*. One plate of sample was incubated for 2-4 days at 37°C under aerobic conditions for aerobic bacteria, under 5% to 10% carbon dioxide (CO_2) atmosphere for aerobic/anaerobic facultative bacteria. Similar identical plates were incubated for 5 to 9 days at 370c under strict anaerobic conditions (N2 85%, H 25%, CO_2 10%) using anaerobic gas jar and gas pack for anaerobe obligate and facultative bacteria. The plates were then incubated and the Colony-Forming Units (CFUs) were visually quantified for each plate.

The results were obtained using the formula:

CFU/ml (10-1)=No. of colonies x dilution factor / Vol. of culture plate.

TABLE 1. Primer Sequence of bacteria used in the study

Bacteria	Primer Sequence
E. faecalis	GTTTATGCCGCATGGCATAAGAGCCGTCAGGGGACGTTCAG
P. propionicum	CTGTAAACCGACCAAAAAGG
Actinomyces	TGGGCCGGCTGCTCCTGGA
F. nucleatum	AGAGTTTGATCCTGGCTCAGGTCATCGTGCACACAGAATTGCTG

Statistical analysis

The data obtained was systematically organized in the form of a master table on Microsoft Excel. Descriptive statistics of mean±SD of all parameter were calculated for all variables.

- The number of positive cultures were evaluated and represented graphically.
- Mann-Whitney Test was used to test the difference in the efficacy of the two disinfection regimens (intergroup comparison).
- Wilcoxon Signed Ranks Test was used to test the difference in reduction at each phase of disinfection protocol (intragroup comparison).

The statistical analysis was done using Statistical Package for Social Sciences (IBM SPSS Inc., version 22.0, Chicago, IL, USA). P value less than 0.05 was considered statistically significant.

RESULTS

All samples were analysed qualitatively for presence or absence of the bacterial species using PCR analysis. The number of positive cultures of Aerobic, Anaerobic, *E. faecalis, Propionibacteria* sp., *Actinomyces* sp. , *F. nucleatum* were quantitatively analysed after use of Disinfection Protocols at various stages of root canal retreatment for both the groups is depicted in Figure 1 and 2. Aerobic [28/28], Anaerobic [28/28], Propionibacterium sp. [20/28] and *F. nucleatum*[24/28] were the most frequently isolated in S1 sample followed by *Actinomyces* sp. [16/28] and *E. faecalis* sp. [19/28]. The mean bacterial reduction was significant (P<0.05) in Aerobic, Anaerobic, *E. faecalis, Propionibacteria* sp., *Actinomyces* sp., *F. nucleatum* in S2 samples in Group 1 (SmearOff) and Group 2 (NaOCl) (Table 2). The mean bacterial reduction was significant in Aerobic, *E. faecalis* and *F. nucleatum* in S3 samples in Group 1 and Group 2 (Table 2). Inter group comparison revealed significant difference (P<0.05) in bacterial reduction after biomechanical preparation between Group 1 and Group 2 for *E. faecalis* and *F. nucleatum*. After medicament placement, significant differences (P<0.05) between the groups were noted only for the *E. Faecalis* group in which the final irrigant as 2 in 1 mix of EDTA and Chlorhexidine as intracanal medicament (Table 3).

DISCUSSION

Endodontic infection is polymicrobial in nature and a community of multispecies have been found responsible in failed treatment (20-22). *E. Faecalis*, *Fusobacterium nucleatum*, *Propionibacterium propionicum* and *Actinomyces* have been commonly isolated in teeth with post treatment periodontitis and were therefore evaluated in the present study at various stages of endodontic retreatment (Initial Samples [S1], Samples after biomechanical Preparation [S2], Samples after medicament placement [S3]) (23, 24).

