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Clinical efficacy of different irrigation protocols in teeth with chronic apical periodontitis: randomized clinical trial – a pilot study

Клиничка ефикасност различитих иригационих протокола код зуба са хроничним апикалним периодонтитисом: рандомизовано клиничко испитивање – пилот студија

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SUMMARY

Introduction/Objective Effective disinfection of the root canal system is essential for successful endodontic treatment. This study aimed to determine the most effective final irrigation protocol.

Methods This randomized clinical trial investigated the antimicrobial efficacy of three irrigation protocols in 30 patients with asymptomatic apical periodontitis in single-rooted teeth. The groups were: (1) 2.5% sodium hypochlorite (NaOCl) + 17% EDTA; (2) NaOCl + EDTA + 2% chlorhexidine (CHX); and (3) the same as Group 2 with sonic agitation (EndoActivator). Microbial samples were collected at three intervals and analyzed using endpoint and real-time PCR targeting seven key endodontic pathogens.

Results Group 3 demonstrated the greatest bacterial reduction across all time points, with CHX-containing protocols significantly outperforming NaOCl + EDTA alone. Sonic activation further enhanced irrigant efficacy, notably reducing *P. gingivalis*. Despite improvements, complete bacterial eradication was not achieved, and *E. faecalis* and *F. nucleatum* remained persistent.

Conclusion While limitations in microbial sampling and profiling exist, the results underscore the importance of combining chemical agents and mechanical activation to manage complex root canal infections effectively.

Keywords: endodontics; irrigation protocols; PCR; clinical trial

САЖЕТАК

Увод/Циљ Ефикасна дезинфекција система канала корена кључна је за успешан ендодонтски третман. Циљ ове студије био је да се утврди најефикаснији коначни протокол испирања канала корена зуба.

Методе У овом рандомизованом клиничком испитивању испитивана је антимикробна ефикасност три различита иригациона протокола код 30 пацијената са асимптоматским апикалним периодонтитисом једнокорених зуба. Групе су биле следеће: (1) 2,5% натријум-хипохлорит (*NaOCl*) + 17% *EDTA*; (2) *NaOCl + EDTA*+ 2% хлорхексидин (*CHX*); и (3) исто као група 2, уз додатак соничне активације (*EndoActivator*). Микробни узорци су прикупљени у три фазе и анализирани помоћу PCR методе (класичне и *real-time*) са циљем детекције седам кључних ендодонтских патогена.

Резултати Група 3 је показала највеће смањење бактеријског присуствау свим фазама, док су протоколи са *CHX* били значајно ефикаснији од комбинације *NaOCl+ EDTA*. Сонична активација додатно је побољшала деловање ириганаса, посебно против *P. gingivalis*. Ипак, потпуна ерадикација бактерија није постигнута, а *E. faecalis* и *F. nucleatum* су остали перзистентни.

Закључак Резултати указују на значај комбинације хемијских агенаса и механичке активације у дезинфекцији комплексних система канала.

Кључне речи: ендодонција; протоколи иригације; *PCR*; клиничка студија

INTRODUCTION

Achieving effective infection control is the primary objective of root canal treatment, as bacteria play a critical role in the development of pulpal and periapical diseases [1]. Due to the complex anatomy of root canals, complete bacterial elimination is nearly impossible [2]. Therefore, endodontic therapy aims to reduce the microbial load to levels that allow periapical healing [1]. This necessitates thorough chemo-mechanical preparation, combining instrumentation with copious irrigation to remove infected tissue and debris [2].

An ideal irrigant would lubricate instruments, dissolve pulp tissue, eliminate the smear layer, and penetrate complex canal areas—while being antimicrobial, non-toxic, and preserving tooth

structure [3]. Since no single irrigant meets all the necessary criteria, it is common to use combinations of irrigants to enhance effectiveness. Sodium hypochlorite (NaOCl) is the most common irrigant due to its strong antimicrobial and tissue-dissolving properties; however, it is highly toxic if extruded beyond the apex [4]. Chlorhexidine digluconate (CHX), while unable to dissolve organic tissue, offers broad-spectrum antibacterial activity, lubrication, and lower cytotoxicity [5]. Ethylenediaminetetraacetic acid (EDTA) aids in smear layer and inorganic debris removal but has limited antibacterial properties [5]. When used together, these irrigants can effectively address each other's limitations.

