

# Prevalence of *Enterococcus faecalis* and *Porphyromonas gingivalis* in Infected Root Canals and Their Susceptibility to Endodontic Treatment Procedures: A Molecular Study

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## SUMMARY

**Introduction** Because apical periodontitis is recognizably an infectious disease, elimination or reduction of intracanal bacteria is of utmost importance for optimum treatment outcome.

**Objective** The prevalence of *Enterococcus faecalis* and *Porphyromonas gingivalis* in infected root canals was studied. Also, the effect of endodontic therapy by using intracanal medicaments, calcium hydroxide paste (CH) or gutta-percha points containing calcium hydroxide (CH-GP) or chlorhexidine (CHX-GP) on these microorganisms was assessed by polymerase chain reaction (PCR) assay.

**Methods** Fifty-one patients with chronic apical periodontitis were randomly allocated in one of the following groups according to the intracanal medicament used: CH, CH-GP and CHX-GP group. Bacterial samples were taken upon access (S1), after chemomechanical instrumentation (S2) and after 15-day medication (S3). PCR assay was used to detect the presence of selected bacteria.

**Results** *E. faecalis* was detected in 49% (25/51) and *P. gingivalis* in 17.6% (9/51) of the samples. Samples which showed no bacterial presence at S1 were excluded from further analysis. Overall analysis of all 29 samples revealed significant differences between S1 and S2 ( $p < 0.001$ ), S2 and S3 ( $p < 0.05$ ), and S1 and S3 ( $p < 0.001$ ). When distinction was made between the intracanal medications, there was a significant difference in the number of PCR positive samples between S1 and S2, S1 and S3, but not between S2 and S3 samples.

**Conclusion** *E. faecalis* is more prevalent than *P. gingivalis* in primary endodontic infection. Intracanal medication in conduction with instrumentation and irrigation efficiently eliminates *E. faecalis* and *P. gingivalis* from infected root canals.

**Keywords:** antibacterial treatment; calcium hydroxide; chlorhexidine; medicated gutta-percha points; polymerase chain reaction

## INTRODUCTION

Chronic apical periodontitis is a lesion formed by the periradicular host defense system as response to microorganisms present in the root canal system [1]. Although no specific microorganism has been identified as the principal etiologic agent of pulpal and periapical pathosis, some species have been more frequently reported in the root canal space. Culture studies have shown that species of the genera *Eubacterium*, *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas* and *Prevotella* are commonly encountered in endodontic infection [1, 2]. Sensitive and accurate molecular biology techniques have provided significant additional knowledge regarding the composition of microbiota associated in root canal infection [2, 3, 4]. Consequently, some uncultivable and difficult-to-cultivate species have been detected in higher prevalence values in samples from the canal with pulp necrosis [2]. The prevalence of

some species in endodontic samples can significantly vary between patients from different locations [5, 6]. Nevertheless, there is only scarce information as to whether these variations are restricted to certain species or involve the whole profile of bacterial communities [7].

It has been confirmed that the presence of cultivable bacteria in the root canal at the time of final obturation plays a role in the failure of endodontic treatment [8]. Thus, the most important objective in the treatment of chronic apical periodontitis is complete elimination or at least maximal reduction of bacterial population from the root canal. Due to inability to achieve a complete disinfection even after vigorous chemomechanical instrumentation, antimicrobial intracanal medication has been suggested for application between therapy sessions [9, 10, 11]. Calcium hydroxide is most commonly used intracanal medicament [9, 10]. Even though it has been shown to exhibit potent antimicrobial effect on the majority of root

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canal bacteria, some microorganisms have proved to be resistant to calcium hydroxide [12]. So, alternative agents capable to predictably eliminate root canal bacteria have been investigated. Chlorhexidine is considered as an effective medicament against endodontic pathogens. It is a broad-spectrum antimicrobial agent with prolonged substantial effect. When used as interappointment dressing, chlorhexidine has been shown as more effective than calcium hydroxide in the elimination of *Enterococcus faecalis* [13], the species that has been implicated in the treatment failures.