For the S1 sample, all the 28 samples were positive for aerobic as well as anaerobic bacteria. *Propionibacterium* sp. and *Fusobacterium* sp. were most frequently isolated followed by Actinomyces, and *E. faecalis*. These findings could be corrob-

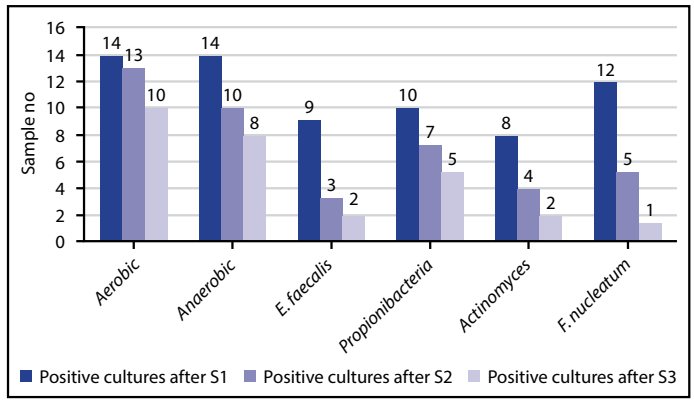

Figure 1. Graph depicting the number of positive cultures after use of Disinfection Protocols in Group -1 (2 in 1 mix EDTA+Chlorhexidine) and Chlorhexidine

EDTA: Ethyelene Diamine Tetra acetic acid.

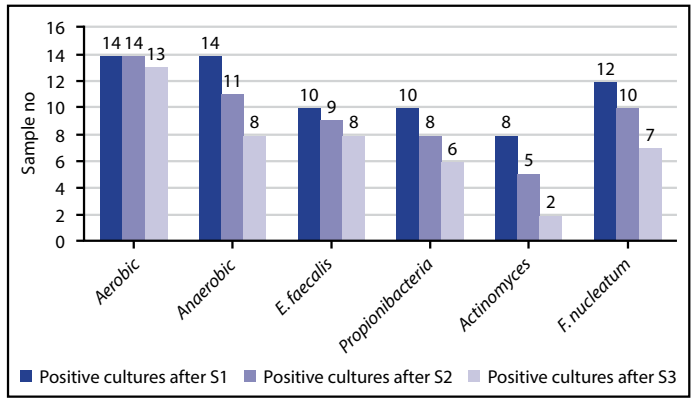

Figure 2. Graph depicting the number of positive cultures after use of Disinfection Protocols in Group -2 (Sodium Hypochlorite+Calcium Hydroxide)

orated with the study by Rôças et al. (25) in which the status of *E. Faecalis* as the major pathogen has been questioned and other potential species (*Propionibacterium* and *Fusobacterium*) have been majorly identified to cause persistent infection. This could also suggest the ecological diversity present within the root canal system.

The study by Pereira et al. (26) evaluated the presence of microbial population in different portions of the root canal system and reported the presence of more facultative organisms in the coronal portion and strict anaerobes in the apical third. The authors concluded that the growth of particular bacterial species depends on the root canal environment and the gaseous conditions. Hence, the variability reported could be attributed to the method of sample collection which were collected more homogenously in our study. The present study used dentin shavings (obtained after filing with H files) that were sampled from the root canal using sterile paper points. This is in contrast to other studies (27-29), in which paper point sampling method has been solely used and therefore it becomes difficult to standardize the samples obtained.

Studies have shown that after biomechanical preparation eradication of bacteria from root canals ranges from 80% to 95% (30, 31). In our study, when S2 samples were analyzed,chemo-

TABLE 2. Intra-group comparison of bacterial reduction (%) at various stages of root canal treatment in Group 1- [final irrigant as SmearOFF+ 2%Chlorhexidine gel) as intracanal medicament, Group 2- (final irrigant as 5.25 % Sodium Hypochlorite+Calcium Hydroxide as intracanal medicament)