Irrigation techniques include syringe irrigation, ultrasonic activation, and sonic agitation [1]. Activation methods enhance the effectiveness of irrigants, particularly in complex anatomies, by improving smear layer removal and bacterial reduction [1]. Newer systems employing plastic rotary instruments apply similar principles [6].

Given the ongoing issues in the field of Endodontology, this study aimed to determine the most effective final irrigation protocol for reducing bacterial presence in root canals after chemo-mechanical preparation.

METHODS

Patient selection and eligibility criteria

A single-blinded, two-arm randomized clinical trial, in the form of a pilot investigation, evaluated the effectiveness of three final irrigation protocols in eliminating root canal bacteria after chemo-mechanical preparation. One clinician with three years of endodontic experience treated 30 patients (mean age 51.2), with asymptomatic apical periodontitis at the Department of Dental Medicine, Faculty of Medicine, University of Novi Sad (April–December 2022). Patients had single-rooted teeth with necrotic pulp and carious lesions. Exclusion criteria included prior endodontic treatment, periodontal pockets >4 mm, active orthodontic treatment, fractures, or recent antibiotic use. Data analysis was blinded. All patients were thoroughly informed about the procedures involved, and written consent was obtained along with an assent document from every participant in this study.

Sample collection and root canal treatment procedures

Samples were collected under strict aseptic conditions. After scaling and cleaning with pumice, a rubber dam was applied, and caries or prior restorations were removed under saline irrigation. The tooth was disinfected with 3% hydrogen peroxide, followed by 2.5% sodium hypochlorite (NaOCl) (i-dental, Šiauliai, Lithuania), and then neutralized with 10% sodium thiosulfate ($Na_2S_2O_3$) (Centrochem, Stara Pazova, Serbia). These steps were repeated during access cavity preparation to prevent contamination. A sterile paper point (Dentsply Maillefer, Ballaigues, Switzerland) was carefully inserted without contacting canal walls [7], left for 60 seconds, and stored in a sterile 1.5 ml tube labeled S1, then frozen at -20°C.

Working length was determined using an apex locator (Propex, Dentsply Maillefer, Ballaigues, Switzerland) and radiographs. The canal was shaped with endodontic rotary instruments (ProTaper Next, Dentsply Maillefer, Ballaigues, Switzerland) and irrigated with 9 ml of 2.5% NaOCl. Upon reaching the working length, one of three randomized irrigation protocols was applied. A second sterile paper point (S2) was inserted 1 mm short of working length for 60 seconds, then stored as described.

A sterile cotton pellet and temporary filling were placed (Orafil-G, Prevest DenPro, India). After 48 hours, the tooth was disinfected again, and the temporary restoration removed. Sampling was repeated (S3). The assigned irrigation protocol was reapplied, the canal dried, and calcium hydroxide medication placed (i-CAL, i-dental, Šiauliai, Lithuania).

Ten days later, if asymptomatic, the medication was removed and the canal obturated with gutta-percha and sealer. If symptoms persisted, the procedure was repeated. A permanent filling was placed 24 hours post-obturation [8].

Irrigation protocols

All irrigation protocols were performed using 5 mL Luer-lock syringes and closed-ended endodontic needles (i-TIPS, i-dental, Šiauliai, Lithuania), and included sterile saline irrigation between irrigants, to prevent mixing of different irrigants and subsequent chemical reactions between them.

Group 1 *(NaOCl, EDTA)*: During treatment, the root canal was rinsed with 9 ml total amount of 2.5% NaOCl. Upon treatment completion, the canal was rinsed with 6 mL of 2.5% NaOCl and 3 ml of 17% EDTA (Cerkamed, Stalowa Wola, Poland), alternately applying 2 mL of NaOCl and 1 mL of EDTA, until the entire amount of both irrigants was consumed. Next, 1

mL of 5% $Na_2S_2O_3$ (Centrochem, Stara Pazova, Serbia) was added to the canal to inactivate the remaining NaOCl.

Group 2 *(NaOCl, EDTA, CHX)*: The previously explained irrigation protocol (Group 1) is enriched with the application of a 2% chlorhexidine (CHX) solution. The irrigation commenced with a combination of 6 ml of 2% CHX (Cerkamed, Stalowa Wola, Poland) and 3 ml of 17% EDTA, alternately applying 2 ml of CHX and 1 ml of EDTA until both irrigants were consumed. Finally, the canal was flushed with 1 ml of 10% $Na_2S_2O_3$ to inactivate the remaining amount of CHX.