Calcium hydroxide has been commonly used as a root canal dressing in the form of paste. However, complete removal of paste may be a challenge, raising concern about the potential influence of calcium hydroxide residue on the setting of root canal sealers [14]. To sidestep that potential difficulty, gutta-percha points impregnated with calcium hydroxide have been introduced as another means for calcium hydroxide delivery. Chlorhexidine containing gutta-percha points that allow easy introduction and retrieval from the root canal have been also marketed. Studies investigating antimicrobial activity of calcium hydroxide and chlorhexidine containing gutta-percha points have generated conflicting results and have been almost exclusively conducted *in vitro* [13, 15-18].

## OBJECTIVE

The purpose of this study was to investigate the prevalence of *E. faecalis* and *Porphyromonas gingivalis* in teeth with pulp necrosis and periradicular lesion and to evaluate the effects of endodontic therapy associated with calcium hydroxide paste or gutta-percha points containing calcium hydroxide or chlorhexidine as intracanal medicaments on these microorganisms by polymerase chain reaction (PCR).

## METHODS

### Patients and teeth

Fifty-one healthy patients (mean age 36.53 years, SD 12.97 years), range 18-76 years, 21 males, 30 females, who have been referred to the Endodontic Clinic of the Faculty of Medicine Foča, University of East Sarajevo, Bosnia and Herzegovina, for the treatment of chronic apical periodontitis were selected for a study according to the following criteria: asymptomatic single-rooted and single-canal teeth with necrotic pulps (as confirmed by negative response to the electric pulp test), and present radiographic evidence of chronic apical periodontitis. In all teeth the presence of periapical radiolucency was assessed using the periapical index (PAI), and teeth with PAI score equal to or greater than 3 were included. Teeth that could not be properly isolated with a rubber dam, had present periodontal pockets (>4 mm), crown or root fracture as well as retreatment cases and patients who received antibiotic therapy

during previous 6 months were excluded from the study. Only one tooth was included from each patient. Before treatment the teeth were randomly assigned into one of the following three groups (17 teeth per each group) according to the intracanal medicament used: calcium hydroxide paste (Calxyl; OCO Products, Dirnstein, Germany; CH group), calcium hydroxide containing gutta-percha points (Calcium hydroxide Plus, Roeko Langenau, Germany; CH-GP group) or chlorhexidine containing gutta-percha points (Active Point, Roeko, Langenau, Germany; CHX-GP group). The study was conducted in accordance with the World Medical Association Declaration of Helsinki of 1975 (revised in 1983) and had been approved by the Ethical Committee of the Faculty of Medicine Foča. The procedures and the purpose of the study were explained to all patients. Informed consents were obtained before entering the study.

### Endodontic treatment and root canal samples

Each tooth was polished with pumice and isolated with a rubber dam. The operative field including the tooth crown, rubber dam and clamps was disinfected with 30% hydrogen peroxide for 60 seconds followed by 2.5% sodium hypochlorite for additional 60 seconds [19]. Caries and/or coronal restorations removal and access cavity preparation were accomplished using sterile high-speed and low-speed burs under sterile saline irrigation. Before entering the pulp chamber, decontamination procedure was repeated in the same way as described above. Disinfecting agents were neutralized with 5% sodium thiosulphate solution and sterility of operative field was checked using sterile paper points. All of these samples were tested negative. The subsequent procedures were performed aseptically.

Upon the initial access into the root canal, the first sample was taken (S1). Three sterile paper points were sequentially introduced to the level approximately 1 mm shorter to the radiographic apex of the tooth and each was maintained in place for 1 min. If the root canal was dry, a small amount of sterile saline was introduced into the canal and a file was used to disperse the canal content. Paper points were transferred in sterile tubes containing 1 mL of RTF and immediately frozen at -20°C until they were further processed.