Bacteria	Treatment phase	Gp-1 Smearoff+CHX (Mean±SD)	Overall reduction % (Mean±SD)	P	GP-2 NaOCl+ $Ca(OH)_2$ (Mean±SD)	Overall reduction% (Mean±SD)	P
Aerobic	S1	911.3±643.4	-	-	818.5±407.1	-	-
	S2	111.6±86.3	87.4±6.1	0.001*	111.5±50.8	82.0±18.5	0.001*
	S3	52.3±54.62	95.1±4.4	0.016*	58.0±38.9	92.7±4.0	0.013*
Anaerobic	S1	651.9±543.7	-	-	705.5±764.0	-	-
	S2	58.7±70.3	90.0±10.0	0.005*	86.7±108.5	84.6±18.1	0.003*
	S3	12.0±21.1	98.3±2.0	0.139	24.0±32.9	94.9±9.6	0.328
E. Faecalis	S1	105.5±96.9	-	-	164.0±180.2	-	-
	S2	4.7±11.2	69.0±45.5	0.007*	76.0±92.9	39.3±27.4	0.646
	S3	0.29±0.7	71.2±46.7	0.003*	30.7±35.9	58.2±38.7	0.008*
Propionibacteria	S1	296.2±356.7	-	-	323.5±386.8	-	-
	S2	30.5±47.8	85.4±28.2	0.028*	45.7±50.2	74.9±32.5	0.012*
	S3	15.9±27.8	88.2±28.5	0.116	25.6±35.2	88.3±20.1	0.208
Actinomyces	S1	138.5±236.0	-	-	174.4±258.6	-	-
	S2	19.2±39.1	90.3±16.0	0.068	13.2±33.0	83.2±28.2	0.043*
	S3	4.79±12.7	97.2±7.0	0.715	2.2±8.2	99.3±1.6	0.225
F. Nucleatum	S1	95.3±110.8	-	-	115.3±125.6	-	-
	S2	7.29±14.5	92.4±12.9	0.043*	34.2±38.5	59.6±31.7	0.005*
	S3	0.57±2.1	99.2±2.3	0.043*	7.4±14.3	84.4±6.2	0.017*

EDTA: Ethyelene Diamine Tetra acetic acid.

TABLE 3. Intergroup comparison of bacterial reduction after use of different disinfection regimens. Group 1- [final irrigant as SmearOFF and 2% Chlorhexidine gel as intracanal medicament. Group 2- (final irrigant as 5.25 % Sodium Hypochlorite + Calcium Hydroxide as intracanal medicament)

Bacteria	Group	S1	S2	S3	S1-S2	P	S1-S3	P
Aerobic	GP-1	911.3±643.4	111.6±86.3	52.3±54.62	87.4±6.1	0.667	95.1±4.4	0.164
	GP-2	818.5±407.1	111.5±50.8	58.0±38.9	82.0±18.5		92.7±4.0	
Anaerobic	GP-1	651.9±543.7	58.7±70.3	12.0±21.1	90.0±10.0	0.667	98.3±2.0	0.376
	GP-2	86.7±108.5	86.7±108.5	24.0±32.9	84.6±18.1		94.9±9.6	
E. faecalis	GP-1	102.6±106.2	1.84±5.6	0.07±0.2	98.2±13.4	0.021*	99.9±4.4	0.027*
	GP-2	111.5±170.9	42.2±39.3	7.37±24.7	62.1±28.6		93.3±6.3	
Propionibacteria	GP-1	296.2±356.7	30.5±47.8	15.9±27.8	85.4±28.2	0.254	88.2±28.5	0.456
	GP-2	323.5±386.8	45.7±50.2	25.6±35.2	74.9±32.5		88.3±20.1	
Actinomyces	GP-1	138.5±236.0	19.2±39.1	4.79±12.7	90.3±16.0	0.573	97.2±7.0	0.762
	GP-2	174.4±258.6	13.2±33.0	2.2±8.2	83.2±28.2		99.3±1.6	
F. nucleatum	GP-1	95.3±110.8	7.29±14.5	0.57±2.1	92.4±12.9	0.007*	99.2±2.3	0.069
	GP-2	115.3±125.6	34.2±38.5	7.4±14.3	59.6±31.7		84.4±6.2	

*: Significant difference.

mechanical preparation was effective in significantly reducing the bacterial levels. This could be a synergistic effect of GP removal system (Reciproc system followed by XP Endo Finisher R) and the effects of irrigants which were activated with PUI. The use of reciprocating file system is more efficient in removal of filling materials than the rotary systems (31). Micro CT study by Silva et al. (32) reported that XP Endo Finisher R touches 59.4% in volume and 61.4% of the root canal surface area and effectively removes gutta-percha. Furthermore, the core diameter and tip angulation of XP - Endo Finisher R makes it the instrument of choice in retreatment cases. The overall reduction was in the range between 71- 99% for Group-1 and 39-99% for Group 2 and these findings are congruent with the culture studies on endodontic retreatment reporting bacterial persistence between 23-67% (33). However, literature is scanty to compare the results obtained with previous data. Considering the complex consortium of bacterial species isolated from failed root canals, the bacterias taken into account in our study have not been studied previously in an *in vivo* setups and therefore, direct comparison cannot be made.