Group 3 *(NaOCl, EDTA, CHX + endoactivation)*: Same as Group 2, but with sonic activation using the Endoactivator® (30 s per irrigant). Activation enhanced irrigant efficacy. Tip size matched prepared canal diameter, one size smaller, operating at 2–3 kHz [9].

Polymerase chain reaction (PCR) analyses

The collected intracanal fluid samples (S1−S3) were used for qualitative and quantitative analysis of the bacterial communities present in the root canal system.

Bacterial DNA was extracted by heating the samples in a 300 µl of 50 mM NaOH solution in a thermoblock (Biosan TS100C, Riga, Latvia) at 95 °C for 5 minutes. After heating, 30 µl of a 1 M tris-(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl) solution (pH 8) was added to the samples to stabilize and preserve the isolated DNA molecules. Isolated DNA samples were stored at -20 °C until further analyses [10].

A qualitative analysis of the bacterial composition of the root canal system was performed using the *end-point* PCR method with specific primers, allowing parts of the DNA molecule that are specific for each tested bacterium to be multiplied (Figure 1). The presence of the following bacteria was assessed: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Porphyromonas endodontalis* (Pe), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn), *Parvimonas micra* (Pm), and *Enterococcus faecalis* (Ef). Five microliters of bacterial DNA were resuspended in 20 µl of aqueous mixture containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U of DNA polymerase (all products from Thermo Fisher Scientific™; Waltham, MA, USA) and 0.2 µM of specific primers and used for DNA amplification on thermal cycler (PeqSTAR 2X®, Peqlab, Erlangen, Germany). The PCR started with initial denaturation step (95 °C for 3 min) followed by 35 cycles of denaturation (95 °C for 45 s), annealing (55 °C for 1 min) and elongation (72 °C for 1 min) steps, and final elongation step (72 °C for 5

min). PCR products were separated on a polyacrylamide gel using vertical electrophoresis system (PerfectBlueTM Twin S system, Peqlab, Erlangen, Germany) and visualized by staining with a 1% ethidium bromide solution on a UV-transilluminator (TCX-15 MX®, Vilber Lourmat, Collégien, France). The list of primer sequences and size of PCR products is given in Table 1.

Real-time PCR was used for the quantitative analysis of the bacterial communities present in the root canal system. This method relies on fluorescence detection and requires primers specific for highly conserved regions of the 16S ribosomal DNA of bacteria. The components of reaction mixture were following: two microliters of bacterial sample, 10 µl of PCR master mix (Luna® Universal qPCR Master Mix 2X, New England Biolabs, Ipswich, MA, USA), 1 µl of forward and reverse primer (final concentration in solution for each primer was 250 nM) and ultra-pure nuclease-free water up to 20 µl in total. The sequences of forward and reverse primer were TCCTACGGGAGCACAGT and GGACTACCAGGGTATCTAATCCTGTT, respectively. A reaction was performed on a fluorescence detection system (Line Gene-K fluorescence PCR detection system, BIOER Technology, Shanghai, China) under following conditions: initial denaturation (95 °C for 5 min), 35 cycles of denaturation (95 °C for 1 min), annealing (95 °C for 1 min) and elongation (72 °C for 1.5 min), and final elongation step (72 °C for 5 min). A standard curve, generated from seven (1:10) serial dilutions of DNA samples of the reference bacterial strain *Prevotella melaninogenica ATCC* 25845 (Microbiologics KWIK-STIK, Manassas, VA, USA) was used for extrapolation of total gene copy number (total bacterial count) [11].

Statistical analysis

Categorical data were presented as absolute numbers; numerical data as means, standard deviations, medians, and ranges. The Chi-square test compared bacterial species frequency across protocols at different time points. The Kruskal-Wallis test assessed total bacterial counts between protocols within each period. The Friedman test analyzed bacterial count changes over time within protocols; if significant, the Wilcoxon test was used for pairwise comparisons. A p-value < 0.05 indicated statistical significance. Data analysis was performed using SPSS 28.0 (IBM, USA).

Ethics: The study received ethical approval from the Faculty of Medicine, University of Novi Sad (01-39/183/1, 2022).