The working length was established using the apex locator (Raypex® 5, VDW, GmbH, Munich, Germany) and confirmed radiographically. The root canal was instrumented using step-back technique with K-type files and Gates-Glidden drills (both from Dentsply/Maillefer, Ballaigues, Switzerland) up to the apical size of at least 35 depending on both the initial size of the root canal and root anatomy. After each instrument change, the canal was irrigated with 2 mL of 1% sodium hypochlorite using a 27G needle. At the completion of instrumentation, the canal was dried with sterile paper points and any remaining sodium hypochlorite was inactivated with 5% sodium thiosulphate. The canal was rinsed with sterile saline solution and the second microbiological sample (S2) was

taken in the same manner as the first sample. To remove smear layer, the root canal was flushed with 17% EDTA followed by 5 mL of 1% sodium hypochlorite. After inactivation of sodium hypochlorite and drying the canal with sterile paper points, intracanal medicament was placed in the root canal. Calcium hydroxide paste was introduced into the canal using the Lentulo spiral and packed with a cotton pellet to the level of canal entrance. A medicated gutta-percha point was selected to the full working length and inserted into the canal with a drop of sterile water, according to the manufacturer's instructions. Following the placement of intracanal medicament, the access cavity was sealed with temporary filling (Cavit, 3M ESPE AG, Seefeld, Germany) and glass ionomer cement (Fuji IX, GC, Tokyo, Japan). A radiograph was taken to assure the proper placement of the medicament.

At the second appointment, 15 days later, a rubber dam was placed and the root canal was accessed respecting the strict aseptic protocol as previously described. Calcium hydroxide paste was removed using a master apical file and sterile saline irrigation while calcium hydroxide and chlorhexidine containing gutta-percha points were removed with tweezers. Neutralization of calcium hydroxide dressing (paste and gutta-percha point) was achieved with 2 mL of 0.5% citric acid. The root canal containing chlorhexidine gutta-percha point was rinsed with 2 mL of 3% Tween 80 and 0.3% L- $\alpha$ -lecithin to inactivate chlorhexidine. The canal was additionally flushed with sterile saline solution and the third, postmedication sample (S3) was obtained. Subsequently, the root canal was obturated with gutta-percha and AH Plus sealer (Dentsply, DeTrey, GmbH, Konstanz, Germany) using the cold lateral compaction technique. All teeth were treated by the same person, an endodontic specialist.

### Microbiological assessment

The detection of bacterial DNA was performed using the PCR assay (polymerase chain reaction). The extraction of potentially present bacterial DNA was performed by boiling the collected material at 100°C for 10 min followed by 5 min centrifugation in a microfuge (mini Spin, Eppendorf, Hamburg, Germany) to pellet the cell debris. Tested bacteria were detected by means of multiplex PCR using the following primers: Universal 16S rDNA forward primer *Escherichia coli* 5' AGA GTT TGA TCC TGG CTC AG

3' and species specific reverse primer 5' CAA TAC TCG TAT CGC CCG TTA TTC 3' for *Porphyromonas gingivalis*. Primers used for detection *Enterococcus faecalis* were: forward, 5' TACTGACAAACCATTCATGATG 3' and reverse, 5' AACTTCGTCACCAACGCGAAC 3'. The size of amplified products for *P. gingivalis* and *E. faecalis* were: 400 bp, and 110 bp, respectively. PCR was performed in volumes of 25  $\mu$ L containing PCR buffer (Fermentas, Vilnius, Lithuania), 0.2 mM of each dNTP, 0.2  $\mu$ M of each primer, 1U Taq DNA polymerase (Fermentas, Vilnius, Lithuania) and 3-5  $\mu$ L of template DNA containing supernatant.

The amplification was carried out in the thermal cycler (PCR Express, Thermo Hybaid, California, USA), and PCR reactions for analysis of *P. gingivalis* under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 5 min. For the detection of *E. faecalis* PCR cycle conditions were as follows: initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and final extension at 72°C for 5 min. In the negative control, DNA sample was replaced by distilled water.

The amplicons were visualized on 8% native polyacrylamide gel stained with ethidium bromide in an UV transilluminator.

### Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows (IBM Corp., Armonk, NY). The Fischer Exact test was used to evaluate reduction in the number of PCR positive samples from S1 to S3. The difference between groups at various sampling points was compared using the chi-square test. The level of significance in all analyses was set at 5% ( $p < 0.05$ ).

### RESULTS

Upon the initial entering into the root canal (S1 samples), 56.9% (29/51) of all cases were PCR positive. Teeth with no initial presence of bacteria being evaluated were excluded from further analysis (Table 1). Of the teeth that yielded tested bacteria at S1, 69% (20/29) were bacteria-free at S2. A significant reduction in the number of PCR positive canals from S1 to S2 was observed ( $p < 0.001$ ). All samples

**Table 1.** Incidence of positive PCR results for the tested bacterial species at various sampling points (S1, S2, S3)

Medicament*	n	Incidence of positive PCR results			p		
		S1†	S2 (%)	S3 (%)	S1:S2	S2:S3	S1:S3
CH	17	11	4 (36.4)	0 (0.0)	<0.01	ns	<0.001
CH-GP	17	8	2 (25.0)	1 (12.5)	<0.01	ns	<0.001
CHX-GP	17	10	3 (30.0)	0 (0.0)	<0.01	ns	<0.001
Total	51	29	9 (31.0)	1 (3.5)	<0.001	<0.05	<0.001

\* No significant difference in bacterial prevalence between the groups at S1, S2 and S3.

† 22 samples did not show initial bacterial presence, and they were excluded from the study.

S1 – initial sample; S2 – postinstrumentation sample; S3 – postmedication sample; CH – calcium hydroxide; CH-GP – calcium hydroxide containing gutta-percha; CHX-GP – chlorhexidine containing gutta-percha; p – statistical significance; ns – not significant

that were PCR positive at S2 were PCR negative at S3, except one sample in CH-GP group. With no distinction of the medication used, significant differences were found between S2 and S3 ( $p < 0.05$ ), and S1 and S3 ( $p < 0.001$ ).

Within the groups, reduction in the number of PCR positive S2 samples for 63.6% in CH, 75% in CH-GP and 70% in CHX-GP group as compared to S1 was observed. After 15-day medication, the number of positive PCR samples further decreased in all groups (Table 1). Intragroup analysis showed that the number of canals yielded significantly decreased tested bacteria from S1 to S2 ( $p < 0.01$  for each group) and from S1 to S3 ( $p < 0.001$  for each group), but not from S2 to S3 ( $p > 0.05$  for each group). Considering the intergroup comparisons, no significant differences were observed at the start of the treatment (S1), after chemomechanical preparation (S2) and intracanal medication (S3).

Regarding bacterial species, *E. faecalis* was found in 49% (25/51) and *P. gingivalis* in 17.6% (9/51) of S1 samples (Table 2). Postinstrumentation samples (S2) and post-medication samples (S3) contained *E. faecalis* only in 31% and 3.4% of cases, respectively.

## DISCUSSION

The present study evaluated the prevalence of *E. faecalis* and *P. gingivalis* in untreated teeth with asymptomatic chronic periradicular lesions. The antimicrobial effects of chemomechanical procedures and various intracanal medicaments were investigated as well.

Endodontic infections are mixed infections of polymicrobial etiology, with a predominance of obligate and facultative anaerobic species. In this study the occurrence of *E. faecalis* and *P. gingivalis* in a tooth with necrotic pulp and periradicular lesions was determined for several reasons. Data concerning molecular detection of these pathogens in primary endodontic infection are diverse [2, 3, 4, 20, 21, 22]. Also, some studies found that the prevalence of some species in infections of endodontic origin may significantly differ from one geographic location to another [5, 6], and no clinical studies have been performed in the Bosnian population detecting the presence of these two pathogens in primary infection. In addition to the gram positive bacteria, at least *P. gingivalis* has been reported for its ability to invade dentinal tubules [23]. Furthermore, facultative bacteria, such as *E. faecalis* have been considered one of the most resistant species in the oral cavity and a possible cause of root canal treatment failure. The effects of endodontic therapy associated with calcium hydroxide paste or medicated gutta-percha points as intracanal medicaments on these pathogens were assessed as well. To the best of our knowledge, no clinical study has investigated antibacterial effects of medicated gutta-percha points against *E. faecalis* and *P. gingivalis*.