The reduction achieved with SmearOFF was higher than NaOCl in all the bacterial groups, although the difference was statistically significant only for *E. faecalis* and F. nucleatum. Thesuperior antibacterial efficacy of SmearOFF could be attributed to its ability to disrupt the interactions involved in cross-linking the biofilm matrix and disrupting the cohesive forces of the extracellular matrix of the bacteria which increases its membrane permeability (34). The composition contains EDTA

that helps in removal of smear layer, detergent that reduces the fluid viscosity and surface tension of the irrigant leading to better antibacterial efficacy (34, 35).

The superior antimicrobial efficacy of SmearOFF against *F. nucleatumas* compared to NaOCl is in similar to the research by Ozok AR et al. (36) and can be attributed to the fact that *F. Nucleatum* coaggregates to each other and other bacteria which promotes their growth as a biofilm and makes it less susceptible to the action of hypochlorite. This is due to the extreme reactivity and faster consumption of the irrigant which reduces its efficacy in deeper layers (37). A recent systematic review by Lim et al. (38) found superior antibacterial efficacy of QMix (having similar composition to SmearOFF) against *E. Faecalis*.

After 7 days of medicament placement, the overall mean reduction achieved in Group 1 (CHX gel) (71.2-99.2%)was higher compared to Group II ($Ca(OH)_2$) (58.2-99.3 %)with respect to all bacterial populations studied. However, significant results were observed only for *E. Faecalis*. 100 % sterility was not obtained for all the samples in either of the disinfection protocols. Superior efficacy of CHX gel could be because of its lower viscosity that enables long contact time with root dentin walls (39).

The use of culture method allows for a quantitative assessment of bacteria, which is specially crucial when prompt effects of antimicrobial therapy are to be evaluated and was therefore, chosen for the present study. However, molecular techniques seems advantageous in providing precise and reliable data on the identification of bacterial species (40). A negative culture may not implicit 100% canal sterility and could be the result of the limitations of the experimental protocol. Another limitation of this design could be the fact that the samples were obtained from the main canal only while the inaccessible regions like the isthmus and accessory canals could not be reached by the sampling procedure. In addition, it is quite possible that the bacteria present in the canal may be present at levels which is below the sensitivity of the culture method and as a result were not detected (40). Also, the sample size was limited and hence, more clinical studies assessing the effect of various disinfection regimens on microbial load reduction could be the scope of future studies.

Considering the limitations of the present *in vivo* study, it may be concluded that Instrumentation with Reciproc followed by XP-Endo Finisher R and final irrigation with SmearOFF lead to a significant reduction in bacterial species and can be recommended in secondary endodontic therapy. Although, significant reduction in bacterial load after placement of intracanal medicament, their usage wouldstill be recommended. In particular, keeping in mind the high prevalence of resistant bacterial species majorly *E. faecalis* and F. nucleatum, CHX intracanal medicament gel should be advised over $Ca(OH)_2$ in retreatment cases.

CONCLUSION

There was significant reduction in bacterial load of Aerobic bacteria, Anaerobic bacteria, *E. faecalis*, Propionibacteria sp., Actinomyces sp., F. nucleatum after chemomechanical preparation with reciprocating kinematics for GP removal combined with supplemental instrumentation with XP Endo Finisher along with irrigant activation with PUI device and this protocol may be recommended for retreatment cases. SmearOFF as the final irrigant caused significantly more reduction in bacterial load for *E. faecalis* and F. nucleatum in comparison to NaOCl.2 % CHX gel may be advisable in retreatment cases as it was reported to besignificantly effective against *E. faecalis*.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The ITS-CDSR Ethics Committee (Date: 10/04/2018, Number: CDSR/IIEC/2018-20/CONS/03).

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