RESULTS

At the initial measurement, no statistically significant differences were found among the three irrigation protocols regarding bacterial prevalence. *Aggregatibacter actinomycetemcomitans (Aa)* was detected in one case per group. *Porphyromonas* gingivalis (Pg) and *Fusobacterium nucleatum (Fn)* were absent in the first protocol (NaOCl + EDTA). In the second protocol (NaOCl + EDTA + CHX), *Pg* appeared in 18.2% and Fn in 45.5% of cases. The third protocol (NaOCl + EDTA + CHX + activation) showed both *Pg* and *Fn* in 36.4% of cases. *Prevotella intermedia (Pe)* was most prevalent in the first group (37.5%), followed by 18.2% and 27.3% in the second and third groups, respectively. *Enterococcus faecalis (Ef)* was the most frequently isolated bacteria: 50% in the first group and 54.5% in both the second and third. *Parvimonas micra (Pm)* was most common in the second group (45.5%), with 25% in the first and 9.1% in the third. *Prevotella intermedia (Pi)* was absent at this time but was detected in 25% of cases in the first group during the third time point.

During the second measurement, no significant intergroup differences were observed. *Aa* was absent in the first group and present in one case in both the second and third. *Pg* appeared in one case each in the first and second groups. *Fn* had its highest prevalence (27.3%) in the third group. *Ef* increased in the first group to 75% and was found in 72.7% of cases in the second group, while remaining unchanged in the third. *Pm* prevalence stayed the same in the first and third groups but dropped significantly in the second (from 45.5% to 9.1%). *Pi* was not detected.

In the third measurement, bacterial prevalence again showed no statistically significant differences between groups. *Aa* was found in 25% of cases in the first group but unchanged in the other groups. *Pg* was absent in the first and third groups but stable in the second. *Fn* increased across all protocols compared to the second period, notably 37.5% in the first group. *Pe* levels remained stable between the second and third periods. *Ef* decreased in the third period, returning to levels seen initially, with a notable drop in the activation group (54.5% to 36.4%). *Pm* prevalence fell in the first group (25% to 12.5%), remained unchanged in the second, and rose in the third group to 18.2%. *Pi* was again found only in the first group (25%) (Table 2).

Overall, there were no statistically significant differences in total bacterial counts between protocols at any time point. Intra-group analysis showed significant reductions in bacterial counts from the first to second and first to third measurements in the second and third protocols. However, no significant changes occurred between the second and third periods in either group. Despite a rise in bacterial counts after the second measurement, the final levels remained significantly lower than at baseline (Figure 2).

DISCUSSION

Biofilms are resilient bacterial communities embedded in a matrix that protects them from host defenses and environmental stressors. Their ability to persist in root canals poses significant challenges during endodontic treatment. This study evaluated three irrigation protocols for biofilm elimination: (1) NaOCl with EDTA, (2) NaOCl with EDTA and CHX, and (3) NaOCl with EDTA and CHX with sonic agitation. Although no statistically significant differences were found among the protocols regarding bacterial prevalence, the second and third protocols reduced the total bacterial load more effectively. The analysis confirmed the presence of an intracanal microbial community characteristically associated with endodontic environments [12]. However, their prevalence remained largely unchanged under the irrigation protocols applied, confirming the strong resilience due to numerous virulence factors they possess [13].

Despite intervention, key pathogens including *E. faecalis* remained prevalent due to factors such as deep dentinal tubule invasion, biofilm maturity, and dentin demineralization, which can mineralize the matrix and reduce irrigation efficacy [14]. *E. faecalis*, a well-known endodontic pathogen [15], resists NaOCl and CHX in biofilms even though both irrigants are highly effective in suspension. High concentrations of NaOCl (5.25%) can eliminate *E. faecalis* rapidly in planktonic form, while CHX at 2% is comparably effective but requires longer contact in biofilms [16].

Combined NaOCl and CHX solutions demonstrated enhanced antimicrobial activity in suspension, but little is known about their synergistic impact on biofilms. In *ex vivo* models, *E. faecalis* penetrates dentin deeply, beyond the reach of conventional irrigants [17]. Agitation increases irrigant penetration, but may also drive bacteria further into tubules [18]. EDTA, though helpful in smear layer removal, has minimal antimicrobial action against *E. faecalis* [19].