Different methods are used for the detection and identification of endodontic microbiota, including cultivation of microorganisms and molecular techniques. Molecular methods have proved to be more rapid, more sensitive, and

**Table 2.** Number of PCR positive samples for the selected species at various sampling points (S1, S2, S3)

Medicament	Bacterial species	PCR positive samples		
		S1	S2	S3
CH	<i>E. faecalis</i>	8	4	
	<i>P. gingivalis</i>	6		
CH-GP	<i>E. faecalis</i>	7	2	1
	<i>P. gingivalis</i>	1		
CHX-GP	<i>E. faecalis</i>	10	3	
	<i>P. gingivalis</i>	2		

S1 – initial sample; S2 – postinstrumentation sample; S3 – postmedication sample; CH – calcium hydroxide; CH-GP – calcium hydroxide containing gutta-percha; CHX-GP – chlorhexidine containing gutta-percha

more accurate than culture and can provide more reliable results with regard to the composition of root canal microbiota and effects of endodontic treatment procedures. PCR assay has been increasingly used to identify endodontic bacteria in clinical samples and is able to detect culture difficult and even as-yet-uncultivated bacteria [2]; therefore it was used in the present study. One should also consider the fact that PCR is unable to differentiate living bacteria and DNA from nonviable or lysed cells, which is particularly relevant when effects of intracanal procedures are being evaluated. However, a previous study suggested that antimicrobial agents such as sodium hypochlorite and calcium hydroxide used during endodontic treatment might destroy DNA from dead cells [24].

Studies using culture methods have reported that *E. faecalis*, closely found in association with root-filled teeth, is present in very low numbers of the untreated canals [21, 25]. Culture-independent molecular studies also failed to isolate this species in higher prevalence from the primary infected root canals. Rôças et al. [20] and Fouad et al. [26] using standard PCR, and Sequeira et al. [4] by DNA-DNA hybridization detected this species in 18%, 14% and 14.3% of cases, respectively. In this study *E. faecalis* was found in about one-half (25/51) of the infected canals. Findings for this species is in line with results from several other recent molecular studies that demonstrate a high presence of *E. faecalis* in untreated root canals and its association with primary endodontic infection. Sassone et al. [3] were able to detect *E. faecalis* in 89.3% of cases using DNA-DNA hybridization, whereas Sedgley et al. [21] and Gomes et al. [25] revealed this species in 67.5% and 82% of cases by real-time PCR and PCR, respectively. It is apparent that prevalence data for *E. faecalis* are quite variable, and is probably influenced by the molecular method of the detection used in each investigation, as well as by existence of geography-dependent variation in the oral microbiota.

Molecular methods usually detected higher prevalence of black-pigmented bacteria in endodontic infection than the traditional culture methods [27]. Sassone et al. [3] and de Souza et al. [2] using DNA-DNA hybridization detected *P. gingivalis* in 67% and 75% of samples, respectively. In this study *P. gingivalis* was observed in 17.6% of patients by PCR, an estimate closer to those obtained by Siqueira et al. [4], who used the DNA-DNA hybridization method (17.9%), and Seol et al. [22], who used PCR (22.5%) to detect this species. Foschi et al. [28] found *P. gingivalis* in

13% of the samples from Italian patients using PCR, while Baumgartner et al. [6] found *P. gingivalis* in 72% and 30% of the endodontic abscesses from patients in the United States and Brazil, respectively. These results suggest that variation in the prevalence of *P. gingivalis* may be caused by the differences in clinical diagnosis, sampling method and sample analysis, as well as differences in the endodontic microbiota and several host and environmental factors, such as genetic background, socioeconomic status, psychological stress, smoking, and the nature of the species colonizing other individuals in the same country [5, 6].