Clinical studies report inconsistent *E. faecalis* prevalence, ranging from 14% to 92% [20]. Our study found a 53% initial prevalence. Notably, in groups 1 and 2, prevalence increased post-treatment, likely due to bacterial release from tubules during instrumentation [21]. This aligns with other studies showing recolonization even after confirmed eradication [20]. Group 3, with sonic activation, showed an 18% reduction after 48 hours, but differences between protocols were not statistically significant. This result aligns with findings from Brito et al., who reported comparable reductions in *E. faecalis* when comparing conventional irrigation to endo-activated methods [22].

F. nucleatum was the second most common species identified. Known for bridging oral biofilms and resisting environmental stress, its eradication is difficult. Found in 30% of initial samples, its prevalence was lower than in some literature, possibly due to antagonism with *E. faecalis* [23]. Although NaOCl and CHX reduce *F. nucleatum* in biofilms, complete eradication remains elusive, potentially due to buffering by tissue fluids or irrigant inactivation upon dentin contact. Its resurgence post-treatment may reflect recolonization [24].

P. endodontalis, another Gram-negative anaerobe, was present in 26.6% of cases—comparable to prior studies [25]. Post-treatment, its prevalence dropped significantly or was undetectable, with no further change after 48 hours, indicating good responsiveness to the protocols used.

P. gingivalis, a major oral and systemic pathogen, showed 20% prevalence. In protocol 3 (with sonic activation), it was completely undetectable after treatment, suggesting sonic agitation may help eliminate this species. In groups 1 and 2, prevalence decreased but persisted.

P. intermedia was undetectable initially and post-treatment across all protocols but emerged in group 1 after 48 hours. This reappearance may indicate recolonization due to instrumentation displacing bacteria [23,24].

The grouping of *P. endodontalis*, *P. gingivalis*, and *P. intermedia* is common due to similar antimicrobial sensitivity. In previous *in vivo* studies, both NaOCl and CHX significantly reduced these species, though CHX was slightly less effective [26]. Differences with our findings may result from varying concentrations and exposure times.

P. micra, an anaerobic Gram-positive bacterium, appeared in 23% of patients. Its reduction was most notable in group 2, suggesting greater CHX sensitivity. Literature supports this, indicating CHX produces a larger inhibition zone for *P. micra* than NaOCl [27].

A. actinomycetemcomitans was rare (10%) and inconsistently affected by irrigation. It reappeared post-treatment in group 1 but remained stable in groups 2 and 3. This pathogen is often found in periodontal infections and has limited interaction with other biofilm species.

Regarding overall bacterial load, protocols 2 and 3 showed significant reductions immediately post-treatment, with further reductions at the 48-hour mark. In contrast, protocol 1 showed possible recolonization. This suggests CHX's prolonged antimicrobial activity and the potential benefit of agitation [28].

While traditional syringe irrigation with NaOCl or CHX effectively reduces bacterial load, several studies, including ours, suggest CHX may offer superior bacterial reduction due to its substantivity and prolonged effect. Some *ex vivo* studies also indicate that sonic agitation enhances

NaOCl effectiveness, particularly in lateral canal areas where bacteria persist [29]. However, results remain mixed, and other studies found no difference between conventional and sonic irrigation [30]. Our findings suggest agitation does not consistently outperform manual irrigation in clinical settings. Nonetheless, enhanced performance may be achieved by optimizing irrigant chemistry, oscillation frequency, and duration, particularly in complex canal systems.

Several study limitations should be noted. First, sampling precision *in vivo* is constrained by root canal anatomy and the use of paper points, which may not reflect the true microbial composition. Additionally, bacteria residing deep in dentin cannot be accessed without more advanced, often impractical techniques. Second, sonic activation loses effectiveness when files contact the canal walls. Third, real-time PCR, while sensitive, only detects specific bacteria and total counts, limiting full microbiome characterization. Finally, ethical constraints precluded the use of negative controls like saline, which are standard in controlled experimental designs.

CONCLUSION

Traditional irrigants like NaOCl and CHX, whether used alone or combined, reduce bacterial prevalence but fail to achieve complete eradication. *E. faecalis* and *F. nucleatum* remain particularly resistant due to their ability to persist in biofilms and dentin. CHX appears more effective than NaOCl due to its longer-lasting effects. Sonic activation modestly improves results, though its benefits are not always statistically significant. Future strategies should focus on optimizing irrigant properties, improving agitation methods, and employing comprehensive molecular techniques to fully characterize and address endodontic biofilms.