Sodium hypochlorite is most widely used root canal solution because of its pronounced antimicrobial activity and tissue dissolving capacity. However, no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25%. In this study the 1% of sodium hypochlorite solution was selected because it provides efficient antimicrobial action with acceptable cytotoxic level [29]. A substantial reduction in the prevalence rate of selected species was observed after irrigation with 1% sodium hypochlorite (S2). This finding is in agreement with previous studies [10, 30], confirming the essential role of chemomechanical procedures in eliminating root canal microbiota. *P. gingivalis* was completely eliminated, corroborating the results from other studies that found gram-negative bacteria usually eliminated after the chemomechanical debridement [24, 30]. However, 17.6% of cases still yielded *E. faecalis*. It has been shown that irrespective of concentration used, sodium hypochlorite is highly effective in eliminating *E. faecalis* including its existence as a biofilm in vitro [31]. For instance, 1% sodium hypochlorite eliminated *E. faecalis* to levels below the detection after contact time ranging from 10 to 30 min [32, 33]; the time that does not exceed the average time usually spent for chemomechanical preparation. Nevertheless, its clinical efficacy may be influenced by the complexity of root canal anatomy and inability of sodium hypochlorite to penetrate into confined areas of the root canal. In addition, the interaction of sodium hypochlorite with tissue fluids, blood, dentine and other organic debris inactivates irrigant and reduces its antibacterial capacity. This may explain the persistence of *E. faecalis* after chemomechanical preparation in the present study.

An interappointment medication has been recommended to supplement antibacterial effect of chemomechanical procedures and eliminate residual bacteria [9, 11]. In our study, the placement of either CH paste, CH-GP or CHX-GP as intracanal dressing successfully eliminated *E. faecalis* (except in one sample of CHX-GP group) but these reductions did not reach statistical significance when compared to the samples obtained after the chemomechanical procedure (S2). Low prevalence of tested species in root canals before medication as well as a small sample size in each group may have influenced the results. Because the bacterial reduction was similar in all treatment protocols, ranging from 87.5% to 100%, a study with a large sample size than the present study would be required. To compensate for this shortcoming, we performed power calculation to determine the number of samples required

in order to reach significance for the observed differences. A sample of 125 teeth per group would have resulted in a significant difference between the groups at the 5% level and 80% power. Since this number is difficult to achieve in a prospective, controlled studies, with stricter inclusion/exclusion criteria for selection of study population, our finding may still be clinically relevant.

Although the observed difference between S2 and S3 within the groups was not significant, decrease in bacterial prevalence from S2 to S3 in overall sample highlights the importance of intracanal medication after chemomechanical procedures to predictably control the root canal infection. Calcium hydroxide is effective in killing the majority of bacteria in the root canal system; however, some controversies exist about its effectiveness against *E. faecalis* [12]. Several theories have been proposed to explain the survival of *E. faecalis* after treatment with calcium hydroxide [12, 34]. It has been shown that *E. faecalis* has ability to maintain pH homeostasis passively and actively by proton pump [34]. Moreover, it can survive harsh environment, including a high pH value of 11.5, and is capable of penetrating into the dentine tubules and escaping from the effective concentration of medication. Furthermore, the buffering effects of dentine may not allow a sufficiently high pH to be achieved in the dentine tubules [35]. Our findings are in accordance with studies done by Sjögren et al. [9] and de Souza et al. [2] who confirmed the effectiveness of calcium hydroxide paste against *E. faecalis*. Considering antibacterial activity of intracanal medicament vehicle may have a significant impact affecting both physical and chemical properties of carrying compounds. Some in vitro studies have shown that CH-GP may not be the effective delivery system of calcium hydroxide [16, 17]. This difference may be explained by a lower hydroxyl-releasing potential of medicated points in comparison to calcium hydroxide paste. In addition, CH-GP may not act as a good physical and chemical barrier as calcium hydroxide paste. However, findings from the present study demonstrate that, in addition to the chemomechanical instrumentation, intracanal medication with calcium hydroxide, despite the vehicle, efficiently eliminates *E. faecalis* from infected root canals.