Conflict of interest: None declared.

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Table 1. Primers used for PCR and their product sizes

Species	Sequence (5'-3')	Product size (bp)
Aggregatibacter actinomycetemcomitans	Fwd GCTAATACCGCGTAGAGTCGG Rv ATTTCACACCTCACTTAAAGGT	500
Porphyromonas gingivalis	Fwd AGGCAGCTTGCCATACTGCG Rv ACTGTTAGCAACTACCGATGT	404
Porphyromonas endodontalis	Fwd GCTGCAGCTCAACTGTAGTC Rv CCGCTTCATGTCACCATGTC	665
Prevotella intermedia	Fwd CGTGGACCAAAGATTCATCGGTGGA Rv CCGCTTTACTCCCCAACAAA	259
Fusobacterium nucleatum	Fwd ATGGTGGCTAAAAATTATAGT Rv ACCCTCACTTTGAGGATTATA	1000
Parvimonas micra	Fwd AGAGTTTGATCCTGGGCTCAG Rv ATATCATGCGATTCTGTGGTCTC	207
Enterococcus faecalis	Fwd TACTGACAAACCATTCATGATG Rv AACTTCGTCACCAACGCGAAC	112

Table 2. Prevalence of bacteria in each group at different time points

Time point	Bacteria	Irrigation Protocol 1: NaOCl + EDTA	Irrigation Protocol 2: NaOCl + EDTA + CHX	Irrigation Protocol 3: NaOCl + EDTA + CHX + activation	p
First	*Aa*	1 (12.5%)	1 (9.1%)	1 (9.1%)	0.963
	Pg	0 (0%)	2 (18.2%)	4 (36.4%)	0.145
	Fn	0 (0%)	5 (45.5%)	4 (36.4%)	0.087
	Pe	3 (37.5%)	2 (18.2%)	3 (27.3%)	0.642
	Ef	4 (50%)	6 (54.5%)	6 (54.5%)	0.976
	Pm	2 (25%)	5 (45.5%)	1 (9.1%)	0.155
	Pi	0 (0%)	0 (0%)	0 (0%)	N/A
Second	*Aa*	0 (0%)	1 (9.1%)	1 (9.1%)	0.677
	Pg	1 (12.5%)	1 (9.1%)	0 (0%)	0.515
	Fn	1 (12.5%)	1 (9.1%)	3 (27.3%)	0.485
	Pe	1 (12.5%)	0 (0%)	1 (9.1%)	0.515
	Ef	6 (75%)	8 (72.7%)	6 (54.5%)	0.560
	Pm	2 (25%)	1 (9.1%)	1 (9.1%)	0.526
	Pi	0 (0%)	0 (0%)	0 (0%)	N/A
Third	*Aa*	2 (25%)	1 (9.1%)	1 (9.1%)	0.526
	Pg	0 (0%)	1 (9.1%)	0 (0%)	0.409
	Fn	3 (37.5%)	5 (45.5%)	5 (45.5%)	0.927
	Pe	1 (12.5%)	0 (0%)	1 (9.1%)	0.515
	Ef	4 (50%)	6 (54.5%)	4 (36.4%)	0.677
	Pm	1 (12.5%)	1 (9.1%)	2 (18.2%)	0.819
	Pi	2 (25%)	0 (0%)	0 (0%)	0.053

EDTA – ethylenediaminetetraacetic acid; CHX – chlorhexidine; *Aa – Aggregatibacter actinomycetemcomitans*; *Pg – Porphyromonas gingivalis*; *Pe – Porphyromonas endodontalis*; *Pi – Prevotella intermedia*; *Fn – Fusobacterium nucleatum*; *Pm – Parvimonas micra*; *Ef – Enterococcus faecalis*