In response to the ambivalent efficacy of calcium hydroxide, chlorhexidine has been proposed as alternative intracanal medicament that can be delivered in a variety of vehicles. In the present study CHX-GP was effective in eliminating *E. faecalis*, corroborating the results of previous in vitro studies. Namely, a complete elimination of *E. faecalis* was observed in simulated root canals after 5 hours [16] and in the infected dentine tubules up to 500  $\mu\text{m}$  [15] after treatment with CHX-GP. When compared to calcium hydroxide based preparation (paste or gutta-percha points), CHX-GP exhibited similar antibacterial effect against *E. faecalis*. Although some studies reported CHX-GP as more effective intracanal medicament than calcium hydroxide [13, 16, 17], our results are in agreement with the results reported by Oztan et al. [18]. The factors complicating the comparison are related to the fact that all abovementioned studies were conducted in vitro and used different methods for pathogens detection.

## CONCLUSION

Within the limitations of the study, the obtained results showed that *E. faecalis* is more prevalent than *P. gingivalis* in untreated teeth with asymptomatic chronic periradicular lesions. In addition, intracanal medication in

conduction with instrumentation and irrigation efficiently eliminate *E. faecalis* and *P. gingivalis* from infected root canals. Further investigation should be conducted to elucidate antimicrobial potential of medicated points against a wider range of root canal bacteria in clinical condition.

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## Преваленција бактерија *Enterococcus faecalis* и *Porphyromonas gingivalis* у инфицираним каналима корена зуба и њихова осетљивост на ендодонтско лечење: молекуларна студија

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### КРАТАК САДРЖАЈ

**Увод** Будући да је апексни пародонтитис обољење које настаје услед постојања инфекције, за постизање оптималног исхода лечења најзначајније је уклањање бактерија из канала корена зуба или барем смањење њиховог броја.

**Циљ рада** Циљ овог рада је био да се испита преваленција бактерија *Enterococcus faecalis* и *Porphyromonas gingivalis* у инфицираним каналима корена зуба и установи ефекат ендодонтског лечења применом интраканалних медикамента – калцијум-хидроксидне суспензије (CH), гутаперка-поена на бази калцијум-хидроксида (CH-GP) или гутаперка-поена на бази хлорхексидина (CHX-GP) – на ове микроорганизме методом ланчане реакције полимеризације (PCR).

**Методе рада** Истраживањем је обухваћена 51 особа која је имала зуб с хроничним апексним пародонтитисом. Испитаници су методом случајног узорка сврстани у три групе у зависности од врсте примењеног интраканалног медикамента (CH, CH-GP и CHX-GP). Бактеријски узорци из канала корена сакупљани су при иницијалном уласку у ка-

нал корена (S1), после хемомеханичке обраде (S2) и после петнаестодневног лечења (S3). PCR анализа је коришћена за одређивање присуства испитиваних бактерија.

**Резултати** *E. faecalis* је изолован из 25 (49%) узорака, а *P. gingivalis* из девет (17,6%). Узорци у којима нису нађене бактерије у S1 фази искључени су из даље анализе. Анализа свих 29 узорака је указала на статистички значајну разлику између S1 и S2 узорака ( $p < 0,001$ ), S2 и S3 ( $p < 0,05$ ) и S1 и S3 ( $p < 0,001$ ). Када се узме у обзир врста интраканалног медикамента, статистички значајна разлика у броју PCR-позитивних узорака забележена је између S1 и S2, S1 и S3, али не и између S2 и S3.

**Закључак** У примарној ендодонтској инфекцији *E. faecalis* се чешће јавља од *P. gingivalis*. Интраканална медикација заједно с инструментацијом и иригацијом ефикасно уклања *E. faecalis* и *P. gingivalis* из инфицираних канала корена.

**Кључне речи:** антибактеријски третман; калцијум-хидроксид; хлорхексидин; медиковани гутаперка-поени; ланчана реакција полимеризације (PCR)

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