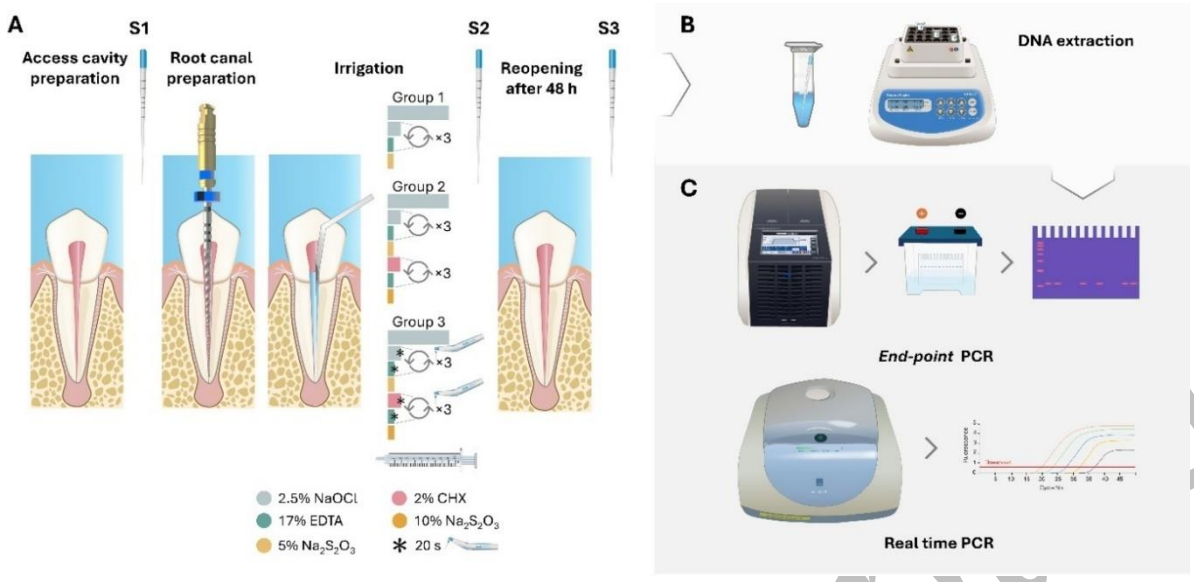

Figure 1. Experimental flow chart: A) sampling; B) DNA extraction; C) DNA amplification and visualization

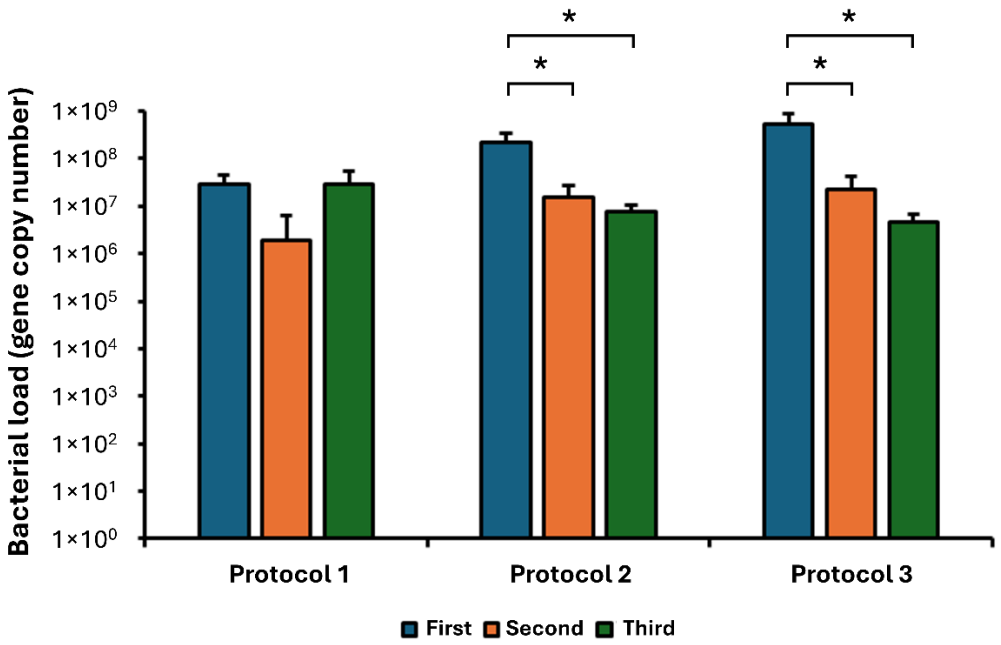

Figure 2. Total bacterial load (gene copy number) in different study groups assessed by real-time PCR;

*statistically significant ($p < 0.05$)

